2020-2021

Compilation of COVID19 Publication of CCRS

Central Council for Research in Siddha
Ministry of AYUSH, Govt. of India
Arumbakkam, Chennai 106.

केंद्रीय सिद्ध अनुसन्धान संस्थान

आयुष मंत्रालय, भारत सरकार

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22nd December, 2021

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MESSAGE

Central Council for Research in Siddha (CCRS) is the apex body pertaining to research in Siddha system of Medicine. CCRS is working towards the scientific validation of Siddha System of Medicine through clinical research, drug research, medicinal plants research, fundamental research, literary research and documentation.

CCRS is carrying out its research activities and health care services mainly through 8 peripheral Institutes/Units – 3 located in Tamil Nadu, one each in Puducherry, Kerala, Karnataka, Andhra Pradesh and New Delhi. CCRS is currently carrying out 33 Intra-mural Research (IMR) Projects funded by Ministry of AYUSH.

CCRS has more than 1000 palm leaf manuscripts and involved in decoding the formulations and other contents and publishing as books. CCRS is also publishing rare Siddha literatures, palm leaf manuscripts and till now 50 books have been published. The research outcomes are converted into intellectual property rights through patents and publications in peer reviewed journals

The importance of evidence based publications reaches the peer group and fellow scientists in a bigger way. Peer reviewed publications help us to overcome the shortcomings and enable us to potray better science and thereby help to improve the contribution of Scientists. Covid19 Pandemic has created a lot of Chance for the scientist world wide to publish their findings in a reliable manner. It is the pride of CCRS that every scientist has utilised this opportunity to publish and that too in International Peer reviewed Journals. The range of publications extend from Insilico to Computer modelling, Case Studies to RCTs, Review to Repurposing. All studies followed GCP and Ethics in a proper manner. This compilation will give a chance to the reader the different dimensions of research Happened in Siddha during Covid Pandemic. Congratulations to all Contributors.

(Prof. Dr. K. Kanakavalli)

Catalogue	
S.No.	Contents
I	LITERATURE REVIEW
1.	Analogy of Kaba Suram with COVID-19 Symptoms - A Siddha Literature Review
2.	A Perspective Review on Siddha System of Medicine in the Management of Corona Virus Disease 2019
3.	Pharmacological Actions of Contents of Kabasura Kudineer- A Siddha Formulation for Fever with Respiratory Illness
4.	Prevention of COVID 19 - Siddha perspective
II	IN-SILICO STUDIES
5.	In Silico computational screening of Kabasura Kudineer - Official Siddha Formulation and JACOM against SARS-CoV-2 spike protein
6.	Repurposing of Medicinal Plants Used in Siddha Formulations As Potential Protease Inhibitors of COVID-19: An in silico Approach
7.	In Silico Docking Analysis of Poly Herbal Formulation Adathodai Kudineer used in Siddha medicine in inhibiting Main Protease and ACE 2 Receptor Spike protein SARS-CoV-2
8.	Siddha Medicine and Computer Modelling: A Treasure for SARS-CoV-2 Treatment – Book chapter
9.	Molecular Docking Studies of Phytoconstituents Identified in Traditional Siddha Polyherbal Formulations Against Possible Targets of SARS-CoV-2
III	PRE-CLINICAL STUDIES
10.	In vitro antiviral activity of Kabasura Kudineer - Siddha polyherbal Formulation against novel coronavirus (SARS-CoV-2)
11.	In-Vitro Immunomodulatory Activity and Thrombolytic Potential of Kabasura Kudineer (KSK), an Official Siddha Polyherbal Formulation
12.	Antioxidative potential of Kabasura Kudineer (KSK), an official Siddha polyherbal formulation.
IV	CLINICAL STUDIES
13.	Survival analysis to assess the length of stay of novel coronavirus (COVID-19) patients under Integrated Medicine - Zinc, Vitamin C & Kabasura Kudineer (ZVcKK)
14.	The efficacy of Siddha Medicine, Kabasura Kudineer (KSK) compared to Vitamin C & Zinc (CZ) supplementation in the management of asymptomatic COVID-19 cases: A structured summary of a study protocol for a randomised controlled trial
15.	A double blinded placebo controlled comparative clinical trial to evaluate the effectiveness of Siddha medicines, Kaba Sura Kudineer (KSK) & Nilavembu Kudineer (NVK) along with standard Allopathy treatment in the

Published online in http://ijam.co.in ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 11 (4), 616-621

Analogy of *Kaba Suram* with COVID-19 Symptoms - A Siddha Literature Review

Review Article

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Abstract

Kaba Suram (phlegmatic fever) is one among the 64 types of *Suram* (Pyrexia/fever), its synonyms, causes, pathology and characteristics have been mentioned crystal clear in Siddha literature. Its clinical features have been mentioned in few literatures such as *Theran karisal*, *Suravagadam*, *Yugi chinthamani* etc., which are almost similar to each other. The present day pandemic disease COVID-19 which still lacks specific drug towards its management or prophylaxis has become the spectrum of research focus in current scenario. Upon keen understanding of the Siddha literature the signs and symptoms of *Kaba Suram* fall in line with COVID-19 and a few other flu like illnesses. Therefore, the herbal decoction *Kaba Sura Kudineer* (KSK) indicated for the management of *Kaba Suram* has been recommended by the Siddha research community towards an integrated approach in tackling this worldwide emergency. The current scientific community demands evidence based explanation to understand the concept of Siddha system of medicine and necessitates International standards to reassure the efficacy of Siddha medicine. In this aspect, this review is aimed at evaluating all the available informations on *Kaba Suram* in Siddha literature and pathophysiology of COVID-19 and to interpret the search results in terms of pathophysiology of *Kaba Suram* based on *Mukkutram theory.* This effort would therefore enable the Siddha research community to opt for effective Siddha medicines to manage the present pandemic situation.

Key Words: *Corona Virus, COVID-19, Traditional medicine, Indian Medicine, Kaba suram.*

Introduction

The Siddha system of medicine is one among the Indigenous medical systems predominantly practiced in South India. The literature of Siddha describes 4448 diseases which can affect the human (Siddha classical text Agathiyar irantaayiram)(1). According to Siddha science, the three subtle entities called *Vali* (*Vatham)*, *Azhal* (*Pitham),* and *Aiyam* (*Kabam)* are said to be the three vital life forces / functional units of the human body. These units are responsible for the general health and well being of an individual when they are present in the ratio of 1:½:¼ respectively(2). Consumption of unhealthy food pattern and habits can alter the ratio of these functional units leading to various ailments (Imbalance of the vital life forces). *Suram* (Pyrexia/fever) is considered as one of the diseases in Siddha literature which has been classified into 64 types. The causes of *Suram* are categorized based on the affected *Mukkuttram* (*Vatham, Pitham* and *Kabam*) by means of unhealthy diet and habits.

According to Siddha concept, the etio-pathogenesis of *Suram* (Pyrexia/fever) is due to the formation of *Amam* (indigested metabolic products) in the intestine favoured by the aggravated *Kabam* humour. This fact has been emphasized in the Siddha literature as *Kudal thannil seetham allaathu Suram varaathu*(3). Though unhealthy food habits have been mentioned as a general cause of *Suram* in majority of selected literatures, it has also included a few practical issues of life towards the etiopathogenesis of fever in common. They are wrong behaviour/ habits, Exposure to excessive and polluted air, walking for a long distance/running hardly, excessive work strain, excessive sexual indulgence, vitiated *Vathathodam*, fear and worries, insomnia, anger and other bad emotions, vitiated *Kabathodam*, wandering in mist and excessive chillness, sensation of coldness due to getting wet in rain or bathing for a long time, indigestion and constipation(3-5). The Siddha concept portray that *Vatham* humour is responsible for conception and creation, *Pitham* for counter action against diseases and *Kabam* for annihilation which is emphasized in the lines of Siddhar *Theran* as "*Vaathamai padaithu pitha vanniyai kaathu sethuma seethamai thudaithu.*"

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Infections and disease progression occur when an individual's immunity is challenged. This immune challenge could be connected with decrease of *Pitham*. The preliminary symptoms of COVID-19 such as fever, dry cough, sore throat might be overcome if there is acceptable measure of immunity when *Pitham* humour is energetic. If not the *Kaba Thodam* raises and further progresses to a critical stage *Sanni* (Delirium)(3).This literature search would provide information regarding disease staging, prognosis and crucial assessment to choose effective *Siddha* medicines based on altered humour and aid in the management of COVID-19. This literature analysis has been focused at analyzing, summarizing and correlating all the available informations on *Kaba Suram* in Siddha literature with that of the various symptoms and pathophysiology of COVID-19 and to derive the Siddha pathophysiology of *Kaba Suram* based on *Mukkutram theory*.

The relevant *Siddha* literatures which has mentioned about the *Suram* were searched from the Library Unit of Siddha Medicine. Siddha Classical books such as *Yugi Vaithya Sintamani, Noi Nadal Noi Muthal Nadal Thiratu (Rendam Paagam), Siddha Maruthuvam (Pothu), Pararasasekaram Sura Roga Nithanam, Theran karisal, Theran yamaka venpa* were identified and collected for this study. Web based search was also carried out using biomedical data bases such as Scopus, Embase, Elseivier, medline, web of science , Google scholar etc. All data were collected, tabulated and analysed for analogy with the symptoms of COVID-19. A flow chart based on the *Kaba Suram* symptoms which parallel with COVID-19 stages was formulated and the *Mukkuttram* (*Trithodam*) based patho physiology was concluded.

Kabam and Kaba Suram

Normally, the predominant seats of *Kabam* in human body has also been described in literature as the region of thorax above the heart, head, tongue, tonsils, fat, cartilage, blood, nose, chest, nerves, brain, eyes, joints, intestine and semen.(6) *Kabam* provides support and substance to the body. It structures everything the musculo-skeletal frame both physical and psychological, and governs human emotions such as love, compassion, forgiveness, loyalty, and patience. *Kabam* can bestow immunity against disease and can act to restrict the two forces *Vatham* and *Pihtam* and prevent their excessive manifestation. Any abnormal increase in humour of *Kabam* would result in aggravated *Kaba thodam* (*Thannilai Valarchi*) in the regions of *Kabam* predominance in the body resulting in ailments. Since Siddha system of medicine deals with the root cause of diseases, the present study *Kaba Suram* is said to occur due to the aggravated humour of *Kabam* much more than its normalcy, facilitating a favourable environment for any respiratory infection(1,7,4). The aggravated humour of Kabam lodges in other parts of the body other than its own seats (*Vaetrunilai Valarchi*).

The above mentioned reasons increases *Kabam* which fluctuates *Kledaka Kabam* and results in *Amam* and fever. This *Amam* is absorbed by the intestinal vessels and distributed all over the body and obstructs the excretion of sweat resulting in fever. Thus the formation of *Amam* is the main reason for increasing the body temperature. The common and major symptoms of *Kaba Suram* mentioned in all the texts are body ache, fever, cough, fatigue, diarrhoea, sore throat, shortness of breath, chest pain and sometimes leading to neurological symptoms like confusion and even death(2).

End stage of *Kaba Suram* (*Sanni* - Delirious stage)

The end stage symptoms of *Kaba Suram* occur due to vitiation of the *Mukkuttram* (*Vatham, Pitham* and *Kabam*) (the abnormal physiological state). In general the vitiation of *Vatham*, *Pitham* and *Kabam* either singly, dually, or triply results in diseases. In *Kaba Suram* the end-stage features such as altered mental status, impaired consciousness, irrelevant speech, emotional instability, agitations etc., is said to occur on the derangement of all the three humours ie *Vali*, *Azhal* and *Aiyam*. This stage is termed as *Sanni* (Delirium) in which quantitative/qualitative changes in *Mukkuttram* takes place and the nomenclature is made accordingly based on the degree of the involvement of each dosha and the dominant dosha. Therefore the conglomeration of vitiated *Mukkuttram* is termed as final stage of disease and the presenting illness becomes more advance and mostly irreversible (3).

Seasonal variation

The Siddha literature also predicts the framework of seasons and the probability of disease occurrence on earth. As the earth resolves around the Sun it gets sunlight at various positions. With reference to the position of the earth towards the Sun, each year is divided into six seasons. They are,

1. *Karkaalam –Avani, Puratasi* (August, September)
2. *Koothirkaalam – Iyppasi, Karthigai* (October, November)
3. *Munpanikaalam –Margazhi, Thai* (December, January)
4. *Pinpanikaalam –Masi, Panguni* (February, March)
5. *Elavenilkaalam –Chithirai, Vaigasi* (April, May)
6. *Mudhuvenilkaalam –Aani, Aadi* (June, July).

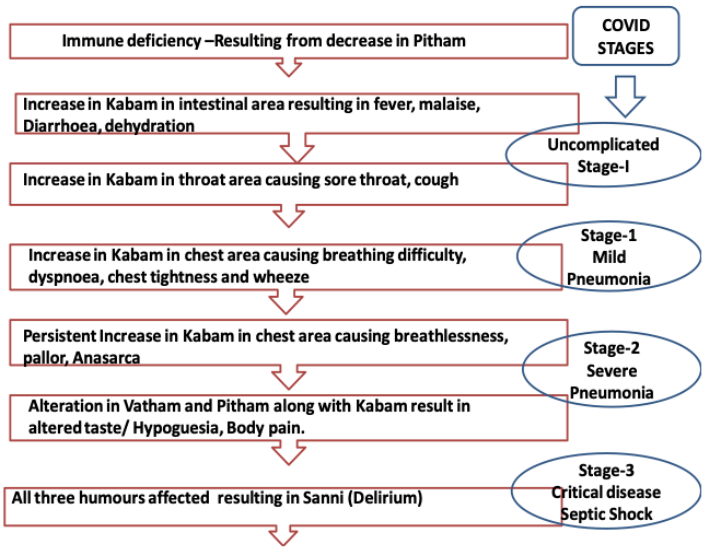
Kaba Suram mainly occurs due to variation of *Kabam*. The period of seasonal aggravation in *Kabam* is called as *Kabam thannilai sirapurum kaalam* which exist during the Tamil months *Karthigai* to *Masi* (November to February)(8).

Literature review on *Kaba Suram*(3).

Table-1. Classification of symptoms of *Kaba Suram* according to COVID-19 Stages

S.No	Symptoms of *Kaba Suram* in Tamil Poetic lines	Analogy with symptoms of various stages of Covid-19
Uncomplicated and mild pneumonia symptoms (COVID-19 stage-1)		
2	*Thondai nothal, irumal, ilaippu untaathal, kozhai kakkal*	Symptoms of sore throat, cough, expectoration of phlegm, wheeze due to upper and lower respiratory tract infections.(11,12)
3	*Thalarchi, udal vanmai kuraidhal, adikkadi kalaithu podhal*	Fatigue - a common symptom associated with respiratory illness in COVID-19. (13)
4	*Malamum neerum kazhidhal,Vayiru kazhidhal.*	ACE2 in modulating intestinal inflammation causing diarrhoea.(14)
5	*Neer vetkai*	Dehydration and thirst commonly recognized causes of dehydration may be due to fever and diarrhoea. (15)
Severe Pneumonia (COVID-19 Stage-2)		
6	*Mael moochu, moochu thinaral, peru moochu vidal*	Breathing difficulty with Diffuse alveolar damage with cellular fibromyxoid exudates, desquamation of pneumocytes and hyaline membrane formation.(16)
7	*Naakku mugam veluthal*	Pallor of tongue, face due to hypoxemia and dyspnoea causing low oxygen saturation.(17)
8	*Oon kollaamai, thookkaminmai*	Poor sleep, loss of appetite may be due to many causes, including cough, excess mucous production, and frequent arousals from sleep caused by hypercapnia.(18)
9	*Vali, udal nonthu*	General muscle pain and fatigue are common symptoms of COVID-9.(19,20)
Critical Disease - sepsis, Neurological deficits (COVID-19 STAGE-3)		
10	*Viyarvai*	Sweating and vasodilatation due to fever and hypercapnia.(21)
11	*Achathaiyum manavetrumaiyaiyum mugathiluntaakkum, kaadhu yiraithal, kaadhu kaelaamai*	Fear and depression (Emotional instability) due to delirium.(17) Hearing loss and ringing of ears due to neurological deficits.(22)
12	*Athatti paesuthal, serukku, Vaai kularal,Nenju ilaguthal, mamam thuvalal, epporulilum veruppadaithal*	Irrelevant speech a neurological symptom due to replication of the virus in the brain.(23)
13	*Maarbu nothal*	Chest pain or tightness due to myocardial injury resulted due to expression of ACE2 in cardiac muscles(24)
14	*Vikkal*	Hiccups may be associated with COVID- 19 pneumonia.(25)
15	*Naachuvaiyariyaamai*	Loss of smell, taste, ataxia and convulsions due to neurological deficits like have been reported in COVID-19. (23),(26)
16	*Muppini(sanni noi) varuvikkum. Appothu mandaiyil miguntha soodu, vaai pithatral, thannilai ariyaamai undaagum*	Delirious stage exhibiting disoriented speech, impaired consciousness due to invasion of SARS-CoV to some specific brain areas, including the thalamus and brainstem.(26)
17	*Udal veenguthal*	Anasarca due to renal impairment with reduced urine output at sepsis stage of COVID-19.(27)

Fig-1. Parallel analysis of humoral pathology of *Kaba Suram* and stages of COVID-19



**Vatham*, *Pitham* and *Kabam* - The three humours are responsible for all the diseases according to the Siddha Pathology. Stepwise alteration of humours have been evaluated and correlated with the stages of COVID-19. (3).

Parallel analysis of humoral pathology of *Kaba Suram* and stages of COVID-19

Based on the severity of the disease, the Chinese CDC report divided the clinical manifestations of the COVID-19 as mild, moderate and severe(27).

Uncomplicated Mild disease (COVID-STAGE-1)

About 81% of COVID-19 cases are of mild symptoms which may contribute as non-pneumonia and mild pneumonia(27). Patients with mild COVID-19 symptoms present with mild fever, sore throat, dry cough, head ache and muscle pain(17). The Siddha literature on *Kaba Suram* has also described these symptoms in its Tamil linguistic purism such as *Suram* (Fever), *Thondai nothal* (Sore throat) , *Irumal* (Cough), *Kozhai kakkal* (Expectoration).

Kaḷaippu (Malaise), vali (Muscle pain), *Vayiru kazhidhal* (diarrhoea) in its literature *Suravaagadam* (Table-1). Recent researches on COVID-19 suggests that fever with headaches are often the major and initial symptom that may occur early in COVID-19 patients(11,12). The Siddha pathophysiology substantiates this fact, according to which, the *Kabam* is said to be the foremost humor responsible for fever (*Kudal thannil seethamalaathu suram varaathu*) which is exhibited on the exterior due to the core pathology(3).

A study conducted in 2019 December also revealed that 98% of patients had symptoms of fever, 76% had dry cough, 55% were presented with dyspnoea, and 3% had gastrointestinal symptoms of diarrhoea(28). The increase in *Kaba* humour is manifested in throat area referred in literature as (*Kandam*) resulting in *Thondai nothal* (Sore throat), *Irumal* (Cough), *Kozhai kakkal* (Expectoration) which are also the symptoms of uncomplicated stage of COVID-19(12,13). Though the symptoms of dyspnoea, and non-respiratory symptoms such as diarrhoea can be rarely observed at the initial stage, however 10% of patients have been found to present with gastrointestinal symptoms initially (e.g. diarrhoea, nausea), before the manifestation of fever and dyspnoea(29).The event of diarrhoea occurs due to impaired protective immune response which paves way for the entry of infection especially in organs that have high Angiotensin-converting enzyme 2 gene (ACE2) expression. This causes inflammation in digestive tract resulting in diarrhoea(14). This fact highly emphasizes the Siddha pathophysiological concept of aggravation of *kaba humour* in the intestine (*Kudal thannil seetham*) which is the prime cause of fever and the step wise disease progression.

Mild Pneumonia

A Taiwan case study on the first COVID-19 subject revealed the symptoms of fever, dyspnoea and mild hypoxemia on 10th day of illness and the X-ray chest indicated invading pneumonia, which advanced diffusely later. This study was additionally in comparative with the COVID-19 case study of United states with similar characteristics on day 9 of disease. Both these cases had mild manifestations of Pneumonia(30). This stage can be compared with the symptoms of increase of *Kabam* in chest area causing *Mael moochu, Moochu thinaral* (Dyspnoea), *Oon kollaamai* (loss of appetite), *Thookkaminmai* (Insomnia), *Kalaitu pothal* (fatigue) mentioned in the texts *Theran karisal* and *Suravaagadam* (Table-1). The Siddha texts emphasize even the minor symptoms concerned with mild respiratory discomforts that may accompany mild pneumonic stage. Poor sleep complaints are normal in individuals with chronic respiratory illness which may be due to incessant cough, mucous production, and frequent sleep arousals due to hypercapnia(18). Previous research studies have discovered that the Viral flu infection can prompt rhabdomyolysis which might be the reason for general exhaustion and myalgia in COVID-19(19).

Severe Pneumonia (Stage-II)

This severe stage of pneumonia is associated with severe dyspnoea, tachypnoea, respiratory distress, and hypoxia followed by Acute Respiratory Distress Syndrome (ARDS). Notwithstanding, the indication of fever can be moderate or even missing. This stage can be suggestive of respiratory failure which is associated with pulmonary edema. In Sidddha literature on Kaba Suram,this severe stage of COVID-19 can be well correlated with the persistent severe increase of *Kabam* in chest area resulting in *Mael moochu* (Severe dyspnoea), *Naakku mugam veluthal* (Pallor of tongue and face) as indicated in the text *Yugimunivar vaithya chintamani*, *viyarvai* (profuse sweat), uṭal vīṅkutal (Anasarca) as mentioned in *Theran Karisal* (Table-1). *Viyarvai* (profuse sweat) can be produced due to high body temperature(22). Hypercapnia may lead to a lower diffusion of carbon dioxide leading to excessive sweating and vasodilatation (33). The reason behind Pallor of tongue and face as mentioned in Siddha texts may be due to ARDS which is characterized by poor oxygenation resulting on hypoxemia and may also present as central or peripheral cyanosis as a result of hypoxemia. *Udal veenguthal* (Anasarca) as mentioned in *Theran Karisal* can occur due to pulmonary edema, renal impairment resulting in reduced output(18).

Critical stage (Stage-III)

The exaggerated inflammatory response induced by infecting organisms results in critical stage of sepsis. At this stage excessive uncontrolled release of proinflammatory cytokines known as Cytokine storm (CS) plays an important role in the pathogenesis leading to multi organ failure and death(31,32). The patients experience a decrease in oxygen saturation and inflammation-induced lung injury followed by sepsis which is the crucial phase of the disease. The clinical symptoms of COVID-19 at sepsis stage are dyspnoea, hypoxemia, tachycardia, adjusted mental status, hyperbilirubinemia, acidosis, high lactate, coagulopathy,

and thrombocytopenia. This situation, is related with high mortality, circulatory, and cell/metabolic variations(29) These features of sepsis described in conventional literature in which there is drastic release of proinflammatory mediators causing multiorgan failure and sepsis has been termed as *Sanni* (Delirious stage) in Siddha texts alerted as the final stage of *Kaba suram*. At this stage all the three humours *Vatham, Pitham* and *Kabam* which are

responsible for the well being of an individual is drastically affected resulting in symptoms of *Acham, Manavetrumai* (Emotional disturbances of fear and depression), *Athatti pesuthal, Vaay Kularal* (Irrelevant speech, agitation), *Thannilai ariyaamai* (Impaired consciousness), *Muppini* (*sanni noi*) (Delirious stage due to neurological disorders/brain infections) and *Vikkal* (Hiccups) (Table-1). *Vikkal* (Hiccups) though seems like a minor symptom, may be the main presenting symptom of pneumonia.(25). Neurological disorders in COVID-19 has been analysed and concluded through previous research studies. According to these studies, the infection of olfactory neurons in the nose may enable the virus to spread from the respiratory tract to the brain. Infection of endothelial cells may allow the virus to pass from the respiratory tract to the blood and then across the blood-brain barrier into the brain and replication of the virus in brain may cause neurological disorders(23).

Besides these, the Siddha literature on *Kaba Suram* highlights even the subtle symptoms of COVID-19 as *Vaay Thuvarthal, Naachuvai ariyaamai* (loss of smell/altered taste) and *kaadhu iraidhal and kaadhu kaelaamai* (Auditory deficits) (Table-1). A recent research study indicated that out of 82 COVID-19 patients 1.22% has coincidence of neurosensory hearing loss.(33) The spread of COVID-19 virus to the brain via the cribriform plate may cause other sensory deficits which involve anosmia (loss of smell). Hence the COVID-19-affected patients who exhibit loss of smell and taste are also accompanied with neurological signs and symptoms. (34) Owing to the systemic inflammatory response and immune system disorders during the disease progression the incidence of cardiovascular symptoms is high, in patients with COVID-19. SARS-CoV-2 and MERS-CoV have similar pathogenicity, and the myocardial damage caused by infection with these viruses can cause acute myocarditis and heart failure(24). Hence *Maarbu nothal* (Chest pain) a symptom of *Kaba Suram* can be well correlated with the cardiovascular symptom of COVID-19. However, it may also accompany severe lung complications such as pneumonia(35).

Conclusion

Through this review, the signs and symptoms of *Kaba Suram* in *Siddha* literature has been validated and found to be in analogy with various stages of COVID-19 symptoms. Also, the pathophysiological concepts of *Kaba Suram* has been derived based on Siddha *Noi Naadal , Noi Mudhal Nadal* (Disease diagnosis from its root cause) and the *Mukkutram* theory (Trihumoural theory) of COVID-19 has been established. This literature analysis would therefore be supportive to Siddha physicians and research scientists and enable them to opt suitable Siddha formulations based on altered humour to combat COVID-19 according to its stages and severity.

Conflict of Interest

None

Abbreviation:

KSK- Kaba Sura Kudineer

Funding source

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgements

The author thankfully acknowledges Prof. Dr. K. Kanakavalli, Director General, Central Council for Research in Siddha (Ministry of AYUSH, Govt. of India), Arumbakkam, Chennai-600106 for her support in carrying out this literature research.

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JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2021/25876
SHORT REVIEW

A Perspective Review on *Siddha* System of Medicine in the Management of Corona Virus Disease 2019

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Abstract

Respiratory Tract Infections (RTIs) are the most common, and potentially most severe, of infections treated by health care practitioners. Lower RTIs along with influenza, are the most common cause of death by infection. The fatality risk is doubled if the person is with other morbid conditions or in extremes of age. These seasonal respiratory infections caused by viruses are of more concern as their spread is very vast than we imagine. COVID-19 (corona virus disease 2019) is such a pandemic respiratory illness caused by severe acute respiratory syndrome corona virus 2 (SARS-CoV-2), a strain of Corona virus. The emerging anti-microbial resistance and easy spread of respiratory pathogens has also increased the challenge for appropriate management of RTI like COVID-19. Drugs with anti-viral property and potency to prevent the co-morbidity stand are the need of the hour. This review article includes information on preclinical studies and clinical studies to add a scientific validation to formulations that are commonly used in *Siddha* system of Medicine. The ingredients of the *Siddha* drugs possessing anti-viral property and immune-modulatory effect particularly against respiratory pathogens are elaborated. Evidence of anti-viral property has been made out to the light for further clinical trials.

Keywords: Antiviral Drug, Immuno-modulator, Influenza, Respiratory Tract Infection, *Siddha* Medicine, COVID-19

1. Introduction

Corona virus disease (COVID-19) is an infectious disease caused by a new virus. COVID -19 is an illness caused due to the novel Corona virus-2 (nCoV-2/2019-nCoV), known as Severe Acute Respiratory Syndrome Corona Virus-2 (SARS-CoV-2)[1]. In the early December 2019, patients with pneumonia of unknown origin were first identified in the city of Wuhan of Hubei province of China[2]. China reported first to the World Health Organization (WHO) Country Office in China on 31 December 2019[3]. WHO declared it as a pandemic on March 11, 2020, as it had confirmed its presence in all the continents[4]. The clinical characteristics of SARS CoV-2 infection vary from mild to severe as reported. The most common symptoms at the onset of illness were fever, cough, myalgia and less common symptoms were sputum production, headache. More than half of the affected patients developed dyspnea and shortness of breath that demanded mechanical ventilation as the disease advanced[5,6]. The incubation period for COVID-19 is thought to extend to 14 days, with a median time of 4–5 days from exposure to symptoms onset. A study reported that 97.5% of patients with COVID-19 who develop symptoms will do so within 11.5 days of SARS-CoV-2 infection[7–9]. According to WHO, it is estimated that 80% of the world's population still depend mainly on traditional medicines for their health care[10]. Complementary and alternative medicines have been used effectively by humans over several centuries for treating various diseases and can be effectively employed

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Article Received on: 13.08.2020 Revised on: 05.01.2021 Accepted on: 09.03.2021

to target the host response during influenza outbreaks[11]. *Siddha* system of medicine is one of the traditional systems of medicines practiced particularly in southern parts of India that has an enormous collection of classical literature, and has in store several herbals, metallo-mineral, aquatic and animal products that are spectacular in the prevention and treatment of respiratory infections. As per the concept of the *Siddha* system of medicine, *SiddharTheran* has defined *Vatham (Vali)* to be responsible for the creation, *Pittam (Azhal)* for prevention and *Kabam (Sethumam / Aiyam)* for destruction[12]. A Person gets infected when his immunity is challenged which could be related to the reduction of *Azhal*.

The clinical features of COVID-19 in the initial stage can be correlated with *KabaSuram* in the pathology of disease as per *Siddha* literature in "*YugiVaithiyaSintha mani800*". The clinical characteristics mentioned are cough, chest discomfort, anorexia, dyspnoea and shortness of breath. So, it has been unanimously agreed to equate the diagnosis to '*Kabasuram*'[13] in *Siddha* in its early stage and further implications proceeding to *Sanni*. *Sanniroganidhanam* of YugiVaithyaSinthamani classifies *Sannirogam* into 13 types, among them *Abinyasa Sanni* can be correlated with the third stage of COVID-19. This will be also reaffirmed through Delphi or other sources of FGD[14]. This paper is a compilation of various research studies conducted on the efficacy of ingredients of *Siddha* formulations and herbs for the symptomatic management of SARS CoV-2 Infection through anti-viral property, Immuno-modulation and preventive aspect.

2. *Siddha* System of Medicine in the Management of SARS COV-2 Infection

The line of treatment will begin with balancing the derangement of three humors such as *Vatham, Pithamand Kabam*. It includes internal and external *Siddha* medicines, Yoga and pranayama is indicated to maintain the physical and mental well-being. Proper diet (*Pathiyam*) also plays a significant role to enhance innate immunity and nutritional requirement. In COVID-19, the novel Corona Virus is symptomatically managed by the medical system. The WHO interim guidance ver.1.2, suggests mainly two ways of clinical management for SARI (Severe Acute Respiratory Infection) in COVID-19[15].

1. Symptomatic management
2. Infection Prevention and Control (IPC)

In SARS CoV-2 infection, there is an initial increase in body temperature, cough and throat pain, which might subside if there is a good amount of immunity which is when *Azhal Thathu* comes into action. If not, it escalates to a phase of *Aiyathodam* which is mentioned as "*Thanamulla sethumanthan ilagilveppu*". If not treated at this stage it slowly moves to the Stage of *Sanni*[12].

This review article includes information on preclinical studies and clinical studies to add a scientific validation to formulations, herbs that are commonly used in *Siddha* system of Medicine. The drugs are selected from Classical *Siddha* literatures based on their indication for ailments like flu like infections, fever, cough, bronchial asthma, pulmonary tuberculosis, and other respiratory infections. The ingredients of the drugs possessing anti-viral property and immune-modulatory effects are also elaborated in Table 1.

On the emergence of COVID-19, guidelines for *Siddha* Clinical Management of COVID-19 were framed and published by the Central Council for Research in *Siddha*. The herbs, the internal medicines for prevention of infection prescribed in the guidelines were selected and the scientific evidence validating their antiviral property has been tabulated below in Tables 2 & 3 respectively.

3. Discussion

Anti-viral are the class of medicines that do not destroy or deactivate the microbes but by arrest the viral replication cycle at various stages. Either they prevent the attachment to host cell or prevent penetration, inhibit neuraminidase activity, or reverse transcriptase enzyme necessary for viral replication. Thus, they decrease the viral load and make it easier for our innate immune mechanisms to neutralize the virus. Moreover, since viruses are intracellular parasite it is difficult to find an antiviral drug without harming the host cells. Lack of effective therapeutics for most of viral diseases, emergence of antiviral drug resistance, high cost are the challenges in the treatment of viral infections and of some antiviral therapies necessitate finding new effective antiviral compounds. In order to circumvent the above said challenges it is the need of the hour to identify and develop new antiviral products. From the results it is evident that the selected medicines have the ingredients that constitute the anti-viral property.

Andrograpis paniculata the key ingredient of *Nilavembu kudineeris* loaded with antiviral and anti-microbial properties[17–26,125–127]. The other ingredients like *Plectranthus amboinicus*[128,129], Sandal wood[130,131], *Cyperus rotandus*[132,133] are effective against *Klebsiella pneumoniae*. *Kaba sura kudineer* medicine having potent anti-viral herbs such as *Tragia involucarata* and *Terminalia chebula* which acts against Influenza virus[134] and also effective against Swine flu[27]. Vasa leaves (*Justicia adhatoda*) the

Table 1. *Siddha* Formulations used in the management of SARS CoV-2 Infection

***Siddha* Formulations and their indications**	**Composition**	**Therapeutic effects**	**Ref.**
Nilavembu Kudineer (NVK) Indication: Suram (Fever)[16].	*Andrographis paniculata, Vetiveria zezonides, Plectranthus amboinicus, Santalum album, Cyperus rotandus, Piper nigrum, Zingiber officinale, Trichosanthes cucumerina*	Stimulates interferon synthesis leading to immunomodulation. Ethanol extract of NVK inhibits inflammation in higher level carrageen-induced paw edema NVK has antipyretic, anti-inflammatory, and analgesic activity. Analgesic and inflammatory properties, reduced COVID-19 associated myalgia and malaise symptoms through inhibiting ACE2‡ Inhibitor, which repels the route of entry of Corona virus. Andrographolide exerts its antiviral effects through inhibiting the dysregulation of signal transduction pathways necessary for viral replication and HIV-1 induced T cell cytopathicity.	17–26
Kabasura Kudineer Indication:Kapa Suram(Fever)[16].	*Zingiber officinale, Piper nigrum, Syzygium aromaticum, Tragiain volucrata, Anacuclus pyrethrum, Terminalia chebula, Adathoda vasica, Costus speciosus, Tinospora cordifolia, Clerodendrum serratum, Andrograpis paniculata, Caesalmphela parera, Cyperus rotandus*	In Siddha system, it used for all types of fever and immune enhancement Kaba Sura Kudineer also neutralize or normalize the 3 humors mentioned in Siddha. Has anti- inflammatory, antipyretic activity. Benzenepropanol, 4-hydroxy-a-methyl, 2-furancarboxaldehyde, and 5-(hydroxymethyl) are the predominant components which have significant immunomodulator and anti-inflammatory action that potentiates the therapeutic effects are also present in Kaba Sura Kudineer.	27,28
Adathodai manapagu Indication: *Kapathikam,Irumal, Suram, vayitrunoi* (deranged Kapham, cough, fever, digestive problem)[16].	*Adathoda vasica*	Leaf is reported to possess expectorant, bronchodilator, antitussive, anti-tubercular, enhancer of platelet count, anti-inflammatory, anti-microbial, anti-viral, anti-helmintic, hepatoprotective activities andalso effective against viral diseases including severe acute respiratory syndrome (SARS). Aqueous and methanolic extracts of *Adhatoda vasica* has marked Hemagglutination(HA) reduction. Extract has been proven to possess strong anti-influenza virus activity.	29–32
Ammaiyar koonthal Kudineer (AAK) Indication: *Vishakaichal, Veekam* (Fever due to toximeia, anasarca)[33].	*Cuscuta reflexa, Hemidesmus indicus, Allium cepa, Acorus calamus, Emblica officinalis, Terminalia chebula, Citrus aurantium, Citrus limon Evolvulus alsinoides, Trichosanthes tricuspidata, Phyllanthus niruri Sida acuta, Diospyros montana Lagenaria siceraria, Cucurbita pepo Asarum europaeum, Asystasia gangetica*	Anti-viral, anti-bacterial, anti-pyretic, anti-inflammatory, anti-HIV activity. Neutralizing virus Inhibit CD4/gp120 from the virus interaction. Anti-HSV-2, anti-proliferative. In Silico analysis of AAK explore the 16 active compounds has a high binding affinity with COVID-19 protease, it may suggest a possibility to protease inhibitor mechanism.	34

Thippili rasayanam Indication: Kaasam, kshyam, Irumal, Iraippu, sethumam 96. (Pulmonary Tuberculosis, Cough, Bronchial asthma, All kind of Kapha diseases[16].	*Piper longum, Piper nigrum, Cuminum cyminum, Nigella sativa, Carum copticum, Syzygium aromaticum, Alpinia galanga, Hyocymus niger, Three myrobalans, Taxsus buccata, Plumbago zeylanica, Eletria cardamom*	Methanolic extract of cumin seeds has inhibitory effect on HSV-1. The essential oil of Carum copticum has anti-viral activity. It also has a relatively Bronchodilator effect on asthmatic airways which was comparable with the effect of theophylline. Ten piperamides isolated from black pepper has been tested against various viral showed effective inhibition of Coxsackie virus type B3 and moderate activity against Human Rhino Virus.	35–45
Swasakudori Mathirai Indication: *Suvasam, Irumal, Kaasam* (Asthma, cough and other respiratory disorders)[16].	*Calotropis flower, Piper nigrum*	Calotropis flower and pepper is widely employed in respiratory diseases as it has antispasmodic activity. The glycoside isolated from the latex of *Calotropis gigantea* showed inhibitory effect against human influenza viruses also in both subtypes A and B while had no effect on avian influenza viruses.	46,47
Thalisathy Chooranam Indication: *Sethumam96, Kamalai, Irumal, Thondaikattu,Kshyam, Atthisuram.(Kaba* diseases 96, Jaundice, Cough, Throat infection,Pulmonary tuberculosis)[16].	*Taxsus bucata, Syzygium aromaticum, Eletria cardamom, Zingiber officinale(dried), Ferula asafetida, Embilica ribes, Costus speciosus, Piper longum, Cuminum cyminum, Myrsticafragarns, Terminalia bellarica, Terminalia chebula, Nardostachys grandiflora, Anethum gravelons, Phyllanthus emblica, Piper nigrum, Mesua fera, Michalia champak buds, Corriandrum sativum seeds, Rhus succedanea, Carum copticum,*	Phytochemical screening of *T. baccata* showed the presence of lignans, flavonoids, sugar derivatives, etc. It acts on lipoxygenase (LOX) enzymes that are potential targets for the treatment of bronchial asthma, inflammation, cancer and autoimmune diseases. The alcoholic extract of the leaves of T. baccata possesses significant anti-asthmatic activity and has beneficial effect in asthma by causing broncho relaxation and decreasing bronchial hyper reactivity. Lignans are known to possess various biological activities including antiviral, antibacterial, antioxidant, anticancer, spasmolytic and anti-inflammatory effects. Flavonoids are known to possess various biological activities including antibacterial, antifungal, spasmolytic, antiviral, anticancer and anti-inflammatory effects.	48–54
RAN therapy Indication: HIV infection	*Rasa Gandhi Mezhugu (RGM), Amukkara Chooranam, Nellikailehyam*[55]	Reduced viral load, improve CD4 and CD8 cells counts, controls symptoms and increase body weight. *Withania somnifera* had shown antiretroviral activity against HIV infection and human respiratory syncytial virus. Has anti-herpes simplex virus type 1 (HSV-1) activity. Anti-inflammatory and neuro protective features and immunomodulatory activity during the post-pyretic phase of CHIKV in an animal model.	56–59

Table 2. *Siddha* herbs used for the treatment of SARS CoV-2 Infection

Herbs in Siddha System of Medicine	Therapeutic effects	Ref
Inji: *(Zingiber officinale)* *Irumal, Thondai pun, Kuralkammal, Iraippu* (Cough, Throat infection, Hoarseness of voice, Bronchial asthma)[33].	Has antiviral, analgesic, and antipyretic properties.Aqueous extract of fresh ginger was found to have antiviral activity against human respiratory syncytial virus on HEp2 and A549 cell line and anti-rhinoviral effect.	60–62
Thulasi: (Ocimum sanctum) Iyyam, Irumal, Kapasuramsuram, (fever, cough)[33].	It gives excellent results for cough caused due to *kabam*, allergic bronchitis, asthma, and eosinophilia. The ethanol extract of *Ocimum sanctum* (EEOS) has antiviral activity against the H1N1pdm virus demonstrated through in-vitro inhibition assay in Madindarby canine kidney cellsMDCK) The crude extract and terpenoid isolated from the leaves of O. sanctum has shown promising antiviral properties against H9N2 virus.	63–65
Milaku: (Piper nigrum) Kulirsuram, Irumal, Kamalai, Seriyamai (fever with rigor, cough, Jaundice, Indigestion)[33].	Piperine the major phytochemical of black pepper is described to have antiviral property. Studies had suggested that aqueous and ethanol extracts from plants used are potential sources of antiviral, antitumor and antimicrobial agents. It is also found to have bioavailability-enhancing properties of other drugs. Ten piperamides isolated from black pepper has been tested against various viral strains which showed effective inhibition of Coxsackie virus type B3 and moderate activity against Human Rhino Virus.	44, 66–68
Karunjeerakam: (Nigella sativa) Sirangu,Irumal, Suram (Scabies, Cough, fever)[33].	Thymoquinone (TQ) is one of the most active constituents and has different beneficial properties. Focus on antimicrobial effects, different extracts of N. sativa as well as TQ, have a broad antimicrobial spectrum including Gram-negative, Gram-positive bacteria, viruses, parasites, Schistosoma and fungi. Oil of *Nigella sativa* was administered continuously for 3 months a dose of (450 mg three times daily) in patient with hepatitis C virus (HCV) infection who were not eligible for IFN-α therapy that significantly improved HCV viral load.	42, 43, 69
Keezhanelli: (Phyllanthus niruri) Suram, Kamalai, Perumbadu (Fever, Jaundice, menorrhagia)[33].	Febrifuge, antiviral.The whole plant inhibits hepatitis B virus DNA polymerase, the enzyme needed for the hepatitis virus to reproduce. The phytochemicals nirtetralin and niranthin has potent antiHBV activity. A novel lignin found in *P. niruri,* nirtetralin B and its two stereoisomers, nirtetralin and nirtetralin A. Nirtetralin significantly had a dose-dependent inhibitory effect on the in-vitro titres of HBV antigens. Aqueous extracts of *P. niruri* containing repandusinic acid, have been shown to exert a significant inhibitory effect on HIV-1 reverse transcriptase (HIV-1-RT). Repandusinic acid competitively inhibits the template primer during the process of reverse transcription. The alkaloid extract of P.niruri has an inhibitory effect on HIV-1 replication and dose-dependent cytoprotectivity against HIV infection. Niruriside inhibits REV/RRE binding during the movement of viral RNA from the cell nucleus to the cytoplasm, the Phyllanthus extract affected the early phases of viral infection such as the viral attachment and entry.	58 70–77

Athimadhuram: *(Glycyrrhiza glabra)* *Irumal, kamalai* (Cough, Jaundice)[33].	Antiviral properties against HSV, Rotavirus, HPV, SARS, Flavivirus, Human immunodeficiency virus, Vaccinia virus, Poliovirus (type 1), Vesicular stomatitis virus, IAV, SARS-related Corona virus, human respiratory syncytial virus, and Arboviruses.	78–94
Vellaipoondu : *(Allium sativum)* *Irumal, Iraippu, Neeretram* (Cough, Bronchial asthma, Sinusitis)[33].	Antiviral, antiprotozoal, disinfectant. Prevent blood clotting and increases the rate of clot dissolution. Garlic extract has been shown to have antiviral activity against influenza B and herpes simplex type 1 virus in cell culture, and influenza virus strain AO/RR8 in infected mice if the garlic extract was administered 15 days before inoculation with the virus. Studies have determined the antiviral activity of garlic and a new commercially available garlic product, garlicin, against seven different animal viruses in two cell lines: herpes simplex virus type 1 and 2 (HSV-1 and -2), vaccinia virus, and vesicular stomatitis virus in vero cells; parainfluenza type 3 (Para-3), poliovirus type 1, and human rhinovirus type 2 in HeLA cells.	60, 95–98
Cittramutti: *(Sidacordifolia)* *Athi suram, Kunmam*(A type of fever, gastritis)[33].	Bronchodilator. *Sida cordifolia* showed promising antiviral activity against DNA (HSV I & II, HSV TK- and Adenovirus type VIII) and RNA (Poliovirus type-I and Influenza virus type A (H1N1).	99,100
Seenthil: *(Tinospora cordifolia)* *Pithasuram, kabapini* (Fever, *kaba* disease) [33].	*Tinospora cordifolia* has shown anti-HIV potential by 3 different mechanisms (interference with the gp120 / CD4 interaction, inhibition of HIV-reverse transcriptase and probable inhibition of HIV-protease enzyme). Anti-HIV activity could be an added advantage along with the immunomodulatory effect of these plants to fight Acquired Immunodeficiency Syndrome (AIDS). Extracts, only petroleum ether and ethanol (successive) extracts of *T.cordifolia* significantly inhibited gp120/CD4 interaction by binding to gp120 and by competitive inhibition and by binding to immobilized CD4. The virus suspensions of HSV1 & HSV2 were incubated with test compounds of concentration 100µg/ml and 50µg/ml of *T.cordifolia* extract and it was observed that at a test concentration level of 100µg/ml and 50µg/ml the percentage protection offered is approximately 61.43% and 23.22% respectively.	101–104
Manjal: *(Curcuma longa)* *Thalaivali,Neeretram, mookuneerpaiythal* (Headache, Sinusitis, Rhinitis)[33].	Curcumin an alkaloid of turmeric inhibits the HIV-I integrase protein, indicating that integrase inhibition may contribute to the anti-HIV activity of curcumin[134]. Curcuminoidshas inhibitory activities on influenza A neuraminidasesand could be used as supplementary materials for battle of influenza virus as neuraminidase inhibitors of influenza A[135] curcumin might be beneficial for the treatment of an influenza virus infection by inhibiting haemagglutinin (HA) protein[136] The study of different bioconjugates of curcumin, against variety of viruses including parainfluenza virus type 3 (PIV-3), feline infectious peritonitis virus (FIPV), vesicular stomatitis virus (VSV), herpes simplex virus (HSV), flock house virus (FHV), and respiratory syncytial virus (RSV) assessed by MTT test showed the potent antiviral activity of curcumin and its bioconjugates. Curcumin was found to be an inhibitor of HIV-1 and HIV-2 protease with IC50 of 100 µM and 250 µM, respectively. The curcumin boron complexes exhibited noteworthy inhibition reduced to the IC50 value of 6 µM with time-dependent activity. The elevated affinity of Curcumin showed the anti-influenza activity against influenza viruses PR8, H1N1, and H6N1. In H1N1 and also H6N1 subtypes, the inhibition of haemagglutinin interaction reflected the direct effect of curcumin on infectivity of viral particles and this has proved by time of drug addiction experiment. Additionally, unlike amantadine, viruses developed no resistance to curcumin[137]. These results proved the significant potential of curcumin for inhibition of influenza. In vitro study of curcumin and its derivatives, namely, gallium-curcumin and Cu-curcumin, exhibited remarkable antiviral activity against herpes simplex virus type 1 (HSV-1) in cell culture.	105–110

Vembu: *(Azadirachta indica)* *Vayitrupuzhu, Nachusuram, Sirangu, amai pun, Kamalai* (Worm infestation, fever due to toxima, Scabies, Herpes)[33]	Neem leaves have considerable antinociceptive, emollient, antiviral, anti-inflammatory, antiseptic, antifungal, astringent, insecticidal, anthelmintic and antibacterial properties. Molecular docking study has shown that Deacetyl-3-cinnamoyl-azadirachtin in neem leaves serve as a potential inhibitor against NS3/4A protease of HCV. In vitro inhibitory potential of Neem leaves NL aq. ext. on DEN virus type-2 in C6/36 cells revealed inhibition of virus replication in a dose dependent response with 100% inhibition being observed by all concentrations. The molecular docking results showed that nimbin, desacetylnimbin and desacetylsalannin have good binding affinity with DENV NS2B-NS3pro.	111–114
Elumitchai: *(Citrus limonia)* Volatile oil Soolai, *Kunmam, Vaanthi* (pain, Gastritis, vomiting)[33]	The antibacterial activity of crude extracts (aqueous and ethanolic) of Citrus limonum fruits against four wound isolates Staphylococcus sp, Pseudomonas sp, Escherichia coli and Klebsiella sp. showed antibacterial effect. The antimicrobial activity of different types and parts of lemon was evaluated against different microbial isolates. The aqueous extracts of peel and juice from fresh and dried citrus and sweet lemon showed various inhibitory effects against 6 Gram-positive and 8 Gram-negative bacteria and one yeast isolates, including Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Enterococcus faecalis, Streptococcus pneumoniae, Streptococcus agalactiae, Pseudomonas aeruginosa, Enterobacter aerogenes, Klebsiella pneumoniae. When Citrus peel extract was added to the CoV infected cells the virus load of corona virus (CoV) decreased, had an effect on IL-8 secretion, TRP gene expression.	115–118

Table 3. *Siddha* Medicines used for the prevention of SARS CoV-2 infection

***Siddha* Formulations**	**Composition**	**Therapeutic effects**	**Ref.**
Nilavembu Kudineer	*Andrographis paniculata, Vetiveria zezonides, Plectranthus amboinicus Santalum album, Cyperus rotandus, Piper nigrum, Zingiber officinale, Trichosanthescucumerina*	*Nilavembu Kudineer* provides protection against CHIKV and DENV-2 during active infection as well can help to prevent virus infection in the cells and it mainly depends on the cellular availability of drugs for maximum protection against both the infections. The ethanol extracts of NVK exhibits antiviral property. *Nilavembu kudineer* and *Adathodai manapagu* has promising effects in treating dengue. It can be used as discreet prophylaxis in conditions of disease outbreaks.	119–133
Kabasura Kudineer	*Zingiber officinale, Piper nigrum, Syzygium aromaticum, Tragiain volucrata, Anacuclus pyrethrum, Terminalia chebula, Adathoda vasica, Costus speciosus, Tinospora cordifolia, Clerodendrum serratum, Andrograpis paniculata, Caesalmphela parera, Cyperus rotandus*	*Kabasura Kudineer* promotes to increase the immunity level of the people and to neutralize or normalize the 3 humors. Aqueous extract of *Kabasura Kudineer* possesses a vital anti-pyretic effect comparable to that of paracetamol (standard drug) from 1 h to 5 h through a possible mechanism of inhibition of prostaglandin. The anti-bacterial activity of the extract was measured by observing bacterial free zones formed around the discs. The extract was found to have significant antibacterial activity. AEKKC was found to have antibacterial activity with MIC of 250 µg/ml for both gram positive and gramnegative organism. *Kabasura Kudineer* Chooranam is widely prescribed for the management and prevention of swine flu.	27,134

ingredient of *Adathodai manappagu* shows antiviral property against Influenza virus [29–32]. In *Ammaiyar Koondhal Kudineer*, Chebulagic acid showed an extremely high binding affinity in inhibiting the SARS CoV2 spike protein in the native conformation and bound state with its target ACE2(Angiotensin-Converting Enzyme) receptor[34]. The ingredients of *Thippili rasayanam* such as *cumin seeds, Carum copticum, Nigella sativa* have antiviral property. Carvacrol present in *Carum copticum* is an efficient smooth muscle relaxant[35–43]. So, it can be used in respiratory infections. *Thalisathi choornam* has the key ingredient *Taxus buccata* it has relaxing effect on bronchial smooth muscles and the lignans present in it have antiviral property[47–55]. *Swasakudorai mathirai* has pepper and flowers of Calotropis with the property of antiviral and antispasmodic effect[46,66]. The major ingredient of *Amukkara chooranam, Withania somnifera* is effective against HSV and HIV[56,57]. Ginger is effective against Respiratory Syncytial Virus[22]. *Ocimum sanctum* has antiviral activity against the H1N1 pandemic virus[64,65]. *Piper nigrum* was found to be antiviral against Human Rhino virus, also found to have bio availability enhancing properties of other drugs[44,136]. *Nigella sativa* has broad spectrum anti-microbialactivity[42,43]. *Phyllanthus niruri* was found to be active against HBV, HIV and Dengue viruses[71–77].

Glycrrhzia glabra has specific antiviral property against the influenza virus[78–94]. Garlic has antiviral property against many viruses including para-influenza type 3 and Human Rhino Virus. *Sida cordifolia* was found to inhibit RNA and DNA viruses including influenza virus type-A[100]. *Tinospora cordifolia* has anti-HIV, HSV1 and HSV 2 activity[101–104]. Turmeric is a potent antiviral herb with the alkaloids providing efficient anti-viral activity against various viral strains including parainfluenza[107,108]. *Azadirachtaindica* inhibits HCV and Dengue virus by destructing the enzymes responsible for viral replication[111–113]. *Citrus limonia* has anti-bacterial activity against *Klebsiella species* and affects IL-8 secretion, TRP (T-Cell Receptor Beta locus) gene expression in Corona virus[115–117].

4. Conclusion

From this review it is evident that the ingredients of the listed *Siddha* formulations and herbs possess potent anti-viral activity, antioxidant property that could enhance the immunity. It is also proven that despite the etiological factor these medicines have the inbound capacity to fight against many respiratory infections, seasonal attacks more specifically of viral origin including COVID-19. Also, the study suggests more literature formulations have to be identified and clinical research could be carried out to identify the molecular level pharmacodynamic targets of *Siddha* medicines to achieve clinical success in the management, prevention of viral respiratory infections and eradication of epidemic and pandemic outbreaks.

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Article *in* Indian Journal of Pharmaceutical Education · February 2021

DOI: 10.5530/ijper.55.1.7

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Review Article

Pharmacological Actions of Contents of Kabasura Kudineer- A Siddha Formulation for Fever with Respiratory Illness

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ABSTRACT

Siddha system of medicine is a distinct therapeutic science with many single drugs and compound formulations used for treating a broad spectrum of ailments. Siddha categorizes the fever manifested by viral infestation into 64 types and it has developed medicines for each type. *Kapacurak / Kabasurak Kutinir* (KK) described in the *Citta Vaittiyattirattu* is the best promising polyherbal formulation of plant origin for curing viral infections especially with flu-like symptoms. As per Siddha system of medicine *Kapacuram* is defined as a fever with upper and lower respiratory catarrh. KK is one of the medicines advised for prevention of Coronavirus (COVID-19) outbreak in India by Ministry of AYUSH, Govt. of India. KK also emerged to be a popular traditional medicine for swine flu as well. The current review recapitulates active phytochemicals of all the 15 herbal drug ingredients in KK with the aim to provide it support for usage in flu-like viral infections spreading over the world in the scenario of having no modern medicines. Interestingly, out of 15 herbal ingredients in KK, *Zingiber officinale* rhizome, *Andrographis paniculata* whole plant, *Syzygium aromaticum* flower bud, *Cyperus rotundus* tuber, *Sida acuta* roots and *Saussurea costus* root have been proved to exhibit antiviral activities. All the ingredients have been proved to possess antinflammatory activities. Three proved to have antipyretic potential and five each proved to possess analgesic and immunomodulatory activities. There are ingredients with antiasthmatic and antispasmodic supporting its use in respiratory illnesses such as that caused by COVID-19. The study supports the usage of KK as a traditional Siddha medicine against respiratory illnesses with flu-like symptoms characteristic of SARS-CoV-2.

Key words: AYUSH, *Citta Vaittiyattirattu*, COVID-19, Flu-like symptoms, Respiratory illness, SARS, Traditional medicine.

INTRODUCTION

Today the human population world over is facing various kinds of virus like corona virus with many of them without an effective medication. These viruses formed due to combination of more than one viral gene in a non-human host are highly contagious when enter into human beings. Scientists and the medical society world over have succeeded in finding medicines for viral infections like malaria, hepatitis, herpes, H1N1 but have not yet achieved success for dengue, chikungunya and novel corona (COVID-19) viruses. This virus has a single stranded RNA as the genetic material surrounded by helical

Submission Date: 24-06-2020;
Revision Date: 09-10-2020;
Accepted Date: 16-12-2020

DOI: 10.5530/ijper.55.1.7

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envelope resembling the shape of corona.[1] Middle East Respiratory Syndrome coronavirus lead to the death of approximately 39% of the infected people.[2,3] As of 4th October 2020 there are 38.4 million cases worldwide with the death rate crossing one million. In India, over 500000 new cases are reported per week since late-August bringing the cumulative counts to an excess of 6.5 million cases and 100000 deaths which accounts to 10% of global total.[4]

Coronaviruses (CoVs) are the largest group of known positive-sense RNA viruses having an extensive range of natural hosts. Since these viruses emerge periodically and unpredictably and spread at a very rapid rate inducing serious infectious diseases, they have turned out to be a serious risk to mankind. The symptoms associated with the upper and lower respiratory tracts like cough, fever and headache are visualized during viral infections.[5]

The herbal medicines which make use of natural products with minimal side effects have made a comeback. They are getting recognized due to pharmacological evidence of safety and efficacy, development of standardized dosage forms and quality control measures.[6] Siddha system of medicine is a distinct science and a unique art of healing originated and flourished in South India and practiced both in traditional and hereditary method. This system recognizes 32 kinds of preparations each for internal administration as well as external applications. There are many single drugs and compound formulations which are based on herbals, animals and metalo-mineral compounds and are used for treating a broad spectrum of ailments. There are more than thousands of authentic works in addition to manuscripts and palm leaf scripts extort. Siddha medicines which do not causes any adverse effects are popular in Southern India for treating several viral diseases like chicken pox, mumps, influenza, dengue and prophylaxis.[7]

In various traditional systems of Indian medicine the poly herbal formulations are favoured with respect to sngle drug therapies.[8,9] The pharmacological actions of herbs are evident only in the combination with other herbs and are described in the classic literatures.[10] *Kapacurak Kutinir* (KK) a poly herbal Siddha formulation is descried in the manuscript *Citta Vaittiyattirattu* and is used for curing phlegmatic fever and fevere with symptoms of flu.[11] KK contains fifteen herbal drugs (Table 1) which are mixed in equal quantities to prepare the decoction. The ingredients are powdered coarsely and mixed well. 35 g of this powder is then mixed with three liters of water, boiled and reduced to its half volume.

Table 1: Ingredients of *Kapacurak Kutinir*.

SN	Botanical Name	Part used	Taste[12]	Therapeutic uses[12]
1.	*Zingiber officinale* Roscoe	Dried Rhizome	Pungent	Anemia, asthma, cough, dyspepsia, diarrhoea, fever flatus, heartburns, peptic ulcer, sinusitis,
2.	*Andrographis paniculata* Burm.f.Nees	Whole plant	Highly bitter	Arthritis, fever, sinusitis, syncope
3.	*Syzygium aromaticum* (L.) Merril. And Perry	Flower bud	Pungent	Diarrhea, dysentery, dyspepsia, earache, sinusitis, toothache, vomiting
4.	*Cypreus rotundus* L.	Rhizome	Bitter	Hypertension, fever, thirst
5.	*Sida acuta* Burm. f.	Root	Astringent	Arthritis, diarrhea, fever, itching, scabies
6.	*Rotheca serrata* (L.) Steane and Mabb.	Root	Slightly bitter	Asthma, fever, myalgia, sinusitis
7.	*Piper longum* L.	Fruit	Pungent	Anemia, asthma, cough, headache, phlegm throat infection
8.	*Justicia adathoda* L.	Leaves	Bitter	Asthma, bleeding dysentery, cough, fever, throat infection
9.	*Plectranthus amboinicus* (Lour.) Spreng.	Leaf	Pungent	Cough pox, phlegm, sinusitis, rhinitis
10.	*Terminalia chebula* (Gaertn.) Retz.	Fruit rind	Bitter astringent followed by sweet	Diabetes, fistula, jaundice, leucorrhea, liver diseases, piles, stomatitis, vitiligo, vomiting
11.	*Tinospora cordifolia* (Willd.) Miers ex Hook.f and Thoms	Stem	Bitter	Diabetes, diarrhoea, fever, hypertension, skin diseases
12.	*Saussurea lappa* (Falc.) Lipsh	Tuber	Slightly bitter	Abscess, asthma, fever, piles, wounds
13.	*Tragia involcurata* L.	Root	Slightly astringent	Asthma, cough, eczema, fever, itching, skin diseases
14.	*Anacyclus pyrethrum* (L.) Lag.	Root	Sweet	Arthritis, dental problem, dryness of tongue, epilepsy, fever, tonsillitis
15.	*Hygrophila auriculata* (K. Schum.) Heine	Root	Slightly bitter	Anemia, edema, sinusitis, urinary tract infection

This is recommended to take twice or thrice daily not exceeding 60 ml.[1] The current study encompasses the scientific details of phytochemical constituents, their pharmacology and the biological activities present in all the fifteen herbals used as the ingredient of KK.

Zingiber officinale Roscoe

Z. officinale (Zingiberaceae) is a perennial herb known as *Chukku* in Siddha, dried drug consists of sympodially branched laterally compressed pieces of horizontal growing rhizome is seen distributed in tropical Asia.[12] Shogaol and gingerols the main constituents of the volatile oil of this rhizome is responsible for its flavor.[13] The rhizome possess anti inflammatory, anti hyperglycemic, anti emetic and immunomodulatory properties[14] (Table 2).

The major phytochemicals reported from essential oil of the rhizome are 6-shogaol, 6-gingerol and *a*-zingiberene. It has anti-inflammatory, hepatoprotective, antioxidant[15] and is used in colic, haemorrhoids, diseases of throat and inflammation.[12] Major Siddha preparations using *Z. officinale* as an ingredient includes *Agathiennei*, *Cukkutailam*, *Kapacurakkutinir*, *Kapadailakam*, *Milakutailam*, *Nellikaiilakam*, *Pooranathiilakam*, *Talicaticuranam*, *Vilvatiilakam* etc.[11]

Andrographis paniculata Burm.f.Nees

A. paniculata (Acanthaceae) is known as *Nilavembu* in Siddha is a herb reaching upto a heigth of 30-110 cm with glaborous leaves and white flowers with purple spotted petals. It is seen as a common weed in South India and also present in states of Assam, Missoram and Himachal Pradesh.[31] It is used to cure malaria, leucoderma, jaundice, abscess, woulds and eczema.[32] Diterpenoids andrographolide are major bioactive components. The compounds from the plant have been reported to have anti-inflammatory,[33] anti-cancer,[34] anti-microbial,[35] and hepatoprotective,[36] anti-viral activities[37] (Table 3). Major Siddha preparations using *A. paniculata* as an ingredient includes *Kapacurak kutinir, Nilavempuk kutinir, Vatacurak kutinir* etc.[11]

Syzygium aromaticum (L.) Merril. and Perry

S. aromaticum (Myrtaceae), is known as *Ilavankam* in Siddha. It is mostly used as a spice to flavor all kinds of foods and has other medicinal values too. The dried flower buds mostly used as a spice having medicinal values occurs throughout South India.[47] The phytochemicals present in this drug belongs to the class sesquiterpenes, monoterpenes and oxygenated compounds.[48] The drug possess anti-carcinogenic,[49] growth inhibitory,[50] anti-thrombic activity,[51] anthelmintic, anti-asthma and other allergic disorders, anti-inflammatory, antioxidant, antiviral and anti-parasitic properties,[52] and insulin like activities[53] (Table 4).

Major Siddha preparations using *S. aromaticum* as an ingredient includes *Amukkara curanam*, *Carapunkavilvatiilakam*, *Elaticcuranam*, *Incivatakam*,

Table 2: Bioactivities of phytochemical constituents in *Zingiber officinale.*

Constituents	Class	Bioactivity	Reference
β-Phellandrene	Monoterpene	Anti-bacterial	16
Zingiberol	Sesquiterpene alcohol	Anti-cancerous	17
α-Zingiberene	Sesquiterpene	Anti-cancerous	18
Ar-Curcumine		Anti-oxidant, anti-microbial	19
β-Bisabolene		Cytotoxicity against breast cancer cells	20
GingerenonesA, B and C	Diarylheptenon	Anti-fungal	21
Isogingerenone B			
Hexahydrocurcumin		Anti-inflammatory, antioxidant	22
Gingerdiols	Phenols	Anti-microbial	23
β-Eudesmol	Sesquiterpene	Anti-inflammatory	24
Nerolidol			25
α-Pinene	Monoterpene	Anti-inflammatory, anti-microbial	26
Farnesol	Alcohol	Apoptotic	27
6-Shogaol	Phenol	Anti-inflammatory, anti-cancerous, antioxidant	22-29
6- Gingerol			30

Table 3: Bioactivities of phytochemical constituents in *Andrographis paniculata*.

Constituents	Class	Bioactivity	Reference
Bis-andrographolide	Terpene	Anti-HIV	38
14-deoxy-11,12-didehyro andrographolide		Anti-fungal	39
Neoxyandrographiside		Anti-fungal	
Ninandrographolide		Immunostimulant	40
Oxygenatedflavones	Flavonoid	Anti-bacterial	41
OroxylinA		Anti-cancer	42
β-Sitosterol-D-glucoside	Phytosterol	Anti-inflammatory	43
Ninandrographolide	Terpene	Immunostimulant	40
Myristicacid	Fattyacid	Anti-bacterial	44
Eugenol	Ether-alcohol	Antiseptic	45
Andrographolide	Diterpene	Anti-inflammatory, anti-cancerous, anti-microbial and hepatoprotective	33,34
Tritriacontane	Hydrocarbon	Antioxidant	46

Table 4: Bioactivities of phytochemical constituents in *Syzygium aromaticum*.

Constituents	Class	Bioactivity	References
p-Cymene	Monoterpene	Antioxidant, hepatoprotective	48
5-Hexene-2 one	Hydrocarbon (HOC)	Anti-cancerous	54
Thymol		Anti-bacterial, antifungal	55
Eugenol		Anti-inflammatory, antioxidant	56
Guaiol	Hydrocarbon (HOC)	Antibacterial, antioxidant	57
Nootkatin	Sesquiterpene	Anticarcinogenic	49
Isolongifolanone			
Hexadecanoic acid	Hydrocarbon (LOC)	Anti-inflammatory	58
Octadecanoic acid butyl ester	Hydrocarbon (HOC)	Acaricidal	59
Dodecatrienoic acid		Anti inflammatory	58
Caryophyllene oxide	Hydrocarbon (LOC)	Anti-cancerous, analgenic	60
Vitamin E acetate	Hydrocarbon (HOC)	Hepatoprotective	61
Gallic acid	Benzoic acid	Anti inflammatory, anti diabetic, anti cancer	62
Kaempferol	Stigma sterol	Antimicrobial	
β Carryophylene	Sesquiterpene	Antiulcer	

Iracameluku, *Kantakarasayanam*, *Nantimeluku*, *Puramattirai*, *Uli mattarai*.[11]

Cypreus rotundus L.

C. rotundus (Cyperaceae) is a perennial sedge plant and is known as *Koraikkizhangu* in Siddha This is seen distributed all over India.[63] The drug is used as anti microbial, cytotoxic, larvicidal,[64] anti-inflammatory[65] and anti-malarial[66] activities (Table 5). This drug also possess analgesic, antispasmodic, astringent, diaphoretic, diuretic properties and is used as tonic and vermifuge.[63]

Major Siddha preparations using *C. rotundus* as an ingredient includes *Athimathura mathirai, Adathodai chooranam, Civatai chooranam, Cukkutailam, KapadaIlakam, Sanjeevi theenir, Chandraprakasa mathirai, Kapacurak kutinir, Thathu pushti kulikai, Parangichakkai chooranam, Milagu thailam*.[11]

Sida acuta Burm.f.

S. acuta (Malvaceae) and is known as *Vattatiruppi* in Siddha.[75] This is a common weed of waste plains and grows gregariously and is present in the tropical regions in India. The whole plant is effective in treating snake bites and haemorrhagic effects of *Bothrops atrox* venom,[76] and is also used for the treatment of urinary infections.[77] The drug possess antimalarial activity,[78] anti-ulcer,[79] wound healing, hepatoprotective activity,[80] cardiovascular activity,[81] hepatoprotective,[82] antioxidant,[83] hypoglycemic

Table 5: Bioactivities of phytochemical constituents in *Cyperus rotundus*.

Constituents	Class	Bioactivity	References
Vitexin	Flavonoid	Anti-viral, anti-cancerous	67,68
Isokobusone	Sesquiterpene	Anti-inflammatory	69
Isocyperol		Anti-inflammatory	70
α-Cyperone		Selective cytotoxic, anti-inflammatory, nueroprotective	71
Cyperene		Apoptotic, anti-oxidant, anti-bacterial	72,73
β-Selinene	Hydrocarbon	Anti-microbial, antioxidant	74
Copadiene	Sesquiterpene	Anti-malarial	66
Kobusone		Anti-inflammatory, analgesic	64
Cyperenone		Antiulcer	54
Eugenol	Ether-alcohol	Antiseptic	45

Table 6: Bioactivities of phytochemical constituents in *Sida acuta*.

Constituents	Class	Bioactivity	Reference
Vasicine	Alkaloid	Antibacterial	77
Ephedrine			
Cryptolepine			
Ecdysterone	Steroid	Anti-diabetic, hepato-protective	93
Sistosterol		Cytotoxic, anti-microbial	94
β- Stigmasterol		Anti-inflammatory	95
Campesterol	Polysterol	Cytotoxic, anti-inflammatory	96
Evofolin	Phenyl propene	Anti-microbial	97
Scopoletin	Hydroxycoumarin	Anti-inflammatory	98
4-ketopinoresinol	Lignan	Anti-oxidant	99
Loliolid	Monoterpenoidhydroxylactone	Oxidative stress protection, anti melanogenic	100

activity,[84] anticancer,[85] analgesic and anti-inflammatory activityies[86] (Table 6).

The phytochemical present in this species are vasicine, ephedrine and cryptolepine, ecdysterone, β-sistosterol, stigmaterol, campesterol, tannins, phenolic compounds, evofolin-A and B, scopoletin, loliolid and 4-ketopinoresinol, polyphenol, sesquiterpene and flavonoids.[87-90] Isolated pure form of alkaloids from *Sida acuta* and their synthetic derivatives have antihypertensive, antiarrhythmic, antimalarial and anticancer activities.[91] Tannin obtained from this plant is used to cure ailments like leucorrhoea, rhinorrhea and diarrhea.[92]

Major Siddha preparations using *S. acuta* as an ingredient is *Kapacurak kutinir.*[11]

Rotheca serrata (L.) Steane and Mabb.

R. serrata (syn. *Clerodendrum serratum*), (Verbenaceae) is a shrub and is known as *Ciruteku* in Siddha.[101] It is found upto an altitude of 1200m in lower Himalayas distributed in Kumaun, West Bengal and Bihar.[102] The major chemical constituents present are D-Mannitol, gamma-sitosterol, stigmasterol, glucose, oleanolic, queretaroic and serratagenic acid.[103-107] It shows antiasthamatic antispasmodic,[108] antinociceptive, anti-inflammatory and antipyretic activities[109] (Table 7).

Major Siddha preparations using *R. serrata* as an ingredient includes *Carapunakavilvatiilakam, Iracakantimeluku, Kappacurak kutinir, Noccit tailam, Parankipattai rasayanam, Vata curak kutinir* etc.[11]

Piper longum L.

P. longum (Piperaceae) is known as *Tippili* in Siddha.[114] The plant is a slender climber distributed in the warmer region of India and lower hills of Bengal.[115] The major phytochemicals present in this drug are piperine, piperlongumine, piperlonguminine, methyl-3,4,5-trimethoxy cinnamate.[116-118] *P. longum* possesses antibacterial,[119] antifungal,[120] anthelmintic,[121] bioavailability enhancing properties,[122] and immunomodulatory effects.[123] The benzene extract of the fruit shows anti fertility activity,[124] ethanol extract shows anti depressent,[125] alkaloids obtained from the fruit shows anti hyperlipedemic,[126] the amides obtained from the plant shows anti platelet activity,[127] and piperedine shows anti obesity[128] activities (Table 8).

Major Siddha preparations using *P. longum* as an ingredients includes *Astapairavam, Atatotainey,*

Table 7: Bioactivities of phytochemical constituents in *Rotheca serrate.*

Constituents	Class	Bioactivity	Reference
D-Mannitol	Alcohol	Diuretic	108
Serratagenic acid	Triterpenoid	Antibacterial	109
Stigmasterol	Steroid alcohol	Anti-inflammatory	110
Queretaroic acid	Triterpenoid	Anti-tumour, anti-fungal, anti-inflammatory	111
Oleanolic acid			
Gamma-sitosterol	Sterol	Antioxidant	112
Verbacoside		Anti proliferative	113
Betulin	Terpene	Anti-fungal	

Table 8: Bioactivities of phytochemical constituents in *Piper longum.*

Constituents	Class	Bioactivity	Reference
Piperine	Alkaloid	Antitumour, antioxidant, anti-inflammatory, anti microbacterial, hepatoprotective	123
Piperlongumine		Analgesic, anti- inflammatory, antimelanogenic	115
Piperlonguminine		Hipolipidemic	
Methyl-3,4,5-trimethoxy cinnamate	Cinnamate	Anti –tubercular	129

Carapunkavlvatiilakam, Cukkut thailam, Culaikkutaram, Kapacurak kutinir, Kecariilakam, Noccitthailam, Pinacatthailam, Uluntutthailam, Vilvatiilakam.[11]

Justicia adhatoda L.

J. adhatoda (Acanthaceae) is known as *Atatotai* in Siddha.[130] A sub-herbaceous perennial shrub found throughout the year in plains and sub Himalayan tracts of India, ascending up to 1200m altitude and is used in almost all traditional medicinal systems in India.[131] It is used for curing cough, cold, bronchial asthma, intestinal worm, skin disease, diarrhea, dysentry and tuberculosis.[132]

The plant possess anti-viral,[133] hypoglycaemic,[134] abortifacient,[135] anti-inflammatory,[136] antibacterial,[137] and cytoxic activities[138] (Table 9).

Major Siddha preparations using *J. adhatoda* as an ingredient includes *Atatotaikkutinir, Atatotaimanappaku, Atattotainey, Kakkuvanilakam and Kapacurakkutinir.*[11]

Plectranthus ambonicus (Lour.)Spreng.

P. ambonicus (syn. *Coleus aromaticus*) (Lamiaceae) and is known as *Karpuravalliillai* in Siddha.[146] It is an errect, succulent, perennial herb arising from horizontal rhizome, found in tropical and sub-tropical regions of India.[147]

The major phytochemicals present are carvacrol, thymol, cyperene, *γ*-terpinene, caryophyllene, terpinolene, *α*-terpinene, *β*-terpineol, ethyl salicylate, quercetin and luteolin.[148-153] The drug shows antibacterial, antiviral,

Table 9: Bioactivities of phytochemical constituents in *Justicia adhatoda.*

Constituents	Class	Bioactivity	Reference
Vasicine	Alkaloid	Antimicrobial, antioxidant, cytotoxic	138
Vasicinone	Quinazoline alkaloid	Neuroprotective	139
Adhavasinone	Quinazole	Antibacterial	140
Kaempferol	Flavanoid Flavonoid	Anticancerous	141,142
Quercetin		Antioxidant	143,144
p-coumaric acids	Cinnamic acid	Anti melanogenic	145

Table 10: Bioactivities of phytochemical constituents in *Plectranthus ambonicus.*

Constituents	Class	Bioactivity	Reference
Carvacrol	Monoterpene	Antimicrobial	157
Thymol		Antibacterial	158
Cyperene	Sesquiterpene	Antimicrobial	157-159
γ-Terpinene	Terpene		
p-Cymene	Monoterpene		
Caryophyllene	Sesquiterpene		
β-Selinene			
1,8-Cineole	Monoterpene	Anti-inflammatory	160,161
Spathulenol	Sesquiterpene	Antioxidant, anti-inflammatory, anti-proliferative, anti-mycobacterial	
Terpinen-4-ol	Terpinol	Insecticidal	
Salvigenin	Flavones	Antimicrobial	161
Cirsimaritin			
Chrysoeriol			
Terpinolene	Monoterpene	Antinociceptive , anti-inflammatory	162
αterpinene	Essential oils	Antifungal	163
B-Terpineol	Terpineol		
Ethyl salicylate	Ester		
6-Methoxygenkwanin	Flavones	Antimicrobial	164
Quercetin			
Luteolin		Anticancerous	165
Apigenin			166

antifungal,[148] antileptic activities.[154] It is used in respiratory disorders, digestive disorders, insect bites, fevers, oral protection and curing of skin diseases[155,156] (Table 10).

Major Siddha preparations using *P. ambonicus* as an ingredient includes *Kanattailam* and *Kapacurak kutinir*.[11]

Terminalia chebula (Gaertn.) Retz

T. chebula (Combretaceae) is known as *Katukkay* in Siddha. Plant is seen distributed throughout the deciduous forests and extends southwards at 300 to 900m altitude.[167]

The major chemical constituents present are tannins, chebulagic acid, ellagic acid, gallic acid, syringic acid etc.[168,169] The fruit pericarp shows cytoprotective, cardiotonic, antimutagenic and antifungal properties.[170-173] The drug shows anti viral activity against HSV 2[174] (Table 11).

Major Siddha preparations using *T. cebula* as an ingredient includes *Anantapairavam, Cittatienney, Incivatakam, Karicalai ilakam, Noccitthailam, Pavanakkatukai, Tutuvalainey, Venpucaniney*.[11]

Tinospora cordifolia (Willd.) Miers ex Hook. F and Thoms

T. cordifolia (Menispermaceae) is known as *Cintiltantu* in Siddha. This climber is found throughout tropical region of India upto 1,200 m elevations.[180]

The major constituents present in this drug are sesquiterpene, tinocordifolin, tinosponone, cordioside, columbin, glycosides, alkaloids etc.[181-185] The drug helps in reduction of liver toxicity,[186] and possesses antiulcer,[187] cardioprotective,[188] immunomodulatory, antimalarial and antileprotic activities.[189,190] It is immunosuppresent, anti-inflammatory, analgesic, antipyretic, antioxidant and hepatoprotective activities[191-194] (Table 12).

Major Siddha preparations using *T. cordifolia* as an ingredient includes *Cintilcuranam, Cintilney, Kapacuranamkutinir*.[11]

Saussurea costus (Falc.) Lipsh.

S. costus (Asteraceae) is known as *Kottam* in Siddha. This perennial herb is distributed in Himalayas, Kashmir, Himachal Pradesh, Uttaranchal and Sikkim.[198]

Table 11: Bioactivities of phytochemical constituents in *Terminalia chebula*.

Constituents	Class	Bioactivity	Reference
Tannins	Polyphenol	Antioxidant	175
Chebulagic acid	Benzopyrantannin	Immunomodulator	176
Ellagic acid	Phenol	Anti-inflammatory, antioxidant	177
Gallic acid	Polyphenol	Antioxidant	178
Syringic acid		Antioxidant, antimicrobial, anti-inflammatory, anti-endotoxic, neuro-hepato protective	179

Table 12: Bioactivities of phytochemical constituents in *Tinospora cordifolia*.

Constituents	Class	Bioactivity	Reference
Tinocordifolin	Sesquiterpene	Antioxidant	195
Tinosponone		Anti-inflammatory	196
Cordioside	Di terpene		
Columbin			197

Table 13: Bioactivities of phytochemical constituents in *Saussurea costus*.

Constituent	Class	Bioactivity	Reference
Costunolide	Sesquiterpene	Anti-inflammatory, anti-viral, anti-tumor	203
Hexadecaterinal	Aldehyde	Anti-ulcer, hepatoprotective	204
Dehydrocostus lactone	Ketone	Anti-microbial, anti –neoplastic	205
Elemol	Alcohol	Immunosupressive	206

The major chemical components present are costunolide and dehydrocostus lactone.[199,200] Antifungal, anthelmintic, anti-asthamatic, anti-diabetic, antiviral, antimicrobial and larvicidal activities are present for this drug[201,202] (Table 13).

Major Siddha preparations using *S. costus* as an ingredient includes *Amirtatikkulikai, Cintilney, Itivallati, Kanatthailam, Kecariilakam, Noccitthailam, Tutwalainey, Vatacurakkutunir, Venpucani Ilakam.*[11]

Tragia involcurata Linn.

T. involucrata (Euphorbiaceae) is an evergreen hispid shrub known as *Cirukoncori* in Siddha. This plant possesses scattered stinging hairs and is seen commonly distributed all over India.[207]

Traditionally the plant is used for curing gastropathy, pruritic skins eruptions, vomiting. The whole plant is analgesic,[208] the root extract have anti-inflammatory,[209] and anti-diabetic activities.[210] The aqueous extracts of the leaves of *T. involucrate* possess antimicrobial and anti-inflammatory activity while the methanol extract possess wound healing property.[211,212] The plant also possesses anti-hyperglycaemic and anti-hyperlipidaemic,[213] antiepileptic,[214] anti-inflammatory activity,[215] hepatoprotective,[216] wound healing,[217] and antihistaminic activities[218] (Table 14).

Major Siddha preparations using *T. involcurata* as an ingredient includes *Kapacurakkutinir, Pitta curakkutinir, Tutuvalainey* and *Vatacurakkutinir.*[11]

Anacyclus pyrethrum (L.) Lag.

A. pyrethrum (Asteraceae) is known as *Akarakaram* in Siddha.[221] It is perennial procumbent herb native to North Africa and is cultivated at the elevation of 900m in Jammu and Kashmir.[222]

The phyto constituents present in this drug beolongs to the class of amides, isoflavones and alkaloids.[223-226] The drug possesses adaptogenic and immunomodulatory,[227] anticonvulsant,[228] antidepressant,[229] antidiabetic,[230] androgenic and spermatogenic,[231] and antibacterial activities[232] (Table 15).

Major Siddha preparations using *A. pyrethrum* as an ingredient includes *Carapunkavivatiilakam, Ematantakkulikai, Kapacurakkutinir, Korocanaimatari, Nantimelaku, Tutuvaliney, Vacantakucumakaram* etc.[11]

Table 14: Bioactivities of phytochemical constituents in *Tragia involucrate.*

Constituents	Class	Bioactivity	Reference
10,13-Dimethoxy-17 tetradecahydro-1*H*-cyclopenta[a] phenanthrene	Methyl ester	Anti-inflammatory	219,220
Stigmosterol	Phytosterol	Antimicrobial	
Quercetin	Flavanol		
Rutin			
3-(2,4- dimethoxyphenyl)-6,7-dimethoxy-2,3- dihydrochromen-4-one	Chromene		
5- hydroxyl-1-methylpiperdin-2-one	Piperdin	Antihistamine	218

Table 15: Bioactivities of phytochemical constituents in *Anacyclus pyrethrum.*

Constituents	Class	Bioactivity	Reference
Pellitorine	Alkaloid	Anti-diabetic, anti-cancerous, anti-bacteria, anti-inflammatory	230
Anacyclin	Amide	Anti-inflammatory	225
Sesamin		Anti-cancerous	
Daidzein	Isoflavone	Anti tumour	233,234
Genistein		Anti-cancerous	
Coumestrol	Sterol	Antioxidant	235
Formononetin	Isoflavone	Anti-inflammatory	236
Biochanin		Anti-inflammatory, neuroprotective	237
Dodeca-2E,4E-dienoic acid isobutylamide	Alkylamide	Anti protozoal	238

Hygrophila auriculata (K. Schum.) Heine

H. auriculata (Acantaceae) is known as *Mulli* in Siddha. The plant is a spiny, stout annual herb with hairy branches, swollen nodes encircled by thorns and leaves. Commonly found growing in marshy areas, wetlands and along water courses throughout the plains in India.[239] The roots and aerial parts of the plant is used and possesses anthelmintic,[240] CNS activity,[241] antimotility,[242] haematinic effect,[243] antipyretic,[244] diuretic effects.[245] The drug also exhibits hypoglycaemic,[246] antinociceptive,[247] antioxidant and hepatoprotective,[248] antibacterial,[249] and antitumour activites[250] (Table 16).

Table 16: Bioactivities of phytochemical constituents in *Hygrophila auriculata.*

Constituents	Class	Bioactivity	Reference
Lupeol	Triterpene	Cytotoxic	251,252
Stigmasterol	Steroid alcohol	Anti-inflammatory	
Betulin	Triterpene	Neuro protective	253,254
Asteracanthine	Alkaloid	Antioxidant	255,256
Palmitic acid	Fatty acid	Antimicrobial	257,258
Stearic acid		Antibacterial	257-259
Uronic acid	Carboxylic acid	Inhibitory	260

Major Siddha preparations using *H. auriculata* as an ingredient includes *Carapunkavilvatiilakam*, *Kapacurakkutinir* and *Tutuvalainey*.[11]

Interestingly all the ingredient which constitutes the KK has been proven to be highly effective against many viruses and has the potential to cure respiratory tract diseases and check inflammations. The studies are summarized in Table 17.

DISCUSSION

The periodic spread of serious infectious viral diseases like ARS-CoV, MERS-CoV and the latest 2019-nCoV has become a serious threat to the human virus. This outbreak becomes more severe as there are no approved vaccines or drugs for the treatment of CoV infections and there exists a range of animal reservoirs for CoVs and recombinant CoVs. The need of the hour is the development of broadly protective universal vaccines which can ensure the ultimate protection but the procedure is time consuming.

There has been an increase in the intensive studies of antivirals from plants which can be successful in

Table 17: Pharmacology of ingredients related to symptoms related to viral infections.

S. No.	Botanical name	Antiviral studies
1.	*Zingiber officinale*	Antirhinoviral sesquiterpene isolated from rhizomes. Anti-viral activity against human respiratory syncytial virus in human respiratory tract cell lines[261]
2.	*Andrographis paniculata*	Neoandrographolide is an immunostimulant agent[262]
3.	*Syzygium aromaticum*	Euginin isolated is used against Human herpes virus[263]
4.	*Cypreus rotundus*	Used in respiratory disorders[264]
5.	*Sida acuta*	Cryptolepine shows anti-inflammatory activity[265]
6.	*Rotheca serrata*	anti-anaphylactic, antipyretic and mast cell stabilizing effect[266]
7.	*Piper longum*	Longumosides and amide alkaloids against Human hepatitis B virus[267]
8.	*Justicia adhatoda*	Treating cold, cough, chronic bronchitis and asthma[268]
9.	*Plectranthus ambonicus*	Anti-HIV [155], anti-inflammatory activity[269]
10.	*Terminalia chebula*	Chebulagic acid and punicalagin (Hydrolyzable Tannins) prevents the entry and spread of Human herpes virus (HSV-1 and HSV-2) entry and spread[270]
11.	*Tinospora cordifolia*	For treating hyperactive respiratory disorders such as asthma and cough[271]
12.	*Saussurea lappa*	costunolide and dehydrocostuslactone showed antiviral activity against Hepatitis B virus (HBV) in Human hepatoma Hep3B cells[272]
13.	*Tragia involcurata*	ethyl ester obtained from root extract has anti-inflammatory activity[273]
14.	*Anacyclus pyrethrum*	Pellitorine possesses anti-inflammatory activity[274]
15.	*Hygrophila auriculata*	Stigmasterol possesses anti-inflammatory activity[275]

Table 18: Pharmcological activities of ingredients of *Kapacurak Kutinir.*

Herbal Drugs in KK	Antibacterial	Anti-viral	Anti-inflammatory	Antioxidannt	Hepatoprotective	Anti-cancer	Neuroprotective	Antimicrobial	Anti-parasitic	Anti-asthmatic	Anti-malarial	Antihelmintic	Antidiabectic	Antifungal	Antispasmodic	Antipyretic	Analgesic	Cytoprotective	Immunomodulatory	Anticonvulsant	Antihyperglycemic	Abortifacient	Wound healing	Hypoglycaemic	Cytotoxic	Antiseptic	Antimelanogenic
Zingiber officinale	✓	✓	✓	✓	✓	✓	✓			✓															✓		
Andrographis paniculata	✓	✓	✓	✓	✓	✓		✓						✓		✓	✓		✓							✓	
Syzygium aromaticum	✓	✓	✓	✓		✓			✓	✓	✓	✓		✓			✓										
Cyperus rotundus	✓	✓	✓		✓		✓	✓	✓		✓		✓		✓		✓	✓									
Sida acuta	✓	✓	✓	✓	✓			✓			✓		✓	✓													✓
Rotheca serrata	✓		✓	✓		✓				✓				✓	✓	✓											
Piper longum	✓	✓	✓	✓		✓		✓		✓		✓		✓		✓	✓		✓								
Terminalia chebula		✓	✓	✓	✓	✓		✓						✓				✓	✓								
Tinospora cordifolia			✓	✓	✓					✓						✓	✓		✓								
Saussurea costus	✓	✓	✓			✓		✓		✓	✓	✓	✓	✓					✓				✓				
Anacyclus pyrethrum	✓		✓	✓		✓	✓	✓	✓		✓		✓						✓	✓							
Plectranthus ambonicus	✓	✓	✓			✓		✓						✓							✓	✓					
Tragia involcurata	✓		✓		✓			✓		✓			✓								✓		✓				
Justicia adhatoda	✓	✓	✓	✓		✓	✓			✓												✓		✓	✓		✓
Hygrophila auriculata	✓		✓	✓	✓	✓	✓	✓								✓								✓	✓	✓	

treating various viral diseases. Billions of people are getting affected all over the world with various viruses like human hepatitis viruses, picornaviruses, human immunodeficiency virus, rota virus, corona virus etc. There are a number of naturally found compounds that have been identified against medically important viruses, which together contribute to annual infections of over billion peoples. In cases of pneumonia associated with CoV, accumulation of cells and fluids occurs in the respiratory tract owing to the cytokine production and inflammatory responses.[5] So its clear that we require a medication which will be helpful to inhibit the immune responses without putting the host defense under strain. The COVID pandemic originated is spreading at an alarming rate world over. The treatment provided by the Chinese medical team includes oxygen therapy, anti viral and antibacterial treatments. Lung clearing detoxification soup prepared by a polyherbal decoction with *Zingiber*, *Ephedra*, *Glycyrrhiza*, *Prunus, Cinnamomum*, *Tussilago*, *Citrus* and *Belamcanda* as the main ingredients is also recommended during clinical treatment period. With the severity of fever associated with the infection different polyherbal decoctions as per the Chineese traditional medicines are also recommended.[276]

Siddha system of medicine emphasizes on neutralizing and normalizing the three humors of the body which makes our immunity in turn increasing the resistant and immunity power of the body in dealing with viral infections. KK has been proven to be very effective in controlling the swine flu and also in boosting the immune system of the body against H1N1.[277] The symptoms of 2019 nCoV virus infection have close resemblance to that of swine flu and other phlegmatic diseases like cough, nasal congestion, fevers, shivering, diarrhea and body pain. The herbs in this decoction are loaded with curative and preventive biological activities. The aqueous extract of KK has proved its anti-inflammatory effects.[278]

The detailed review of the phytochemical constituents and the pharmacological activities of the fifteen herbal drugs used for the preparation of this decoction tabulated data makes it evident that they possess antibacterial, anti-viral, anti-inflammatory, antioxidant, antimicrobial, anti-parasitic, anti-asthmatic, anti-malarial, anthelminthic, anti-diabetic, antifungal, antispasmodic, antipyretic, analgesic, anti-cancer, antidepressant, anticonvulsant, anti-hyperglycemic, abortifacient, cytoprotective, hepatoprotective, immunomodulatory, larvicidal, neuroprotective, hypoglycaemic and wound healing activities (Table 18). This Siddha formulation which is safe, cheap and efficient with multiple benefits can be proposed to be an ideal choice of preventive measure for a broad spectrum of viral infections affecting respiratory system. Kutineer is an aqueous extraction prepared by heat. The compounds used for elucidation of pharmacological properties in this study were extracted using different organic solvents. Hence compounds may slightly vary when a kutineer is prepared as the polarity of organic solvents and water at high temperatures will be different. The various compounds from individual ingredients might also react and produce complex artifacts while they are boiled with water. Such chemical changes occuring during preperation of decoctions are unknown till date and need research by hyphenated analytical and statistical tools.

There are new evidences that active constituents of KK are effective in combating coronavirus due to their interactions with spike protein, as proved by *in silico* models and also molecular docking studies.[279-282] The said study has used major components of the medicine for finding the efficacy; while this study is collection of every constituent reported from ingredients of KK. This paper will serve as platform for further research in lines of docking to find more evidences supporting KK for its therapeutic efficacy. During this study it has been observed that there is difference of opinion among Siddha experts about botanical source of some of the ingredients used to make KK; this study followed the composition as mentioned in Siddha Formulary of India.[11]

CONCLUSION

To deal with the demanding situation such as pandemic we can propose effective therapeutic measures using the accumulated traditional knowledge of that particular region. Traditional therapies derived from herbs are usually complex of various phytochemicals and hence their pharmacolgy and the synergistic activity may give relief from symptoms when used either alone or in combination with antiviral therapies available. *Kapacurak Kutinir* can be an efficient drug in managing the ill effects of viral diseases affecting respiratory system due to promising anti-inflammatory, antiviral, immunoprotective and analgesic activities of the ingredient herbal along with several other medicinal benefits giving synergistic healing.

ACKNOWLEDGEMENT

Bayyaparavu Lakshmanudu, Lab Attendant, Siddha Central Research Institute has been acknowledged for helping in the preparation of manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

KK: *Kapacurak kutinir*; **nCoV:** Novel Corona Virus; **CNS:** Central Nervous System.

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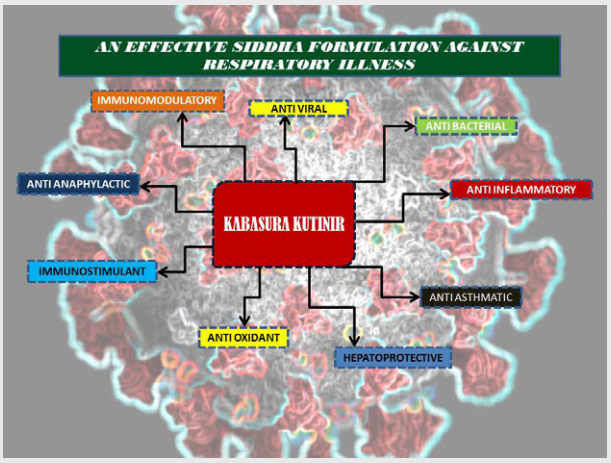
PICTORIAL ABSTRACT



SUMMARY

Presently periodic spread of severe infectious viral diseases is seen all over the world and posing threats to human population. Siddha system of medicine has a variety of poly herbal formulations used to treat a variety of diseases. *Kapacurak kutinir* a poly herbal Siddha formulation is used for curing phlegmatic fever and fever with symptoms of flu. Fifteen herbal drugs which constitute this kutinir have an immense potential with effective bioactivities. KK helps in increasing the immunity power of the body making it more resistant to viral infections. Majority of the ingredients possesses anti-asthmatic and antispasmodic activities which supporting its usage in respiratory illnesses such as that caused supports by COVID-19. This review substantiates the effectiveness of *Kabasura kutinir* in managing viral infections causing flu like symptoms and respiratory distress.

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Cite this article: Kumar KNS, Divya KG, Rubeena M, Erni B, Sathiyarajeswaran P, Kanakavalli K. Pharmacological Actions of Contents of Kabasura Kudineer-A Siddha Formulation for Fever with Respiratory Illness. Indian J of Pharmaceutical Education and Research. 2021;55(1):36-55.

Published online in http://ijam.co.in ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 11 (4), 594-615

Prevention of COVID 19 - Siddha perspective

Review Article

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Abstract

Introduction: Siddha is one of the ancient traditional medicine systems originated in South India which incorporates the extensive use of herbs, inorganic substances and animal products for maintaining a healthy life. Siddha system of medicine (SSM) has diverse and extensive use of natural resources for the prevention and management of comorbid conditions, widespread epidemic or pandemic diseases. Methodology: This article summarizes on Siddha methodologies and practices that are obtained from major scientific databases such as SciFinder, Pubmed, Scopus, Science Direct, Google Scholar and Springer using primary search terms as COVID-19, SARS-CoV-2, epidemic, immune-modulatory, antiviral, environmental sanitization and Siddha. The collected data's are extracted as SARS-COV-2 outline, Basic concepts, communicable diseases and preventive measures revealed in Siddha system of Medicine. Moreover the authors have tabulated the herbs used as health promoters and immune-modulators in Siddha, herbs used for fumigation and sanitization and the herbal ingredients used in important Siddha formulations for the management of infectious diseases. Conclusion: Many of the active principles present in the herbs are studied, proven to be effective immune-modulators, antivirals, anti-asthmatic and anti-inflammatory agents, which may also be effective towards the control of COVID-19. However, further scientific studies and data are required to support the use of Siddha medicines and herbs.

Key Words: *COVID-19, SARS-CoV-2, Siddha, Immune-modulatory, Antiviral, Sanitization.*

Introduction

The novel coronavirus pneumonia (coronavirus disease 2019, COVID-19) has now infected a total of 21,294,845 people and has claimed 761,779 deaths globally as on August 16, 2020 (1). Despite all the advancements in the 21st century in the field of medical sciences and advanced research, health problems and diseases have again led humankind to great distress. It has witnessed three major viral outbreaks in the current century- SARS-CoV, MERS and SARS-COV-2 (2) whereas the latter SARS-CoV-2, highlights the need for control in this highly pathogenic epidemic.

Due to the high infectivity of COVID-19, than its predecessors, more and more of the population are susceptible to higher respiratory infections and death (3). The high chance of human to human transmission is due to the presence of spike protein, which has a 10-20 times affinity for human angiotensin converting enzyme-2 (ACE2) for viral replication as compared to other SARS-CoVs (4).

Currently, there is no proven drug or vaccine for the treatment of COVID-19. The available methods include the use of supportive measures aimed at managing the symptoms such as fever, dehydration, respiratory disorders and other clinical symptoms. Due to the morbid and fatal nature of COVID-19, and the absence of any treatment measures, many of the clinically available antivirals, ACE2 inhibitors, immune-modulators, non-steroidal anti-inflammatory agents (NSAIDS) and corticosteroids, etc. are being repurposed.

Because of the numerous complications involved in the drug and vaccine development processes, the traditional systems of medicine are explored for their preventive and supportive care to the infected patients. In addition, some of the uncontrolled studies containing herbal products from traditional medicines claim a direct effect on the virus. Siddha system of medicine, practiced in South India has a close affinity towards Ayurveda and yet maintains a distinctive identity of its own by considering humans as a universal entity and implies that any change in the environment will have an influence on human existence and living. This system of medicine emphasizes the usage of botanical drugs for many severe respiratory diseases (5). Several of these drugs and formulations have been scientifically proven to be pharmacologically active against specific viruses such as Dengue and Chikungunya (6–8), and also possessing significant immune-modulatory effects (9). In this review, we present a brief outline on SARS-COV-2, and the Siddha concept of infectious and epidemic diseases along with their preventive measures in Siddha.

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Methodology

The authors searched the Siddha medicinal literatures available in Siddha Regional Research Institute and CSIR library for information related to infectious and epidemic diseases and a total of nine important textbooks- *Siddha Vaidya Thirattu*, *Therayar Maha Karisal*, *Therayar Yamagam*, *Brahma Muni Karukkadai*, *Yogi Vatha Kaviyam*, *Agasthiyar Vallathi, Yugi vaidhya cinthamani, Noi illa neri* and *Agasthiyar Kanma Soothiram* were reviewed: Other research articles, literatures and books were also consulted for more details. The latest information regarding SARS-CoV-2 and COVID-19, were obtained through systematic search from major scientific databases such as SciFinder, Pubmed, Scopus, Science Direct and Springer. The keywords used were SARS-CoV-2, COVID-19, treatment, transmission. In addition to the same search engines, Google Scholar search was also performed to obtain evidence regarding Siddha drugs or formulations prescribed during epidemics. For this search, we used the terms Siddha, environmental sanitization, dengue, influenza, epidemic, antiviral, immunomodulatory and phytochemicals along with the names of drugs, according to their use. Time restriction was not made to extract the most useful information.

Brief outline on SARS-COV-2

Till date, seven human CoVs (hCoV) have been identified, capable of infecting humans: 229E and NL63 (alpha coronaviruses); OC4, HKU1, MERS-CoV and SARS-CoV (beta coronaviruses); and SARS-CoV-2 or COVID-19**.** Coronaviruses are enveloped, single stranded positive RNA viruses belonging to Coronaviridae family, consisting of a large club- or petal-shaped surface projections or spikes which resembles that of the solar corona (10). Member viruses of this family can cause respiratory failure, enteric and hepatic dysfunction as well as neurological disorders in different animal species including cattle, cats, camels and bats.

Preliminary genetic analysis studies of the earlier cases from China designated L and S strains of SARS-CoV-2, where the L-type was found to be more frequent (11). In addition, the virus was first discovered through the use of high-throughput sequencing and broncho-alveolar lavage fluid samples from infected patients (12). In addition, the extensive transmission of the virus over several continents summarize the fact for genetic diversity and the presence of three prominent sites in Orf1ab polyprotein in encoding Nsp6, Nsp11, Nsp13, and one in the Spike protein designate recurrent mutations, thereby suggesting convergent evolution and possible cause for adaptation in the human body (13).

The coronaviruses consist of four structural proteins, namely spike, membrane, envelop and nucleocapsid. Spike protein is composed of two functional subunits: S_1 and S_2 which are responsible for binding and fusion of the viral and cellular membranes (14). In 2003, it was identified that ACE2 was a functional receptor for SARS-CoV (4) and the structural and functional analysis revealed the high affinity of spike for SARS-CoV-2 to ACE2 (15,16). ACE2 expression was also found to be high in lung epithelial tissue as compared to other organs such as heart, ileum, kidney and bladder, indicating SARS-CoV-2 primarily affects the respiratory system, in spite other organs are also involved (17). The other notable feature is the cleavage of polybasic cleavage site (RRAR) at the junction of S_1 and S_2 subunits by furin and other proteases which is effective for the determination of viral infectivity as well as host range (18).

In addition, it is estimated that adults are more prone to COVID-19 than infants or young children (19). Some of the hypotheses include (i) the expression of ACE2 was more abundant in adults as the human lung epithelial cells continue to develop following birth (20) (ii) with ageing, the pro-inflammatory mediators also increase, that govern the neutrophil functions and the low capability of the T-cells at early stages of birth (21) (iii) the concurrent presence of other viruses also in the lung mucosa and airways are frequent in children and can lead to competition with SARS-CoV-2 and thereby limiting its growth (22).

Basic Concepts and infectious diseases in Siddha System

According to Siddha system, all substances in the universe are composed of five basic primal elements; earth, water, fire, air and space (23); where the human body is considered as an assortment of three humors and seven physical components. Any changes in the environmental factors- air, water, habitat and season are considered accountable for disease emergence (24). These environmental, epidemiological, seasonal and water-borne diseases can be compared and treated with the use of *Noi Nadal* (Siddha Pathology) concepts (23). In *Agasthyar Pallu*, 82 Siddha drugs are described which are extensively used to fight infectious and communicable diseases (24).

The Siddha system of medicine involves the concepts and relationships of humors within the body- *vali*/*vata* (wind), *azhal*/*pitta* (bile), and a*iya*/*kapha* (phlegm), where the respective pathological condition can be determined by the investigation of *nati* (pulse) to determine the nature of the humor responsible. According to *Siddha* concept, pulsation and its movement is exhibited as different types such as movement similar to that of a swan or peacock for cases of deranged *vata* humor and that of a hen or ant, if there is *pitta* imbalance; that of a fly or vulture in cases of vitiated *kapha* (24). An experienced Siddha physician is able to differentiate and identify status of humors through the pulse movements whether in right- or left-side body parts (25), thereby identifying the nature and cause of disease.

Epidemics/pandemics are mentioned as '*Uzhi Noi*' or '*Kothari Noi*' in Siddha system. In general, they are classified under "*Kollai Noikal*" which most commonly occur at the time of "*Ayana Santhi*" months (end a month of *Uthara Ayanam* & *Thatchana Ayanam*) fall on *Aadi* (mid of July to August) and *Margazhi* (mid of December to January) month in Tamil Calendar. It is believed that in those days, the immunity of human beings will be low; based on *Trithodam* or *Mukkutram*

theory (depended on three humors *vata, pitta* and *kapha*) the occurrence of diseases are raised based on the derangement of *Mukkutram*. Usually, *Thottru Noigal* (communicable diseases), associated with *Aiya kutram* (Respiratory-related illness), gets affected due to its *Sthiram gunam* (stability factor). *Guru Naadi* quoted that, *Thottru Noigal* is generally caused by *Kirumi* (Pathogens or Microbes). The symptoms are due to *Noiyinan vanmai* (immunity of an individual); if it is good, the individual will not be affected. Hence the Siddha formulations or habits are designed to neutralize the *Aiya kutram* (24,26) and to maintain the immuno-modulatory mechanism during this period.

In accordance to the Siddha system of medicine, COVID-19 can be effectively described as a *Thotru Noi* (communicable disease) which is caused due to the derangement of immune system of the body to fight the invading *Kirumi* (virus or pathogen) which directly causes *Aiya noigal* (respiratory related illness) due to changes in food, behaviour and surroundings. In addition, the symptoms of COVID-19 such as mild fever, sore throat, malaise, headache, shortness of breath, pneumonia and respiratory distress can be compared to that of *Kapha suram*. Also, people with low immune power or immunity are also susceptible to epidemics as described by *Tirumantiram* by Saint *Tirumular* (24).

In Siddha, all types of pyrexia including vector-borne diseases such as malaria, dengue etc. are classified as a total of 64 types and collectively called as *Suram*. Among them, Siddha equates dengue to *Pitta Suram,* because the symptoms such as haematuria, anorexia, vomiting, nausea, myalgia, dysentery, fever followed by chills are similar to those described in *Sura Vadagam,* which explains the treatment as well. Literatures such as *Siddha maruthuvam* also describe similar symptoms for dengue fever. Whereas *Agastiyar sura nool 300* describes that the 'Pitta suram' can cause bleeding correlating to haemorrhages (*kuruthi azhal*) in dengue fever and the symptoms described above corresponds with the definition of dengue fever by WHO (8,27).

In the same way Siddha equates COVID-19 to *Kapha suram*, because the symptoms of *Kapha suram/ Slethma suram* are fever, cough, throat pain, anosmia, dysgeusia, shortness of breath and fatigue which can be correlated with mild stage SARS-CoV-2. In severe stage, the symptoms are related with *Sanni* staging of *Kapha suram* or *Kabavatha suram* (28). The literatures in Siddha system explores various formulations for the treatment of *Kapha suram* or *Kabavatha suram* and *Sanni noi.*

Therefore, it is evident that without identifying the microbes and other detrimental substances, Siddha healers or scholars could recognize and apprehend the reason, source and mode of transmission of contamination, thereby managing infectious epidemics. Thus the theories and observations made above supports the effective use of Siddha medicine as a significant therapy against current health problems.

Prevention of epidemics as per SSM

From Siddha literatures, it is found apparent that the "*Pini anugaa vidhi*" (keeping diseases afar) are more important than the cure for the particular disorder. Therefore, the Siddhars have recommended some basic life guidelines that are to be followed for a healthy life and wellbeing. Some of the concepts such as *Thinai/ Nilam ozhukkam* (habitat disciplines), *Kaala ozhukkam* (seasonal discipline), *Naal ozhukkam* (daily regimen) and *Unavu* (diet) are still being followed along with *Kayakalpam* as a preventive measure against diseases.

Thinai ozhukkam stresses the importance of habitat in ancient Tamil literature depending on landscape and landforms- *Kurinchi* (mountains and surroundings), prone to fevers affecting hematopoietic systems; *mullai* (forest), prone to diseases affecting joints and nerves; *neithal* (sea shores and beaches), prone to liver and intestinal diseases, *palai* (dry and desert lands) are considered prone to all kinds of diseases; and *marutham* (agricultural land) is said to have all the humors in equilibrium and hence considered the best for living (23).

Kaala ozhukkam is based on the seasonal changes that affect the humors. The diet and drugs that help in normalizing these humors are also recommended by the *Siddhars*. During monsoon season, buttermilk mixed with dried ginger, root of *Piper longum* L. and *Plumbago zeylanica* L. are considered suitable along with food rich in fiber content; walking on wet surfaces with bare foot are to be strictly avoided. In the autumn season, diet containing pulses, rice, goat milk, amla, green and leafy vegetables are deemed essential and day time sleeping is to be avoided. In the winter season, urududal (split black gram) and a diet consisting of wheat should be included with breakfast regimens. The medicated oils that minimize *vata* should be applied on the head and body as recommended. In case of spring season, rice, ghee, *kezhvaragu* (ragi), fruits like drupe, plantains along with honey are to be included in the diet. Dried roots of *Vetiveria zizanioides* (L.) are highly recommended to be included while boiling water for drinking. During this season, sleeping at daytime are restricted and wakefulness at night are to be avoided. In case of summer season, easily digestible foods rich in high water and fiber content such as grapes, pomegranate, cardamom, ilupaipoo (flower of *Madhuca longifolia* (J. Konig) J.F. Macbr.), and palm jaggery are recommended. Moreover, alcohol consumption is to be strictly avoided during this season (24,26). The diet regimens and sleep patterns are to be strictly maintained in order for healthy wellbeing, according to Siddha concept.

Naal ozhukkam involves the daily discipline that has to be followed on a day; where an entire day is divided into six minor individual parts based on time, called *sirupozhuthu* (small periods) as each part is dominated by a particular humor. Daily lifestyle regimens such as waking up between 4 to 6 am, drinking pure water immediately after waking and use of twigs from *Asoku* (*Saraca asoca* (Roxb.) Willd), *Vembu* (*Azadirachta indica* A. Juss.), *Aathi* (beedi leaf

tree), *Aal* (Indian banyan), *Vael* (Gum Arabic tree) for brushing are considered to be very healthy and hygienic. Application of *Pancha karpam* which is a mixture of five herbal ingredients such as *Kasthuri manjal* (aromatic turmeric), *Milagu* (pepper), *Vembin vithu* (seed of neem), *Kadukkai thol* (*Terminalia chebula* Retz.. fruit) and *Nelli paruppu* (gooseberry seed) mixed with boiled milk, on head before bathing helps for keeping the body cool and balance the humors. Eating only when required and on a right quantity is essential to maintain the humor. The sleeping beds should be used according to the season and pathological conditions. For example, in cases of giddiness, vomiting and aggravated *pitta*, sleeping on *Thazhampai* (screw pine leaves) *padukkai* (mat) is advised (24).

Unavu involves the dietary regimens for a safe and healthy life. According to Siddha, *Unavae marunthu (Food is Medicine) as proclaimed* by the great Siddhar *Thiruvalluvar* in his famous work *Thirukkural.* The dietary regimen during the intake of Siddha medicine is termed as *pathyam* and *apathyam* (23,29,30).

Medicinal ingredients such as Velavarai (*Dolichos lablab* L.), Manathakkali (*Solanum nigrum* L.), Nerunchil (*Tribulus terrestris* L.), Mookiratai (*Boerhavia diffffusa* L. nom. cons.), Musumusukai (*Mukia maderaspatana* (L.) M. Roem.) etc. are recommended for the balancing of *Vata* humour. Whereas, Cucumber (Cucumis sativus), Vallarai (*Centella asiatica* (L.) Urban), Puliarai (*Oxalis corniculata* L.), Kovai (*Coccinia grandis* (L.) Voigt), Ponmusuttai (*Sida acuta* Burm.f.), Oritazhttamarai (*Ionidium suffrutiocosum* Ging.), Sundai (*Solanum torvum* Sw.) etc. are useful in the maintenance of *pitta* and; Brinjal, Peipudal (*Trichosanthes cucumerina* L.), Bittergourd, Pepper, Turmeric, Mustard, Sundai (*Solanum torvum* Sw.), Gooseberry etc. are essential for the control of *kapha* as per Siddha literature. Also, *Kayakalpa* drugs such as *Citrus limon* (L.) Burm. (elumicchai), *Terminalia chebula Retz.* (kadukkai), *Strychnos potatorum* Linn. (thettran), *Phyllanthus emblica* L. (nelli), *Aegle marmelos* (L.) Correa (vilvam) help in providing essential nutrients to the body and act as a preventive against most of the diseases (23). Meat and fish are strictly restricted in certain type of diseases and during Siddha treatment period. A number of immunomodulatory drugs that are encouraged during epidemics are given in **Table 1**.

Role of SSM in environmental sanitization

Fumigation, called "*Pugai"* in Siddha represents the artificial saturation of surroundings with fumes or smoke of any herb or aromatic substance. The purpose of this procedure is to control the microbial infections. *Pugai* is one among the 32 types of external therapies explained in Siddha literature *Theriyar Tharu* and was used as a preventive measure to protect themselves from communicable diseases (24). It is considered as one of the best methods employed for disinfection and sterilization of the environment and surroundings instead of chemical fumigants. During fumigation, the surroundings as well as the persons involved in the process are benefited by the usage of medicinal herbs (31).

Pugai involves fumigating the surroundings by burning dried medicated herbal juices or extracts over a cloth, where the cloth acts as *Thiri* (wick). Similarly, Thippili (*Piper longum* L.), Manjal (*Curcuma longa* L.*),* Omam (*Trachyspermum ammi* (L.) Sprague), and Milagu (*Piper nigrum L.*) are ground, then applied on a cloth and soaked with Neem oil. This mixture was allowed to smolder and the smoke was allowed to be inhaled by the patient and/or used as an environment sanitizer (31). Ellu (*Sesame indicum* L.), Payaru (*Vigna* radiate (L.) R. Wilczek), Kadugu (*Brassica juncea* (L.) Czern.), egg shell, fecal matter of dog, outer skin of the Poondu (*Allium sativum* L.), Kattamanaku (*Jatropha curcas* L.), Thulasi *(Ocimum sanctum L.),* Devadaru (*Cedrus deodara* (Roxb.) G.Don) are also used for the same purpose. *Karuvelam pisin* (Gum of Acacia Arabica), roots of Murungai (*Moringa oleifera* Lam.), Erukku (*Calotropis gigantea* (L.) Dryand.), azhinjil (*Alangium salviifolium* (L.f.) Wangerin) and Sivanar vembu (*Indigofera aspalathoides* Vahl. ex. DC.) are made into a fine powder, and a pinch is added to burning charcoal and was used to fumigate the surroundings as well as the patient (24).

Suruttu (medicated cigar) is made by rolling dry medicated leaves, for eg., *Adathodai suruttu*. The fumes of Sathakuppai (*Anethum graveolens* L.) dry leaves are extensively used around the patient's surroundings as a disinfectant. These fumes not only cleanse the affected areas but also relieves the mental stress of the subjects (31). In Siddha literature, Sage *Agasthiyar's Maanidakkirigai-64* explains the use of various drugs for *Pugai* in the treatment of Psychiatric ailments (24).

Nowadays, Padigara neer (alum) and turmeric water (*Curcuma longa L.*) are also used as an effective hand sanitizer. *Agasthiyar kuzhambu* was extensively used in the dose of 3 to 5 paddy weight to fumigate before the origination of chemical sprays or fumigants (31). Some of the other common herbs that are used for fumigation as well as sanitization in Siddha are: *Shorea robusta* Roth*., Argemone mexicana* L.*, Costus speciosus* (J. Konig) C. Specht*, Anethum graveolens* L.*, Boswellia serrata* Triana & Planch.*, Santalum album* L.*, Abutilon indicum* (Link) Sweet*, Cedrus deodara* (Roxb.) G. Don*, Nicotiana tabacum* L.*, Mangifera indica* L.*, Crinum asiaticum* L., *Aquilaria agallocha* Roxb.*, Lawsonia inermis* L., *Ruta chalepensis* L., *Justicia beddomei* (C.B. Cl) S.S.R. Bennet, *Madhuca longifolia* (J. Konig) J. F. Macbr., *Datura metel* L., *Solanum surattense* Burm. f., *Saccharum officinarum* L., *Rhus succedanea* L. (31). The information regarding these medicinal drugs along with other natural fumigants are available in **Table 2**.

Many of the medicinal herbs contain volatile and essential oils which can act as excellent antimicrobial, for example Manjal (*Curcuma longa* L.), Vembu (*Azadirachta indica* A. Juss.), Sadakuppi (*Anethum graveolens* L.), Milakukkirai (*Mentha piperita* L.), Karupuramaram (*Eucalyptus globulus*

Labill.), Thulasi (*Ocimum sanctum* L.), etc. provide pleasant aroma when burnt or incinerated.

It is evident that from **Table 2**, almost all of the medicinal herbs proposed for fumigation in Siddha literature are found to be antimicrobial or antiviral agents. In-spite of their pharmacological actions, there are no evidence based clinical studies suggesting the use of these herbs as fumigants. However, there are several sporadic studies that compared the herbal disinfectants which are recommended by Siddha to that of the chemical agents and found equally efficacious, safe and available at low cost (32,33). Yet clinical studies in a controlled manner and data reports are required to prove the efficacy and safety of these herbal fumigants (34,35).

Prevention of COVID-19: Siddha perspective

The data collected from the literatures and Manuscripts reveal the following preventive measures of COVID-19 in Siddha system of medicine.

According to the Siddha system of medicine, food is considered as medicine and proper intake of food can provide immunity to the body. A number of immunomodulatory herbs are reported in Siddha as a preventive during epidemics are given in **Table 1.** Immunomodulation is the ability to alter the immune response in humans and animals against infectious agents, stimulation of immune system is preferred for patients those who have compromised immunity and immune-suppressants are required for patients having inflammatory diseases.

In general, an infusion made up of half teaspoon of chukku (dried ginger) in two litres of water can be used for drinking; the use of inji (ginger) thenooral / inji tea / adhimaduram (liquorice) tea is recommended for immunomodulation. It is highly advised to avoid drinking milk before bedtime and if needed for children, adding a quarter teaspoon of manjal (turmeric) with milagu (pepper) is advised. Steam inhalation therapy by using *Tulasi* / *Nochi* / *Manjal* and gargling with a pinch of salt and turmeric is also highly recommended. The use of *karappan pandam* (allergic food) should be devoid from the daily diet. Also, the diet may include Nandu kanji (Crab soup), Pancha mutti kanji (5-grain gruel) and Irumurai vadittha kanji (double-cooked gruel) are highly recommended to include in the daily regimen as per the Siddha literatures (36,37) and the Ministry of AYUSH, Govt. of India (38).

In spite of the other resources, 108 medicinal herbs called as *karpa mooligaikal* are predominantly used in Siddha system for treating certain diseases and as an antioxidant & immunomodulator which were used extensively in case of dengue and HIV epidemics (8,39). These plants are also rejuvenators to boost health and thereby prevent chronic diseases and reduce ageing (40).

Kayakalpa (Kaya- Body, mind and psyche and Kalpa- Transmutation) is one of the significant and exclusive methods of Siddha system which combines both human and natural sources (herbs) for rejuvenation and transformation as prevention from diseases (23,24). The treatment regimen involves lifestyle measures and routines involving breathing regulation, sperm conservation, administration of carefully processed mineral drugs or potent herbs such as *Katrazhai* (*Aloe indica* Royle), *Bhringaraja* (*Eclipta alba* L. Hassk) and *Neem* (*Azadirachta indica* A. Juss.) etc. which are beneficial to the human internal system as a whole (41). Also, the intake of *Muppu*, which is a meticulously prepared mixture of three salts are considered to have prophylactic action besides the rejuvenation of body (13).

In addition, the effectiveness of a number of Siddha medicinal formulations or drugs are being scientifically validated and proven, thereby supporting and promoting the value of Siddha system of medicine. For example, *Brahmananda bairavam mathirai*, *Nilavembu Kudineer*, *Vishnu chakram* are effective against chikungunya infections (7,8,42). Evidence based Siddha medications such as *Nivalembu kudineer*, *Adathodai kudineer*, *Veppilai chooranam*, *Ammukkara chooranam*, *Amman Pachirisi karkam* for dengue infections (8,39); Urai mathirai for its immunomodulatory effect (9); *Kapa Sura*, *Sarva Sura* and *Visha Sura Kudineers* (decoctions) from the Siddha literature *Kaaviya Sura Nool* are also useful against Swine flu fever, as these formulations are found to contain major medicinal herbs and phyto-constituents that are proven to be antiviral and antipyretic agents (28,39,43,44). Other polyherbal Siddha preparations such as *Chitramutti Kudineer, Chukku kudineer, Adathodai manapagu* are some of the classical medications used by Government of Tamil Nadu, India, in the year 2012 when the state was plagued by dengue fever (45).

Urai mathirai is a Siddha formulation made up of 10 herbal ingredients of hot-potency and pungent taste, extensively used for the prevention of recurrent respiratory infections. The medication as a whole after the digestion in stomach gets transformed into a fire moiety which increases the *Azhal* (immunity) of the body. As according to Siddha system *"Vatamaai Padaithu, Pitta Vanniyai Kathu, Sethuma Seethamai Thudaithu"* meaning *Vata* is responsible for creation, *Pitta* for prevention and *Kapha* for destruction. Infection occurs if the immunity is challenged and it can be rectified with the increase of *Azhal.* The ingredients of Siddha polyherbal formulation *Urai mathirai* are chukku (*Zingiber officinale* Roscoe), adimathuram (*Glycyrrhiza glabra* L.), akkirakaram (*Anacyclus pyrethrum* (L.) Lag.), vashambu (*Acorus calamus* L.), catikkai (*Myristica fragrans* Houtt.), katukkai (*Terminalia chebula Retz.*.Retz.), masikkai (*Quercus infectoria* G.Olivier), acanam (*Allium sativum* L.), tippili (*Piper longum* L.) and perunkayam (*Ferula assa-foetida L.*) which are having immunomodulatory activity (7,8).

As COVID-19, is considered as a *Thotru noi* having similar symptoms as that of *Kabasuram* and *Sanni noigal* in Siddha literature, the effective management may be acquired by the use of anti-inflammatory, antiviral, antipyretic, immunomodulators in order to reduce or control the symptoms (24). Some

of the most extensively used Siddha formulations which are said to contain the above said pharmacological activities are (i) *Kaba Sura Kudineer* (ii) *Nila Vembu Kudineer* (iii) *Visha Sura Kudineer* (iv) *Sarva Sura Kudineer* (28,38,39,42,44). The contents of the said Siddha preparations are given in **Table 3**.

The four Siddha formulations contain a blend of medicinal herbs which are to be administered as a liquid at a dose of 60 ml twice a day before food. These formulations contain specific immunomodulators for respiratory care, antivirals, antipyretic and anti-inflammatory agents such as *Sitrarathai* (*Alpinia galanga* (L.) Willd.), *Amukkara* (*Withania somnifera* (L.) Dunal), *Kodiveli* (*Plumbago zeylanica* L.), *Charanai* ver (*Trianthema decandra* L.), *Peyputtal* (*Trichosanthes cucumerina* L.), *Koraik kilanku* (*Cyperus rotundus* L.), *Parpatakam* (*Mollugo cerviana* (L.) Ser.), *Nilavembu* (*Andrographis paniculata* (Burm.f.) Nees), *Keezhanelli* (*Phyllanthus niruri L.), Seenthil* (*Tinospora cordifolia* (Thunb.) Miers) which are individually proven for their respective pharmacological actions (8,28,39,43,44).

Siddha system of medicine also contains different formulations such as *Adathodai Managapu*, *NellikkaiIlagam*, *Vasantha Kusumakaram*, *Thalisathi Vadagam*, *Bramananda Bairavam*, *Thirithoda Mathirai*, *Seenthil Chooranam*, *Pachaikarpoora Mathirai*, *Swasakudori Mathirai*, *Thippili Rasayanam,* etc which are the effective herbal formulations in Siddha which can be employed for the control of *Kabasuram* – fever with Respiratory illness (38,46,47).

Conclusion

It can be summarized that prevention of epidemics are possible through the methods of Siddha system of medicine by maintaining a stable and healthy relationship with the human body and the environment. This review is aimed to update the readers about the classical Indian system of medicine, which is built on the vast experiences on observation and treatment regimens of the ancient saints of India. The Siddha concepts of sanitation, diet and immunomodulation are completely dependent on the mind and natural surroundings of human population. Therefore, Siddha system of medicine is a bundle of desirous information that has to be explored, explained and implemented to understand the ancient knowledge of maintaining a relationship with the environment for better wellbeing.

The medicinal herbs which are being long used in ancient cultures are now being proven to be effective through scientific studies although more controlled clinical data are of utmost importance. The concept of *pugai* as a mode of fumigation and sanitization of surroundings and environment, the use of herbal hand washes such as turmeric and the use of *kayakalpa* technique and various other polyherbal decoctions with respect to the daily and seasonal variations with a strict and proper diet lead to a long and healthy life to our ancestors.

Therefore, it can be concluded that the increase in infectious diseases or pandemics will continue to transpire with the emergence of severe organisms or microbes; and an effective method of control and prevention will be of importance. Hence, the herbal drugs and concept of Siddha medicine and lifestyle may prove effective and cheap products in the development of medications. This review suggests the use of traditional system of Indian medicine such as Siddha to shed light along with the modern system of medicine to maintain and stable and healthy lifestyle remarkably during pandemics.

Tables

Table 1: Herbs used as health promoters and Immunomodulators according to Siddha.

Botanical name and family	Siddha name	Part used	Location	Traditional uses	Constituent responsible	Mode of action or rationale	References
Cedrus deodara (Roxb.) G. Don, Pinaceae	Devadaru	Wood	Jammu and Kashmir, Himachal Pradesh, Nepal, China	Essential oil as insect repellant, aromatherapy, astringent, antifungal	Volatile oil constituents	Immunostimulant, anti-inflammatory, antioxidant	(48–50)
Cinnamomum tamala (Buch. Ham.) T. Nees & C.H. Eberm., Lauraceae	Lavanga pathiri	leaves	Bangladesh, Nepal, China, India	Antidiabetic, carminative, sedative, antidepressant, antidiarrhoeal, astringent, stimulant	A-type procyanidin oligomers	Immunomodulatory, antimicrobial	(37,51)
Alpinia galanga (L.) Willd., Zingiberaceae	Arathai	Rhizome	West Bengal, Assam, Cambodia, Thailand, Japan	Cold, sore throat, anti-emetic, analgesic	Neolignans and sesquineolignans	Immunomodulatory, antioxidant	(52–54)

Withania somnifera (L.) Dunal., Solanacea	Amukkara	Root	Tamil Nadu, Rajasthan, Andhra Pradesh, China, Yemen	Blood tonic, treat irregular menstruation, anaemia, erectile dysfunction	Withaferin A	Immunostimulant, anti-inflammatory	(55,56)
Tinospora cordifolia (Thunb.) Miers, Papilionaceae	Seenthil	Leaves	Haryana, Madhya Pradesh, Assam, China, Bangladesh, Vietnam, Malaysia, Sri Lanka	Memory booster, antispasmodic, antidiarrhoeal, stomachic, tonic, bronchitis, promotes longevity, anti-allergic	Alkaloids, flavonoids, saponins	Immunomodulatory, antiviral	(57,58)
Cynodon dactylon (L.) Pers., Poaceae	Arugampul	Juice	Madhya Pradesh, Himalayas, Pakistan, Indonesia, United States	Haemostatic, diuretic, antipyretic, treatment of bronchitis, leucoderma, diarrhea, hypertension	Protein fraction	Immunomodulatory, antioxidant	(50,59, 60)
Curcuma longa L., Zingeberaceae	Manjal	Rhizome	Most states in India, Pakistan, Malaysia, Indonesia, Ethiopia, Japan, China	Natural antiseptic, disinfectant, analgesic, remedy for skin diseases, indigestion, arthritis	Aqueous rhizome powder	Immunostimulant, anti-inflammatory	(51,61, 62)
Plumbago zeylanica L., Plumbaginaceae	Kodiveli	Root	Assam, Australia, Oman	Expectorant, astringent, laxative, abortifacient, memory booster,	Seselin	Immunomostimulant, anti-inflammatory	(63,64)
Justicia adhatoda L., Acanthaceae	Adathodai	Leaves	Eastern Ghats, Assam, Himalayas, China, Nepal	Cough, fever, asthma, dysentery	Vasicine	Immunostimulant, antioxidant, antimicrobial	(55,63, 65)
Enicostemma axillare Lam., Gentianaceae	Vellarugu	Whole	Tamil Nadu, West Bengal, Kerala	Stomachic, tonic, antipyretic, remedy for dyspepsia, malaria, leprosy	Swertiamarin	Immunomodulatory, antoxidant	(66,67, 68)
Indigofera aspalathoides Vahl. ex. DC.,Fabaceae	Sivanar vembu	Aerial part	Pakistan, Indonesia, Malaysia, Sri Lanka	Demulcent, sedative, analgesic, antispasmodic, remedy for leprosy, malaria, kidney stones	Kaempferol 5-O-β-D-glucopyranoside	Immunostimulant, anti-inflammatory	(61,69)
Senna alexandrina Mill., Fabaceae	Nilavarai	Seeds	Tamil Nadu, Egypt, South Africa, China	Remedy for dysuria, diabetes, night blindness, epilepsy	Rhein	Immunostimulant, antimicrobial	(70,71)

Morus alba Linn., Moraceae	Kambli chedi	Leaves	Haryana, China, Pakistan, Africa, South America	Tonic, laxative, reatment for cough, catarrh, fever, sore throat, dizziness, vertigo.	Methanol extract	Immunomodulatory, antioxidant	(36,59)
Terminalia arjuna (Roxb.) Wight & Arn., Combretaceae	Marudha maram	Bark	Madhya Pradesh, Rajasthan	Cough, cold, skin diseases, inflammation, asthma, excessive perspiration, remedy for viral and blood diseases	Tannins, arjunolic acid	Immunostimulant	(72,73)
Allium sativum L., Amaryllidaceae	Poondu	Garlic bulb	Asia, Iran, Egypt, Mexico	Stomachic, fever, cough, antibacterial, anti-inflammatory	Lectins	Immunomodulatory	(32,61, 58)
Boerhavia diffusa L. nom. cons., Nyctaginaceae	Mukkarattai	Root	Asia, South America and Africa	Remedy for reproductive disorders, jaundice, kidney problems, skin diseases, eye problems	Punarnavine	Immunomodulatory, Anti inflammatory, Antioxidant	(74,75)
Pteridium aquilinum (L.) Kuhn., Dennstaedtiaceae	Parnai	Bud	China, India, Indonesia	Treatment of tuberculosis, antiemetic, antiseptic, appetizer, tonic	Aqueous extract	Immunomodulatory, antioxidant	(55,76, 77)
Andrographis paniculata (Burm.f.) Nees., Acanthaceae	Nilavembu	Whole plant	Eastern Ghats, Sri Lanka, China, United States	Treatment of dyspepsia, influenza, dysentery, malaria, respiratory infections	Andro-grapholides	Immunomodulatory, antipyretic, antioxidant	(55,76, 78)

Table 2: Herbs used for fumigation and environmental sanitization as per Siddha system.

Botanical name and family	Siddha name	Part used	Location	Traditional use	Phytochemistry	Mode of action or rationale	References
Curcuma longa L., Zingeberaceae	Manjal	Rhizome	Most states in India, Pakistan, Malaysia, Indonesia, Ethiopia, Japan, China	Natural antiseptic, disinfectant, analgesic, remedy for skin diseases, indigestion, arthritis	Turmerone, zingiberene	Fumigation, antimicrobial, sanitizer	(51,79,80)
Azadirachta indica A. Juss., Meliaceae	Vembu	Leaves	India, China, Malaysia, Caribbean, South East Asia	Antibacterial, anti-inflammatory, natural antiseptic, antipyretic	Azadirachtin, Nimbin	Fumigation, insecticidal, antimicrobial	(51,61,81)
Anethum graveolens L., Apiaceae	Sadakuppai	Leaves	Central Asia, Mediterranian, USSR	Abdominal pain, eye diseases, uterine pains	Scopoletin, umbelliferone	Insecticidal, antibacterial	(66,82,83)

Piper longum L., Piperaceae	Thippili	Seeds	India, Pakistan, China, Europe	Relieve muscular pains, anti-inflammatory, stimulant, stomachic, antidiabetic	Piperine, piperlongumine	Antiparasitic, antimicrobial, antibacterial	(9,59,84)
Carum copticum (L.) Sprague ex Turrill., *Apiaceae*	Omam	Leaves, seeds	Kerala, West Bengal, Gujarat, Iran, Afghanistan, Pakistan	Carminative, antiseptic, expectorant, antimicrobial bronchodilatory, antitussive	Carvacrol, γ-terpinene	Insecticidal, antimicrobial	(66,85)
Piper nigrum L., Piperaceae	Milagu	Seeds	South India, Vietnam, Brazil	Cough, sinusitis, throat pain, infections, ear ache, gastrointestinal disorders	β-pinene, p-cymene	Antimicrobial, antibacterial	(86-88)
Sesamum indicum L., Pedaliaceae	Ellu	Seeds	Asia, Africa, Japan	Wound healing, antiviral, analgesic, hypolipidaemic	Sesamin, sesamol, sesaminol	Fungicide, antimicrobial	(36,66,89, 90)
Vigna radiata (L.) R. Wilczek,* Fabaceae	Cherupayaru	Seeds	India, China, Bangladesh	Antipyretic, anti-inflammatory	Catechin, gallic acid	Antimicrobial, antibacterial	(55,91,92)
Brassica juncea (L.) Czern., Brassicaceae	Kadugu	Seeds	Pakistan, India, Bangladesh, Japan, China, America	Stimulant, expectorant, diuretic, used as spice	Zeaxanthin, lutein	Fumigation	(61,93-95)
Allium sativum L., Amaryllidaceae	Poondu	Outer skin	Asia, Iran, Egypt, Mexico	Stomachic, fever, cough, antibacterial, anti-inflammatory	Allicin, alliin, diallyl sulfide	Fumigation	(32,96,97)
Jatropha curcas L.,* Euphorbiaceae	Kattamanaku	Leaves	Pakistan, India, South America, China	Lactogogue, stomachic, rubefacient, remedy for diabetes, arthritis, jaundice, malaria	Vitexin, isovitexin, gallic acid	Insecticidal	(51,61,98)
Ocimum sanctum L., Lamiaceae	Thulasi	Leaves	India and Southeast Asia	Bronchitis, malaria, diarrhea, dysentery, skin diseases, arthritis, eye diseases, insect bites, antibacterial, common cold	Methyl eugenol, carvacrol	Insecticidal	(70,97,99)
Cedrus deodara (Roxb.) G. Don, Pinaceae	Devadaru	Wood	Jammu and Kashmir, Himachal Pradesh, Nepal	Essential oil as insect repellant, aromatherapy, astringent, antifungal	Deodarin, cedeodarin, cedrusin	Antiparasitic, antimicrobial	(48,49,55, 64)

Moringa oleifera Lam., Moringaceae	Murungai	Roots	Asia and Africa	Immune booster, anti-inflammatory, abortifacient, cough, common cold	Isotrifolin, quercetin	Fumigation, antimicrobial	(66,86, 100)
Calotropis gigantea (L.) Dryand., Apocynaceae	Erukku	Root	Haryana, China, Thailand, Sri Lanka	Antipyretic, purgative, antimicrobial, wound healing activity	Calotropagenin, calotoxin	Fumigation, insecticidal	(51,101, 102)
Alangium salviifolium (L.f.) Wangerin,* Cornaceae	Azhinjil	Root	India, China, Phillipines	Emollient, anthelmintic, laxative, diuretic, antidote, purgative	Alangidiol, alangicine	Antifungal	(55,57, 103)
Indigofera aspalathoides Vahl. ex. DC.,* Fabaceae	Sivanar vembu	Root	India, Pakistan, Indonesia, Malaysia, Sri Lanka	Demulcent, sedative, analgesic, antispasmodic, remedy for leprosy, malaria, kidney stones	Afromosin, genistein	Insecticidal, antibacterial	(61,69,79, 104)
Shorea robusta Roth.,* Dipterocarpaceae	Kungiliyam	Resin	India, China, Russia	Astringent, detergent, wound healing effect, remedy for cold, piles, bronchitis and leucorrhoea	Fischinidol, Afzetechin tannins	Insecticidal	(51,105)
Argemone mexicana L.,* Papaveraceae	Kudiyotti poondu	Seeds	Madhya Pradesh, Eastern Ghats, Himalayas, South America, West Africa	Treatment of tumors, warts, skin diseases, inflammation, rheumatism, jaundice, leprosy, microbial infections, malaria	Oxyhydrastinine, Mexicanol, Mexicanic acid	Insecticidal, antibacterial	(55,57, 106, 107)
Costus speciosus (J. Konig) C. Specht., Costaceae	Kottam	Roots and rhizomes	Nagaland, Assam, Sri Lanka, Malaysia	Astringent, aphrodisiac, purgative, anthelmintic, depurative, febrifuge, expectorant, tonic, stimulant	Cycloartenol, diosgenin, lupeol	Fumigation	(108,109)
Boswellia serrata Triana & Planch., Burseraceae	Kuntharikkam	Resin	Assam, Bihar, China	Remedy for bronchitis, asthma, cough, diarrhea, dysentery, fever	β-Sitosterol, α-Thujene	Antifungal, fumigant	(32,57, 110)

Santalum album L., Santalaceae	Santhanum	Wood	Eastern Ghats, China,	Tonic for heart, stomach, liver, fever; treatment of diarrhea, piles, vomiting, eye infections and inflammation	Nuciferol, α-Curcumone	Fumigation	(35,55,57, 111)
Abutilon indicum (Link) Sweet,* Malvaceae	Thuthi	Seed	Tamil Nadu, Africa, Australia	Diuretic, laxative, demulcent, analgesic, antiulcer	Gossypetin, Vanillic acid, β-Sitosterol	Insecticidal	(70,75, 112)
Tinospora cordifolia (Thunb.) Miers.,* Papilionaceae	Seenthil	Leaves	Haryana, Madhya Pradesh, Assam, China, Bangladesh, Vietnam, Malaysia, Sri Lanka	Memory booster, antispasmodic, antidiarrheal, stomachic, tonic, bronchitis, promotes longevity, anti-allergic	Quercetin, monocrotaline	Insecticidal, antibacterial	(57,58,68)
Nicotiana tabacum L.,* Solanaceae	Pugayilai	Leaves	India, China, North America, Europe, Africa	Treatment of rheumatism, pulmonary ailments, conjunctivitis, bronchitis and pneumonia	Solavetivone, Nor-nicotine	Antibacterial	(55,113,114)
Mangifera indica L.,* Anacardiaceae	Maa	Leaves, flower	India, Africa, Middle East	Dentifrice, antiseptic, astringent, diaphoretic, stomachic, laxative, vermifuge	Mangiferin, fisetin	Insecticidal, antimicrobial	(59,68, 115)
Crinum asiaticum L.,* Amaryllidaceae	Vidamoongil	Leaves	Tropical Asia	Analgesic, antimicrobial, antiemetic, anthelmintic, laxative, skin infections, expectorant, wound healing property	Lycorine, isocraugsodine, criasbetaine	Insecticidal	(31,116, 117)
Aquilaria agallocha Roxb.,* Thymeleaceae	Akil	Wood	India, China, Indonesia	Mouth freshner, aphrodisiac, astringent, bitter, cardiotonic, stimulant, carminative, fragrant	Aromatic oil	Antimicrobial	(61,79, 118)
Lawsonia inermis L.,* Lythraceae	Alavanam	Seed	Eastern Ghats, Assam, China	Treatment of headache, hemicranias, lumbago, bronchitis, syphilis, scabies, dysuria, skin diseases	Lawsone, scopoletin, esculetin	Insecticide, antioxidant	(55,63, 119)

Ruta chalepensis L., Rutaceae	Aruvatha	Leaves	Kerala, China, North Africa	Anti-inflammatory, analgesic, antipyretic	Chalepin, graveoline, arborinine	Insecticide, fumigant	(66,120)
Justicia adhatoda L., Acanthaceae	Adathodaai	Leaves	Eastern Ghats, Assam, Himalayas, China, Nepal	Cough, fever, asthma, dysentery	Vasicine, Vasicinone, β-Sitosterol	Fumigation, antioxidant	(55,121)
Madhuca longifolia (J. Konig) J.F. Macbr., Sapotaceae	Illupai	Crushed cake of seed	Madhya Pradesh, Eastern Ghats	Emollient, skin diseases, rheumatism, headache, laxative, piles, hemorrhoids	A-Spinasterol, Betulinic acid	Fumigation	(51,68, 122)
Datura metel L.,* Solanaceae	Oomathai	Leaves	Haryana, Jammu and Kashmir, China, Brazil	Anti-fungal, anti-bacterial, antirheumatic, anti-inflammatory	Daturine, atropine	Antifungal, antimicrobial	(58,116, 123)
Solanum surattense Burm. f.,* Solanaceae	Kandangat hiri	Seed	Himalayas, Pakistan, Malaysia, Southeast Asia	Remedy for inflammatory problems, leprosy, dropsy, cough, hernia, dental caries and swelling	Solasonine Solamargine, esculin	Insecticidal	(61,113, 124)
Saccharum officinarum L.,* Poaceae	Karumbu	Sugar	Assam, Pakistan, New Guinea, Taiwan, China	Whooping cough, canes for broken bones, catarrh	Apigenin, Orientin, Ferulic acid	Insecticidal	(59,63, 125)
Rhus succedanea L.,* Anacardaceae	Karkadaka singi	Gall	Himalayas, Australia, New Zealand	Antidote, cholagogue, febrifuge, treatment of phthisis	Aromatic oil, Tannins	Insecticidal	(96,126)

*Studies regarding the fumigation effect of these herbs were not found on any of the search engines. However, scientific studies suggest the presence of bioactives that may provide santization and air-purifying effects on fumigation. Hence, these herbs provide a rationale for future research.

Table 3: Herbal ingredients used in important Siddha formulations for *Kabasuram*.

Botanical name and family	Siddha name	Part used	Location	Traditional uses	Major Pharmacological action	Siddha Formulation	References
Santalum album L., Santalaceae	Chandanam	Heart wood	Eastern Ghats, China	Tonic for heart, stomach, liver, fever; treatment of diarrhea, piles, vomiting, eye infections and inflammation	Anti-pyretic, treatment of common cold and bronchitis	Nilavembu, Visha Sura Kudineer	(55,57,111)
Terminalia chebula Retz., Combretaceae	*Kadukai*	Fruit	Arunachal Pradesh, Nagaland, China, Europe	Detoxifying agent, purgative, digestant, rejuvenator, wound healing property	Antiviral, antinociceptive	Visha Sura Kudineer	(87,127)
Anisochilus carnosus (L.f.) Wall., Lamiaceae	*Katpooravvalli*	Leaves	India, China, Indonesia	Gastric ulcer and skin diseases	Anti-pyretic, antimicrobial	Visha Sura Kudineer	(51,116,12 8)

Plectranthus vettiveroides (Jacob) N.P. Singh & B.D. Sharma, Lamiaceae	Vilamichai	Root	South India, Sri Lanka	Deodorant, treatment of headache, diarrhea, fever, hyperdipsia, leprosy, ulcer, nausea, giddiness	Analgesic, anti-inflammatory, antimicrobial	Nilavembu, Kaba Sura Kudineer	(66,129,130)
Tragia involucrata L., Euphorbiaceae	*Sirukaannchonri Ver*	Root	Assam, West Bengal, Kerala	Treatment of wounds, scabies, skin infections	Bronchodilator, anti-pyretic, anti-inflammatory	Visha Sura Kudineer	(66,131)
Cyperus rotundus L., Cyperaceae	Koraikilanku	Stem bark	Haryana, Africa, central Europe	Remedy for diarrhea, diabetes, inflammation, malaria, stomach, bowel disorder, fever	Antipyretic, anti-hypertensive	Nilavembu, Sarva Sura Kudineer	(43,132,133)
Piper nigrum L., Piperaceae	Milaku	Seed, Fruit	South India, Vietnam, Brazil	Cough, sinusitis, throat pain, infections, ear ache, gastrointestinal disorders	Antimicrobial, anti-pyretic, anti-inflammatory	Nilavembu, Sarva Sura Kudineer	(42,88)
Hygrophila auriculata Schumach., Acanthaceae	Neermulli ver	Root	South India, Sri Lanka	Treatment of cough, anal fistula, blood disorders, jaundice, anaemia, dropsy, aphrodisiac	Haematopoietic, anti-inflammatory	Visha Sura Kudineer	(39,67,134)
Sida acuta Burm.f.., Malvaceae	Vattathiruppi Ver	Root tuber	Tamil Nadu, Central America	Treatment of fever, skin diseases, diarrhea, dysentry	Antibacterial, antipyretic	Visha Sura Kudineer	(39,135)
Andrographis paniculata (Burm.f.) Nees., Acanthaceae	Nilavembu	Stem, leaves	Eastern Ghats, Sri Lanka, China, United States	Treatment of dyspepsia, influenza, dysentery, malaria, respiratory infections	Immunostimulant, anti-inflammatory	Nilavembu, Sarva Sura, Kaba Sura Kudineer	(8,28,44)
Hedyotis corymbosa (L.) Lam., Rubiaceae	Parpadaakam	Whole plant	India, Sri Lanka, East Asia	Antiviral, treatment of acne, hepatitis, eye diseases, skin ailments, bleeding, promotes diuresis	Antimicrobial, antioxidant, immunostimulant	Nilavembu Kudineer	(55,136,137)
Trichosanthes cucumerina L., Curcubitaceae	Peipudol	Gourd	Eastern Ghats, Sri Lanka, Malaysia	Treating headache, alopecia, fever, abdominal tumors, boils, diarrhea, haematurian skin allergy	Anti-inflammatory, antipyretic	Nilavembu, Sarva Sura Kudineer	(42,44,55, 61,138)
Zingiber officinalis Roscoe, Zingiberaceae	Sukku	Rhizome	Kerala, Andhra Pradesh, tropical Asia	Antiemetic, stomachic, expectorant, aphrodisiac	Antipyretic, anti-inflammatory	Nilavembu, Sarva Sura, Visha Sura, Kaba Sura Kudineer	(28,39,42, 44)

Vetiveria zizanioides (L.) Nash, Poaceae	Vettiver	Root	India, Senegal, Sri Lanka, Brazil	Cooling agent, tonic, blood purifier, treatment of skin disorders, indigestion, loss of appetite	Antioxidant, anti-inflammatory	Nilavembu, VIsha Sura Kudineer	(39,42,129)
Hemidesmus indicus (L.) R.Br., Apocyanaceae	Nannari ver	Root	Eastern Ghats, China	Demulcent, astringent, diaphoretic, diuretic, tonic, antipyretic, blood purifier, leprosy, bronchitis, syphilis, pruritis, urinary diseases	Anti-inflammatory, antipyretic	Visha Sura Kudineer	(39,55)
Justicia adhatoda L., Acanthaceae	Aadathodai	Leaves	Eastern Ghats, Assam, Himalayas, China, Nepal	Cough, fever, asthma, dysentery	Immunostimulant, antimicrobial, antitussive	Sarva Sura, Kaba Sura Kudineer	(55,63,139)
Glycyrrhiza glabra L., Fabaceae	Adimaduram	Root	Kerala, China, Central and South Western Asia	Sweetening and flavoring agent, expectorant, anti-tussive agent,	Anti-inflammatory, immunomodulatory	Sarva Sura, Visha Sura Kudineer	(57,66,140)
Aristolochia bracteolata Lam., Aristolochiaceae	Eechuramooli	Root	Nigeria, Ethiopia, India	Prevent seizures, immune booster, treatment of snake bites, intestinal pain, gall bladder pain, arthritis, gout, rheumatism	Antibacterial, anti-inflammatory	Visha Sura Kudineer Kudineer	(61,66,141)
Anacyclus pyrethrum (L.) Link, Asteraceae	Akkarakaram	Stem bark	Himalayas, Jammu and Kashmir, West Bengal, Spain, North Africa	Aphrodisiac, antidiabetic, antiasthmatic, throat problems, rejuvanant, carminative, diuretic, muscle relaxant	Antipyretic, anti-inflammatory, immunostimulant	Sarva Sura, Kaba Sura Kudineer	(53,93,116)
Vitis vinifera L. Vitaceae	Grapes/Plums	Fruit	Europe, Western Asia	Laxative, purgative, diuretic, aphrodisiac, appetizer, asthmatic, treatment of diarrhea, bleeding.	Antipyretic, anti-inflammatory	Sarva Sura Kudineer	(61,136,142)

Indigofera tinctoria L., Fabaceae	Neeli	Root	India, Pakistan, China	Treatment of epilepsy, nervous disorders, asthma, bronchitis, fever, stomach ache, rabies, skin diseases, wounds, sores, ulcers	Immunoprotective, anti-inflammatory	Visha Sura Kudineer	(39,61,143)
Syzygium aromaticum (L.) Merr. & L.M. Perry, Myrtaceae	*Karampu*	Fruit	Haryana, China, Indonesia	Anxiolytic, expectorant, antimicrobial, decongestant	Antipyretic, antiviral	Visha Sura Kudineer	(39,48)
Phyllanthus niruri L., Phyllanthaceae	Keezhkainelli	Whole plant	Central and south India, China, Nigeria, Guam	Treatment of jaundice, gonorrhea, antidiabetic, antiviral, skin ulcers, sores, antiallergic	Antiviral, antipyretic, analgesic	Sarva Sura Kudineer	(6,57)
Costus speciosus (J. Konig) C. Specht, Costaceae	Koddam	Root	Nagaland, Assam, Sri Lanka, Malaysia	Astringent, aphrodisiac, purgative, anthelmintic, depurative, febrifuge, expectorant, tonic, stimulant	Antipyretic, anti-inflammatory	Sarva Sura, Kaba Sura Kudineer	(63,93,109)
Elettaria cardamomum (L.) Maton, Zingiberaceae	Elam	Fruit	India, Nepal, Sri Lanka, Mexico, Tanzania	Culinary uses, remedy for asthma, gum infections, kidney disorders, cataracts, nausea, diarrhoea	Antibacterial, anti-inflammatory	Visha Sura Kudineer	(39,59,96)
Azadirachta indica A. Juss., Meliaceae	Vembu	Stem bark	India, China, Malaysia, Caribbean, South East Asia	Antibacterial, natural antiseptic, antipyretic	Antibacterial, anti-inflammatory, antiviral	Visha Sura Kudineer	(51,55,68)
Smilax chinensis L., Smilacaceae	Parankilanku	Stem bark	India, China, Taiwan, Japan	Used as energy tonic, remedy for impotency, seminal disorders, arthritis, syphilis, schizophrenia, epilepsy	Antipyretic	Sarva Sura Kudineer	(55,116,144)
Mollugo cerviana (L.) Ser., Molluginaceae	Parpadaakam	Aerial part	Rajasthan, south India	Fever, stomach ache, jaundice, gout, rheumatism	Antioxidant, antipyretic, spasmolytic, hypolipidemic	Sarva Sura Kudineer	(42,44,145)

Tinospora cordifolia (Thunb.) Miers, Papilionaceae	Seenthil kodi	Leaves	Haryana, Madhya Pradesh, Assam, China, Bangladesh, Vietnam, Malaysia, Sri Lanka	Memory booster, antispasmodic, antidiarrheal, stomachic, tonic, bronchitis, promotes longevity, anti-allergic	Antimicrobial, antioxidant, anti-inflammatory	Sarva Sura, Kaba Sura Kudineer	(57,68,132)
Alpinia galanga (L.) Willd., Zingiberaceae	Sitrarathai	Rhizome	West Bengal, Assam, Cambodia, Thailand, Japan	Cold, sore throat, anti-emetic, analgesic, antioxidant	Anti-inflammatory	Sarva Sura Kudineer	(53,54)
Abies webbiana Lindl., Pinaceae	Talisapathiri	Leaves	Himalayas, Northeast India	Carminative, expectorant, stomachic, tonic, as a remedy for respiratory problems, cold, tuberculosis, indigestion	Anti-tussive, anti-inflammatory	Sarva Sura Kudineer	(87,93,146)
Piper longum L., Piperaceae	Thipally	Root	India, Pakistan, China, Europe	Relieve muscular pains, anti-inflammatory, stimulant, stomachic, antidiabetic	Anti-inflammatory, analgesic	Sarva Sura, Kaba Sura Kudineer	(61,66,84)
Evolvulus alsinoides (Linn.) Linn., Convolvulaceae	Vishnukiraanthy	Whole plant	Eastern Ghats, Africa, Philippines	Cure fever, cold, venereal diseases, adenitis, depression, dementia	Antipyretic, anti-inflammatory, antidiarrheal	Sarva Sura Kudineer	(55,79,131,147)
Clerodendrum serratum (L.) Moon., Verbenaceae	*Siruthekku*	Root	Assam, Himalayas, Sri Lanka, South Africa, Australia	Remedy for cough, asthma, malaria, fever, urinary tract infections, itches, ulcerated wounds, dysmenorrheal, epilepsy	Anti-allergic, antipyretic, anti-inflammatory	Visha Sura Kudineer	(59,148,149)

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Journal of Ayurveda and Integrative Medicine xxx (xxxx) xxx

Contents lists available at ScienceDirect

Journal of Ayurveda and Integrative Medicine

journal homepage: http://elsevier.com/locate/jaim

Original Research Article (Experimental)

In Silico computational screening of *Kabasura Kudineer* - Official Siddha Formulation and JACOM against SARS-CoV-2 spike protein

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ARTICLE INFO

Article history:
Received 21 April 2020
Received in revised form
6 May 2020
Accepted 8 May 2020
Available online xxx

Keywords:
SARS-CoV-2
Spike protein
Kabasura Kudineer
JACOM
Molecular docking

ABSTRACT

Background: Siddha Medicine is a valuable therapeutic choice which is classically used for treating viral respiratory infections, this principle of medicine is proven to contain antiviral compounds.
Objective: The study is aimed to execute the *In Silico* computational studies of phytoconstituents of Siddha official formulation *Kabasura Kudineer* and novel herbal preparation - JACOM which are commonly used in treating viral fever and respiratory infectious diseases and could be affective against the ongoing pandemic novel corona virus disease SARS-CoV-2.
Method: Cresset Flare software was used for molecular docking studies against the spike protein SARS-CoV-2 (PDB ID: 6VSB). Further, we also conducted *in silico* prediction studies on the pharmacokinetics (ADME) properties and the safety profile in order to identify the best drug candidates by using online pkCSM and SwissADME web servers.
Results: Totally 37 compounds were screened, of these 9 compounds showed high binding affinity against SARS-CoV-2 spike protein. All the phytoconstituents were free from carcinogenic and tumorigenic properties. Based on these, we proposed the new formulation called as "SNACK–V"
Conclusion: Based on further experiments and clinical trials, these formulations could be used for effective treatment of COVID-19.

1. Introduction

The novel Coronavirus disease-2019 (COVID-19) is an ongoing pandemic caused by Severe Acute Respiratory Syndrome Corona-Virus 2 (SARS-CoV-2) [1]. COVID-19 has been declared a pandemic disease by WHO which has severely affected the livelihood of the population. SARS-CoV-2 has spread across the continents, as of April 11, 2020, has led to a total of 16,99,676 cases with a mortality of 1,02,734 among the registered cases. Presently, quarantine and symptomatic treatment protocol for disease management exists and there are no specific antiviral drugs available to combat this virus. As per Ministry of Health and Family Welfare, Govt. of India, in India there are 7447 Active cases and 239 deaths as on April 11, 2020; these data commensurate the impending risk facing the country. This pandemic is still ongoing, hence there is an urgent need to find new preventive and therapeutic agents as soon as possible [2].

Knowledge of Microbes and their Disease spread is clearly mentioned in Siddha which is evinced by "*Kirumiyal vandha thodam perugavundu lines mentioned in Guru naadi*"[3]. Siddha holistic approach will be helpful in combating COVID 19 using both therapeutic and non-therapeutic interventions. Siddhar's have advised evidence based treatment approach to understand a disease (*Noi naadi*), its etiology (*Mudhal Naadi*) based on those, fix a treatment (*Athu Thanikka Vainaadi*). As per basic Siddha Concept, Siddhar

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Peer review under responsibility of Transdisciplinary University, Bangalore.
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https://doi.org/10.1016/j.jaim.2020.05.009

Please cite this article as: Kiran G et al., *In Silico* computational screening of *Kabasura Kudineer* - Official Siddha Formulation and JACOM against SARS-CoV-2 spike protein, J Ayurveda Integr Med, https://doi.org/10.1016/j.jaim.2020.05.009

Theran has defined *Vatham* is responsible for creation, *Pittam* for prevention and *Aiyam* for destruction. Infections happen to a person when his immunity is challenged which could be related with reduction of *Pitham*. According to Siddha theory, in a COVID-19 infection there is initial increase of body temperature, cough and throat pain which may subside if there is good amount of immunity and these symptoms subside when *Pitta thathu* (Humor) come into action. If not, it escalates to a phase of *Kapha Dosham* (Disorder) which is said as "*Thanamulla sethumanthan ilagil veppu*". If not treated at this stage it slowly moves to a Stage of *Sanni* (Severe Pneumonia- Respiratory failure). It has been unanimously agreed to have equated diagnosis as *Kaphasuram* in Siddha in early stages moving towards *Sanni* and which is also reassured through Delphi or other sources of FGD (Focus group discussion).

The control and treatment of a viral infection depends mainly on the availability of antiviral drugs, which are few in numbers and usually are not directly acting on virus but prevent replication in the host. The Siddha herbal formulations having medicinal importance have proved to be potentially active against a wide range of causative agents as Influenza, Dengue, Chikungunya, Tuberculosis, etc [4–6]. Siddha medicines have been used effectively by human civilization over several centuries for treating various diseases and can be effectively employed to target the host response, like *Kabasura Kudineer* during influenza outbreaks [7]. Besides, during Dengue outbreak in India, a herbal formulation of Siddha medicine, *Nilavembu Kudineer* is used to prevent and control the morbidity level of public on contacting this viral fever [8].

Kabasura Kudineer, an official Siddha formulation described in Siddha manuscript '*Citta Vaittiyattirattu*' is used for *Aiyacuram* (phlegmatic fevers) and is a dependable Siddha prescription for fever with flu-like symptom [9]. Further, we choose another herbal formulation called "JACOM" a coded novel drug due to its Neuraminidase inhibition potential against inactivated influenza virus H1N1(Patent no.201741016901 A, dated 18.05.2018) [10].

Moreover, to screen out large number of herbs for compounds with antiviral activity against novel corona virus will be a challenge in very short period. Drug discovery is a time consuming, slow and challenging process [11,12], so it is necessary to depend on computational tools (Computer-aided drug design) to overcome these pitfalls to an extent. Of late, the impact on these tools for new drug development had made the drug discovery process very cost effective and time efficient [11]. For searching compounds, this ligand-based virtual screening tool is used to identify most probable molecule with pharmacological activity using molecular docking [13–15]. Similarly, for studies pharmacokinetics, toxicity, and drug-likeness prediction many algorithms exist which makes the job easier [16]. There are lots of evidence which prove the application of computational tools in the discovery of natural-derived drugs [17–20]. Hence, the aim of the current study is to apply this incredible *in-silico* screening methodology for the official Siddha formulation *Kabasura Kudineer* and the novel formulation JACOM against SARS- CoV-2 spike protein.

2. Methods

2.1. Ligand preparation

Kabasura Kudineer Chooranam is a polyherbal formulation containing fifteen herbal drugs (Table 1) mixed in equal quantities and decoction is prepared. To prepare *Kabasura Kudineer Chooranam* all the fifteen ingredient drugs are coarsely powdered and mixed; 35 g of this powder is boiled with three liters of water and reduced to the volume of 1/12th. This has to be taken 30–60 mL twice or thrice daily [9]. The bioactive constituents used for docking were obtained from *Kabasura Kudineer Chooranam* are β-Sesquiphellandrene, β-Bisabolene, Geranial, Piperine, Piperlonguminine, Eugenol, β-Caryophyllene, Stigmosterol, 3-(2,4- dimethoxyphenyl)-6,7- dimethoxy-2,3- dihydrochromen-4-one, Squalene, γ-Sitosterol, Andrograpanin, 5-Hydroxy-7,8-dimethoxyflavanone, Lupeol, Betulin, Chebulagic acid, Gallic acid, Vasicinone, Carvacrol, Cirsimaritin, Chrysoeriol, 6-Methoxygenkwanin, Luteolin, Costunolide, Elemol, Tinosponone, Bharangin, Scutellarein, Magnoflorine, Cycleanine, Cyperene, β-Selinene [21–23] The bioactive constituents from JACOM are Vasicine, Andrographolide, Ursolic acid, Quercetin and Meliacine. The 2D structures of ligands are summarized in Supplementary Table S1. All the ligands were obtained from PubChem and prepared a single.sdf file, further optimization and minimization of all ligands were done in Cresset Flare software with default settings. The ligands file read in Autodetect under full protonation mode.

2.2. Protein preparation

To investigate the phytochemical analogs of Siddha formulation *Kabasura Kudineer Chooranam* and JACOM against SARS-CoV-2 virus, we have selected novel spike glycoprotein (PDB ID: 6VSB), a key target for therapeutics, vaccines and diagnostics in SARS-CoV-2. This spike glycoprotein 2019-nCOV S protein is a single receptor-binding domain (RBD) which binds to ACE2 (Angiotensin converting Enzyme-2) receptor on the host cell with high affinity, which makes it a key target for the novel coronavirus therapy development. The 3D structure of novel spike glycoprotein (PDB ID: 6VSB) were downloaded from Protein Data Bank (https://www.rcsb.org/structure/6VSB). The target protein was downloaded in PDB format and protein preparation was carried out in Cresset module Flare software with default settings. Missing residues, hydrogen's and 3D protonation were carried out on the target protein and minimized for the selected active residues [24].

2.3. Molecular docking studies

Molecular docking was carried for 32 phytochemical constituents of Siddha formulation *Kabaura Kudineer Chooranam* and 05 phytoconstituents of JACOM. The phytochemical analogs were docked with spike protein SARS-CoV-2 (PDB ID: 6VSB) by using Cresset Flare Docking software with default settings and the grid box was defined based on trial and error and carried out in normal mode [25,26]. The crystal structure of protein was obtained from protein data bank. The structures of phytochemical constituents were downloaded from the PubChem and the structures were converted into a single database file in sdf file format in Data warrior software. Best poses were generated and visualized in pose viewer and 3D images stored in storyboard. Analysis of docking results was done with Flare Software and the results are shown in Tables 1 and 2. Best score generating phytoconstituents in the largest cluster was analyzed for its interaction with the protein and 2D poses were obtained from LigPlus.

3. Results

3.1. Molecular docking studies

The molecular docking studies were carried out for the 32 phytochemical constituent's of Siddha formulation *Kabasura Kudineer Chooranam* and 05 phytochemical constituent's JACOM against coronavirus spike protein to identify the molecular interactions between target protein with ligands. All the phytochemical analogs were docked with spike protein SARS-CoV-2 (PDB ID: 6VSB) by using Cresset Flare Docking software.

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Table 1
In silico docking studies of phytoconstituents of Siddha formulation *Kabasura Kudineer Chooranam* and JACOM against spike Protein SARS-CoV-2 (PDB ID: 6VSB) using docking software Cresset Flare.

Plant Name	Compound name and Code	LF dG	LF VSscore	LF Rank Score	LF LE
Kabasura Kudineer Chooranam					
Zingiber officinale Rosc	β-sesquiphellandrene **(1)**	−6.638	−6.846	−2.658	−0.443
	β-bisabolene**(2)**	−6.562	−6.713	−2.8	−0.437
	Geranial**(3)**	−5.099	−5.319	−2.121	−0.464
Piper longum L	Piperine**(4)**	−6.768	−7.445	−4.143	−0.322
	Piperlonguminine**(5)**	−7.078	−7.7	−4.245	−0.354
Syzygium aromaticum	Eugenol**(6)**	−4.818	−5.559	−6.182	−0.402
	β-Caryophyllene**(7)**	−5.654	−5.918	−3.203	−0.377
Tragia involucrata L	Stigmosterol**(8)**	−9.724	−10.39	−7.466	−0.324
	3-(2,4- dimethoxyphenyl)-6,7- dimethoxy-2,3-dihydrochromen-4-one**(9)**	−6.433	−7.316	−9.011	−0.247
Anacyclus pyrethrum	Squalene**(10)**	−9.722	−10.187	−1.389	−0.324
	γ−Sitosterol**(11)**	−9.956	−10.521	−7.679	−0.332
Andrographis paniculata	Andrograpanin**(12)**	−6.819	−7.678	−7.854	−0.296
	5-Hydroxy-7,8-dimethoxyflavanone**(13)**	−7.356	−7.966	−9.035	−0.334
Hygrophilla auriculata (Schum.)Heine	Lupeol**(14)**	−8.337	−8.917	−6.41	−0.269
	Betulin**(15)**	−7.984	−9.117	−7.02	−0.249
Terminalia chebula Retz.	Chebulagic acid**(16)**	−10.769	−11.138	−9.723	−0.158
	Gallic acid**(17)**	−5.549	−6.602	−6.916	−0.462
Justicia adhatoda L.	Vasicinone**(18)**	−5.753	−6.272	−8.164	−0.384
Plectranthus amboinicus (Lour) Spreng	Carvacrol**(19)**	−5.322	−5.696	−6.923	−0.484
	Cirsimaritin**(20)**	−6.42	−7.227	−9.228	−0.279
	Chrysoeriol**(21)**	−7.954	−8.352	−11.392	−0.362
	6- Methoxygenkwanin**(22)**	−6.415	−7.527	−9.293	−0.279
Costus speciosus	Luteolin**(23)**	−8.149	−8.584	−11.159	−0.388
	Costunolide**(24)**	−6.081	−6.607	−3.799	−0.358
	Elemol**(25)**	−6.587	−6.696	−5.43	−0.412
Tinospora cordifolia (Willd.) Miers ex Hook.f&Thoms	Tinosponone**(26)**	−7.043	−7.434	−8.145	−0.293
Clerodendrum serratum L.	Bharangin**(27)**	−7.418	−7.744	−6.682	−0.309
	Scutellarein**(28)**	−7.805	−9.148	−10.277	−0.372
Sida acuta Burm. f.	Magnoflorine**(29)**	−7.635	−8.527	−9.762	−0.305
	Cycleanine**(30)**	−6.184	−8.214	−3.432	−0.134
Cypreus rotundus L.	Cyperene**(31)**	−6.024	−6.225	−3.558	−0.402
	β-selinene**(32)**	−6.33	−6.587	−3.412	−0.422
JACOM Formulation					
Justicia adathoda L.	Vasicine**(33)**	−5.19	−6.1	−7.67	−0.37
Carica Papaya	Quercetin**(34)**	−8.408	−8.59	−11.478	−0.382
Andrographis paniculata Burm.f.Nees	Andrographolide**(35)**	−7.74	−8.45	−7.85	−0.31
Ocimum tenuiflorum	Ursolic acid**(36)**	−7.08	−7.71	−5.1	−0.21
Melia azedarach	Meliacine**(37)**	−4.2	−8.76	−5.14	−0.88

The crystal structure of protein was obtained from pdb bank. The structures of phytochemical constituents were downloaded from the PubChem and the structures converted into a single database file in sdf file format in Data warrior software. To fight against this deadly virus, many X-ray crystal structures of proteins were reposited in pdb bank for Receptor-binding protein (RBD, trimer) with PBD ID 6CRV and 6VSB; Heptad repeat 2(HR2) with PBD ID 2FXP.

The SARS-CoV-2 virus binds to human cells through its spike glycoprotein, making this protein as key target to design potential therapeutics. In this regard, we have selected potential phyto constituents with previously reported antiviral activity for carrying out the docking studies with the viral spike glycoprotein.

Binding affinities of phytocompounds of siddha formulation *Kabasura Kudineer Chooranam* and JACOM towards active site of spike protein SARS-CoV-2 was studied in detail. Biological interaction analyses of phytoconstituents with spike protein SARS-CoV-2 were carried out to identify the compound having highest binding affinity with target proteinin the Flare software docking analysis.

The LF rank score is an indicator of the binding affinity of protein-ligand complex. The LF rank for each phytocompound is described in Tables 1 and 2 The binding orientation for each phytocompounds into the active site of SARS-CoV-2 spike protein is identified based on the molecule having the least LF rank score. The more the negative LF rank score represent the better affinity of the phytocompound against target SARS-CoV-2 spike protein.

Among the docking studies performed on phytocompound, all the analogs had effective binding interactions with SARS-CoV-2 spike protein (LF rank score range from −5.75 to −11.03). From the results it reveals that Phytoconstituents with highest docking LF rank score were seen for Chrysoeriol and Luteolin from *Kabasura Kudineer Chooranam* and Quercetin from JACOM with LF rank score values −11.478, −11.392 and −11.159, respectively. Whereas, 5-Hydroxy-7,8-dimethoxyflavanone, Cirsimaritin, Scutellarein with LF rank score of −9.035, −9.228, and −10.277, show moderate binding affinity against the target protein. Remaining analogs also show lower binding affinity towards SARS-CoV-2 spike protein. We further studied detailed binding orientation of top 11 phytocompounds in the active site of spike protein and best poses in 2D and 3D were generated.

The number of hydrogen bond and the number of amino acid residues of SARS-CoV-2 interacting with each phytocompounds are given in Table 2. From the detailed docking analysis, it is observed that Chrysoeriol, Luteolin, and Scutellarein show a high binding affinity with target protein SARS-CoV-2 spike protein. It is found that, these three compounds have formed H-bond contact with more than four amino acid residues in spike protein showing that it forms more number of H-bonds resulting in increased binding affinity with target protein Figs. 1–3.

The interaction analysis of Chrysoeriol, Cirsimaritin, and Magnoflorine - SARS-CoV-2 spike protein complex reveals that amino acids Cys336, Asp364, Ser373, Asn343, Cys336, Gly339, Asp364,

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Table 2
Amino acid residues of SARS-CoV-2 spike protein participated in H-Bond and hydrophobic interactions with ligands.

Compound Code	LF Rank Score	Interactions	
		H-Bonding	Hydrophobic
β-sesquiphellandrene **(1)**	−2.65	NHB	Ser373, Phe374
β-bisabolene**(2)**	−2.8	Phe342, Ser373,	Phe338, Gly339
Geranial**(3)**	−2.12	NHB	Ser373, Phe374,
Piperine**(4)**	−4.14	Phe374, Trp436	Phe338, Ser373,
Piperlonguminine**(5)**	−4.24	Phe338	Ser373, Phe342, Cys336, Leu335, Val367
Eugenol**(6)**	−6.18	Asn343, Phe342,	Ser373
β-Caryophyllene**(7)**	−3.20		Phe338, Gly337
Stigmosterol**(8)**	−7.46	Cys336, Gly336,	Phe342, Asn343, Ser373,
3-(2,4- dimethoxyphenyl)-6,7- dimethoxy-2,3- dihydrochromen-4-one**(9)**	−9.01	Arg509, Trp436,	Phe374, Phe342, Asn343, Thr345, Ala344, Leu441
Squalene**(10)**	−1.38	NHB	Thr345, Asn643, Phe342, Asn343, Phe338, Leu335
γ−Sitosterol**(11)**	−7.67	Cys336, Gly339	Ser373, Phe374, Val510
Andrograpanin**(12)**	−7.85	Asn343	Phe342, Leu335, Asp364
5-Hydroxy-7,8-dimethoxyflavanone**(13)**	−9.03	Asp 364, Gly339	Cys336, Phe337, Leu335, Phe342, Phe338, Leu368
Lupeol**(14)**	−6.41	Thr345	Asn343, Ser373, Thr345, Arg509
Betulin**(15)**	−7.02	Thr345, Ser373	Asn422, Val341, Arg509, Phe373, Thr345
Chebulagic acid**(16)**	−9.72	Tyr369, Asn370, Tyr369, Phe377, Cys379, Lys378	Lys378, Phe337, Phe342, Cys336
Gallic acid**(17)**	−6.91	Lys356, Val341	Ala397, Val341, Lys356
Vasicinone**(18)**	−8.16	Cys336, Gly339	Val397, Cys336, Phe338, Leu335, Asp364
Carvacrol**(19)**	−6.92	Asp364	Cys336, Leu335, Asp364
Cirsimaritin**(20)**	−9.22	Cys336, Asp364, Ser373, Asn343	Phe338, Phe342, Phe374, Ser373
Chrysoeriol**(21)**	−11.39	Cys336, Gly339, Asp364,	Phe338, Phe342, Phe374, Leu335, Val367, Ser373
6- Methoxygenkwanin**(22)**	−9.29	Cys336, Phe342	Ser373, Phe342, Leu368, Phe338, Leu335
Luteolin**(23)**	−11.15	Asp364, Val367, Ser371, Ser373, Cys336, Val362	Phe338, Gly339, Phe374, Phe342
Costunolide**(24)**	−3.79	Phe515, Gly431	Val511, Phe515, Gly431
Elemol**(25)**	−5.43	Asp364, Asp364	Phe374, Phe342, Asn343,
Tinosponone**(26)**	−8.14	Phe342, Gly339	Trp436, Asn343, Leu368, Val367
Bharangin**(27)**	−6.68	Phe338, Gly339,	Phe337, Phe342, Ser373
Scutellarein**(28)**	−10.27	Cys336, Phe338, Gly339, Asp364, Val362	Ser373, Phe374, Leu335, Asn343
Magnoflorine**(29)**	−9.76	Arg346, Val341, Thr345	Ala344, Lys356, Ala397
Cycleanine**(30)**	−3.43	Ser373	Phe374, Trp436,
Cyperene**(31)**	−3.55	NHB	Ser373
β-selinene**(32)**	−3.41	NHB	Phe342, Ser373
JACOM Formulation			
Vasicine**(33)**	−7.67	Phe 338, Asn343	Gly339
Quercetin**(34)**	−11.47	Asp364	Phe338, Leu335, Gly339, Leu368, cys336, he374
Andrographolide**(35)**	−7.85	Asp364, Phe368, Gly339, Asn343	Cys336, Phe342, Leu368, Phe374
Ursolic acid**(36)**	−5.1	Val367	Leu368
Meliacine**(37)**	−5.14	Phe338	Val367, Ser371, Leu368, Phe338
Hydroxychloroquine**(38)**	−8.35	Phe342, Asn343	Gly339, Phe338, Leu368, Trp436, Ser373, Phe374

NHB: No Hydrogen Bond Interactions.

Arg346, Val341, and Thr345 have played important role in the formation of H-bond network. The possible binding orientation of phytocompounds from Siddha formulation *Kabasura Kudineer Chooranam* and JACOM into the active site of SARS-CoV-2 spike protein and corresponding hydrophobic interaction models, number of hydrogen bonds are shown in Tables 1 and 2 and Figs. 1 and 3. The Docking studies of all the phytochemicals from two formulations were compared with positive control Hydroxychloroquine and found that all docked ligands were interacting with the same amino acid residues. The validation docking and Hydroxychloroquine has LF rank score −8.35 and forms two H-bond interactions with Phe342 and Asn343 Fig. 4.

Flare was used to perform *in silico* computational studies, prediction of cavity, assigning bond orders, structure refinement, defining the active sites of the SARS-CoV-2 and structure preparation. The protein preparation was carried out with Flare and the chain was treated to add missing hydrogen, assign proper bond orders. The structure output format was set to pose viewer file so as to view the output of resulting docking studies and hydrogen bond interactions of different poses with the protein. The 2D and 3D interactions were generated with Ligplus and storyboard in Cresset. All the studied Phytoconstituents have showed excellent free energy of binding interactions with SARS-CoV-2 Figs. S1−S8.

3.2. In Silico *prediction of drug likeliness, and synthetic accessibility*

Rule of 5 by Lipinski is a significant criterion to evaluate drug likeliness and if a specific chemical compound with a certain biological activity has physio-chemical properties that would make it a likely orally active drug in humans. Lipinski's rule evaluates the different descriptors which are important for a drug design. Lipinski's rule of five states that (i) molecular mass less than 500 Da, (ii) no more than 5 H-bond donors, (iii) no more than 10 H-bond

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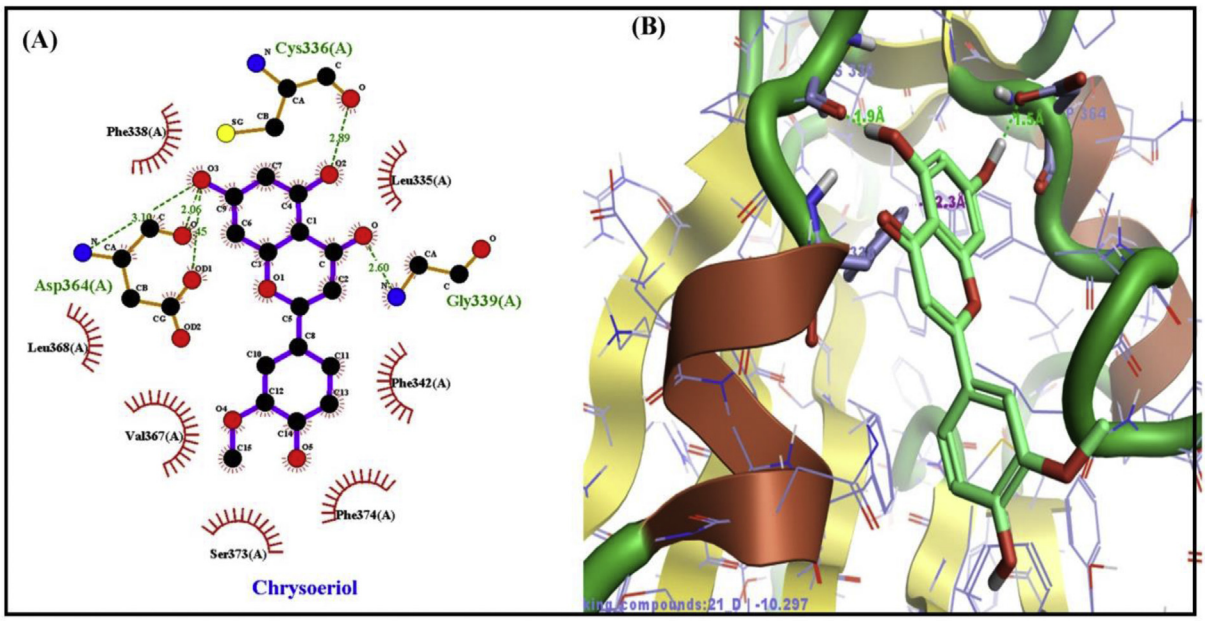

Fig. 1. Molecular docking results of Chrysoeriol into SARS-CoV-2 spike protein. (A) Hydrophobic interaction of Chrysoeriol with SARS-CoV-2 Spike protein (B) Binding mode of Chrysoeriol in SARS-CoV-2 Spike protein. Amino acid residues involved in H-bond formation and H-bond networks are shown.

acceptors, (iv) O/W partition coefficient log P not greater than 5. If the molecule violates more than 3 descriptor parameters, it will not fit into the criteria of drug likeliness and it is not considered in order to proceed with drug discovery.

Supplementary Tables S2 and S3 depicts the drug likeliness and various rules like Lipinski rule of five, Veber Ghose, Muegge and Egan rules were applied to all phytochemical constituents. From the data, most of the Phytoconstituents obeyed the rules only few analogs violated. The low value of synthetic accessibility indicates that all the phytoconstituents could be synthesized. These results indicate the active ingredients of two Siddha Formulations of *Kabasura Kudineer Chooranam* and JACOM have drug like properties.

3.3. In Silico *simulation of Pharmacokinetic Properties*

In silico pharmacokinetics properties of phytochemical constituents of Siddha formulation *Kabasura Kudineer Chooranam* and JACOM were carried out with online pkCSM webserver.

From the data of pharmacokinetic properties shows that Lupeol, Betulin, Cycleanine, β-selinene, Quercetin, Andrographanin and Tinosponone have the highest gastrointestinal absorption, tissue distribution (Vd), and respectable total clearance

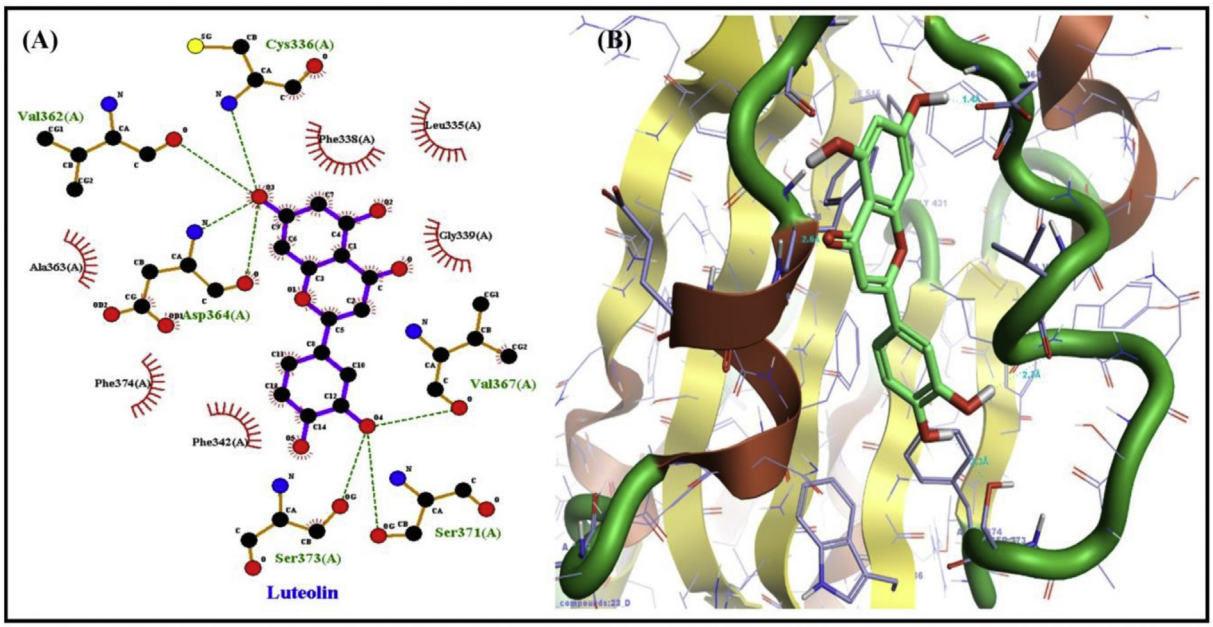

Fig. 2. Molecular docking results of Luteolin into SARS-CoV-2 spike protein. (A) Hydrophobic interaction of Luteolin with SARS-CoV-2 Spike protein (B) Binding mode of Luteolin in SARS-CoV-2 Spike protein. Amino acid residues involved in H-bond formation and H-bond networks are shown.

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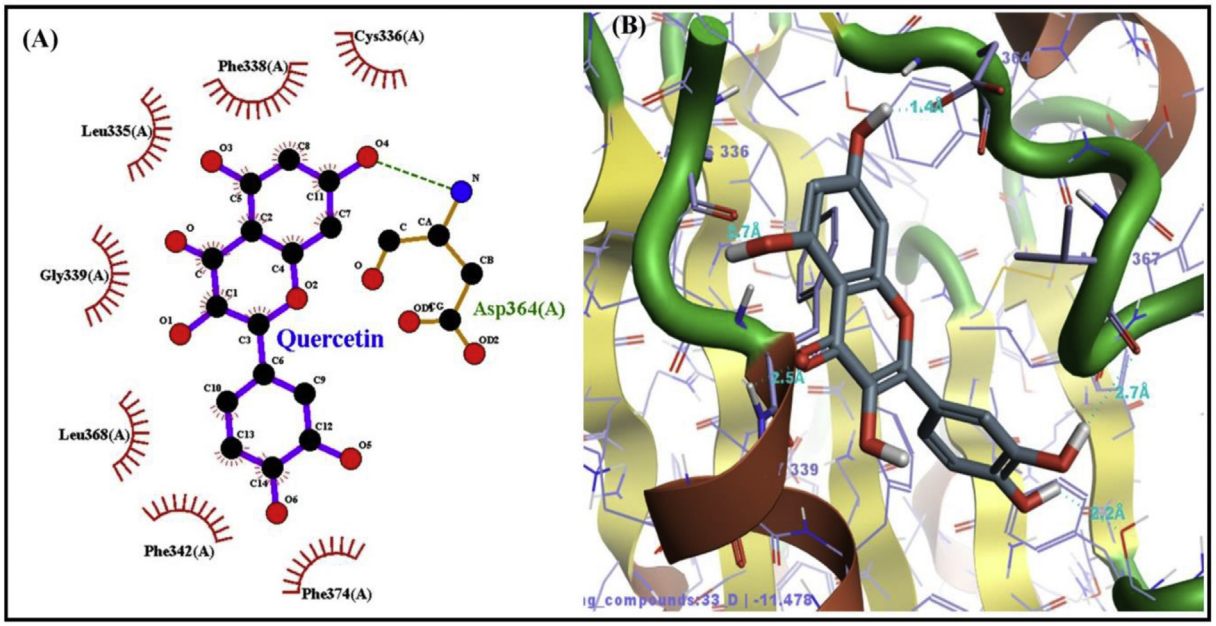

Fig. 3. Molecular docking results of Quercetin into SARS-CoV-2 spike protein. (A) Hydrophobic interaction of Quercetin with SARS-CoV-2 Spike protein (B) Binding mode of Quercetin in SARS-CoV-2 Spike protein. Amino acid residues involved in H-bond formation and H-bond networks are shown.

Supplementary Table S4. The Lupeol and Betulin ingredients of *Kabasura Kudineer Chooranam* formulation have 100% bioavailability and other ingredients also having oral bioavailability >80%. For JACOM formulation Ursolic acid has 100% bioavailability and other ingredients also having >80% bioavailability.

The Cytochrome P450 and P-glycoprotein simulation studies for substrate and inhibition were performed for all selected Phytoconstituents of two Siddha formulations by using online webserver. The results show that most of the Phytoconstituents has less CYP inducing and P-gp compatibility property Supplementary Table S5. Piperine, piperlonguminine, Stigmosterol, 3-(2,4-dimethoxyphenyl)-6,7-dimethoxy-2,3- dihydrochromen-4-one, Squalene, γ-sitosterol, Andrograpanin, 5-Hydroxy-7,8-dimethoxyflavanone, Lupeol, Betulin could undergoes metabolism via CYP3A4 enzyme Supplementary Table S5 Moreover, β-Sesquiphellandrene, β-Bisabolene, Geranial, Gallic acid, Carvacrol, Costunolide, and Elemol were free from drug–drug interaction via the inhibition of cytochrome-P (CYP) or P-glycoprotein (P-gp) I and II enzymes Supplementary Table S5.

3.4. In Silico *toxicity prediction*

Toxicity assessment was performed for the selected phytoconstituents of Siddha formulations and results show that very few analogs have deviated toxicity prediction. Overall the study indicates, the ingredients of these two formulations are free from carcinogenic, teratogenic, and tumorigenic properties Supplementary Table S6.

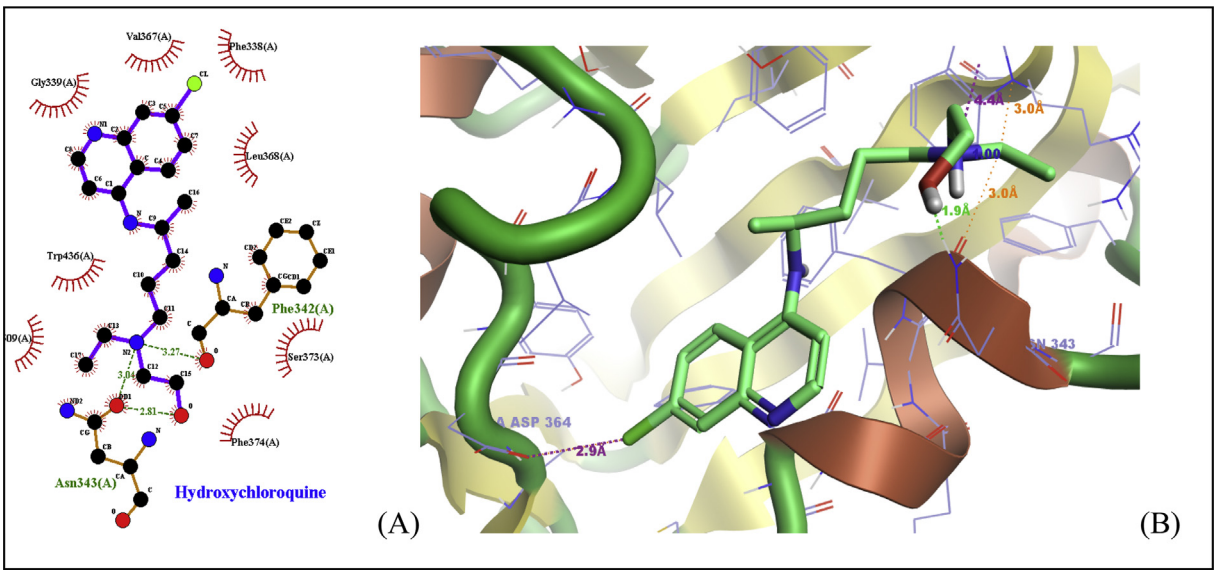

Fig. 4. Molecular docking results of Hydroxychloroquine into SARS-CoV-2 spike protein. (A) Hydrophobic interaction of Hydroxychloroquine with SARS-CoV-2 Spike protein (B) Binding mode of Hydroxychloroquine in SARS-CoV-2 Spike protein. Amino acid residues involved in H-bond formation and H-bond networks are shown.

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Table 3
Proposed SNACK –V formulation containing plants and their phytoconstituents with Dock score.

S.No	Plant Name	Phytoconstituents	LF Rank Score
1	*Sida acuta* Burm. f.	Magnoflorine	−9.76
2	*Andrographis paniculata*	5-Hydroxy-7,8-dimethoxyflavanone	−9.03
3	*Tinospora cordifolia*	Tinosponone	−8.14
4	*Plectranthus amboinicus*	Cirsimaritin	−9.22
		Chrysoeriol	−11.39
		6- Methoxygenkwanin	−9.293
5	*Justicia adhatoda* L	Vasicinone	−8.16
		Quercetin	−11.47
6	*Costus speciosus*	Luteolin	−11.15

4. Discussion

In this work, we have chosen Official Siddha Formulation *Kabasura Kudineer Chooranam* and JACOM (patented formulation). Modern medicines focus on killing the virus but not on increasing the host immunity. In case of Siddha medicine, herbs like *Amukkara*, *Nilavembu* are immuno-modulator and having the capacity to inhibit the virus by enhancing and restoring immunity of human. So, we are utilizing this strength of Siddha medicine to arrive upon a potent formulation that is both anti-viral and Immuno-modulatory with minimum side effects on patients who are immuno compromised as well as those who have co-morbid conditions.

The *Kabasura Kudineer* increases the immunity and could act as immuno modulator as this virus is adversely affecting the immune response by effecting signaling pathway of TNF production as recent findings shows [27]. The formulation chosen are aimed at increasing immunity and also to expel out the *kapham* and reinstate respiratory health. Drugs in these formulations majorly possess Bitter taste or pungent taste. These drugs on post digestive transformation get converted to hot potency which increases and normalizes *pitham* and expel out excessive *kapham* out of lungs, which is the rationale behind selecting these formulations.

Based on these results, nine phytoconstituents (6 plants) were found to be the best lead and drug candidates with good synthetic accessibility. The nine phytoconstituents with the LF rank Score viz., Magnoflorine (−9.76), 5-Hydroxy-7,8-dimethoxyflavanone(-9.03), Tinosponone(-8.14), Cirsimaritin(-9.22), Chrysoeriol(-11.39), 6-Methoxygenkwanin(-9.293), Vasicinone(-8.16), Quercetin(-11.47) and Luteolin(-11.15) are having highest binding affinity with spike protein and the plants associated with the Phytoconstituents were chosen for novel "SNACK-V" formulation. These 6 plants containing 9 phytochemicals have interaction score higher than the positive control Hydroxychloroquine. Based on these results, we proposed a novel herbal formulation called "SNACK -V" (*Sida acuta*, *Adhatoda vasica*, *Andrographis paniculata*, *Tinospora Cordifolia, Costus speciosus*, *Plectranthus ambonicus*) it may have high probability of directly inhibiting the novel corona virus (2019-nCoV), possibly providing instant help in the prevention and treatment of the pneumonia that it can cause Table 3. This formulation having herbs that possess bitter taste increases *pittam* and expels out *kapham* for their properties of immunomodulation, expectorant and antipyretic. These effects reinstate Gaseous exchange normalizing *trithodam* and *Sanni* Symptoms are wiped away thereby restoring normal health.

Tinospora cordifolia is a one of the drug of choice in conditions wherever pitta is diminished and *kapha* dominates [28]. Due to its bitter taste in post digestive transformation it turns into hot potency as a pungent active molecule and helps in reinstating *pitta* to normalcy and eliminates *kapha* slowly out of the body. It is useful also in settling fever. Later studies had proved its efficacy as an antiviral and an immunomodulator. Its effect against HIV has been documented via clinical evaluation [29].

A. paniculata by its bitter taste and hot potency helps in all fevers by precipitating diaphoresis [28], in dengue out break and during other disaster mitigation interventions it was the drug of choice even by public health authorities [5]. By possessing anti-inflammatory, analgesic, anti pyretic and immuno - modulatory activity [30] this has also proven to inhibit dengue virus [5].

Adhatoda vasica is bitter in taste and turns into hot potency. It is also an expectorant and very useful in *kapha* disorders [28]. Studies suggest that extracts have strong anti-influenza virus activity that can inhibit viral attachment and/or viral replication, and may be used as viral prophylaxis [31].

P. ambonicus is a plant having pungent taste and gets converted to hot potency post transformation, possess diaphoretic and expectorant property [28]. Many antimicrobial studies have established its effectiveness in lower respiratory symptoms like pneumonia [32].

C. speciosus is bitter in taste and turns into hot potency [28], indicated in fever and used as an expectorant. Studies have proved that it inhibits *Herpes simplex* and Varicella virus [33].

S. acuta is bitter in taste and turns into hot potency. It is also an expectorant and very much useful in *kapha* disorders [28]. Studies show this herb inhibits the replication of dengue viruses in cell cultures and protected mice against dengue infection. It also showed antipyretic and anti-inflammatory effects [34]. To summarize, these above mentioned 6 plants possess both anti viral and immuno-modulatory property, also all the bioactive compounds are non-toxic and non-carcinogenic. However, further experimental studies and clinical studies are required to validate the results.

Siddha medicine is one of best way to control the COVID-19. The docking studies of bioactive compounds from *Kabasura Kudineer* and JACOM showed that stronger binding affinity with good ADMET properties. Further we propose a new formulation as SNACK-V. Given their binding affinity towards SARS-CoV-2 spike protein and *in silico* safety studies, these two formulations qualify as a potential therapeutic for further *in vitro, in vivo* and clinical studies.

5. Conclusion

Spike protein is an important target for binding with the ACE2 of the host cell, and the inhibitors of this protein could be a potential target for COVID-19 infection. In this study, we have done the *in silico* molecular docking studies for the 37 phytoconstituents against the spike protein of SARS-CoV-2 (PDB ID: 6VSB). The results shown that Chrysoeriol and Luteolin from *Kabasura Kudineer Chooranam* and Quercetin from JACOM have high binding affinity and good binding interactions with spike protein. Further, *In silico* pharmacokinetic and toxicity prediction shown that all the phytoconstituents have good oral bioavailability and free from toxicity. Based on these, we proposed the new formulation called as

Please cite this article as: Kiran G et al., *In Silico* computational screening of *Kabasura Kudineer* - Official Siddha Formulation and JACOM against SARS-CoV-2 spike protein, J Ayurveda Integr Med, https://doi.org/10.1016/j.jaim.2020.05.009

"SNACK–V" which contains nine phytoconstituents from the six plant herbs.

Conflict of interest

None.

Source of funding

None.

Acknowledgements

The authors thanks to management of all the institutes for their support to carry out this research and also thanks to Central Council for Research in Siddha, Ministry of AYUSH, Chennai, Tamilnadu, India and Salem Microbes Private Limited, Salem, Tamilnadu, India.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaim.2020.05.009.

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Repurposing of Medicinal plants used in Siddha formulations as Potential Protease Inhibitors of COVID-19: An in silico approach

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Background:

The Coronavirus disease (COVID-19) caused by the virus SARS-CoV-2 has become a global pandemic in a short time has infected about 1,203,959 patients and brought forth death rate about 64,788 among 206 countries as mentioned by WHO in the month of April 2020. Currently, there is no specific treatment or vaccine for fighting against this infectious disease and scientists agree that possible therapeutic may arise through drug repositioning. Herbal medicine are achieving attention because of the extensive therapeutics like potent antiviral, immunomodulatory, anti-inflammatory, and anti-oxidant properties.

Materials and methods

This study was planned to screen herbs from Siddha that have the potential to increase host immune system as well as blocking virus entry in host cells. Official Siddha formulation Kabasura Kudineer, Nilavembu Kudineer, and Novel Siddha formulation – JACOM are already being in use as antiviral, immunomodulatory, anti-inflammatory, and antioxidant. 54 molecules identified and surveyed via docking study. Docking study was performed using Maestro interface (Schrödinger Suite, LLC, NY).

Results:

Out of these 54 Phytoconstituents, 30 Phytoconstituents were found to interact with > 2 protein structures of COVID-19. The docking results indicate that amongst the reported molecules 4 out 5 protein structure (PDB ID: 5R7Y, 5R7Z, 5R80, 5R81 and 5R82) showed promising results of binding to COVID-19 enzyme. So this formulations may be useful as a therapeutic and/or prophylactic agent for restricting viral attachment to the host cells.

Discussion:

The drug repurposing study provide an insight in terms of binding of active ingredients present in different plants used in formulations and targets enzymes for treatment of the COVID19

Key words:

COVID-19, SARS-CoV-2, Siddha Medicine, Medicinal plants, AYUSH.

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Introduction:

COVID–19 disease caused by the novel coronavirus SARS-CoV–2 has been declared as a global pandemic by WHO first emerged in China [1]. SARS-CoV-2 has spread across all continents, as of latest situation report on June 30, 2020 by WHO, a total of 87, 08 008 cases with a mortality of 4, 61,715 have been reported [2]. In India there are 4, 10 461 cases and 13, 254 deaths as on June 30, 2020 these data correspond the imminent risk facing the country. Transmission of virus spreads via droplets, physical contact with infected individuals, contaminated surfaces [3]. COVID-19 commonly reported symptoms are fever, headache, vomiting, chills, dyspnea, nausea, sore throat, coughing up blood, shortness of

breath, myalgia, diarrhea, and malaise. The severe infection leads to pneumonia, acute respiratory distress syndrome (ARDS) and sometimes multi-organ failures such as kidney failure, and even death [4]. Coronaviruses (CoVs) are the family of Coronaviridae with four gene era (alpha, beta, gamma and delta), and only the alpha and beta- strains are identified to be pathogenic to human [5, 6]. Middle East respiratory syndrome (MERS) virus (MERS-CoV) and SARS-CoV are the other similar agents previously known Coronaviruses [7, 8]. In whole genome sequence analysis SARS-CoV-2 showed higher SARS-CoV genome sequence homology than that of MERS-CoV [9]. Siddha holistic approach will be helpful in combating COVID 19 using both therapeutic and non-therapeutic interventions with disease condition [10]. Presently, quarantine and symptomatic treatment protocol for disease management exists and there is no antiviral drugs or vaccines available. Therefore, it is necessary to develop a treatment for COVID-19. Based on the Siddha Medicine advisory given by Ministry of AYUSH for COVID-19 mentioned stage of medicines for treatment, prophylaxis, and convalescence. These medicines are indicated for symptom management and prophylaxis. However these medicines are in practice for viral diseases like Dengue and Chikungunya in vogue for the past two decades. Kabasura Kudineer and Nilavembu Kudineer, an official Siddha formulation described in Siddha manuscript Citta Vaittiyattirattu [11] is used for *Aiyacuram* (phlegmatic fevers) *Pitthacuram* (hemorrhagic fevers) and is a dependable Siddha prescription for fever [11]. Further, we choose another herbal formulation called "JACOM" a coded novel drug due to its Neuraminidase inhibition potential against inactivated influenza virus H1N1(Patent no.201741016901 A, Dated 18.05.2018) [12]. With the introduction of new and more efficient screening assays and prediction methods, the efficacy of herbal drugs can be effectively used during viral outbreaks [13]. In-silico studies happened in Kabasura Kudineer, and JACOM [14] Nilavembu Kudineer [15] against SARS-CoV-2 spike protein which supports and increase the scope of these medicines in drug repurposing areas.

Till precise treatment is available for COVID-19, the use of known antiviral, immunomodulatory, anti-inflammatory, and antioxidant property herbal drugs is a useful approach. In this study, docking studies were performed over binding pocket of COVID-19 to find the potential small molecule to combat COVID-19.

Materials and methods:

Platform for molecular modelling

The computational investigations were performed using the Schrodinger software (Maestro 11.4, Schrodinger 2017-4).

Ligand preparation

Total 54 Phytoconstituents were selected to perform the molecular docking studies to screen and identify the potent antiviral agents specifically for COVID-19 [16]. PubChem database was used to extract out the 3D chemical structures of the selected molecules. 3D and geometry optimizations with energy minimization of ligands were executed using algorithms monitored in Schrödinger Maestro v 11.4 [17]. LigPrep module (Schrodinger, LLC, NY, USA, 2009) was used from the Maestro builder panel to prepare ligand and generate 3D structure of the ligands by adding hydrogen atoms and removing salt and ionizing at pH (7 ± 2) [18]. Energy minimization was performed using OPLS_2005 force field by using the standard energy function of molecular mechanics and RMSD cut off 0.01 Å to generate the low-energy ligand isomer [19].

Preparation of protein structures and grid generation

To combat the current situation of COVID-19 protein structure of COVID-19 main protease with co-crystallized structure(PDB IDs:5R7Y, 5R7Z, 5R80, 5R81, 5R82, having resolution < 2 Å, R-Value Free < 0.30, R-Value Work < 0.25) were selected and obtained from Protein Data Bank (http://www.rscb.org) with good resolutions [20-24].Protein structure was prepared using protein preparation wizard in Maestro panel. During preparation of protein bond orders were assigned and hydrogen atoms were added as well. Water molecules were removed within 3 Å of het groups [25]. Finally, OPLS-2005force field was applied to minimize the structure of protein (Schrodinger, LLC, NY, USA, 2009) [26]. Further receptor grid boxes were generated using “Glide's Receptor Grid Generation” module at the active site (with the radius of 20 Å around the crystal structure) of co-crystallized ligand with the computing cubic box of 10Å × 10Å × 10Å [27].

Molecular docking

Molecular docking is a structure-based drug design approach to identify the essential amino acid interactions between the selected protein and generated ligands with low energy conformation [28]. Minimum interaction of the ligands characterized by the scoring function which used to foretell the binding affinity with the receptor. Glide Standard precision (SP), docking protocol was applied without smearing any constrain. Flexible docking with Glide Standard precision (SP) protocol was performed to predict the binding affinity and ligand efficiency as inhibitor of COVID-19 target [29]. Concluding energy assessment was done with the dock score. Visualization of docked ligands was done by Maestro interface (Schrödinger Suite, LLC, NY) [30].

$$\text{Dock score} = a \times vdW + b \times Coul + Hbond + Metal + Lipo + BuryP + RotB + Site$$

Where, a and b are co-efficient constant for vdW and Coul, respectively. vdW = van der Waals energy; Coul = Coulomb energy; Hbond = Hydrogen bonding with receptor; Metal = Binding with metal; Lipo=Constant term for lipophilic; Bury P=Buried polar group penalty; Rot B = Rotatable bond penalty; Site = active site polar interaction [31].

3. Results

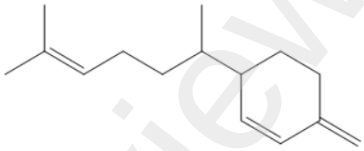
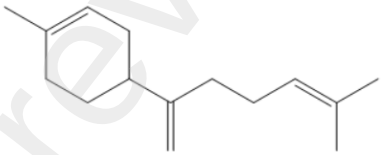
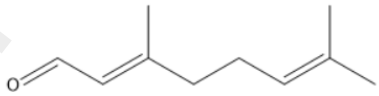
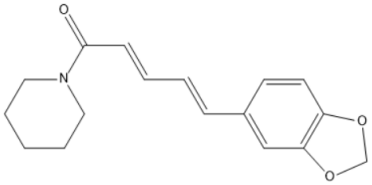
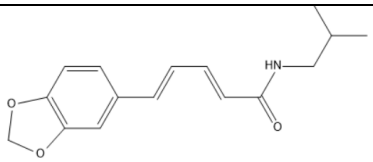
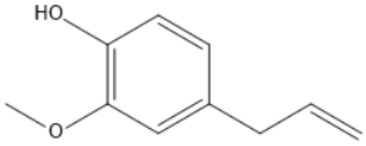
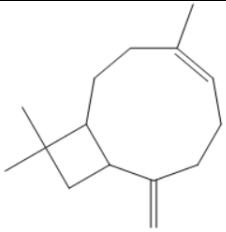
Docking studies were performed to find a potential Phytoconstituents for treating COVID-19, molecular docking studies were performed over three polyherbal Siddha formulation which includes 56 Phytoconstituents on the binding pocket of enzyme COVID-19 (PDB ID: 5R7Y, 5R7Z, 5R80, 5R81 and 5R82). Pharmacological action of each plant included in the formulation was given in Table 1. Also, the list of Phytoconstituents which included in docking study is depicted in Table 2, 3, 4.

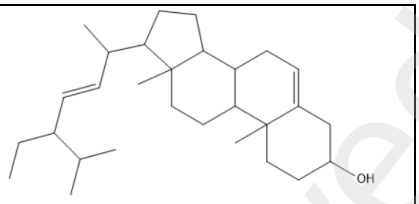
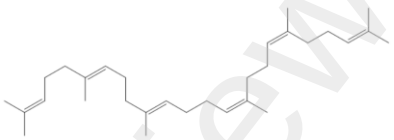
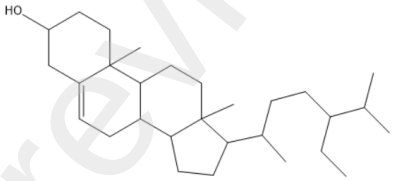
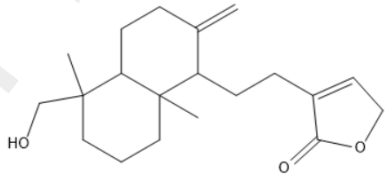
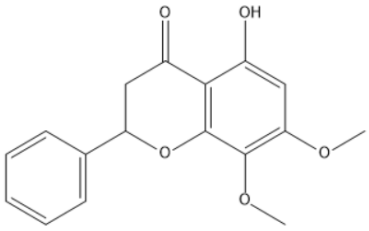
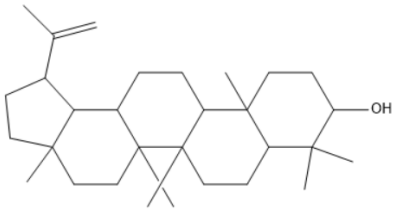
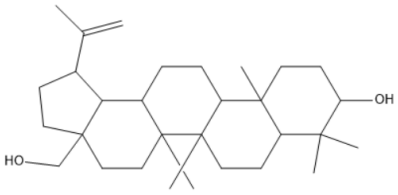
Table 1: List of Individual plants and their Pharmacological activity [32,33,34,35,36,37,38,39,40,41,42,43,44,45]

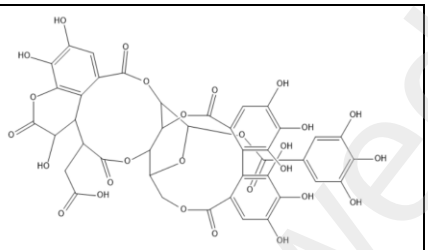
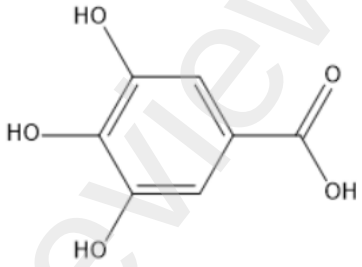
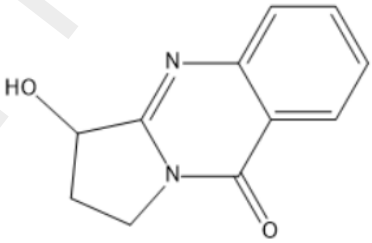
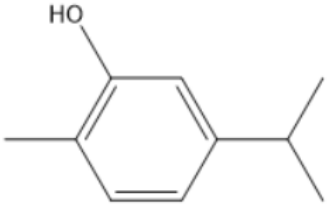
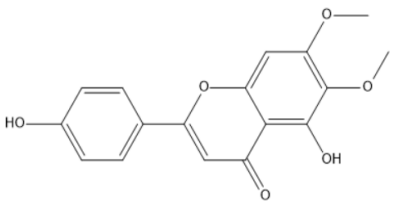
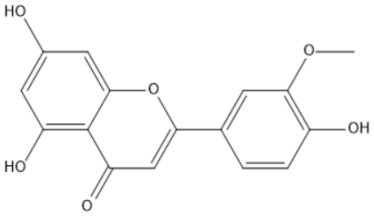
Botanical Name	Pharmacological activity
Zingiber officinale	Antioxidant, Anticancer, Anti-Inflammatory, Antiemetic, Antipyretic, Analgesic, Anti-Inflammatory, Antiviral, Antimicrobial, Immunomodulatory, Expectorant, Hepatoprotective
Piperlongum L	Antifungal, Ant Amoebic, Antimicrobial, Respiratory Stimulation, Antiasthmatic, Antioxidant, Immunomodulatory, Bioavailability Enhancement, Antiviral, Expectorant, Hepatoprotective

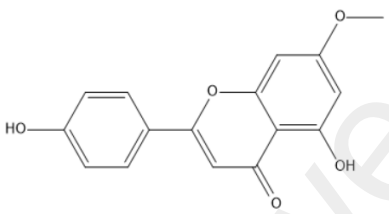
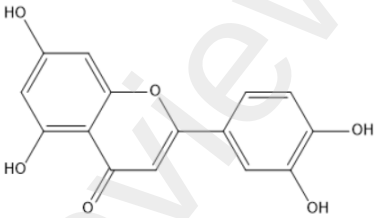
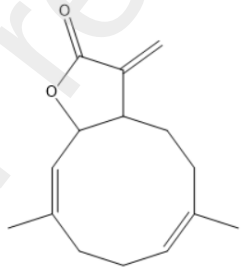
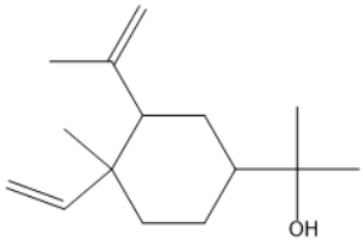
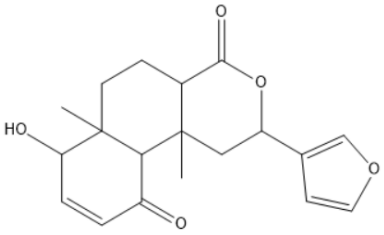
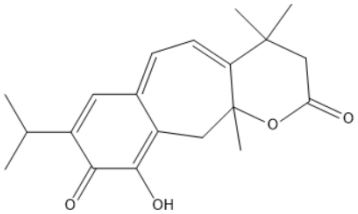
Syzygiumaromaticum	Antimicrobial, Analgesic, Antioxidant, Anticancer, Anthelmintic, Antiulcer, Anti-Inflammatory, Anti-Depressant, Bone Preserving, Antipyretic, Antithrombotic, Expectorant
*Tragiainvolucrata*L	Anti-Inflammatory, Analgesic, Diuretic And Anthelmintic, Antimicrobial
Anacycluspyrethrum	Anti-Inflammatory**,** Antioxidant, Antimicrobial, Immunomodulatory, Antipyretic
Andrographispaniculata	Anticancer, Anti-Inflammatory, Angiogenic, Antivenom, Antidiabetic, Antimalarial, Antimicrobial
Hygrophilaauriculata	Antimicrobial, Antipyretic, Anti-Inflammatory
Terminaliachebula	Antiviral, Antimicrobial, Immunomodulatory, Expectorant
*Justiciaadhatoda*L.	Antimicrobial, Antipyretic, Expectorant, Bronchodilator
Plectranthusamboinicus	Antimicrobial
Saussurealappa	Antiviral, Antimicrobial, Antipyretic
Tinosporacordifolia	Antiviral, Antimicrobial, Immunomodulatory, Antipyretic,
Clerodendrumserratum	Antimicrobial, Expectorant
*Sidaacuta*Burm.f.L	Antiviral, Antimicrobial, Antipyretic
*Cypreusrotundus*L.	Antiviral, Antimicrobial, Antipyretic
*Justiciaadathoda*L.	Antimicrobial, Expectorant
CaricaPapaya	Antibacterial, Antioxidant, Antipyretic, Insecticidal, Antimicrobial
Andrographispaniculata	Antiviral, Antipyretic
Ocimumtenuiflorum	Antibacterial, Antiviral, Antifungal, Antiprotozoal, Antimalarial, Anthelmintic, Antidiarrheal, Analgesic, Antipyretic, Anti-Inflammatory, Antiallergic, Antihypertensive, Cardio Protective, Central Nervous System (CNS) Depressant, Memory Enhancer, Anti-Hyper Cholesterolaemic, Hepatoprotective, Antidiabetic, Antiasthmatic, Antithyroid, Antioxidant, Anticancer, Chemopreventive, Radioprotective, Immunomodulatory,
Vetiveria zizanioides	Inflammatory, Antibacterial, Antifungal, And Anti-Malarial, Anti-Tubercular, Anti-Hyperglycaemic, Anti-Hepatoprotective And Antioxidant Activity.
Santalum album	Antipyretic, Antiscabietic, Diuretic, Expectorant, Stimulant, Anti-Inflammatory, Anti-Mitotic, Antiviral,
Piper nigrum	Antihypertensive, Antiplatelet, Antioxidant, Antitumor, Anti-Asthmatics, Analgesic, Anti-Inflammatory, Anti-Diarrheal, Antispasmodic, Antidepressants, Immunomodulatory,
Hedyotis corymbose	Antibacterial, Antioxidant, Analgesic, Hepatoprotective, Anticancer
Plectranthus vettiveroides	Antioxidant Activity, Anticancer
Trichosanthes cucumerina	Anti-Inflammatory

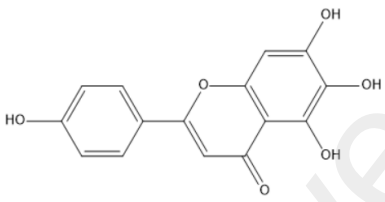
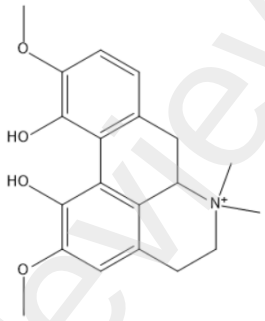
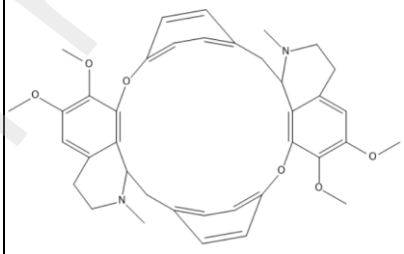
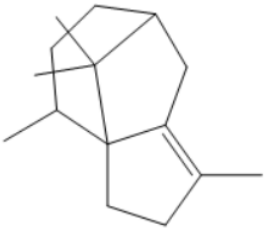
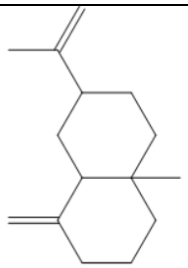
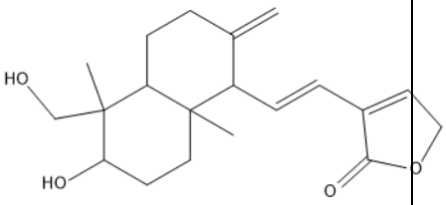
Table 2: List of Phytoconstituents of Medicinal plants used in Siddha formulations docked against COVID-19

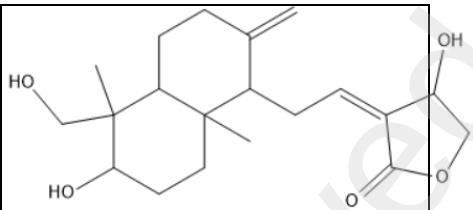
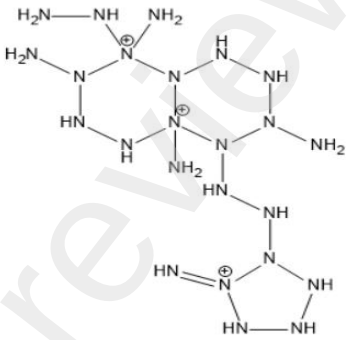
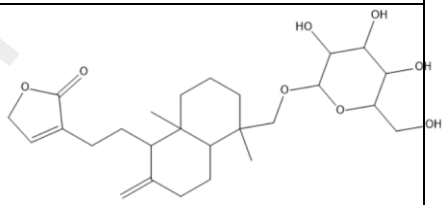
Plant name	Compound Name	Structure
Zingiber officinale Rosc	b-sesquiphellandrene	
	b-bisabolene	
	Geranial	
*Piperlongum*L	Piperine	
	Piperlonguminine	
Syzygiumaromaticum	Eugenol	
	b-Caryophyllene	

*Tragiainvolucrata*L	Stigmosterol	
Anacycluspyrethrum	Squalene	
	g-Sitosterol	
Andrographispaniculata	Andrograpanin	
	5-Hydroxy-7,8-dimethoxyflavanone	
Hygrophilaauriculata (Schum.)Heine	Lupeol	
	Betulin	

Terminaliachebula Retz.	Chebulagicacid	
	Gallicacid	
*Justiciaadhatoda*L.	Vasicinone	
Plectranthusamboinicus(Lour) Spreng	Carvacrol	
	Cirsimaritin	
	Chrysoeriol	

	6-Methoxygenkwanin	
Saussurealappa(Falc.)Lipsh	Luteolin	
	Costunolide	
	Elemol	
Tinosporacordifolia(Willd.)MiersexHook.f&Thoms	Tinosponone	
Clerodendrumserratum	Bharangin	

	Scutellarein	
*Sidaacuta*Burm.f.L	Magnoflorine	
	Cycleanine	
*Cypreusrotundus*L.	Cyperene	
	b-selinene	
Andrographis paniculate	14-deoxy-11,12-didehydroandrographide	

	Andrographolide	
	Deoxyandrographolide	
	neoandrographolide	
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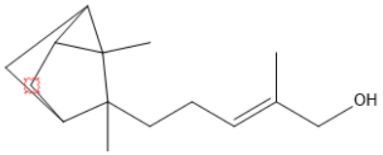
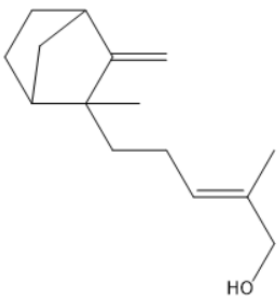
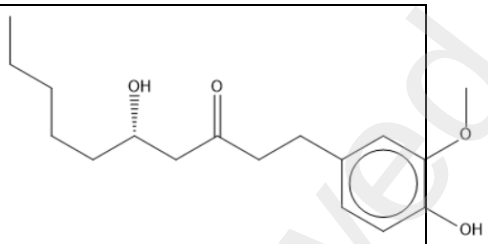
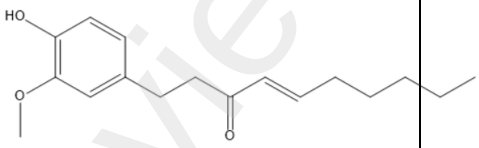
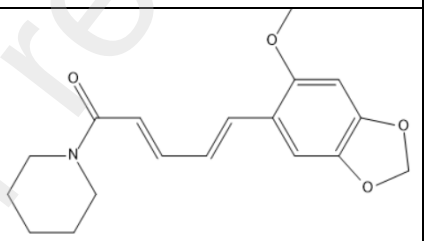
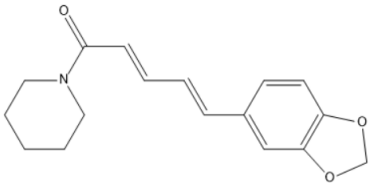
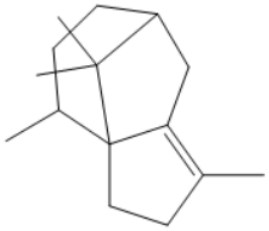
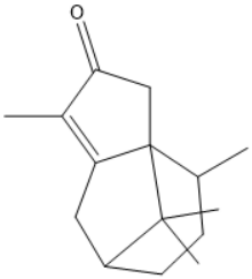
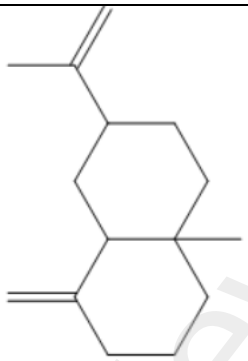
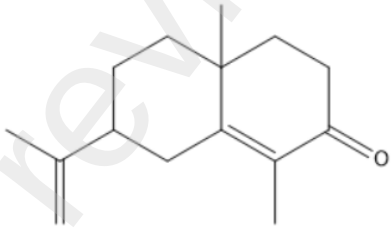
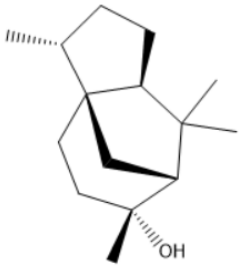
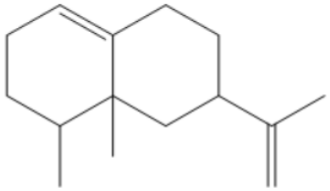
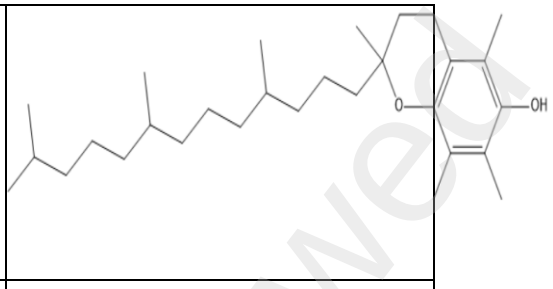
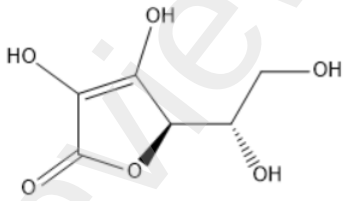
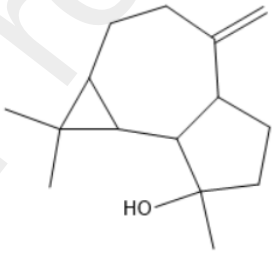
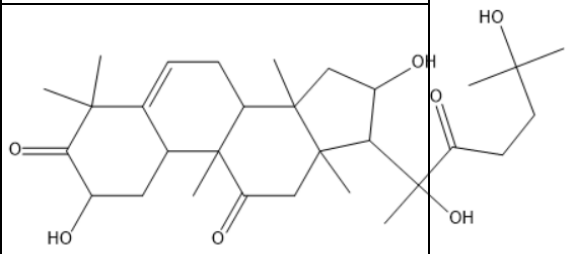
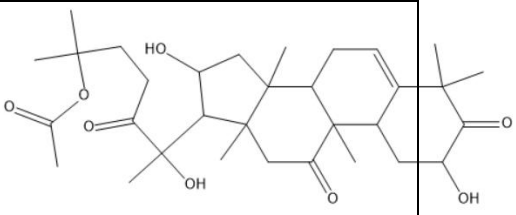
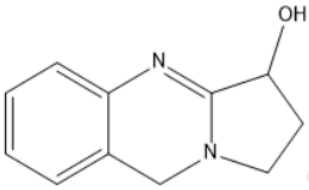
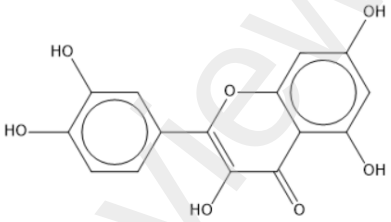
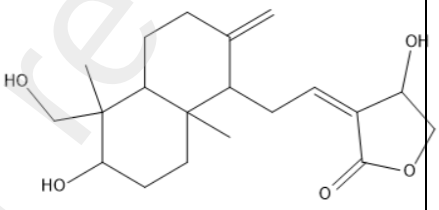
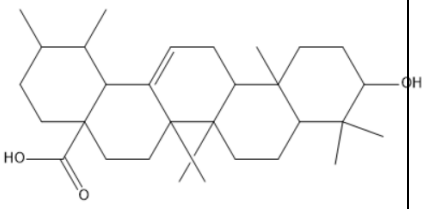
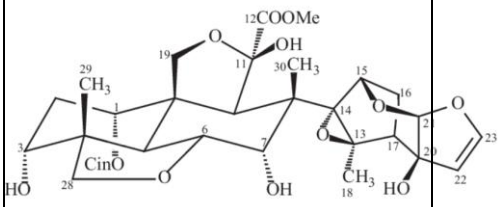
Santalum album	A Santalol	
	B Santalol	

Zingiber officinale	Gingerol	
	Shagaol	
Piper nigrum	Wisanine	
	Piperine	
Cyperus rotundus	Cyperene	
	Cyperotundone	

	ß-selinene	
	a-cyperone	
	Cedrol	
	Valencene	
Hedyotis corymbose	Hexadecanoic acid	

	Vitamin B	
	Vitamin C	
Plectranthus vettiveroides	Spathulenol	
	androstan-17-one 3-ethyl-3-hydroxy- (5a)	
Trichosanthes cucumerina	23, 24-dihydrocucurbitacin D	
	23,24-dihydrocucurbitacin B	

*Justiciaadathoda*L.	Vasicine	
CaricaPapaya	Quercetin	
Andrographispaniculata Burm.f.Nees	Andrographolide	
Ocimumtenuiflorum	Ursolicacid	
Meliaazedarach	Meliacine	

All these 54 Phytoconstituents were docked against the target enzyme COVID-19 and ranked based on their dock score. Phytoconstituents having dock score of -6 or less are considered better agent for inhibition of the COVID-19. A comparative analysis can be done by referring to Table 3. This table represents the list of active Phytoconstituents obtained after docking studies. These active Phytoconstituents have dock score value of

-6 or lower. Total 30 Phytoconstituents showed binding interactions with different COVID-19 structures of PDB ID 5R7Y, 5R7Z, 5R80, 5R81 and 5R82.

Table 3: Comparative docking study on COVID 19 enzymes of all Phytoconstituents

PDB ID	**5R7Y**	**5R7Z**	**5R80**	**5R81**	**5R82**
Phytoconstituents	**docking score**	**docking score**	**docking score**	**docking score**	**docking score**
Vasicinone	-6.492	-7.498	-6.778	-7.445	-6.098
Vasicine	-6.828	-7.522	-6.765	-7.31	-6.111
Chebulagic acid	-4.951	-7.641	-6.609	-6.646	-7.202
Piperine	-6.097	-7.166	-6.26	-7.098	-5.696
Cirsimaritin	-5.553	-7.398	-6.021	-6.834	-6.481
Luteolin	-7.129	-6.625	-6.764	-6.469	-6.925
5-Hydroxy-7,8-dimethoxyflavanone	-5.607	-6.611	-7.09	-6.572	-6.145
Scutellarein	-6.635	-6.932	-6.557	-6.585	-7.031
Chrysoeriol	-6.843	-6.81	-5.649	-6.579	-6.552
6- Methoxy genkwanin	-6.532	-6.611	-6.037	-6.434	-5.72
Bharangin	-6.379	-6.434	-5.152	-6.457	-4.943
Piperlonguminine	-6.28	-6.098	-5.325	-6.364	-5.192
Magnoflorine	-6.03	-5.419	-5.754	-6.409	-5.343
Carvacrol	-6.026	-6.568	-6.06	-6.594	-5.898
Tinosponone	-5.979	-6.5	-5.128	-5.608	-5.822
Gallic acid	-5.715	-5.677	-5.418	-6.12	-5.555
b-Caryophyllene	-5.679	-5.749	-6.002	-6.198	-5.421
Quercetin	-6.446	-6.811	-6.552	-6.525	-6.653
Ursolic acid	-5.329	-6.433	-3.694	-4.262	-4.7
androstan-17-one 3-ethyl-3-hydroxy- (5a)	-6.686	-6.677	-5.096	-5.635	-5.59
23,24-dihydrocucurbitacin B	-6.609	-6.594	-4.715	-6.532	-6.299
cucurbitacin B	-6.427	-6.335	-4.24	-6.11	-6.408
Cedrol	-6.384	-5.566	-6.061	-6.22	-5.744
Vitamin B	-6.328	-6.769	-5.024	-5.861	-4.182
Wisanine	-5.495	-7.247	-6.146	-6.645	-5.064
14-deoxy-11,12-didehydroandrographide	-4.853	-6.46	-5.118	-6.434	-6.331
deoxyandrographolide	-4.811	-5.99	-5.126	-5.677	-5.584
b-selinene	-5.014	-5.811	-5.194	-6.305	-4.726
Spathulenol	-5.726	-5.961	-6.501	-5.766	-5.212
Andrographolide	-5.014	-5.811	-5.194	-6.305	-4.726
Lupeol	-5.513	-5.138	-4.036	-3.576	-4.282

Betulin	-5.334	-5.03	-3.324	-4.626	-5.639
Cyperene	-5.286	-5.049	-5.048	-5.037	-4.924
Elemol	-5.146	-4.673	-5.263	-4.478	-5.099
Eugenol	-5.034	-4.704	-5.126	-5.307	-4.474
Stigmosterol	-4.723	-5.299	-5.443	-5.415	-4.979
Cycleanine	-4.634	----	-4.537	-4.037	-----
Squalene	-4.61	-5.84	-5.156	-5.558	-4.329
b-sesquiphellandrene	-4.575	-5.107	-4.998	-5.002	-4.288
b-bisabolene	-4.338	-5.156	-5.451	-5.342	-4.044
Costunolide	-4.317	-5.616	-5.667	-5.701	-4.839
g-Sitosterol	-4.137	-5.232	-5.261	-4.677	-4.14
Geranial	-3.486	-3.833	-4.005	-3.434	-3.66
Vitamin C	-5.643	-5.354	-4.505	-4.998	-4.767
a-cyperone	-5.271	-4.875	-5.5	-5.186	-5.276
B Santalol	-5.235	-5.062	-5.411	-5.151	-4.127
23, 24-dihydrocucurbitacin D	-5.038	-4.994	-4.417	-4.652	-5.357
Valencene	-4.994	-5.246	-5.477	-5.509	-4.736
Cyperotundone	-4.991	-5.011	-5.197	-5.234	-5.127
A Santalol	-4.899	-5.028	-5.824	-5.294	-4.414
Gingerol	-4.276	-4.82	-4.066	-3.47	-3.749
Shagaol	-3.861	-3.903	-3.734	-4.317	-2.778
9-octadecenamide	-1.91	-2.069	-1.676	-2.016	-0.541
Hexadecanoic acid	-1.05	-0.521	-0.018	-0.044	0.368

Out of these 30 Phytoconstituents, Vasicinone, Vasicine, Chebulagic acid, Piperine, Cirsimaritin, Luteolin, 5-Hydroxy-7,8- dimethoxyflavanone and many more were found to interact with more than 2 protein structures of COVID-19. Importantly amongst all Phytoconstituents Vasicine, Vasicinone, Luteolin, Scutellarein and Quercetin binds to all the protein structures of COVID -19 with dock score of less than -6. Moreover, Chebulagic acid, Piperine, Cirsimaritin, 5-Hydroxy-7,8- dimethoxyflavanone, Chrysoeriol, 6-Methoxy genkwanin, Carvacrol, 23,24-dihydrocucurbitacin B and cucurbitacin B also showed promising results in 4 out of 5 protein structures of COVID-19. Interestingly, half of the ingredients of the Kabasura Kudineer, Nilavembu Kudineer, and JACOM formulations showed excellent results in in silico docking studies.

Docking interactions of some of active Phytoconstituents based on docking studies are depicted in Figure 1 to 4. Vasicinone, Luteoline and Scutellarein interacted with all the

protein structures of COVID 19. Binding interaction of them with PDB ID 5R7Z, 5R7Y and 5R82 is showed in figure 1, 2 and 3 respectively.

Hydroxyl group of Vasicinone forms H-bond with amino acid Glu 166 and Pi-pi stacking interaction is also visible between amino acid Hie 41 and phenyl ring which is same as binding in case of Lopinavir and Remdesevir [44]. Four hydrogen bonds can form between OH group of Luteoline with Asn 142, Hie 41, Gln 192 and Thr 190. Also pi-pi stacking is observed between Phenyl ring and amino acid Hie 41.

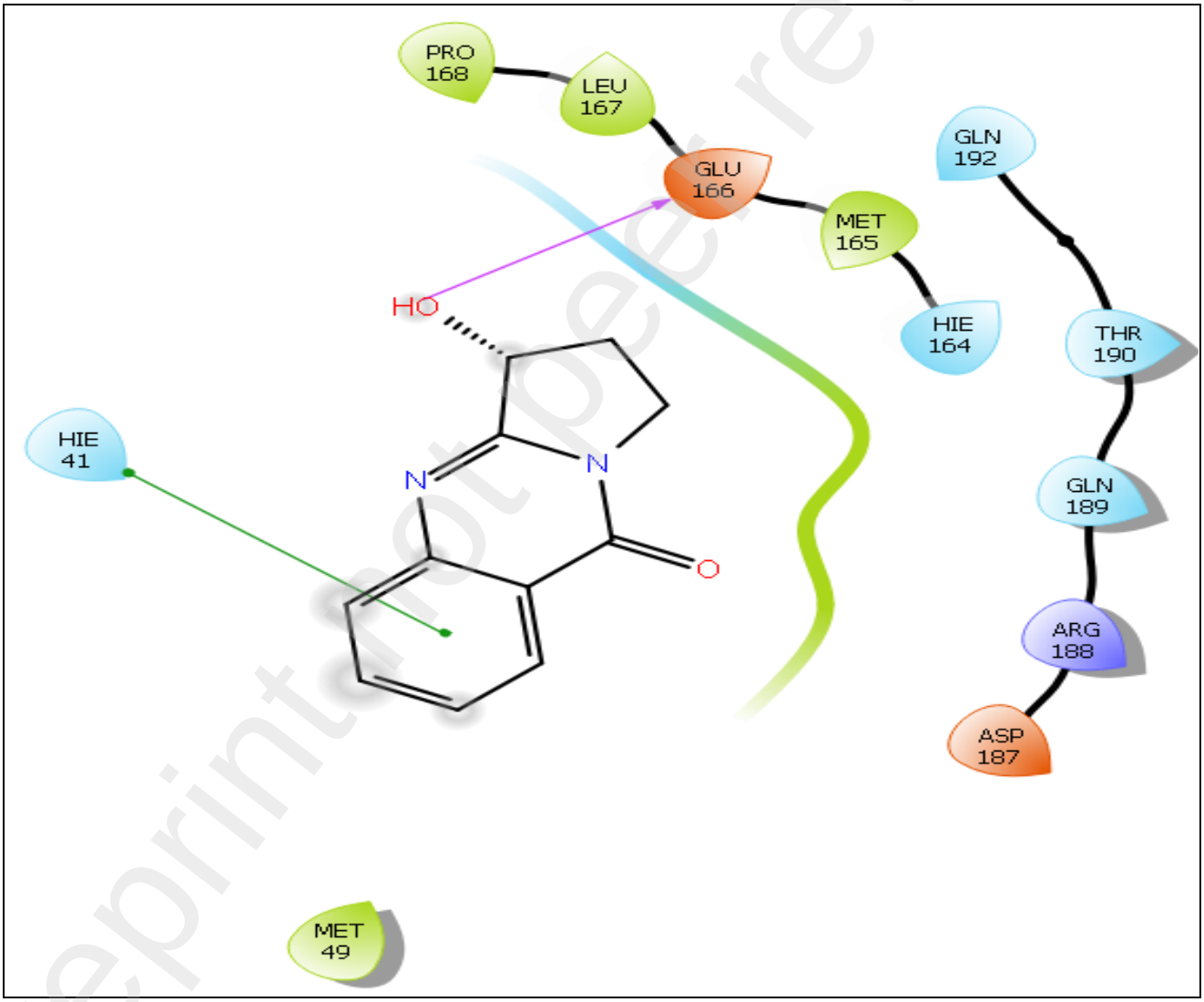


Figure 1: Docking interaction of Vasicinone with 5R7Z

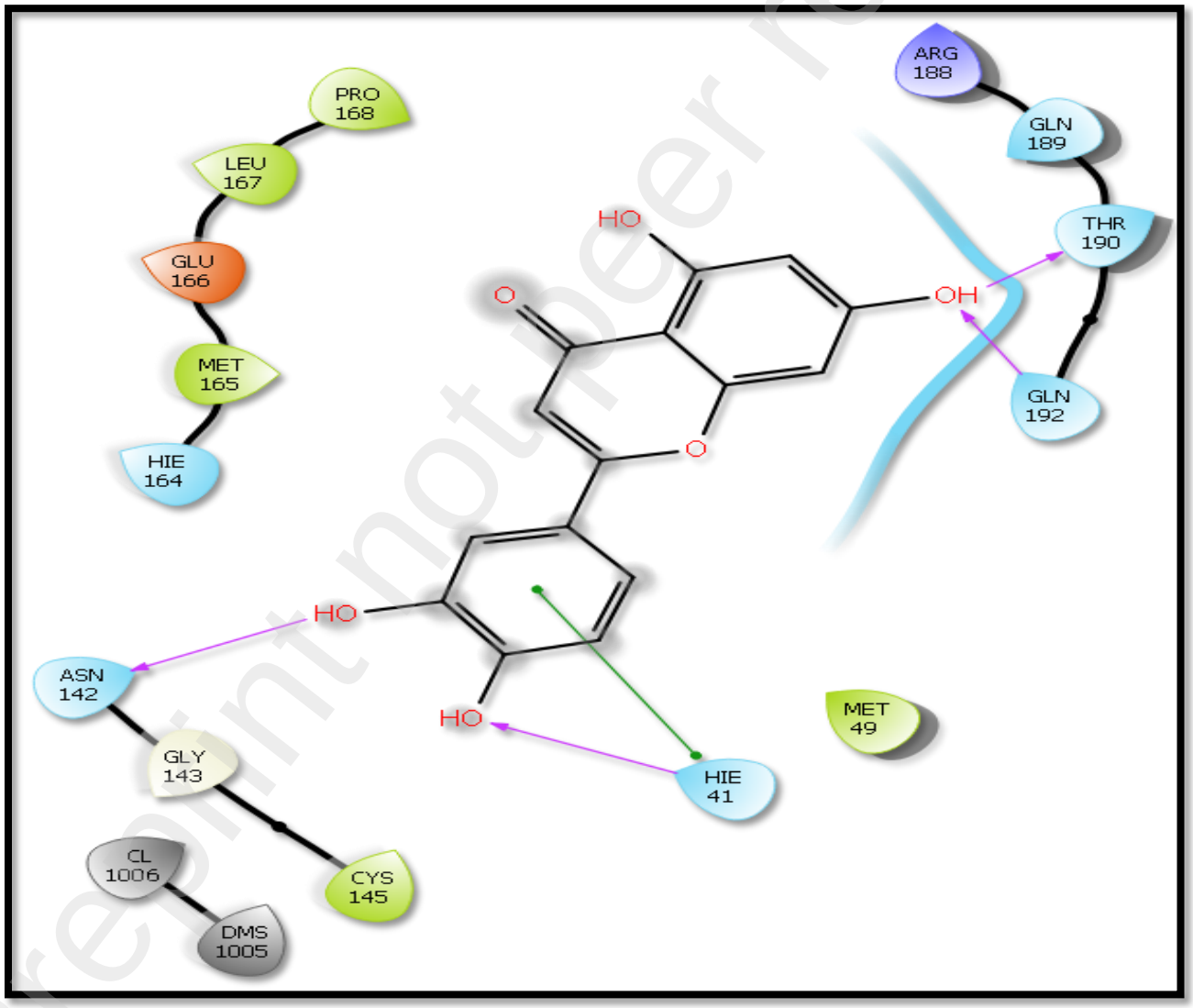


Figure 2: Docking interaction of Luteoline with 5R7Y

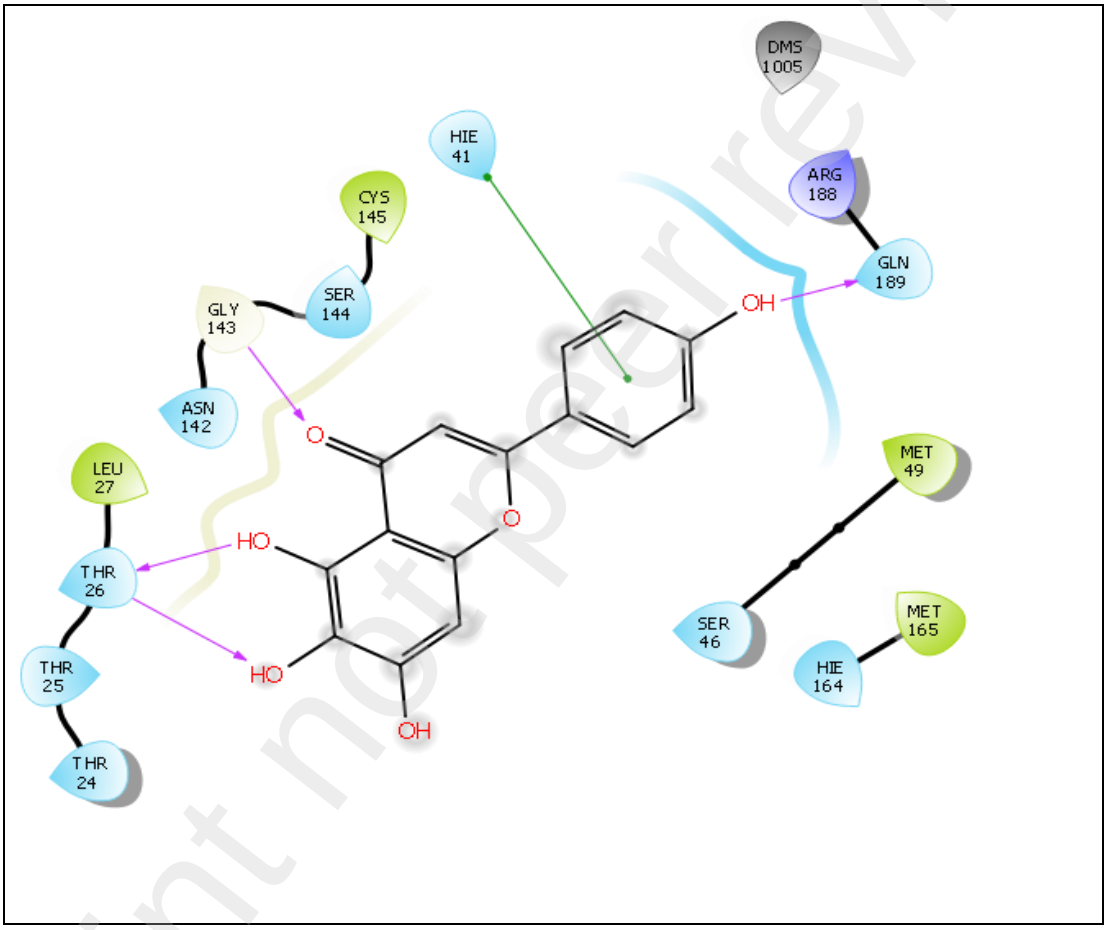


Figure 3: Docking interaction of Scutellarein with 5R82

As Scutellarein also exhibit good binding interaction with COVID 19 enzyme, a binding image of Scutellarein with 5R82 is given in Figure 3. Hydroxyl group of Scutellarein forms H- bond with amino acids Thr 26, Gln 189. Also 4-oxo chromene group of Scutellarein forms H-bonding interaction with Gly 143. More over phenyl ring of scutellarein is responsible for the pi-pi stacking interaction with Hie 41.

Docking interaction diagram of Protease inhibitor Chebulagic acid, is shown in Figure 4 with PDB ID 5R7Z. Chebulagic acid shows Many H- Bonding interaction with protein 5R7Z. In this case, Carbonyl group of acid form H- bond with Gln 189. Additionally, Ser 46 and Asn 142 froms H- bond with Carbonyl group of Ester. Further OH group on Phenyl ring can form H-bonding interaction with different Amino acids like Thr 26, Asn 142, Glu 166.

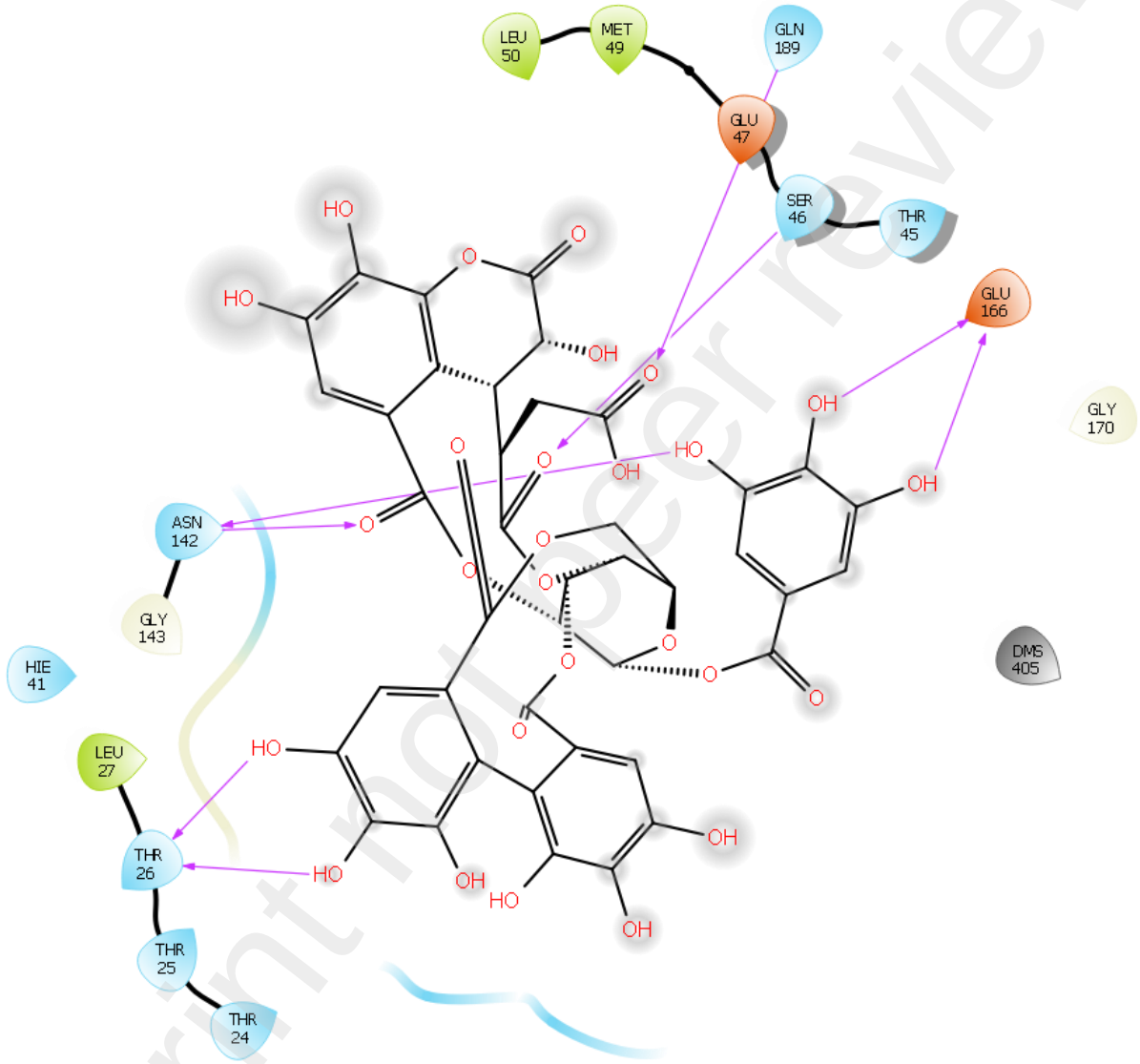


Figure 4: Docking interaction of Chebulagic acid with 5R7Z

Based on these docking score and interactions, it is estimated that Kabasura Kudineer, Nilavembu Kudineer and JACOM Siddha polyherbal formulations can be effective in treating the COVID 19 infections.

Conclusion:

To combat the life-threatening COVID-19 infection, Several Pre-clinical and Clinical studies are ongoing using antiviral and immunomodulatory drugs. Protease inhibitors plays a important role as potential candidate for COVID-19.

In this work, docking studies were performed on 54 Phytoconstituents known for its antiviral activities. In this study also many protease inhibitors showed remarkable binding interactions with COVID-19 enzymes. Vasicinone, Luteoline and Scutellarein found be more useful. Vasicinone inhibits protein synthesis and interacts with 5R7Z and 5R81 enzymes with dock score values of -7.498 and -7.445. The dock score values of Luteolin with 5R7Y are -7.129. The dock score values of Scutellarein with 5R82 are -7.031 respectively. As per docking score half of the ingredients of the formulations showed excellent results in in silico docking studies. These phytoconstituents showed promising results in Insilco, however pre-clinical and clinical studies should be impart in COVID-19 patients.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Published online in http://ijam.co.in ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 11 (4), 765-772

In Silico Docking Analysis of Poly Herbal Formulation *Aadathodai Kudineer* used in Siddha medicine in inhibiting Main Protease and ACE2 Receptor Spike protein SARS-CoV-2

Research Article

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Abstract

Corona virus disease (COVID-19) is an infectious pandemic disease caused by the newly discovered novel corona virus. World Health Organization has declared the global health emergency due to COVID19 outbreak. Currently, there is no specific treatment or vaccine for fighting against this infectious disease. *Aadathodai Kudineer* is a drug indicated for *Iya Erumal, Kozhai Kattu, Kabasuram.* Upon the mortality and severity of the disease COVID19, we tried to identify the possible inhibition of phytocomponents of *Aadathodai Kudineer* in inhibiting Main Protease and ACE2 Receptor Spike protein SARS-CoV-2 through molecular docking studies. Methodology: In Silico molecular docking analysis was performed for phytocomponents present in the *Aadathodai Kudineer* formulation for targets main protease and ACE2 Receptor Spike protein, PDB ID: 6LU7 and PDB ID: 2AJF using Autodock tool. ADME properties was also predicted for all the above compounds. Results: Among the 9 active Phytocompounds present in the *Aadathodai Kudineer* formulation, Lupeol showed high binding affinity with COVID19 main protease and ACE2 receptor which shows the promising contrivance of protease inhibition. The ADME suggested that the formulation is free from toxic. Conclusion: The phytocomponents showed possible affinity towards these targets and has the lead molecules that inhibits COVID19 main protease and ACE2 receptor.

Key Words: *Siddha formulation, Aadathodai Kudineer, SARS-CoV-2, COVID19, Molecular docking, ADME.*

Introduction

On 31st December 2019, 27 cases of pneumonia of unknown aetiology were recognized in Wuhan City, Hubei area in China. The causative organism was recognized from throat swab tests led by the Chinese Centre for Disease Control and Prevention (CCDC) on seventh January 2020, and was along these lines named Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2). The World Health Organization (WHO) named this infection as COVID-19. On 30th January 2020, the WHO proclaimed the Chinese episode of COVID-19 to be a Public Health Emergency of International Concern representing a high hazard to nations with weak wellbeing frameworks (1).

As of 27 July 2020, following data has been reported throughout the world, India & Tamil Nadu. More than 16.4 million cases of COVID-19 have been reported in 185 countries and territories, resulting in more than 6,46,641 deaths. More than 9.51 million people have recovered (2). The Ministry of Health and Family Welfare of India has confirmed a total of 14,35,453 cases, 9,17,567 recoveries (including 1 migration) and 32771 deaths in India. The Department of Health and Family Welfare of Tamil Nadu has confirmed a total of 2,13,723 cases, including 3493 deaths and 1,56,526 recoveries. Around 53703 active cases are reported (3).

The World Health Organization (WHO) welcomes innovations around the world including repurposing drugs, traditional medicines and developing new therapies in the search for potential treatments for COVID-19 (4). In China, traditional Chinese medicine is very useful to control and prevention and treatment for COVID 19 patients. In integrated approach is very success full treatment in COVID 19 patients in china (5).

Among six recognized streams of Indian Medicine System, Siddha medicine is one such traditional medicine originating in Tamil Nadu, India and practiced over centuries (6). Siddha system of medicine has played a major role in treating the diseases such as dengue, chikungunya. Both the TN Govt and union ministry of AYUSH has recommended an herbal siddha medicine called *Nilavembu Kudineer* as a treatment for dengue (7). This COVID 19 Pandemic Ministry of AYUSH publish the "Guidelines for Siddha Practitioners for COVID 19".

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Aadathodai Kudineer, a classical Siddha Formulation described in Siddha manuscript *Gunapadam Mooligai Vaguppu* and is used for *Iya Erumal, Kozhai Kattu, Kabasuram*. This poly herbal medicine contains three different ingredients such as *Aadathodai, Kandankathiri, Seenthil* (8). In Siddha treatment these three ingredients have been used extensively for the treatment of respiratory diseases such as cold, cough, whooping cough, chronic bronchitis, asthma, pneumonia and tuberculosis. *Aadathodai* has its own Anti-viral property (9), Anti-asthmatic, Bronchodilator activity, Anti-tubercular activity, Anti-bacterial activity (10 – 11), Antipyretic Activity (12). *Seenthil* has its own Antiviral property (13), Immunomodulatory activity (14), Anti-malarial, Anti-inflammatory, Antioxidant, Hepatoprotective, Immunomodulatory properties (15). *Kandankathiri* has it owns Anti-asthmatic activity, Anti-inflammatory activity, Cardio-protective activity (16), Antimicrobial activities, Anti-inflammatory activity, Anti-pyretic activity (17). Immunomodulatory activity, Anti-oxidant activity, Anti-pyretic activity, Hepatoprotective activity (18).

Materials and methods

Aadathodai Kudineer is a polyherbal Siddha formulation and active compounds in the formulation is indicated for *Iya Erumal, Kozhai Kattu, Kabasuram*. This formulation is having the following Siddha medicinal plants (Table 1).

Table 1: *Aadathodai Kudineer* their botanical name and its source.

S. No	Common Name	Botanical Name
1	Aadathodai	*Justicia adhatoda* L
2	Kandankathiri	*Solanum surattense* BURM. F.
3	Seenthil	*Tinospora cordifolia* (WILLD.) HOOK.F. & THOMS

Protein ligand docking

Protein preparation

The three-dimensional crystal structure of SARS-CoV-2 main protease (3-chymotrypsin-like protease (3CL pro) [PDB ID - 6LU7] and Angiotensin-converting enzyme 2 (ACE2) receptors – [PDB ID - 2AJF] was retrieved from the Protein Data Bank (PDB) and energy minimized and then converted into their corresponding PDBQT formats.

Ligand preparation

The phytocomponents identified in *Aadathodai Kudineer* formulation such as Adhatodine, Anisotine, Berberine, Tinosporide, Apigenin, Diosgenin, Lupeol, Vasicinone, Vasicoline were selected for docking from the *Aadathodai Kudineer* formulation. Ligand molecules were downloaded from Pubchem (19) in sdf format. Optimization was done with the force field type MMFF94 using Openbable softwares and saved as pdbqt format and used in docking studies.

Molecular Docking

Docking was performed with Autodock tool (20) a prevalent molecular screening tool for identifying binding energy between the 3D structures of each ligand and target proteins. Two target proteins PDB ID - 6LU7 and PDB ID - 2AJF were +9 selected, a Grid-free docking performed, and the binding energies of each ligand found.

ADME properties prediction

The ADME (absorption, distribution, metabolism, excretion and toxicity) properties of above mentioned phytocomponents predicted using SwissADME an online tool for ADME prediction (21).

Results and Discussion

Aadathodai Kudineer Chooranam is one among the poly herbal formulation used for the treatment of COVID-19, Molecular docking studies carried out for the nine phytocomponents present in *Aadathodai Kudineer.* SARS-CoV-2 use ACE2 receptors to gain entry to the target cells causing Corona Viral disease 19 in humans (22) and the main protease 3CLpro is highly essential for cleavage of polyprotein to get16 non-structural proteins (called nsp1-nsp16). These non-structural proteins are highly essential for viral replication. For this purpose, AutoDock4 docking program was employed, which uses Lamarckian genetic algorithm. The results of the binding affinity of these compound with their respective targets are given in (Table 2).

Table 2: Binding energy of various compounds involved in *Aadathodai Kudineer*

S. No.	Compounds	Binding Free energy Kcal/ mol 3CLPRO	Binding Free energy Kcal/ mol ACE2
1	Adhatodine	-7.47	-5.13
2	Anisotine	-7.42	-4.18
3	Berberine	-5.99	-5.344
4	Tinosporide	-5.77	-3.86
5	Apigenin	-4.55	-2.23
6	Diosgenin	-7.96	-6.27
7	Lupeol	-8.38	-6.18
8	Vasicinone	-5.38	-3.59
9	Vasicoline	-7.69	-5.32

Among all the docked compounds that showed a range of binding affinity Lupeol showed 8.38 kcal/ mol for 3CLPRO and 6.27 kcal/mol for ACE2 receptor respectively. Adhatodine, Anisotine, Tinosporide, Apigenin and Vasicoline possess 100% binding efficacy by interacting with both the core target amino acids (31 LYS and 353 LYS) present on the target. Followed by this other phytocompounds such as Berberine, Diosgenin, Lupeol and Vasicinone possess 50% affinity by binding with one of the target amino acid either with

31 LYS or with 353 LYS present on the target receptor ACE-2. Anisotine has maximum of 6 interactions with the core active amino acid residues present on the target. Followed by this the compounds such as Adhatodine, Tinosporide and Lupeol ranked second with the maximum of 5 interactions. Similarly, the compounds Berberine, Apigenin and Vasicinone ranks third with the maximum of 4 interactions with the active site of the target enzyme 3CLpro. The ADME properties predicted using swissADME suggested that did not show any hepatocellular toxicity. The compound did not also show any blood-brain barrier crossing suggesting low toxicity induced upon intake. The compound is shows 90% solubility concentration of this phytocompound.

Table 3: Ligand Properties of the Compounds Selected for Docking Analysis

Compound	Molar weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds
Adhatodine	337.416 g/mol	$C_{20}H_{21}N_3O_2$	1	2	4
Anisotine	349.4 g/mol	$C_{20}H_{19}N_3O_3$	1	5	4
Berberine	336.4 g/mol	$C_{20}H_{18}NO_4$	3	0	4
Tinosporide	374.4 g/mol	$C_{20}H_{22}O_7$	1	7	1
Apigenin	270.24 g/mol	$C_{15}H_{10}O_5$	3	5	1
Diosgenin	414.6 g/mol	$C_{27}H_{42}O_3$	1	3	0
Lupeol	426.7 g/mol	$C_{30}H_{50}O$	1	1	1
Vasicinone	202.21 g/mol	$C_{11}H_{10}N_2O_2$	1	3	0
Vasicoline	291.4 g/mol	$C_{19}H_{21}N_3$	0	2	2

Table 4: Summary of the molecular docking studies of compounds against Angiotensin-converting enzyme 2 (ACE2) receptor- PDB 2AJF

Compounds	Binding Free energy Kcal/mol	Inhibition constant Ki µM (*mM) (**nM)	Electrostatic energy Kcal/mol	Intermolecular energy Kcal/mol	Total Interaction Surface
Adhatodine	-5.13	172.73	-0.82	-5.04	530.48
Anisotine	-4.18	867.69	-0.06	-5.03	503.55
Berberine	-5.344	103.4	-1.14	-4.88	551.99
Tinosporide	-3.86	1.47*	-0.16	-4.62	444.65
Apigenin	-2.23	23.08	-0.12	-4.05	430.24
Diosgenin	-6.27	25.36	-0.02	-6.57	582.39
Lupeol	-6.18	29.59	-0.16	-6.77	655.34
Vasicinone	-3.59	2.34*	-0.17	-3.89	344.17
Vasicoline	-5.32	126.28	-0.60	-5.23	463.48

Table 5: Amino acid Residue Interaction of Lead against Angiotensin-converting enzyme 2 (ACE2) receptor- PDB 2AJF

Molecule	Interactions	Amino Acid Residue- Binding						
Adhatodine	2	31 LYS	34 HIS	35 GLU	37 GLU	38 ASP	353 LYS	-
Anisotine	2	31 LYS	34 HIS	35 GLU	37 GLU	38 ASP	353 LYS	-
Berberine	1	33 ASN	34 HIS	37 GLU	38 ASP	353 LYS	389 PRO	393 ARG
Tinosporide	2	31 LYS	34 HIS	35 GLU	37 GLU	38 ASP	353 LYS	-
Apigenin	2	31 LYS	34 HIS	38 ASP	353 LYS	-	-	-
Diosgenin	1	33 ASN	34 HIS	37 GLU	38 ASP	353 LYS	389 PRO	393 ARG
Lupeol	1	30 ASP	33 ASN	34 HIS	37 GLU	353 LYS	389 PRO	393 ARG
Vasicinone	1	31 LYS	34 HIS	35 GLU	38 ASP	-	-	-
Vasicoline	2	31 LYS	34 HIS	35 GLU	37 GLU	38 ASP	353 LYS	-

Table 6: Summary of the molecular docking studies of compounds against COVID-19 main protease (3-chymotrypsin-like protease (3CL pro) – PDB 6LU7

Compounds	Binding Free energy Kcal/mol	Inhibition constant Ki µM (*mM) (**nM)	Electrostatic energy Kcal/mol	Intermolecular energy Kcal/mol	Total Interaction Surface
Adhatodine	-7.47	3.36	-0.36	-8.29	766.16
Anisotine	-7.42	3.66	-0.03	-8.11	790.62
Berberine	-5.99	40.33	-0.17	-6.71	726.25
Tinosporide	-5.77	59.28	-0.07	-6.59	758.09
Apigenin	-4.55	45.50	-0.21	-6.39	650.78
Diosgenin	-7.96	1.46	-0.18	-8.29	832.88
Lupeol	-8.38	716.67**	-0.03	-8.98	911.38
Vasicinone	-5.38	113.14	-0.10	-5.68	493.23
Vasicoline	-7.69	2.31	-0.32	-7.61	687.35

Table 7: Amino acid Residue Interaction of Lead against COVID-19 main protease (3-chymotrypsin-like protease (3CL pro) – PDB 6LU7

Molecule	Interactions	Amino Acid Residue- Binding									
Adhatodine	5	41 HIS	49 MET	54 TYR	142 ASN	144 SER	145 CYS	163 HIS	165 MET	166 GLU	189 GLN
Anisotine	6	27 LEU	41 HIS	144 SER	145 CYS	163 HIS	165 MET	166 GLU	-	-	-
Berberine	4	41 HIS	49 MET	145 CYS	165 MET	166 GLU	189 GLN	-	-	-	-
Tinosporide	5	41 HIS	142 ASN	144 SER	145 CYS	163 HIS	165 MET	166 GLU	189 GLN	-	-
Apigenin	4	41 HIS	49 MET	52 PRO	54 TYR	145 CYS	163 HIS	166 GLU	189 GLN	-	-
Diosgenin	3	25 THR	41 HIS	49 MET	143 GLY	145 CYS	166 GLU	189 GLN	-	-	-
Lupeol	5	41 HIS	142 ASN	145 CYS	165 MET	166 GLU	168 PRO	189 GLN	-	-	-
Vasicinone	4	27 LEU	41 HIS	144 SER	145 CYS	163 HIS		-	-	-	-
Vasicoline	3	165 MET	166 GLU	167 LEU	168 PRO	189 GLN	192 GLN	-	-	-	-

Table 8: *In silico* pharmacokinetics properties of phytochemical constituents of *Aadathodai Kudineer*

Phytochemical constituent	Intestinal absorption	BBB permeability	Human Vd (L/kg)	Total clearance (mg/kg/ day)	Renal OCT2 substrate
Adhatodine	86.22	-0.127	0.08	0.58	No
Anisotine	94.97	-0.381	-0.092	0.675	No
Berberine	97.147	0.198	0.58	1.27	No
Tinosporide	100	-0.482	0.15	0.414	No
Apigenin	93.25	-0.734	0.822	0.566	No
Diosgenin	96.565	0.2	0.426	0.328	Yes
Lupeol	95.782	0.726	0	0.153	No
Vasicinone	92.532	-0.206	0.142	0.568	No
Vasicoline	93.064	0.614	0.76	0.609	Yes

Table 9: The predicted pharmacokinetics properties of phytochemical constituents for Cytochrome Inhibition and P-glycoprotein studies for *Aadathodai Kudineer*

Phytochemical constituent	CYP2D6 and CYP3A4 substrate	CYP enzymes inhibition	P-gp substrate	P-gp I or II inhibition
Adhatodine	No	CYP1A2	No	No
Anisotine	CYP3A4	CYP1A2 CYP2C19 CYP2C9 CYP3A4	substrate	P-gp I and II
Berberine	CYP3A4	CYP1A2 CYP2D6 CYP3A4	substrate	P-gp II
Tinosporide	CYP3A4	No	No	No
Apigenin	No	CYP1A2 CYP2C19	substrate	No
Diosgenin	CYP3A4	No	No	P-gp I and II
Lupeol	CYP3A4	No	No	P-gp I and II
Vasicinone	No	CYP1A2	substrate	No
Vasicoline	CYP3A4 CYP2D6	CYP1A2 CYP2C19 CYP2C9 CYP2D6	No	No

Table 10: The predicted toxicity of phytochemical constituents of *Aadathodai Kudineer*

Phytochemical constituent	AMES tox.	hERG I or II inhibition	Hepatotoxicity	Skin sensitization	Carcinogenicity	Human maximum tolerated dose (mg/kg/day)	Oral rat acute toxicity (mol/kg)	Oral rat chronic tox. (mg/kg_bw/day)
Adhatodine	Yes	No	No	No	No	0.15	1.956	2.564
Anisotine	NO	NO	Yes	No	No	0.158	2.268	1.758
Berberine	Yes	No	Yes	No	No	0.144	2.571	1.89
Tinosporide	No	No	Yes	No	No	-0.373	3.165	0.967
Apigenin	No	No	No	No	No	**0.328**	2.45	2.2983
Diosgenin	No	hERG II	No	No	No	-0.559	1.921	1.452
Lupeol	No	hERG II	No	No	No	-0.502	2.563	0.89
Vasicinone	No	No	No	No	No	0.332	1.91	1.708
Vasicoline	Yes	hERG II	Yes	No	No	0.088	2.621	0.728

Figure 1a: 3D- Structure of Angiotensin-converting enzyme 2 (ACE2) receptor- PDB ID 2AJF

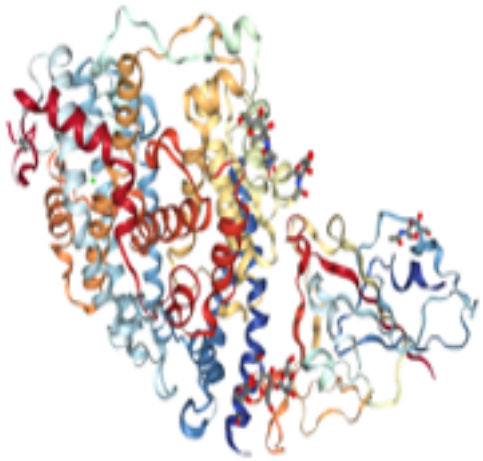

Figure 1b: 3D crystalline structure of the target protein COVID-19 main protease (3-chymotrypsin-like protease (3CL pro) – PDB ID 6LU7

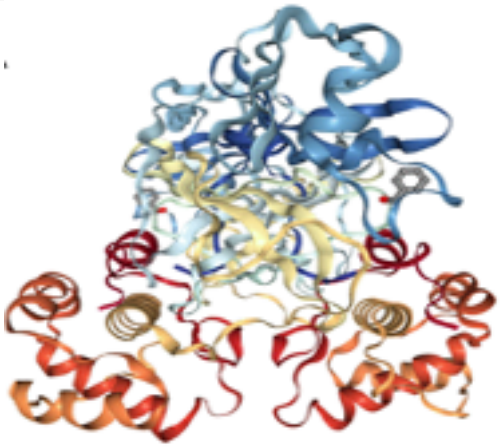

Figure 2: 2D and 3D Structure of Selected Ligands

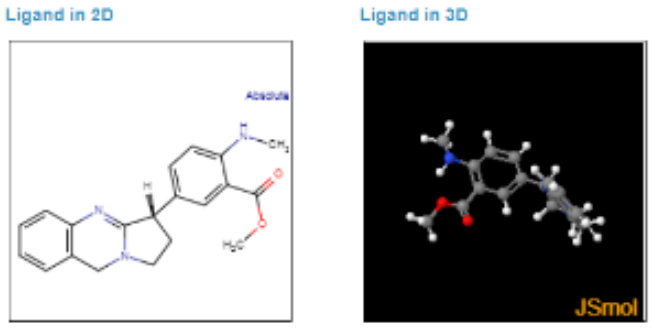

Figure 2a : Adhatodine

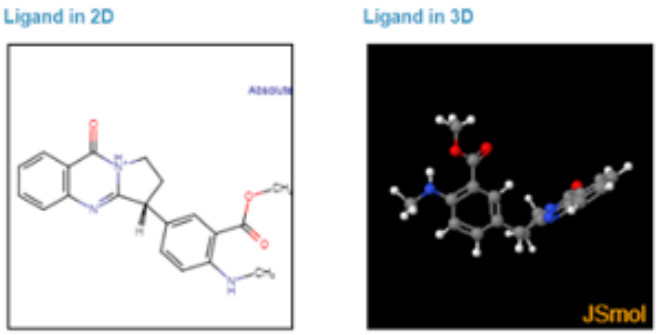

Figure 2b : Anisotine

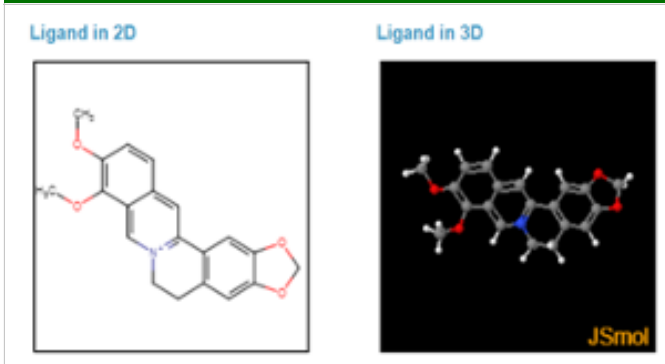

Figure 2c : Berberine

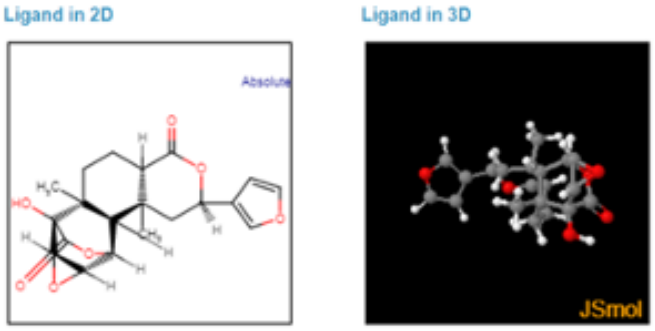

Figure 2d : Tinosporide

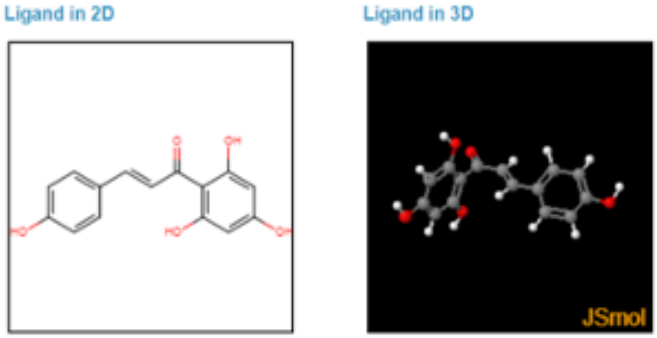

Figure 2e : Apigenin

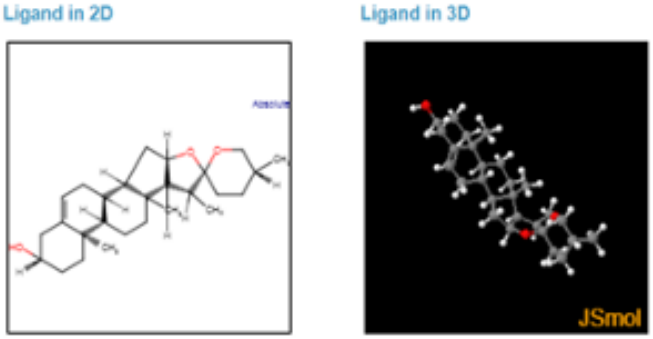

Figure 2f : Diosgenin

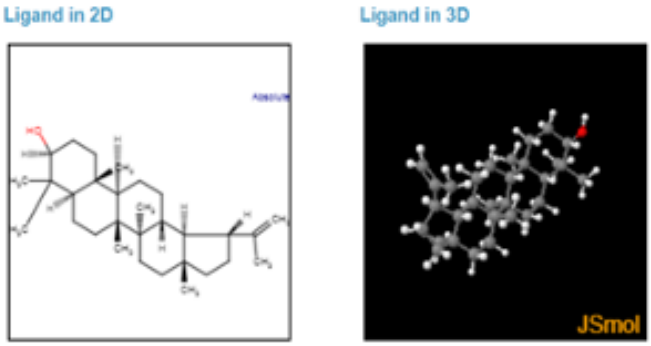

Figure 2g : Lupeol

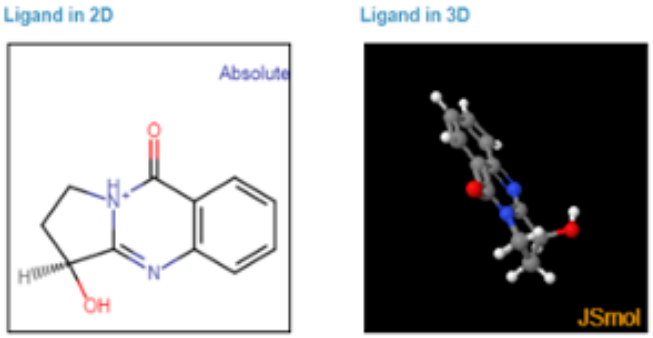

Figure 2h : Vasicinone

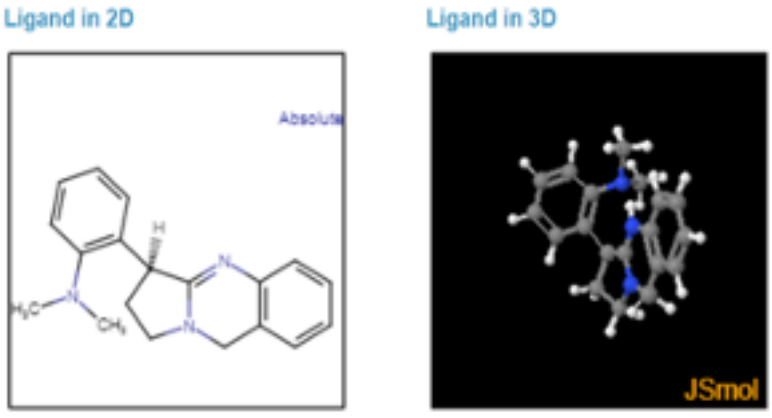

Figure 2i : Vasicoline

Conclusion

Various Phytoconstituents of the *Aadathodai Kudineer* Chooranam such as Adhatodine, Anisotine, Tinosporide, Apigenin, Vasicoline, Berberine, Diosgenin, Lupeol and Vasicinone present in the herbs of the formulation *Aadathodai Kudineer* revels significant binding against the target protein thereby it was concluded that these compounds may exerts promising inhibiting against ACE-2 receptor and hereby halt the host-viral interface and exerts promising inhibiting against 3 CL pro enzyme and hereby halt the formation of 16 non-structural proteins (nsp1-nsp16) that are highly essential for viral replication and there by prevents the viral survival in the host environment. This formulation have high binding affinities to the C3-like protease and ACE2 Receptor Spike protein of COVID-19 and can possibly be future therapeutics against this coronavirus if in silico studies are confirmed with antiviral activity studies. It remains a possibility that this formulation on treatment may contribute to the lower incidences of COVID-19 cases in India. Further, preclinical and clinical trials have to be conducted in order to know the exact mechanism and efficacy of *Aadathodai Kudineer* in SARS-CoV-2.

Conflict of Interest

Nil.

Funding Information

Nil.

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Studies in Systems, Decision and Control 366

Ahmad Taher Azar
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Modeling, Control and Drug Development for COVID-19 Outbreak Prevention

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Modeling, Control and Drug Development for COVID-19 Outbreak Prevention

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Siddha Medicine and Computer Modeling: A Treasure for SARS-CoV-2 Treatment

M. S. Shree Devi, P. Sathiyarajeswaran, D. Thirumal Kumar, S. Udhaya Kumar, R. Siva, George Priya Doss, and K. Kanakavalli

Abstract The SARS-CoV-2 was identified in December 2019 and spread quickly around the globe. Around 218 countries with 61,468,916 cases have been diagnosed as of November 27, 2020. The epidemic quarantine and symptomatic care plan control are the first step of the treatment in the absence of medicines and vaccines. The need for treatments/therapeutics is in high demand. Pharma companies are working around the clock to develop these treatments/therapeutics. Several traditional medicines are also followed as the treatments/therapeutics across the globe. In India, the traditional medicine system ranks to be one of the topmost promising treatments/therapeutics for ages. In COVID-19, the medicinal products for treatment, prophylaxis, and convalescence were listed based on the Siddha Medicine advisory provided by the Ministry of AYUSH. These drugs are recommended for the treatment and prophylaxis of symptoms. In reality, however, these medications have been in vogue for infectious diseases like Dengue and Chikungunya for the past two decades. In parallel, the in silico studies are positively helping in the drug discovery and unravel the drug mechanisms. The computational protein modeling techniques also play a significant role in identifying all the reference genome's proteins. This chapter discussed Siddha and Quarantine's traditional insight in viral diseases, Virus-based drug repurposing for coronaviruses, and various treatment, including significant drug repurposing and BSAA combination therapy. We have also used computational modeling techniques to identify and model the individual protein structures from the whole genome of SARS CoV-2. Finally, this chapter will explain the steps

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A. T. Azar and A. E. Hassanien (eds.), *Modeling, Control and Drug Development for COVID-19 Outbreak Prevention*, Studies in Systems, Decision and Control 366, https://doi.org/10.1007/978-3-030-72834-2_15

taken to develop and repurpose Kabasura Kudineer as a drug to inhibit the COVID-19 pandemic.

Keywords Siddha medicine · AYUSH · COVID-19 · Computational modelling · Drug repurposing · Kabasura kudineer

1 Introduction

The novel corona-virus disease-2019 (COVID-19) is an ongoing corona-virus ailment pandemic prompted with the aid of corona-virus two (SARS-CoV-2) as a Severe Acute Respiratory Syndrome [1]. WHO coined the phrase COVID-19 after it used to be widely agreed [2–4]. The disorder was first detected in Wuhan in China in December 2019 and had unfolded globally, ensuing in the modern-day pandemic [5, 6]. World Health Organization (WHO) reported on January 30 that the 2019-nCoV outburst is a public fitness emergency of global difficulty [7]. Around 218 countries with 61,468,916 cases have been diagnosed as of November 27, 2020, with the numbers rising by means of the hour. The following things need to be implemented (1) slow the spread of illness; (2) provide time better to prepare health care systems and the general public to be ready if widespread transmission with substantially associated condition arises; and (3) better illustrate 2019-nCoV to monitor public health commendations and the expansion of therapeutic countermeasures including diagnostics and vaccines [7–10]. Isolation, treatment based on symptoms, is the current management pattern because of the non –availability of suitable antiviral drugs [11]. Repurposed therapeutic agents targeting COVID-19 is essential [12]. The Indian government recorded 93,51,224 confirmed cases and 1,36,238 deaths on November 11, 2020. Despite allopathic medicines, Siddha Medication could also be a safer option for this COVID-19 infection as an alternative/supplement treatment [13]. The potential for these forms lies in the herbs used in the Siddha medicine system. During earlier epidemics of vector-borne diseases and HIV, Siddha literature's compound formulations were tested [14–16]. Herbal remedies have made a comeback, making use of herbal ingredients with limited or no side effects. Due to pharmacological proof of protection, effectiveness, the manufacturing of standardized dosage forms, and high-quality manipulate measures, and they are recognized [17]. The Siddha remedy is also described as science. In South India, the outstanding restoration artwork originated, flourished, and practiced by each normal and ancestral method [18]. Classically, relying on the patient's medical symptoms, indication, and adjuvant remedy is referred to in traditional literature. It states that the Siddha method of medicine (sastric preparation) can also be clinically used for viral infections. Treatment can differ according to its behavior and symptoms, referring to a category of diseases [19]. The selection of the specific Siddha medicine (sastric preparation/novel formulation) would depend on the disease's indication [20]. COVID-19 correlated with kaphasuram [21]. Based on the symptoms, germ theory is not new to Siddha Medicine as terms like kirumi existed significantly earlier [22]. The diseases like

Journal of Molecular Docking
Vol 1 Issue 1 June 2021
Page 15 – 24

http://journal.umpalangkaraya.ac.id/index.php/jmd/article/view/2264
DOI: https://doi.org/10.33084/jmd.v1i1.2264
e-ISSN: 2798-138X

Original Research Article

Molecular Docking Studies of Phytoconstituents Identified in Traditional Siddha Polyherbal Formulations Against Possible Targets of SARS-CoV-2

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Keywords:
ACE2
ADME
Molecular docking
Phytochemicals
Spike protein

Abstract

The Indian Traditional Medicines System has long used Siddha polyherbal formulations for different viral diseases. The ingredients of these formulas have been proven to be antiviral. The study focuses on *in silico* computational evaluation of phytoconstituents of the official Siddha formulation Kabasura, Thonthasura, and Vishasura Kudineer, which were widely used in treating viral fever and respiratory infections and may influence the current SARS-CoV-2 coronary virus pandemic. Maestro interface (Schrödinger Suite, LLC, NY) was used for molecular docking studies against M^{Pro} (PDB ID 5R82, 6Y2F, and 6LU7), Nsp15 endoribonuclease (6W01), RNA-dependent RNA polymerase (6M71), and spike protein (6VW1) of SARS-CoV-2. In addition, pharmacokinetics (ADME) and safety profile prediction studies were performed to identify the best drug candidates using Qikpro and Toxicity Estimation Software Tool (T.E.S.T). A total of 36 compounds were screened, of which nine displayed strong binding affinity and drug-likeness. Luteolin and chrysoeriol produced stronger results. These nine compounds were free of oral toxicity as evaluated by the Toxicity estimation software. Based on further *in vitro*, *in vivo*, and clinical effectiveness trials, these compounds may be used for the prevention or treatment as per the Indian system of traditional medicines.

Received: May 2nd, 2021
Accepted: June 7th, 2021
Published: June 30th, 2021

INTRODUCTION

Ever since the outbreak of COVID-19 caused by Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in Wuhan, China, the world has witnessed the rapid spread of the pandemic across the world[1]. World Health Organization (WHO) reported approximately 82,579,768 COVID-19 cases and 1,818,849 deaths as of January 2nd, 2021, with cases reported in more than 222 countries/territories. This novel coronavirus outbreak has posed a severe burden to the global economic, medical, and public health infrastructure[2].

The COVID-19 is primarily a droplet-spread infection, and patients exhibit various symptoms of which fever, dry cough, and fatigue are predominant[3]. In some cases, the symptoms had rapidly developed to acute respiratory distress syndrome, metabolic acidosis, septic shock, coagulation dysfunction, eventually leading to multiple organ failure[4-6]. However, mild or asymptomatic COVID-19 patients can recover shortly after isolation and healthy lifestyle and food habits[7].

There is no particular treatment available for COVID-19 infection except for comprehensive support by the combination of broad-spectrum antibiotics, antiviral and anti-malarial drugs, corticosteroids, and convalescent plasma therapy[8]. Numerous clinical trials are in progress, including identifying vaccines against SARS-CoV-2. Researchers and health care professionals are in desperate search of an effective

cure for this pandemic. In the current scenario where the conventional drugs do not prove to be much efficacious, exploring the traditional system of medicine could be a feasible and hopeful strategy[9]. Traditional, complementary, and alternative medicine has a long history of providing primary beneficial health care to the population[10].

India has an unmatched alternative system of medicine in the form of Ayurveda, Yoga, and Naturopathy, Unani, Siddha, Homeopathy, which is now jointly referred to as Ayush, recognized by the Government of India[11]. Siddha Medicine is one of India's oldest (5000 years old) and well-documented medical systems and is practiced mainly in South India, especially in Tamil Nadu and Sri Lanka, Malaysia, Singapore, and Mauritius, where Tamils live[12]. In the current pandemic situation, many strategies would be highly critical to combat the rapid virus spread and treat the infection. Ministry of Ayush, Government of India has issued an "Advisory on Coronavirus" to manage this outbreak and broadly comprises of preventive and prophylactic symptom management of COVID-19 like illnesses and also insights to interventions based on Ayush systems of medicine through the evidence for immunity boosting as well as help in improving the respiratory symptoms[13].

Drug discovery and development involve a long time, a vast number of individuals, high prices. *In silico* screening approaches allow researchers to explore new and potentially active lead compounds in less time, expense, and humans[14]. Siddha polyherbal formulations are potent against several causative agents such as influenza, chikungunya, dengue, tuberculosis, and others[15-17]. Siddha medicines have been used successfully by Siddha practitioners and ordinary citizens for the treatment of many diseases for several years, such as Kabasura Kudineer during influenza outbreaks, Nilavembu Kashayam for dengue fever. Kabasura kudineer, Thonthasura kudineer, and Vishasura kudineer are polyherbal formulations that have long been used in Siddha medication for different health problems, including currently being developed for COVID-19 therapy[18-19]. These polyherbal formulas are made up of different medicinal plants.

This study aims to evaluate the activity of phytoconstituents in Siddha polyherbal formulations against various potential SARS-CoV-2 targets using *in silico* methods. Thirty-six phytoconstituents were selected from these medicinal plants and docked against all potential SARS-CoV-2 targets, including M^{Pro}, Nsp15 endoribonuclease, RNA-dependent RNA polymerase (RdRp), and spike protein, utilizing Maestro 11.8 (Schrodinger 2018-4 package).

METHOD

Hardware and Software

Software used includes Maestro 11.8 from Schrödinger, Inc (https://www.schrodinger.com/products/maestro) and Toxicity Estimation Software Tool (T.E.S.T.) 4.2.1 from United States Environmental Protection Agency (https://www.epa.gov/chemical-research/toxicity-estimation-software-tool-test).

Ligands

Hygrophila auriculata, Piper longum, Syzygium aromaticum, Tragia involucrata, Clerodendrum serratum, Anacyclus pyrethrum, Terminalia chebula, Adhatoda vasica, Coleus amboinicus, Saussurea lappa, Tinospora cordifolia, Andrographis paniculata, Sida acuta, Cyperus rotundus, and *Zingiber officinale* were the 15 ingredients of Kabasura Kudineer[20,21]. The Thonthasura Kudineer contains ten ingredients, including *Z. officinale, A. vasica, A. paniculata, T. cordifolia, Elettaria cardamomum, Solanum xanthocarpum, Trichosanthes cucumerina, Tephrosia purpurea, Mollugo cerviana*, and *Vitis vinifera*[22]. While the Vishasura Kudineer consists of nine ingredients, including *Azadirachta indica, Z. officinale, Hemidesmus indicus, Indigofera tinctoria, Aristolochia bracteolata, E. cardamomum, Vetiveria zizanioides, Santalum album*, and *Glycyrrhiza glabra*[23].

The major active phytoconstituents present in those plants were selected. The selected 36 phytoconstituents including β-sesquiphellandrene (PubChem ID 11106487), β-bisabolene (10104370), geranial/citral (638011), piperine (638024), piperlonguminine (5320621), eugenol (3314), β-caryophyllene (5281515), stigmasterol (5280794), squalene (638072), γ-sitosterol/clionasterol (457801), andrograpanin (11666871), moslosooflavone/5-hydroxy-7,8-dimethoxyflavone (188316), lupeol (259846), betulin (72326), chebulagic acid (442674), gallic acid (370), vasicinone (10242), carvacrol (10364), cirsimaritin (188323), chrysoeriol (5280666), luteolin (5280445), costunolide (5281437), elemol (92138), tinosponone (15215479), bharangin (194464),

scutellarein (5281697), magnoflorine (73337), cycleanine (121313), cyperene (99856), β-selinene (442393), zingiberene (92776), vasicine (442929), cucurbitacin B (5281316), andrographolide (5318517), apigenin (5280443), pyrethrin I (5281045), and the reference drugs (chloroquine, hydroxychloroquine, ivermectin, lopinavir, remdesivir, and ritonavir) were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/).

Receptors

All potential SARS-CoV-2 targets, including M^{Pro}, Nsp15 endoribonuclease, RdRp, and spike protein, have been selected to evaluate the optimum ligand. The 3D structure of selected proteins has been downloaded from Protein Data Bank (https://www.rcsb.org). The PDB ID of the selected proteins was M^{Pro} (5R82, 6Y2F, 6LU7), Nsp15 endoribonuclease (6W01), RdRp (6M71), and spike protein (6VW1)[24-29].

Docking protocol

Preparation of ligands

The ligand minimization was carried out by the LigPrep module in Maestro 11.8. The 3D ligand structure was generated, and hydrogen atoms were introduced. Salt reduction and ionization (pH 7.0±2.0) were conducted, and the minimization was performed utilizing the OPLS-2005 force field[30,31].

Preparation of protein

Protein Preparation Wizard was used to prepare protein structures. Bond orders were assigned, and hydrogen atoms were inserted. Within 3 Å of the het groups, the water molecules were removed, and the missing side chains were filled with prime. As a result, hydrogen bonds (H-bond) were optimized and reduced using the OPLS 2005 force field. The co-crystallized ligand binding sites have been identified after elimination. The receptor grid was then created using the "Glide's Receptor Grid Generation" module with a 20 Å radius[30,31].

Molecular docking and free energy calculation

The molecular docking between receptor binding sites and ligands was conducted using the Glide Module of Maestro 11.8, and the lowest binding pose of each ligand was maintained. Glide docking scores were performed in three high-throughput virtual screening (HTVS), standard precision (SP), and extra precision (XP) modes. Firstly, docking was performed with reference molecules of respective proteins to validate the docking protocol. We used the XP mode for docking. After XP mode docking, compounds were sent to Prime MMGBSA from Maestro 11.8 for free energy calculations.

ADME and toxicity analysis

Out of the 36 compounds, ten compounds were chosen based on the docking performance. The chosen compounds were used in the ADME study using the QikProp module from Maestro 11.8, and the following parameters were determined.

1. The molecular weight of the molecule.
2. Predicted octanol/water partition coefficient.
3. Predicted brain/blood partition coefficient.
4. Percent human-oral absorption
5. Lipinski's rule of five.
   a. mol_MW <500
   b. QPlogPo/w <5
   c. donorHB ≤5
   d. accptHB ≤10
6. Jorgensen's rule of three
   a. QPlogS >-5.7
   b. QP PCaco >22 nm/s
   c. # Primary Metabolites <7

Toxicity was measured using T.E.S.T. 4.2.1. Oral rats LD_{50}, developmental toxicity, and Ames mutagenicity were conducted using four methods: Consensus system, Hierarchical clustering method, FDA method, and Nearest neighbor method[32].

1. Hierarchical method [HM]: Using the weighted average of estimates from several separate models, the toxicity of a specified question compound was determined. Using the Ward approach to fragment the training set into a sequence of structurally linked clusters, the separate models were obtained. A genetic algorithm-based approach was used to create models for each cluster. Models were created before runtime.
2. FDA Method [FM]: For and test product, the prediction was produced using a new model appropriate for chemicals closest to the test compound. Each model was generated at runtime.
3. Nearest neighbor method [NM]: The predicted toxicity was calculated by taking an average of the three chemicals most comparable to the research chemicals in the training kit.

4. Consensus Method [CM]: The predicted toxicity was calculated by taking an average of the predicted toxicity from the QSAR as mentioned earlier methods (provided the predictions were within the respective applicability domains).

RESULTS AND DISCUSSION

Molecular docking and free energy calculation

Compounds with a docking score of less than -6.0 were deemed possible candidates against SARS-CoV-2, as represented in **Table I** for a comparative study. Out of 36 molecules, luteolin, chrysoeriol, and cucurbitacin B have been associated with more than two receptor structures. Luteolin displays a docking score less than -6 with M^{Pro}, Nsp15 endoribonuclease, and RdRp, as seen in **Figure 1**.

Table I. Comparative docking analysis of ligands against M^{Pro}, Nsp15 endoribonuclease, RdRp, and spike protein

Compounds	Receptors (PDB ID)					
	5R82	6Y2F	6LU7	6W01	6M71	6VW1
Remdesivir	-5.478	-5.306	-7.189	-7.829	-8.643	-7.206
Hydroxychloroquine	-5.395	-2.741	-4.438	-4.814	-4.177	-8.748
Chloroquine	-4.203	-1.587	-3.98	-5.896	-2.191	N/A*
Lopinavir	-5.373	-3.5	-4.535	-5.953	-7.797	-6.702
Ritonavir	-3.927	-5.233	-6.79	-5.848	-1.198	-6.493
Ivermectin	-3.037	-3.427	-4.44	-4.187	-3.558	N/A
Luteolin	-7.408	-6.036	-7.47	-7.314	-6.304	N/A
Scutellarein	-6.807	-6.081	-7.587	-7.191	N/A	N/A
Chrysoeriol	-6.473	-6.394	-7.342	-6.43	-6.174	N/A
Cucurbitacin B	-6.267	N/A	-6.946	-7.021	-6.488	N/A
Apigenin	-6.065	N/A	-6.22	-6.41	N/A	N/A
Andrographolide	-6.042	N/A	N/A	N/A	N/A	N/A
Cirsimaritin	-6.031	N/A	-6.743	-6.461	N/A	N/A
Moslosooflavone	-6.003	N/A	-6.973	N/A	N/A	N/A
Gallic acid	N/A	N/A	N/A	-6.379	N/A	N/A
Pyrethrin	N/A	N/A	N/A	N/A	-6.704	N/A
Cycleanine	N/A	N/A	N/A	N/A	N/A	-6.907

*N/A: Not available

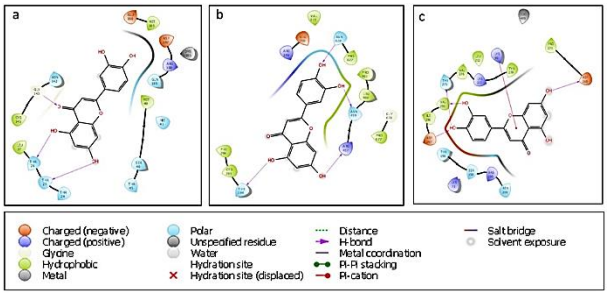

Figure 1. Binding-interaction analysis of luteolin with (**a**) M^{Pro}, with (**b**) RdRp, and with (**c**) Nsp15 endoribonuclease.

Chrysoeriol also displays a docking score less than -6.0 with M^{Pro}, Nsp15 endoribonuclease, and RdRp, as seen in **Figure 2**. The associations of luteolin and chrysoeriol with various SARS-CoV-2 target forms were comparatively analyzed, in which H-bond and hydrophobic pockets were presented in **Tables II** and **III**. Luteolin shows hydrogen bonding with nearly four amino acids of most of the targets. This finding shows its high binding potency towards the SARS-CoV-2.

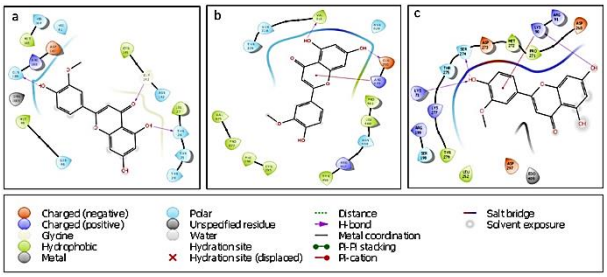

Figure 2. Binding-interaction analysis of chrysoeriol with (**a**) M^{Pro}, with (**b**) RdRp, and with (**c**) Nsp15 endoribonuclease.

Table II. Binding interactions of luteolin with the active sites of different targets in SARS-CoV-2

Target	PDB ID	H-Bond	Hydrophobic pocket
M^{Pro}	5R82	GLY 143, THR 26, THR 25	CYS 145, MET 165, MET 49, LEU 27
	6LU7	THR 26	LEU 27, CYS 145, CYS 44, MET 49, PRO 52, TYR 54, MET 165
	6Y2F	GLU 166, LEU 141, HIE 163, HIE 41	LEU 27, VAL 42, CYS 44, TYR 54, MET 49, PHE 140, LEU 141, LYS 145, MET 165
RdRp	6M71	THR 394, ARG 457, ASN 628, ASN 459	PHE 396, CYS 395, VAL 315, PRO 627, PRO 461, LEU 460, PRO 677
Nsp15 endoribo-nuclease	6W01	ASP 268, ASP 297, THR 275, VAL 295	PRO 271, LEU 252, VAL 295, ILE 296, VAL 276, TYR 279

Table III. Binding interactions of chrysoeriol with the active sites of different targets in SARS-CoV-2

Target	PDB ID	H-Bond	Hydrophobic pocket
M^{Pro}	5R82	GLN 189, GLY 143, THR 26	CYS 145, LEU 27, MET 49, MET 165
	6LU7	THR 26	CYS 44, PRO 52, MET 49, TYR 54, MET 165, CYS 145, LEU 27
	6Y2F	ASP 187, GLU 166, LEU 141	CYS 44 , LEU 141, CYS 145, MET 165, TYR 54, MET 49
RdRp	6M71	VAL 315, GLU 350	VAL 315, PRO 461, LEU 460, PHE 396, CYS 395, TYR 456, PRO 677, VAL 675
Nsp15 endoribo-nuclease	6W01	LYS 71, SER 275, LYS 90	TYR 279, MET 272, PRO 271, LEU 252

In the molecular docking of phytoconstituents with M^{Pro} (5R82), luteolin had a higher affinity with a docking score of -7.408, followed by scutellarein and chrysoeriol with docking scores of -6.807 and -6.473, respectively. These phytoconstituents had a higher affinity to M^{Pro} (5R82) than remdesivir, displaying a docking score of -5.478. Chrysoeriol had a higher affinity with a docking score of -6.394, followed by scutellarein and luteolin with docking scores of -6.081 and -6.036, respectively with the target M^{Pro} (6Y2F). These phytoconstituents had a higher affinity to M^{Pro} (6Y2F) than remdesivir, with a docking score of -5.306. Scutellarein had a greater affinity with a docking score of -7.587, followed by luteolin and chrysoeriol with -7.470 and -7.342, respectively, for molecular docking of phytoconstituents with M^{Pro} (6LU7). These phytoconstituents had a higher affinity than remdesivir, which had a docking score of -7.189.

Remdesivir shows greater affinity with a docking score of -7.829, followed by scutellarein and cucurbitacin B with a score of -7.314 and -7.191, respectively, in the docking analysis with Nsp15 endoribonuclease (6W01). With RdRp (6M71), remdesivir had a higher affinity with a docking score of -8.643, followed by pyrethrin and cucurbitacin B with docking scores -6.704 and -6.488, respectively. Hydroxychloroquine had a higher affinity with a docking score of -8.748, followed by remdesivir and cycleanine, which had a docking score of -7.206 and -6.907, respectively, with the target spike protein (6VW1). Most phytoconstituents exhibited similar reference drugs in binding energies and binding pockets, except gallic acid, pyrethrin, chebulagic acid, and cycleanine.

Chrysoeriol shows less hydrogen bonding than the luteolin but better than other phytoconstituents. The hydrogen bonding of both luteolin and chrysoeriol could be increased by substitute better chemical groups. The prime MM-GBSA was generally accepted for the re-scoring of docked complexes. Both of the chosen complexes were subjected to prime MM-GBSA measurements after XP Docking[33]. MM-GBSA DG-bind scores for all chosen compounds were displayed in **Table IV**. The negative DG-bind values indicate that the selected compounds associate favorably with the receptor. Ligand binding energies for both substances vary from -40.0 to -100.0 kcal/mol. The binding energies of several of the substances were relatively close to those of the reference drug binding energy. These findings indicate that the selected compounds would inhibit SARS-CoV-2.

Table IV. MM-GBSA DG-bind values of selected compounds

Compounds	Receptors (PDB ID) (kcal/mol)					
	5R82	6Y2F	6LU7	6W01	6M71	6VW1
Remdesivir	-63.6	-74.01	-79.74	-61.48	-73.53	-47.55
Hydroxychloroquine	-77.77	-94.09	-64.02	-46.21	N/A*	-64.72
Chloroquine	-62.03	-87.32	-78.62	-36.65	N/A	-66.13
Lopinavir	-59.92	-52.27	-48.39	-47.87	-93.51	-70.62
Ritonavir	-93.22	-88.95	-96.23	-69.29	N/A	-31.95
Ivermectin	-62.33	-59.89	-55.15	-66.39	N/A	-54.7
Luteolin	-45.23	-26.87	-54.84	-41.3	-48.75	N/A
Scutellarein	-43.21	-41.37	-50.83	-44.21	N/A	N/A
Chrysoeriol	-56.63	-23.2	-56.63	-39.5	-54.83	N/A
Cucurbitacin B	-82.11	N/A	-63.88	-58.96	-79.78	N/A
Apigenin	-45.79	N/A	-52.44	-43.85	N/A	N/A
Andrographolide	-69.79	N/A	-51.5	N/A	N/A	N/A
Cirsimaritin	-53.92	N/A	-55.73	-50.21	N/A	N/A
Moslosooflavone	-51.89	N/A	-56.77	N/A	N/A	N/A
Gallic acid	N/A	N/A	N/A	-18.15	N/A	N/A
Pyrethrin	N/A	N/A	N/A	N/A	-79.94	N/A
Cycleanine	-63.6	-74.01	-79.74	-61.48	-73.53	-47.55

*N/A: Not available

ADME analysis

The absorption, distribution, metabolism, and elimination of substances play an essential role in the drug development phase. *In silico* ADME analysis would save thousands of dollars spent in the drug development phase by producing fewer new compounds[34]. The ADME parameters, such as mol MW, QPlogPo/w, QPlogBB, percent human oral absorption, Rule of Five, and Rule of Three using QikProp showed a better score for the docked compounds[35]. Both of the chosen nine compounds have enhanced ADME properties and drug-likeness according to the spectrum as shown in **Table V**. All of the nine phytoconstituents have enhanced ADME properties. Cucurbitacin B violates a rule of 1 of 5, which was appropriate. Gallic acid and pyrethrin were in breach of a law of three that was fitting. Luteolin and chrysoeriol display improved drug-likeness and high binding capacity, all of which were essential to the drug candidate.

Table V. ADME prediction for the selected compound

Compounds	Mol MW	QPlogBB	QPlogPo/w	Percent human oral absorption	Rule of Five	Rule of Three
Andrographolide	350.454	-1.222	1.437	79.068	0	0
Apigenin	270.241	-1.411	1.624	73.955	0	0
Chrysoeriol	300.267	-1.409	1.81	76.672	0	0
Cucurbitacin B	558.711	-1.964	2.92	67.293	1	0
Gallic acid	170.121	-1.659	-0.585	41.441	0	1
Luteolin	286.24	-1.91	0.941	62.05	0	0
Pyrethrin	372.46	-1.157	4.385	100	0	1
Scutellarein	286.24	-1.819	1.001	63.924	0	0
Moslosooflavone	298.295	-0.43	3.165	100	0	0

In silico toxicity study

The oral rat LD_{50}

The endpoint of the oral rat LD_{50} was the amount of the chemical (chemical mass per rat body weight) that destroys half of the rats when administered orally[36]. The oral rat LD_{50} was conducted in four methods for all of the chosen compounds, and the findings were comparatively evaluated in **Table VI**. All substances have been shown to have an acceptable toxicity limit for drug production and preclinical and clinical assessment.

Developmental toxicity

Developmental toxicity includes embryonic and fetal mortality, miscarriage, and other abnormal developmental symptoms such as liver toxicity, lowered body weight, growth, developmental retardation, and physical abnormalities (teratogenic effects)[37]. Developmental toxicity was performed in four approaches with all of the chosen compounds, and the findings were comparatively analyzed in **Table VI**. A predicted value greater than 0.5 indicates toxicity. Except gallic acid, all other compounds show developmental toxicity.

Ames mutagenicity

In Ames assay, frame-shift mutations or base-pair substitutions could be identified by exposure of histidine-dependent strains of *Salmonella typhimurium* to the test compound. When these strains were exposed to a mutagen, reversing mutations that restore the functional capacity of the bacteria to synthesize histidine would cause the bacterial colony to develop on a medium histidine deficiency (revertant)[38].

A compound was labeled Ames positive if it significantly induces the development of the reverting colony in at least one of the five strains. If a compound was positive for the Ames test, it could be a possible mutagen[39]. Ames mutagenicity was conducted in four methods for all of the chosen compounds, and the findings were comparatively analyzed in **Table VI**. A predicted value greater than 0.5 indicates mutagenicity. All the nine phytoconstituents except pyrethrin were not mutagens based on the results on the Ames mutagenicity as predicted by T.E.S.T software.

Table VI. Predicted value for oral rat LD_{50} – Log^{10} (mol/kg), developmental toxicity, and Ames mutagenicity

Compounds	Oral rat LD_{50} - Log^{10} (mol/kg)				Developmental toxicity				Ames mutagenicity			
	HM	FM	NM	CM	HM	FM	NM	CM	HM	FM	NM	CM
Andrographolide	2.82	3.87	5.32	4.00	0.76	0.81	1.00	0.82	0.32	0.49	0.33	0.38
Apigenin	2.17	2.28	2.14	2.20	0.94	0.35	N/A	0.65	0.06	0.47	0.33	0.29
Chrysoeriol	2.30	3.11	3.05	2.82	1.04	1.13	N/A	0.95	0.04	0.18	0.33	0.18
Cucurbitacin B	3.87	4.18	3.37	3.81	0.77	0.69	1.00	0.83	0.02	0.00	0.00	0.01
Gallic acid	1.88	1.35	1.69	1.64	0.34	0.39	0.33	0.34	0.29	0.64	0.00	0.31
Luteolin	2.37	1.85	2.14	2.12	0.89	1.20	N/A	0.88	0.31	0.62	0.67	0.53
Pyrethrin	3.28	3.36	2.77	3.14	0.70	0.98	N/A	0.84	0.92	1.12	0.67	0.90
Scutellarein	2.38	2.05	2.14	2.19	0.89	1.30	N/A	0.92	0.28	0.17	0.67	0.37
Moslosooflavone	3.35	3.20	3.37	3.31	0.99	1.09	0.67	0.89	0.65	0.07	0.00	0.24

HM: Hierarchical method; FM: FDA method; NM: Nearest neighbor method; CM: Consensus method; N/A: Not available

Our current research has chosen three Official Siddha Formulation Kabasura, Thonthasura, and Vishasura Kudineer to test its potential against SARS-CoV-2 targets. Siddha medicine is one of the oldest Indian systems of medicine. The methods of Siddha emerged in India, and it was most commonly practiced in India, especially in southern regions. Siddha medicinal plants were a promising area for the treatment of a wide variety of diseases. Siddha medicinal plants might also be considered a new choice for their role in overcoming viral transmission[40,41].

Mekala and Krishnamurthy[42] performed the phytochemical screening and pharmacological update on Kabasura Kudineer Choornam and Nilavembu Kudineer Choornam. Kabasura Kudineer was found to have alkaloids, carbohydrates, glycosides, heart-glycosides, flavonoids, phenols, saponins, and hydrolyzable present in Kabasura Kudineer Choornam. In addition to the broad range of other pharmacological operations, the ingredients in Kabasura Kudineer show that most of the components were antipyretic, anti-inflammatory, antimicrobial, and immunostimulant[43]. Therefore, it was scientifically rational to use it in respiratory viral infection.

The molecular docking study of Thonthasura Kudineer ingredients demonstrated affinity with the Coronavirus Spike (S) glycoprotein, carried out by Kumar *et al*[22]. Vishasura Kudineer was a polyherbal formulation from the Siddha literature 'Kaaviya Sura Nool'. Vishasura Kudineer was traditionally used for symptoms associated with viral fever. Its portion demonstrates antiviral activity against a wide variety of viruses. It might also be antipyretic, antiasthmatic, anti-inflammatory, antioxidant, hepatoprotective, and immunostimulant[18].

Various research studies have been performed on different formulations of Siddha and its phytoconstituents against selective targets for SARS-CoV-2[19,22,44]. The main protease (M^{Pro}, $3CL^{Pro}$, Nsp5) proteolytically cleaves the overlapping pp1a and pp1ab polyproteins to functional proteins, crucial in viral replication. In the viral replication cycle, the M^{Pro} acts as the primary enzyme. Its inhibition could thus interfere with the production of infectious virus particles and reduce disease symptoms[45].

The SARS-CoV-2 spike protein mediates the binding of the virus to its receptor angiotensin-converting enzyme 2 (ACE2) and facilitates the integration of viral and host cell membranes and the entrance of the virus into the host cell. Thus, the Spike protein was vital in neutralizing and T-cell reactions and maintaining immunity during SARS-CoV-2 infection. Given the essential role of the S-protein in viral infection and adaptive immunity, most methods and therapies were based on the S-protein[46]. RNA-dependent RNA Polymerase was an enzyme that replicates RNA from an RNA template. RNA-dependent RNA Polymerase was one of the Nsp (Nsp12) that plays a key role in the coronavirus life cycle[47].

Nsp15 was responsible for protein interaction with the innate immune response, although other studies suggest that the mechanism was independent of endonuclease activity. In order to conceal it from the host's immune system, there were also reports that Nsp15 degrades viral RNA[48]. Nevertheless, in coronavirus biology, Nsp15 was important. The active site, located in a shallow groove between the two β-sheets, carries six key residues conserved among SARS-CoV-2, SARS-CoV, and MERS-CoV proteins: His235, His250, Lys290, Thr341, Tyr343, and Ser294[27].

CONCLUSION

The present research was planned to classify potential drug candidates exhibiting potential binding affinity to all possible SARS-CoV-2 targets (M^{Pro}, Nsp15 endoribonuclease, RdRp, and spike protein). Based on the findings obtained from molecular docking, free energy measurement, ADME analysis, as well as toxicity analysis, luteolin and chrysoeriol exhibit stronger docking score, binding energy, ADME properties, and lower toxicity than all other compounds.

CONFLICTS OF INTEREST

There are no conflicts of interest to declare.

FUNDING

None.

DATA AVAILABILITY

All data are available from the authors.

ACKNOWLEDGMENTS

The authors thank Sri Ramachandra Institute of Higher Education and Research (Deemed University) management for providing us with all the facilities for completing the project.

AUTHORS' CONTRIBUTIONS

Logesh Kumar Selvaraj: conceptualization, data curation, formal analysis, investigation, methodology, project administration, software, visualization, writing – original draft. **Geethanjali Thayumanavan**: data curation, investigation, writing – original draft. **Srikanth Jeyabalan**: conceptualization, investigation,

project administration, software, supervision, validation, writing – review & editing. **Sugin Lal Jabaris**: supervision, validation, writing – review & editing.

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In vitro antiviral activity of Kabasura Kudineer - Siddha polyherbal formulation against novel coronavirus (SARS-CoV-2)

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Abstract:

Kabasura Kudineer (KSK) is a Sastric Siddha polyherbal formulation has been used to treat fever with influenza like indication. The purpose of this study is to validate the antiviral activity of KSK against the novel SARS-CoV-2 virus. The antiviral activity of KSK against SARS-CoV-2 was assessed in Vero E6 cells and followed by RT-PCR assay. KSK significantly inhibited SARS-CoV-2 replication in Vero E6 cells. These data indicate that KSK prevents against the attack of its virus, rendering the use of these a novel COVID-19 infectious diseases strategy.

Keywords: Kabasura Kudineer, Coronavirus, SARS-CoV-2, Siddha Medicine

Introduction:

A viral disease named as a novel coronavirus Since December 12, 2019, a novel coronavirus disease (SARS CoV-2) has caused an international outbreak of acute respiratory syndrome. WHO named the virus as Coronavirus Disease 19 (COVID-19) and declared a pandemic on 11th March 2020 (https://covid19.who.int/dg/) Communication of viruses spread via droplets, physical contact with infected individuals, contaminated surfaces (Bai et al., 2020). Coronaviruses are one of the large group of viruses (Li, 2016). Different types of coronaviruses that cause respiratory and sometimes gastrointestinal symptoms (Li, 2016). They consist of a core of genetic material surrounded by an envelope with protein spikes. This gives it the appearance of a crown. Crown in Latin is called Corona; that is how these viruses get their name (Schoeman et al., 2020). The novel coronavirus is a single-stranded, positive-sense RNA genome surrounded by double-layer lipid derived from the intracellular Rough endoplasmic reticulum and Golgi membrane of the infected cells. CoVs are structured into three groups: α-CoVs, β-CoVs, γ-CoVs (https://www.cdc.gov/coronavirus/2019) based on genetic and antigenic benchmarks. The corona viral genome encodes four major structural proteins: the spike (S) protein, nucleocapsid (N) protein, membrane (M) protein, and the envelope (E) protein, all of which are required to produce a structurally complete viral particle. More recently, however, it has become clear that some CoVs do not require the full ensemble of structural proteins to form a complete, infectious virion, suggesting that some structural proteins might be dispensable or that these CoVs might encode additional proteins with overlapping compensatory functions (https://www.cdc.gov/coronavirus/2019). COVID-19 commonly reported symptoms are fever, headache, vomiting, chills, dyspnoea, nausea, sore throat, coughing up blood, shortness of breath, myalgia, diarrhoea, and malaise. The severe infection leads to pneumonia, acute respiratory distress syndrome (ARDS) and

sometimes multi-organ failures such as kidney failure, and even death (Huang et al., 2020) and significant mortality rates, particularly among elderly populations and people with co-morbidities (Li et al., 2020). Presently, quarantine and symptomatic treatment protocol for disease management exists, and there are no specific antiviral drugs available to combat this virus. As this pandemic is still ongoing; hence there is an urgent need to find new preventive and therapeutic agents as soon as possible (WHO, 2005). The new intervention will require more years to develop; based on the pandemic situation, we focus on the potential existing drugs to repurpose for treating viral infections. The Siddha herbal formulations having medicinal importance have proved to be potentially active against a wide range of causative agents as Influenza, Dengue, Chikungunya, Tuberculosis, etc. (Jain et al., 2018; Jain et al 2020; Jain Jaspreet et al., 2018). Traditional and complementary medicines always give an answer for the pandemics from time immemorial. Siddha system of medicine has its footprints during infections caused by Hepatitis -A, Hepatitis – B, HIV, Dengue and Chikungunya formulations which are used during these pandemics have undergone reverse pharmacology to prove drug efficacy, i.e. Pre-clinical studies for evidence providing. Recent example is proving of Nilavembu Kudineer decoction inhibit Dengue virus through invitro studies (Jain et al., 2020). Siddha medicines have been used effectively by human civilization to treat various diseases and effectively target the host response. During global epidemics of HIV on par with other medical systems, Siddha came out with a solution, RAN therapy (Fritts et al., 2008). During the Chikungunya epidemic in India, Siddha formulations have been successfully used in public health (https://www.ccras.nic.in/). When the entire state of Tamil Nadu suffered out of Dengue morbidity, Siddha medicine - Nilavembu Kudineer has been employed in public health in the year 2012 (https://www.cms.tn.gov.in/). Nilavembu Kudineer is used to prevent and control the public's morbidity level on contacting this viral fever. The same formulation has been successfully used to prevent infections in post-Chennai flood mitigation (Selvavinayagam. 2016). Nilavembu Kudineer has been found to inhibit the Dengue virus (Jain et al., 2020). Siddha formulations like Amukkara Chooranam and Brahmananda Bairavam have effectively inhibited the Chikungunya virus (Jain et al., 2018). Kabasura Kudineer is a drug which has been used as a tool to combat Swine flu. Swine flu epidemic brought back Kabasura Kudineer by Govt. of Tamilnadu during the H1N1 influenza outbreak (https://www.nhp.gov.in/). Based on its usage of the same drug-using drug repurposing technique, Kabasura Kudineer was included in the advisories of Govt. of India and followed by Govt. of Tamil Nadu as the same has been endorsed by the experts (https://www.ayush.gov..in/). Globally drugs are being repurposed in COVID-19 mitigation

Hydroxychloroquine, Remdesiver, and Ivermectin or some. Remdesiver based on its benefit in MERS AND SARS- CoV -1, and SARS-CoV-2 has been used clinically (Wang et al., 2020). Its efficacy has been reported from various stakeholders in containment zones, quarantine facilities, and in COVID care centers, which are reported by and large in media of south India. As per the reports, KSK formulation could significantly relieve prime symptoms and reduce the course of the COVID-19. There are two randomized control trials to check its efficacy against the standard of care medication (CTRI/2020/05/025215 & CTRI/2020/06/025874). To support all the above-said claims, data related to inhibition of SARS-CoV-2 becomes essential. In the present study, we evaluated the antiviral efficiency of KSK against a clinical isolate of SARS-CoV-2 by *in vitro*.

2. Materials and methods

2.1. Cell lines and virus

The African green monkey kidney epithelial (Vero E6) cells were cultured in Dulbecco's Modified Eagle's medium (DMEM, Gibco, USA) supplemented with 10 % fetal bovine serum (FBS) at 37°C. A clinical isolated SARS-CoV-2 virus (Gen bank accession no. MT123290.1) was propagated in Vero E6 cells, and viral titre was determined by 50 % tissue culture infective dose (TCID50) according to the cytopathic effect. All the infection experiments were performed in a biosafety level-3 (BSL-3) laboratory in Regional Centre for Biotechnology, Faridabad, India.

2.2. Extract preparation

KSK was purchased from Tamil Nadu Medicinal Plant Farms and Herbal Medicine Corporation Limited (TAMPCOL), India. KSK water and DMSO extract is prepared as per standard protocol (Sathiyarajeswaran et al., 2020).

2.3. Cytotoxicity assay

The cytotoxic effects of the KSK on Vero E6 were evaluated in a 96-well plate format in 3 wells for each sample. $1x10^4$ VeroE6 cells were plated per well and incubated at 37°C overnight for the monolayer formation. The cells were infected with SARS-CoV2 at an MOI of 0.01. The next day, cells were incubated with the KSK at 2ul (water extract) and 1ul (DMSO extract). The water and 1% DMSO were kept as a control. After 24 and 48 hr, the cells were stained with Hoechst 33342 and Sytox orange dye. Images were taken at 10X, 16 images per well, covering 90% of the well area using Image Xpress Micro confocal (Molecular Devices) and reading the plates spectrophotometrically.

The viral RNA was extracted from 100 µl culture supernatant and subjected to qRT-PCR (duplicates) where Ct values for N and E gene sequences were determined. Inhibition of virus

replication is determined based on the fold change in the Ct value in KSK-treated cells compared to the control. Remdesivir was used as a positive control for viral inhibition. The IC_{50} value was calculated using GraphPad prism (Corman et al., 2020; Caly et al., 2020).

3. Results and Discussion:

After KSK treatment, the cell viability was determined by MTT assay in Vero E6 cells. KSK water extract showed unnoticeable cytotoxicity for cell lines at concentrations up to 2 µg/mL and KSK DMSO extract concentrations up to 1 µg/mL. The positive control Remdesivir showed no cytotoxicity to cells at a concentration of 10 µM (Table 1).

At 24 h, the water extract of KSK showed 76.71% and 80.44% reduction in viral RNA of E and N respectively and the DMSO extract of KSK showed 88.18% and 85.99% reduction in viral RNA of E and N respectively Similarly, remdesvir treated cells showed 72.26% and 77.61% reduction in viral RNA. By 48h, this effect was increased. the water extract of KSK showed 99.50% and 99.48% reduction in viral RNA of E and N respectively and the DMSO extract of KSK showed 99.56% and 99.56% reduction in viral RNA of E and N respectively. Similarly, remdesvir treated cells showed 99.64% and 99.76% reduction in viral RNA. The IC_{50} value of Remdesivir is 0.13 µM (E gene) 0.12 µM (N gene) and KSK is IC_{50} = 0.01 mg extract (E gene) 0.01 mg extract (N gene) (Fig 1 & Table 2).

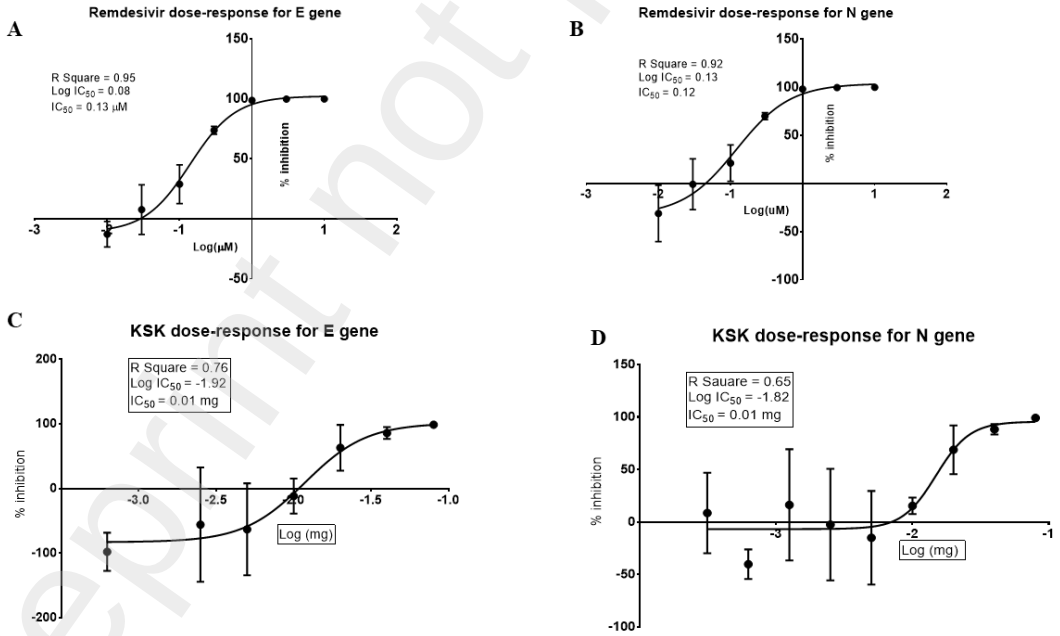
Kabasura Kudineer (KSK), an official Siddha formulation described in Siddha manuscript Citta Vaittiyattirattu, it is used for Aiyacuram (phlegmatic fevers) and is a dependable Siddha prescription for fever with flu-like symptom (Siddha, 2011). Our team already demonstrated the efficacy of Kabasura Kudineer against SARS-CoV-2 through the *In-silico* method and demonstrated that Chrysoeriol and Luteolin from Kabasura Kudineer Chooranam inhibit ACE2 spike protein of SARS-CoV-2. It is also stated that the formulation increases oral bioavailability and less toxic (Kiran et al., 2020). Kabasura Kudineer has been reported to have antipyretic, anti-inflammatory, and antibacterial activity (Saravanan et al., 2018). Further, KSK also showed significant Immunomodulatory, thrombolytic activity (Sathiyarajeswaran et al., 2020), Antioxidant and anti-atherogenic activity (Rajalakshmi et al., 2020). Also, it has Neuraminidase inhibition potential against inactivated influenza virus H1N1 (Unpublished Data). Based on these results, it clearly indicates that KSK is potential SARS-CoV-2 antiviral. At present, KSK is king of siddha medicine used for the treatment of SARS-CoV-2. In future, the mechanism of action should be studied detailed manner.

Compound Name	Concentration (µM)	% Cell viability		% inhibition of virus replication			
		24 hr	48 hr	24 hr Post-infection		48 hr Post-infection	
				E	N	E	N
Remdesivir	**10 µM**	**-**	**-**	**72.26**	**77.61**	**99.64**	**99.76**
KSK_Water extract	**2µl added**	**83.30**	**62.00**	**76.71**	**80.44**	**99.5**	**99.48**
KSK_DMSO extract	**1 µl added**	**85.13**	**60.38**	**88.18**	**85.99**	**99.56**	**99.56**

Table:1 Cytotoxicity and Inhibition of Viral replication by KSK

KSK_Water extract	% Cell viability
0.16 mg in 2µl of extract	67.77
0.08 mg in 2µl of extract	76.87
0.04 mg in 2µl of extract	90.44
0.02 mg in 2µl of extract	95.53
0.01 mg in 2µl of extract	95.44

Table 2: IC_{50} calculation of KSK



(Figure 1: Cytotoxicity activity of KSK. A. Remdesivir IC_{50} = 0.13 µM (E gene); B. Remdesivir IC_{50} = 0.12 µM (N gene); KSK IC_{50} = 0.01 mg extract (E gene); KSK IC_{50} = 0.01 mg extract (N gene))

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In-vitro Immunomodulatory activity and Thrombolytic potential of Kabasura Kudineer (KSK), an official Siddha polyherbal formulation

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Abstract:

Coronavirus disease 2019 (COVID-19) caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), a pandemic, which has led to the spread of mortality and morbidity all over the globe. In this dire situation, there is an urgent requirement for the development and immediate dissemination of treatment against COVID-19. The traditional medicine system of Siddha can be utilized as preventive care to boost the immune system. The vast treasure of knowledge found in Siddha medicine can help in the betterment of humankind. Kabasura Kudineer (KSK) is from the ancient times present as an immune-boosting agent against several diseases. The present experimental setup to investigate the immunomodulatory and thrombolytic potential of KSK. The *in vitro* immunomodulatory models of phagocytosis of *Candida albicans* assay and nitro blue tetrazolium have demonstrated that KSK is giving better results compared with the controls (pooled serum, lipopolysaccharide, and streptokinase). The KSK at the concentrations of 12.5, 25, 50, and 100 µg/ml showed % immune-stimulations of 12.40 %, 20.81 %, 33.53 %, 43.20 % and for NBT showed 19.00 %, 25.50 %, 64.00 %, 71.00 % respectively. And similarly, the thrombolytic activity showed 50 and 100 µg/ml concentration showed 43.83 %, 71.83 % clot lysis respectively; and the control value for the streptokinase showed 83.78 %. Hence, it can be confirmed that KSK has immunomodulatory and thrombolytic properties in *in vitro* models, although the in vivo and the identification of KSK are to be discovered.

Keywords: Kabasura Kudineer (KSK), Immunomodulatory, Thrombolytic, COVID-19, Siddha formulation.

Introduction

Current human healthcare services are as a rule significantly tested and challenged by the SARS-CoV-2 with its indisputable complex biochemical architecture. Its present momentum is very much persistent, making a predictable second wave (Cyranoski 2020). The human respiratory system is very much vulnerable to different viral infections starting from coronavirus, rhinovirus, human metapneumovirus etc. and the human immune system is very affected during COVID-19 progression in the infected host (Schmidt and Varga 2018). Skowronski et al. (2005) had reported respiratory disease result in cytokine-chemokine reaction resulting in serious damage to the host.

The role of immunology was the most rapidly developing scientific area and showed an evolving opportunity in the treatment and prevention of disorders, inflammatory reactions of different parts of the human body. Similarly, the infections are considered immunological diseases, whereas the neoplastic and autoimmune diseases are occur in immunosuppressed state (Ziauddin et al. 1996). It is reported that many of the synthetic, semi-synthetic and natural therapeutic agents have the suppressive and cytotoxic nature which support the immune system (Cyranoski 2020; Manderville 2001; Ren and Kinghorn 2019; Shavit et al. 1984).

In todays, health wellness commerce, the role of immunomodulators is well-established as a key component. These immunomodulators are grouped into three main classes: immunosuppressants, immunostimulants, and immunoadjuvants, and their applications in medicine and pharma industries as for stimulation and suppression of the immune system. And used as both prodrugs and prophylactic drugs for the healthy populace (El Enshasy and Hatti-

Kaul 2013; Shukla et al. 2014). In addition, the immunomodulators from the plant kingdom seem to be a good substitute for the synthetic chemical compounds (Patwardhan et al. 1990).

The World Health Organization has put a public health emergency by putting COVID-19 as an transnational threat (WHO 2020; Wu et al. 2020). Until, today there is no medicine or prophylactic treatment for this disease and has been constrained towards the palliative help to the affected people. Hence, there is dire need to produce a safe and stable COVID-19 immunization.

The recent trending strategies for the COVID-19 treatment plan have been focused in the immunization against the virus and head on attack on the virus particle. This makes host as a vital factor in ailment's subtleties. The Siddha medicine is always aimed towards healthy routine rather than just issue of medicine.

Immunity is termed *Vanmai* in Siddha and it has a direct association with *Uyir thathukkal* (*VALI*, *AZHAL*, and *AIYAM*) and Seven *Udal thathukkal* (Body tissues). Natural immunity of the human body by birth is called *Iyarkai Vanmai*, its improvement with the help of intake of balanced food and medicines is called *Seyarkai Vanmai* and *Kala vanmai*, which is further defined as the change of physical state under the effects of seasons and in their affected state there might be possibilities of disease (Govindammal 2016; Rajeshwari 2017; Srinivasan 2007). The human beings are the subtotal of *Uyir thathukkal* and *Udalthathukkal* forming his/her strong physical and mind results in a strong immune system. Individuals with *Vazhi* trait have lesser immunity, while persons of *Azhal* have moderate immunity, and persons having *Aiyam* are having stronger immunity. The Siddha medicinal system has tested thoroughly the herbs and the polyherbal formulations via *in vitro* and *in vivo* which include the Urai mathirai, Saya chooranam, and Nilavembu kudineer, etc., which are very much beneficial (Dayanand et al. 2019; Kavinilavan et al. 2017; Santhammal et al. 2011). The botanicals used in Kayakalpa are

effective in immunomodulation and restoration of immune homeostasis (Vaidya 2010). The docking studies carried out by us for better understanding KSK extract revealed a pathway to understand the Siddha in scientific manner (Kiran et al. 2020).

The COVID-19 infection cycle has two distinct phases in which the first protective phase of the adaptive immune response in host which might eliminate the virus (Shi et al. 2020). In the current situation, hydroxychloroquine is considered as a candidate for COVID-19 treatment due to its Immunomodulatory and antiviral effects (Chen et al. 2020; Liu et al. 2020). The COVID-19 leads to blood clots in people with severe form of the COVID-19 disease. Blood clots causes a severe problem in blood circulatory system. Blood clots in the form of thrombus hampers the flow of blood in blood vessels, reducing the oxygen intake to the tissues. The fibrinolytic drug dissolves clot trapped in coronary vessels, restoring the blood of heart, limiting the necrosis (Mucklow 1995). The tissue plasminogen activator, urokinase and streptokinase are drugs prescribed as thrombolytic agent nowadays by physicians. The Indian population have been prescribed streptokinase and urokinase due to its low cost, (Collen 1990; Martin et al. 1996) in comparison to other drugs which have hyper risk of hemorrhage (Liu et al. 2018; Rouf et al. 1996).

The COVID-19 is the trending research topic which is being researched again in every developed country. The research papers are being published in almost every branching scientific fields from biotechnology, bioinformatics, physics, chemistry and many other. The traditional medicines are also involved in this research race track to curb the pandemic COVID-19. Siddha Medicine is a treasured healing desire that is classically used for treating viral pulmonary infections, this precept of drugs is confirmed to incorporate antiviral compounds. The Siddha medicine is prescribing KSK for the treatment of fever and as prophylactic antiviral agents

(AYUSH 2020). At present, the guidelines issued by the Ministry of AYUSH, Government of India, KSK is given for boosting immunity among the common people (AYUSH 2020) but not limited to prophylaxis too. So that we can take to the integrative model of therapeutics. For selecting Siddha Medicine's safety, efficacy and availability have to be addressed. However, immunomodulatory activity and thrombolytic activity of Kabasura Kudineer has not been reported or scientifically investigated. Therefore, the present study focused on investigate the immunomodulatory and thrombolytic potential of KSK.

Materials and Methods

Kabasura Kudineer Chooranam is a compound formulation consisting of fifteen ingredients which are given in **Table 1**. Kabasura Kudineer Chooranam was purchased from Tamil Nadu Medicinal Plant Farms and Herbal Medicine Corporation Limited (TAMPCOL). All the chemicals and solvents are of analytical grade, obtained and used in the same condition. The *Candida albicans* suspension (MTCC-183) was purchased from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India.

Extraction of the KSK and sample preparation

The dried KSK powder was weighted and was packed in Soxhlet apparatus and refluxed with distilled water. The extracts were pooled, filtered, dried, and stored below 5 °C till further use. Doses such as 12.5, 25, 50, and 100 µg/ml were prepared in the isotonic solution for *in vitro* immunomodulatory activity.

In vitro immunomodulatory activity by Phagocytosis of *Candida albicans* assay

Phagocytosis of *Candida albicans* test was carried out according to method (Ponkshe and Indap 2002; Ramesh and Padmavathi 2010; Rawat et al. 2018). The Sabouraud's dextrose broth was inoculated with *C. albicans* (MTCC-183) and was incubated overnight. The *C. albicans* was

then washed with Hank's balanced salt solution and was subjected to centrifugation for four times and the final cell pellet was again mixed sterile Hank's balanced salt solution and human serum ratio of 4:1. In the present experimentation, the concentration of cells used was $1x10^8$.

Evaluation of Phagocytosis

As per Ponkshe and Indap (2002), the estimation of the phagocytosis was performed. the finger prick method was employed to assess the phagocytosis, by placing a drop of blood sterile glass slide, which was preincubated at 37° C for 25 min. Sterile saline was used to isolate clot, care was taken not to wash away adhered neutrophils. The KSK extract was tested in 12.5, 25, 50, and 100 µg/ml concentrations and pooled serum was used a standard and were incubated at 37° C for 15 min. This step was followed by predetermined C. albicans suspension concentrations and was further incubated at 37° C for 60 min. After this slides were drained, fixed using methanol and were stained using Giemsa stain. The assessment of phagocytosed number of *C. albicans* cells by neutrophils was carried out microscopically. The number of *Candida* cells phagocytosed/engulfed by a neutrophil are Phagocytic index (PI) and the study was performed in triplicates. Immunostimulation was calculated in percentage using the following equation.

% of Simulation = PI (samples) - PI (control) / PI (control) x 100 ----------(1)

Where, the Immunostimulation % = PI (samples) - PI (control) / PI (control) x 100. Where, PI of samples: Phagocytic index of the test sample, PI of control: Phagocytic index without the test sample (i.e., normally by neutrophils).

Nitroblue Tetrazolium Assay

The test was performed as described as Mali et al. (2008) described with minor modification. Leucocyte suspension ($5x10^6$/ml) in phosphate buffer saline (PBS) was taken in all

Eppendorf tubes as per Dagur and McCoy (2015). 100 µl of PBS was added into first Eppendorf tube and was used as control, second Eppendorf tube was added with 100 µl of lipopolysaccharide (10 µg/ml) was used as standard and the remaining Eppendorf tubes were added with 100 µl of different concentration (12.5, 25, 50, and 100 µg/ml) of the Kabasura Kudineer extract. All these Eppendorf tubes were further added with 200 µl of 0.15% NBT solution and were incubated for 20 min at 37°C. After incubation the Eppendorf tubes were centrifuged for 3-4 min at 400 g and the supernatant was discarded. Further, the cells were treated with small volume of PBS solution and a thin film was made with the drop on the clean glass slide. The slides were then dried, fixed by heating, and were countered stained with carbol-fuchsin for 15s. The percentage of NBT positive cells with blue lumps or granules was determined by observing the stained slides for blue colour cells/lumps/granules under 40 X objective for 200 cells. All the experiments were carried out in triplicates and the results are expressed as mean ± SD.

% of NBT positive cells = observing blue color cells / 200 cells ×100 ------- (2)

***In vitro* thrombolytic activity of KSK**

Preparation of streptokinase (SK)

The lyophilized SK vial of 15,00,000 I.U was mixed properly with 5 ml phosphate buffered saline. This suspension was labelled stock from which dilutions were made to thrombolytic activity as per the in vitro model developed in our lab (Khan et al. 2011; Prasad et al. 2006).

Determination of thrombolytic activity

Three millilitres of venous blood were distributed in four different Eppendorf tube. The thrombolytic activity was performed by preincubating the Eppendorf tubes at 37 °C for 45

minutes. Subsequently, the clot formation was followed with removal of serum without disturbing clot. The clot weight was determined using the formula, Clot weight = Weight of clot filled tube - Weight of empty tube alone. To these tubes, with pre-weighted clot, 100 µl of KSK extract was added and for the standard, 100 µl of streptokinase and negative nonthrombolytic control - 100µl of distilled water were separately added to the control Eppendorf tubes. Incubation followed for 90 minutes at 37 °C and were observed for clot lysis. After which, the fluid was removed and the tubes were weighted to observe weight difference (Prasad et al. 2006). The difference obtained in weight taken before and after clot lysis was expressed as the percentage of clot lysis is shown below:

$$\% \text{ of clot lysis} = (\text{Weight of lysis clot/weight of clot before lysis}) \times 100$$

Statistical analysis

Tests were carried out in triplicate for three separate experiments. Results were expressed as graphically with mean± standard deviation values.

Results and Discussion

An immunomodulatory agent from the plant or animal kingdom increases the responsiveness of immune system of human body with activation of non-specific immune responses. Different plants have tested for their immunostimulant and immunosuppressive properties. In the support of this statement; many of the traditional medicine system concepts of preventive health care and the therapeutic potential have been tested and reviewed in detail (Rawat et al. 2018; Upadhyay 1997). The Ministry of AYUSH has issued guidelines for the Siddha practitioners for COVID-19 for different antiviral and immunity booster formulations which includes KSK, and NilaVembu Kudineer (AYUSH 2020). We have reported docking studies of bioactive compounds from KSK (Kiran et al. 2020), which confirmed that this extract

has a good binding efficiency with spike protein of SARS-CoV-2. Further, in this study we also explored the immunomodulatory and thromolytic activity of KSK.

The *in vitro* immunomodulatory activity of the KSK extract have illustrated in **Figure 1**. The percentage of killed *C. albicans* have found to be near to the control sample (serum). This graph substantiates the immunomodulatory property of KSK. Similar results have been observed in *Rhododendron arboreum* leaves (Rawat et al. 2018), *Euphobia hirta* (Ramesh and Padmavathi 2010), Similarly, many plant isolated compounds have been reported to immunomodulating nature. The vincristine as immunosuppressant have been employed for treating thrombotic thrombocytopenic purpura or chronic idiopathic thrombocytopenic purpura (Qweider et al. 2007). Also, this alkaloid compound has utilized for the treatment of many more diseases idiopathic thrombocytopenia purpura, bladder cancer, cervical cancer, non-small-cell lung cancer, autoimmune hemolytic anemia, neck cancer, and head cancer (Dhayalan et al. 2015; Qweider et al. 2007).

Nitroblue tetrazolium test is to assess the immunomodulatory activity of the test compound by determining its ability to stimulate the phagocytic activity in leucocytes. Once stimulated, the membrane permeable, water soluble, yellow-colored, nitroblue tetrazolium is reduced to blue NBT formazan crystals by the leucocytes. The KSK extract stimulated phagocytic activity of the leucocytes in a concentration dependent manner as seen by the increased percentage of NBT positive cells, results shown in **Figure 2**. The immunomodulatory effect with the aid of nitroblue assay have been observed in *Ficus glomerata* Roxb.(Heroor et al. 2013), *Nelumbo nucifera* Gaertn.(Mukherjee et al. 2010), *Pouteria cambodiana* (Manosroi et al. 2006). The result of the study indicates the functionality of the neutrophils in the process of phagocytosis is high creating a proactive environment from the infection.

The COVID-19 patients have shown thrombosis as one of the symptoms (Connors and Levy 2020; Panigada et al. 2020). And the formation of thrombus leads to progressive respiratory failure (Ackermann et al. 2020), and myocardial infarction, systemic arterial embolism in COVID-19 patients (Klok et al. 2020). The effective thrombolytic percentages with different concentration of the KSK extract, Control, 50 and 100 µg/ml and standard (SK) showed 22.36, 43, 71.83 and 83.75 %, respectively has been illustrated in **Figure 3**. From the **Figure 3**, it is evident that the percentage of the thrombolytic activity was 71.83 % at 100 µg/ml when compared to the 100 µl Streptokinase. From the different samples the 50µg/ml showed 43% thrombolytic activity, which is higher than the distilled water (negative control). The phytoconstituents of Siddha formulation KSK have been already reported by Kiran et al. (2020). These compounds have been detailed of their biological activities, some them were found to have thrombolytic, immunomodulatory, ant inflammatory, and fibrinolytic activity for example β-bisabolene (Akbar 2020; Aruna et al. 2014; Li et al. 2019; Sharma 2018), piperine (Meghwal and Goswami 2013; Rather and Bhagat 2018; Zheng et al. 2016), Squalene (Fox et al. 2011; Fox et al. 2012), Chebulagic acid (Shanmuganathan and Angayarkanni 2018), Carvacrol (Ezz-Eldin et al. 2020; Kianmehr et al. 2016), Luteolin (Maatouk et al. 2017; Wangchuk et al. 2018), Magnoflorine (Bala et al. 2015; Sharma et al. 2012; Xu et al. 2020).

*A*s per Siddha stickiness, mucilaginous, rounded, little hard, are listed as characters of *kabam*. A thrombus has all the qualities of increased *kabam*, majority of the drugs which are thrombolytic are pungent and bitter in taste. Kabasura Kudineer has already been screened for its anti-atherogenic property. The data from the results also suggests the thrombolytic potential of Kabasura Kudineer owing to its fire-based elements in the ingredients. We have reported

docking studies of bioactive compounds from Kabasura Kudineer (Kiran et al. 2020), which confirmed that this extract has a good binding efficiency with spike protein of SARS-CoV-2.

CONCLUSION

Siddha medicine is one of best way to control the COVID-19. The immunomodulatory and anti-thrombolytic are the stepping stones towards the development of a stable, safe and working cure for COVID-19. Kabasura kudineer is a polyherbal decoction with fifteen different components, and each of them are in themselves strongly established herbal plants, whose synergistic activity might probably improve human immune response and lead the human body to healthiness. The immunomodulatory property and thrombolytic activity of this miracle Siddha medicines has been studied using *in vitro* experiments but still requires *in vivo* animal model experiments for better understanding. This research paper has clearly indicated and has supported the notion of using the KSK extract for improving the immune response in this COVID-19 infected time.

Acknowledgements

The authors thanks to management of all the institutes for their support to carry out this research and thanks to Central Council for Research in Siddha, Ministry of AYUSH, Chennai, Tamilnadu, India and Salem Microbes Private Limited, Salem, Tamilnadu, India.

Conflicts of Interest

None

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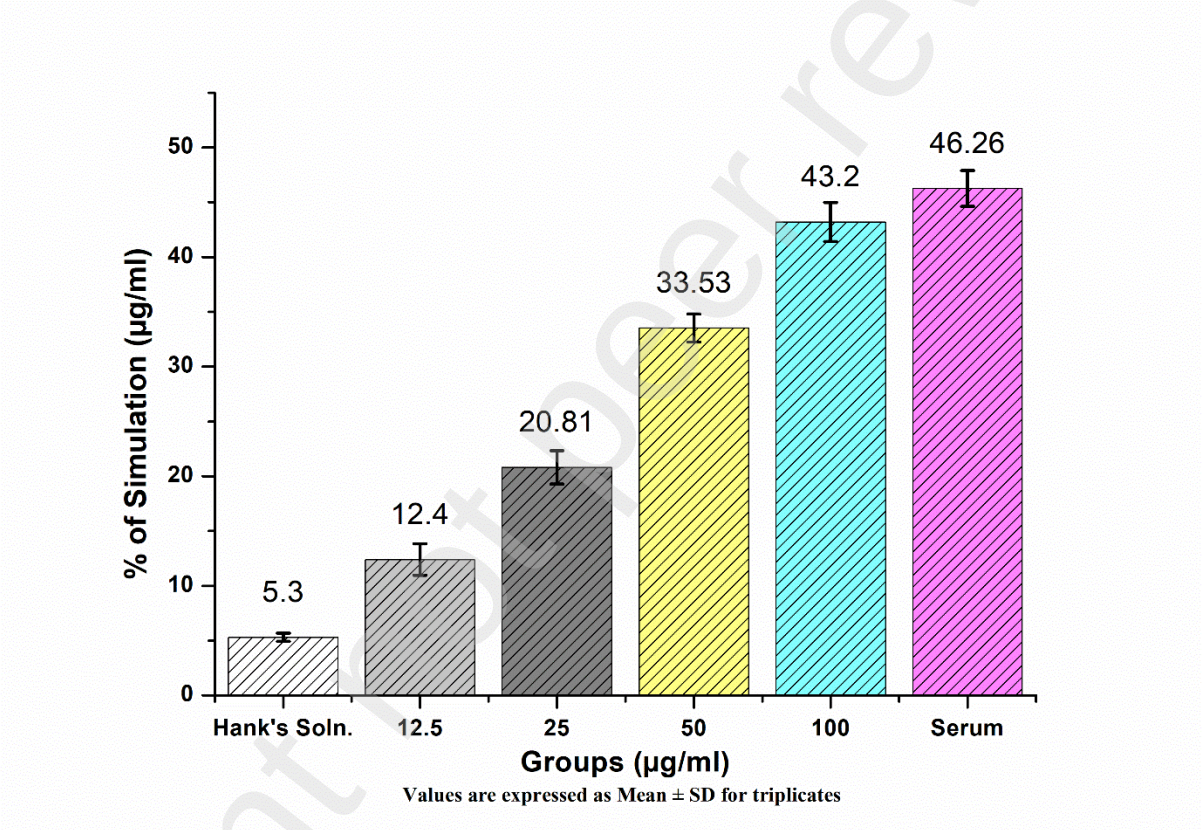

Figure 1. Percentage of killed *Candida albicans* after treatment with KSK extract by Phagocytosis stimulation.

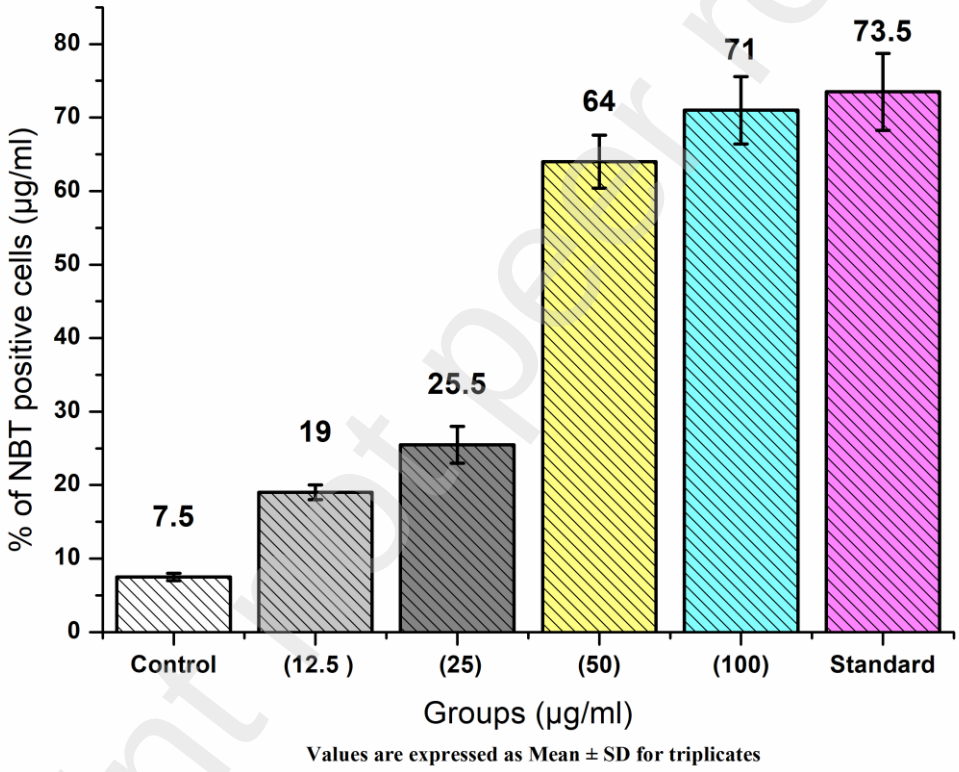

Figure 2. Percentage of NBT positive cells after treatment with KSK extract by Nitroblue Tetrazolium Test (NBT)

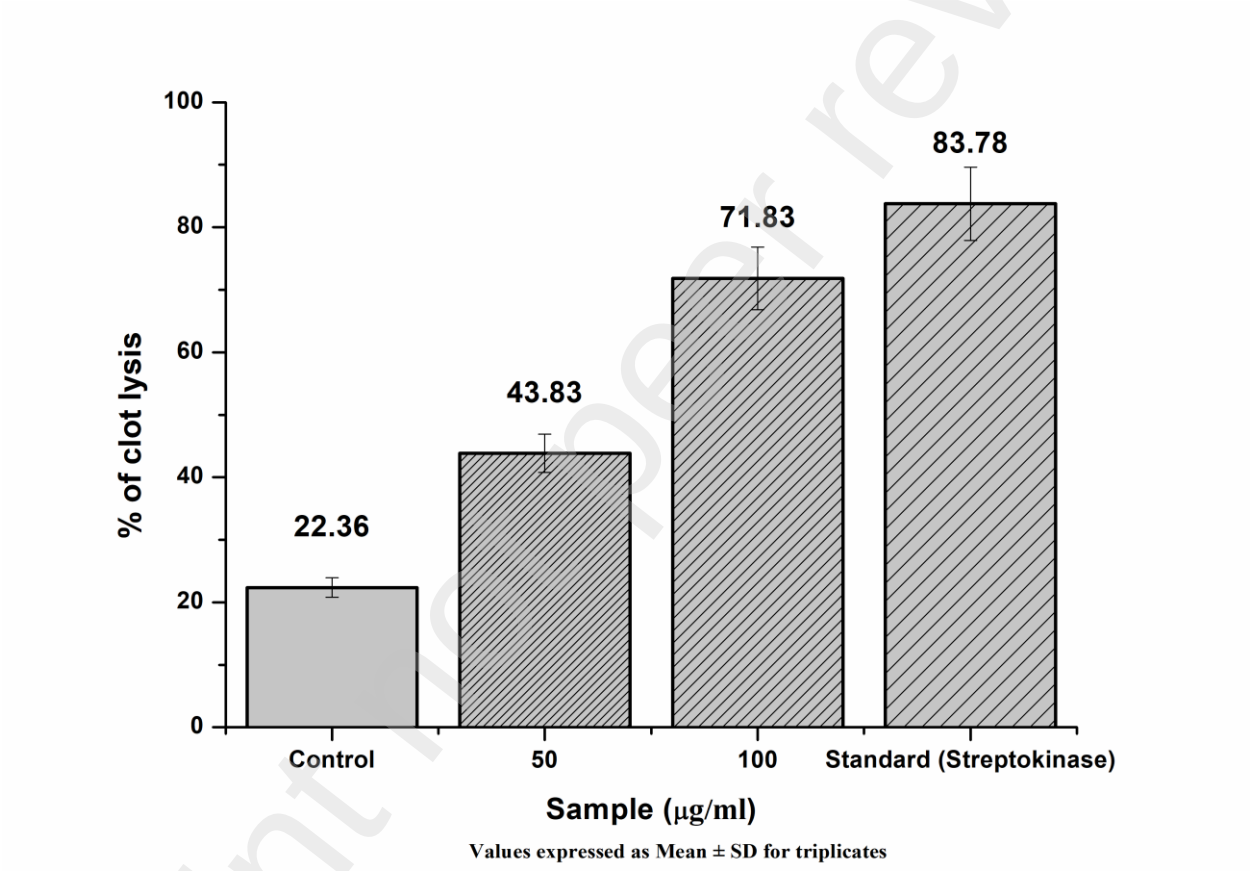

Figure 3. Thrombolytic activity (in terms of % clot lysis) of KSK extract

S.No.	Ingredients
1.	*Zingiber officinale Rosc*
2.	*Piper longum L*
3.	*Syzygium aromaticum*
4.	*Tragia involucrata L*
5.	*Anacyclus pyrethrum*
6.	*Andrographis paniculata*
7.	*Hygrophilla auriculata (Schum.)Heine*
8.	*Terminalia chebula Retz.*
9.	*Justicia adhatoda L.*
10.	*Plectranthus amboinicus (Lour) Spreng*
11.	*Costus speciosus*
12.	*Tinospora cordifolia (Willd.) Miers ex Hook.f&Thoms*
13.	*Clerodendrum serratum L.*
14.	*Sida acuta Burm. f.*
15.	*Cypreus rotundus L.*

Table.1: Kabasura Kudineer ingredients

ISSN 0975-2366
DOI: https://doi.org/10.31838/ijpr/2021.13.02.399

Research Article

Antioxidative potential of Kabasura Kudineer (KSK), an official Siddha polyherbal formulation.

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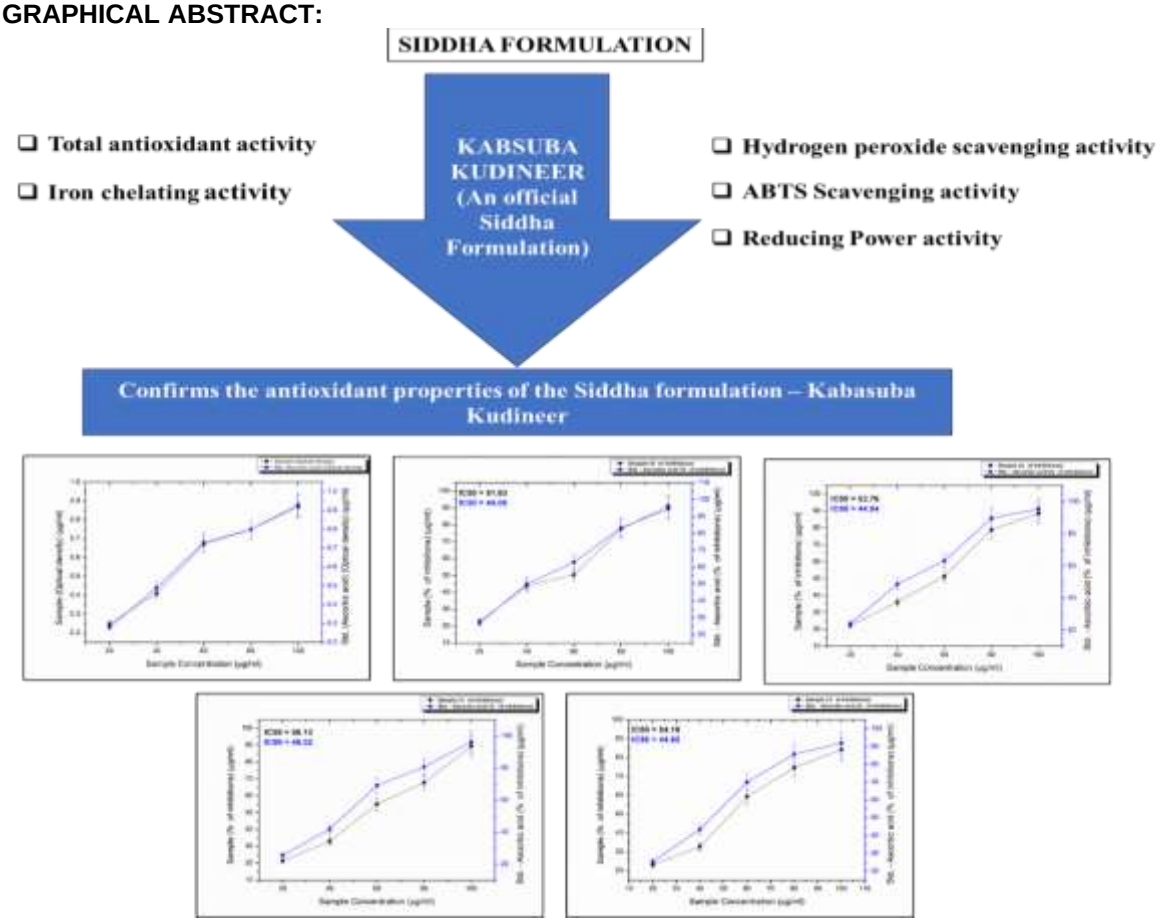
Received: 11.01.21, Revised: 10.02.21, Accepted: 19.03.21

ABSTRACT
Siddha'sfolkmedicinesystemis usedtoenhanceimmuneresponsesaspreventive medicine.Siddhamedicine'senormousplethoraofdatawillenhancehumanwellbeing. Sincethe oldyears,KabasuraKudineer(KSK)has beenanimmunedrugtoseveralillnesses. Aqueous extract was employed for preparing the extract and analyzed for Total Antioxidant Capacity, Fe^{2+} chelating activity assay, Hydrogen peroxide scavenging activity, ABTS Scavenging assay and Reducing power assay. Thiswas found to possess high antioxidant properties of aqueous extract of KSK and their derivatives have been confirmed with the different tests. This experimentation further affirms the antioxidative nature of KSK extract, which further aid in the fight in this COVID-19 pandemic situation.

Keywords: Kabasura Kudineer (KSK), Siddha formulation, antioxidant assay, DPPH assay.

GRAPHICAL ABSTRACT:



INTRODUCTION

Some of Siddha formulations possess phytochemicals such as flavonoids and vitamins that exhibit significant amounts of antioxidant activity and scavenge the excess free radicals from the human body[1,2]. Siddha system of medicine contributes profoundly to the wellness, curative, and preventive aspects of the diseased conditions. Many of today's diseases are due to the "oxidative stress" that results from an imbalance between the formation and neutralization of free radicals [3]. Antioxidants prevent the reactive oxygen species (ROS) from being formed or removedby complex forming mechanisms before it damages the cell's vital components [4]. Oxidative stress is initiated by free radicals, which seek stability through electron pairing with biological macromolecules such as proteins, lipids, and deoxyribonucleic acid in healthy human cells and cause protein deoxyribonucleic acid damage along with lipid peroxidation. These changes contribute to cancer formation, atherosclerosis, cardiovascular diseases, many other inflammatory diseases, and ageing [5,6].Sometimes, various pathological processes disrupt these protective mechanisms. Hence, antioxidant supplements are vital to combat oxidative damage—attention directed towardsdeveloping"Ethno-medicines" that possess potent antioxidant properties and beneficially less toxicity comparatively to chemical molecules. Kayakarpam is a unique branch of Siddha Medicine, and the drugs mentioned in this have been described to both cure disease and promote health by physically and mentally. [7].In general, Kayakarpam drugs promote healthy longevity, memory and intellect, preserve youthfulness, the lustre of skin, and clarity of voice, and strengthen all body organs.ThirumoolarThirumanthiramquoted the theory of “Tissue damage and prevention of the same for longevity and preventing mortality”. Theyemphasizedthat only through the preservation of body life could attain ‘Eternal bliss’. Practices of Kayakarpam are recommended by him in the 12th century, which has two significant divisions Karpaaviztham and Karpayogam.

Karpaavizhthamcomprises food-based practices thatmust be taken in a regulated pattern, inKalpayogamspecific breathing exercises and Yogasanas are advocated. These theories were introduced late in the earth in the name of antioxidant theory in 1950. Oxidants are free radicals circulating in the body, causing considerable damage to tissues and antioxidants to scavenge these free radicals. Plants are composed of thousands of chemical compounds that act as antioxidative components[8-12], anti-inflammatory[8,13-15], antiparasitic [16-18]and many other plant activities. The KSK is one of the ommonly used medicinein Siddha system of Medicine. Countless compounds are yet characterized and used for the betterment of the humanpopulace. Other critical biological activities also exist in the natural compounds, which are responsible for antioxidant activity and therefore, the use of natural antioxidants is continuously promoted by the scientific community as well as by the general desire that healthy and natural foods make the industry an alternative to the exploitation of synthetical additives by natural antioxidants.

Antioxidant and antioxidative stress properties of the plants acting as components of KSK are very well known in the scientific world in which the plants like Zingiber officinale [19-23], Piper longum [24]; Syzygiumaromaticum[25-27]; Anacyclus pyrethrum [28]; Tragiainvolucrata[29-30]; Solanum anguivi[31], Terminalia chebula [32 – 34]; Justicia adathoda[32-34]; Costusspeciosus[35]; Tinospora cordifolia [36-38]; Clerodendrumserratum[39]; Andrographis paniculate [40-41]; Cyperus rotundus[42 – 45]; Sida acuta [46]. The composition of KSK has been detailed already in the earlier reports; thus, making it an ample concoction for antioxidant 'super-product'. Therefore, the affirmation of the notion of utilizing KSK as an antioxidant material.The Siddha medicinal system has a lot of such that can support the fight against diseases like cancers, and preventive aspects of viral diseases like the common cold, and COVID-19.

MATERIALS AND METHODS

Kabasura Kudineer Chooranam plant concoction extract with fifteen ingredients **Table 1**. The raw drugs were procured from Tamil Nadu Medicinal Plant Farms and Herbal Medicine Corporation Limited (TAMPCOL) Chennai, Tamilnadu.

Table1: KabasuraKudineer ingredients

S.No.	Ingredients
1.	Zingiber officinale Rosc
2.	Piper longum L
3.	Syzygiumaromaticum
4.	Tragiainvolucrata L

5.	Anacyclus pyrethrum
6.	Andrographis paniculata
7.	Hygrophilla auriculata (Schum.)Heine
8.	Terminalia chebula Retz.
9.	Justicia adhatoda L.
10.	Plectranthusamboinicus (Lour) Spreng
11.	Costusspeciosus
12.	Tinospora cordifolia (Willd.) Miers ex Hook.f&Thoms
13.	Clerodendrumserratum L.
14.	Sida acuta Burm. f.
15.	Cypreusrotundus L.

Extraction of the KSK and sample preparation

The dried KSK powder was weighted and was packed in a Soxhlet apparatus and refluxed with distilled water. The extracts were pooled, filtered, dried, and stored below 5 °c till further use. Doses such as 12.5, 25, 50, and 100 μg/mL prepared in the isotonic solution for antioxidant activity assays.

In vitro Antioxidant Activity

Preparation of extract

The extract was prepared as stated earlier and dried with a water bath. The test dose was 20, 40, 60, 80, and 100μg/mL.

Total Antioxidant Capacity

The total antioxidant capacity of KSK extract was evaluated by the phosphomolybdenumbased method [47]. 300 μL extract was added to 3 mL of reagent solution, comprised of 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The mixtures were placed in test tubes and incubated at 95◦c for 90 min. The UV absorbance was measured at 695 nm using UV-visible spectrophotometer against blank after cooling room temperature. The total antioxidant capacity was expressed as the number of equivalents of the ascorbic acid. The scavenging activity was calculated according to the following equation:

$$\%\ ofInhibition = \frac{(A_0 - A_1)}{A_1} \times 100$$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the KSK aqueous extract.

Fe^{2+} chelating activity assay

The chelating activity of the extracts for ferrous ions Fe^{2+} was carried as per [48]. 0.5 mL of KSK aqueous extract (20 μg/ml, 40 μg/ml, 60 μg/ml,80 μg/ml, 100 μg/ml), 1.6 mL of deionized water and 0.05 mL of $FeCl_2$ 2 mM was added together. After about 30 s, to the mixture 0.1 mL ferrozine (5mM) was mixed. After 10 min at room temperature, the absorbance of the Fe^{2+}-Ferrozine complex was measured at 562 nm. Ferrozine reacted with the divalent iron to form stable magenta complex species that were very soluble in water. The chelating activity of the extract for Fe^{2+} was calculated as:

$$Chealatingrate\ (\%) = \frac{(A_0 - A_1)}{A_1} \times 100$$

A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the extract's presence.

Hydrogen peroxide scavenging activity: Hydrogen peroxide scavenging activity of the extract as estimated [49]. To 1mL aliquots of 0.1 mM H_2O_2, 1 mL of different concentrations of the KSK were added, and 2 drops of 3% ammonium molybdate, 10 mL of 2M H_2SO_4 and 7.0 mL of 1.8M potassium iodide. The concoction was titrated with 5.09 mM NaS_2O_3 till the yellow color is disappeared. The percentage scavenging of hydrogen peroxide was calculated with

$$\%\ ofInhibition = \frac{(V_0 - V_1)}{V_1} \times 100$$

V_0 was the volume of NaS_2O_3 solution used to titrate the control sample in the presence of hydrogen peroxide (without extract), V1 was the volume of NaS_2O_3 solution used in the presence of the extract.

ABTS Scavenging assay

KSK extractantioxidant effect was studied using ABTS (2,2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) radical cation decolorization assay according to the method of [50]. 7 mM ABTS solution was added to 2.45 mM potassium persulphate resulted in the production of the ABTS radicals. The solution was placed at room temperature in dark for 12-16 h to form dark colored solution. The radicals were diluted for an initial absorbance of 0.700 (±0.02)at wavelength of 734 nm. Different aliquots of varying concentrations of extract were added to 1mL of ABTS solution. The UV absorbance was measured at 734 nm with L-Ascorbic acid was used as the standard in triplicates. The percentage inhibition was calculated with the formula:

$$\%\ ofInhibition = \frac{(Control - Test)}{Control} \times 100$$

Reducing power assay: The Fe^{3+} reducing power of the KSK extract was determined by method of Oyaizu[51]. The extract (0.75 mL) at various concentrations (20 µg/ml, 40 µg/ml, 60 µg/ml,80 µg/ml, 100 µg/ml),was mixed with 0.75 mL of phosphate buffer (0.2M, pH 6.6) and 0.75 mL potassium hexacyanoferrate (1%, w/v), followed by incubating at 50 °C in a water bath for 20 min. The reaction was stopped with 0.75 mL of trichloroacetic acid solution (10%) and then centrifuged at 3000 rpm for 10 min. 1.5 mL of the supernatant was mixed with 1.5 mL of distilled water and 0.1 mL of ferric chloride solution (0.1%, w/v) for 10 min. The absorbance at 700 nm was measured as the reducing power. Higher absorbance of the reaction mixture indicated increased reducing power.

Statistical analysis: Tests were carried out in triplicate for 3 separate experiments. The amount of extract needed to inhibit free radicals' concentration by 50%, IC_{50}, was graphically determined by a linear regression method using Graphpad software. Results were expressed as graphically/mean±standard deviation.

RESULTS AND DISCUSSION

An imbalance in the production of the reactive oxygen species and the human body's antioxidant defense modules is termed as the state of oxidative stress. The state is a widely accepted feature of many diseases from bacterial, cancer and fungal diseases [52]. In **Figure 1**, the total antioxidant assay of the KSK extract is shown with standard control ascorbic acid. The assay is carried out with 20, 40, 60, 80, and 100 µg/mL dose concentrations with the values of 21.25±0.85, 33.12±2.31, 55.00±3.85, 67.81±4.74, and 89.37±6.25, respectively.

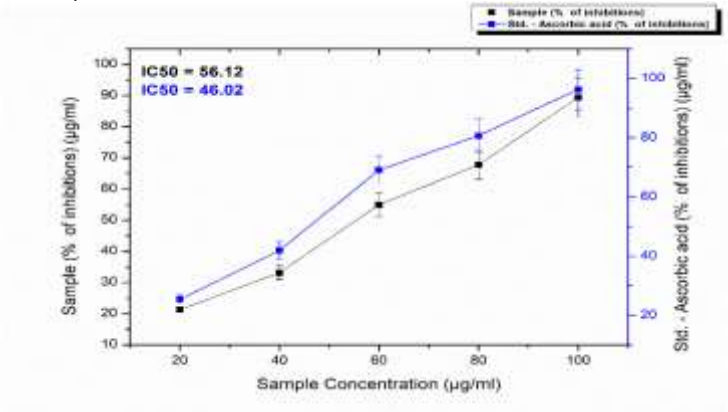


Fig.1: Total antioxidant activity of KSK extract and standard as ascorbic acid at different concentrations. Inlet is showing the IC50 value.

The transition metal ion, Fe^{2+}, possesses the ability to move single electrons by allowing the formation and propagation of many radical reactions, even starting with relatively non-reactive radicals [53]. In **Figure2**, the Iron chelating activity of the KSK extract was illustrated. The experiment is carried out in a dose concentration of 20, 40, 60,80 and 100 µg/mL, showing their corresponding iron-chelating activity as 23.07±1.61, 32.69±2.28, 59.23±4.14, 74.61±5.22, and 84.23±5.89, respectively. Therefore, the iron chelation is dose-dependent compare to the control.

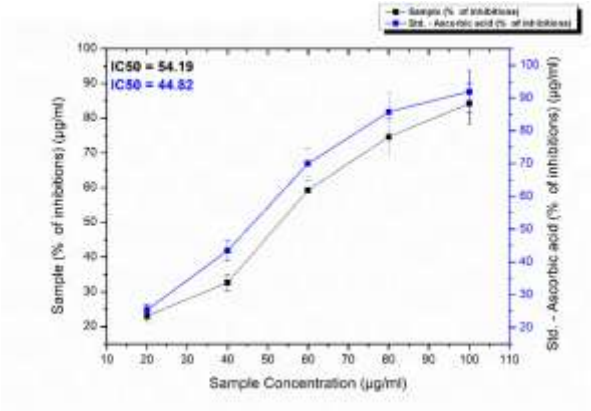


Fig.2:Iron chelating activity of KSK extract and standard as ascorbic acid at different concentrations. Inlet is showing the IC50 value.

In **Figure 3**. Hydrogen peroxide scavenging activity of KSK extract has been shown. The experiment is carried out in dose concentration of 20, 40, 60,80 and 100 μg/mL, showing their corresponding iron-chelating activity as 22.36±1.56, 36.18±2.53, 51.31±3.59, 78.94±5.52, and 88.48±6.19, respectively.The weak oxidizing agent, hydrogen peroxide is responsible for the inactivation of the enzymes containing thiol (-SH). It can pass through the cell membranes of cells, resulting in hydroxyl radicals' formation by reacting with Fe^{2+} and Cu^{2+} ions [54-55].

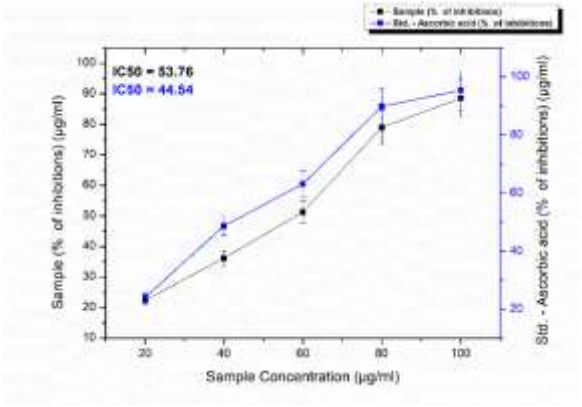


Fig.3:Hydrogen peroxide scavenging activity of KSK extract and standard as ascorbic acid at different concentrations. Inlet is showing the IC50 value.

The 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid), also known as the ABTS scavenging assay, is most commonly used to measure different phenolic compounds. The assay comprises a radical, is chemically produced and used to identify the screening complex [56], as depicted in **Figure 4**. ABTS Scavenging activity for the KSK extract has been shown. The experiment is carried out in dose concentration of 20, 40, 60, 80 and 100 μg/mL, showing their corresponding iron-chelating activity as 22.67±1.58, 43.78±3.06, 50.45±3.53, 77.56±5.42, and 91.34±6.39, respectively.The results imply the presence of antioxidant properties of the KSK extract compared to the standard.

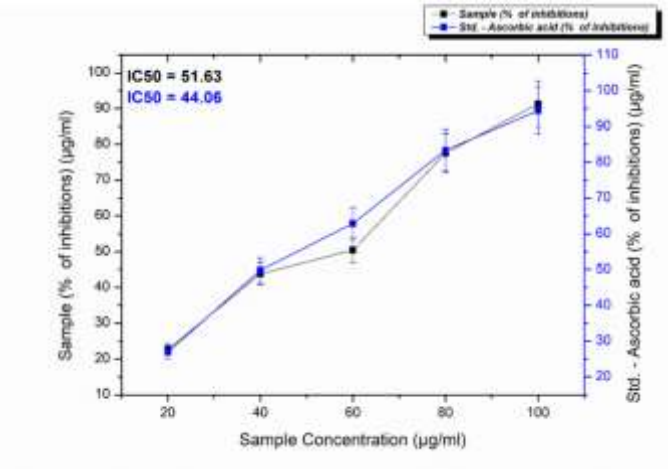


Fig.4:ABTS Scavenging activity of KSK extract and standard as ascorbic acid. Inlet is showing the IC50 value.

Reducing power assay

The reducing capacity of a compound may be a significant indicator of its potential antioxidant activity [57]. A compound's capacity to reduce is found to be due to reductones [58], thereby showing the antioxidant potential. The test sample's reductants are attributed to the reduction of Fe3+/ferricyanide complex to the ferrous form [59]. In **Figure 5**, the reducing power assay is illustrated. The experiment is carried out in dose concentration of 20, 40, 60, 80 and 100 μg/mL, showing their corresponding reducing power activity as 0.25±0.01, 0.41±0.02, 0.67±0.04, 0.75±0.05, and 0.87±0.06, respectively.

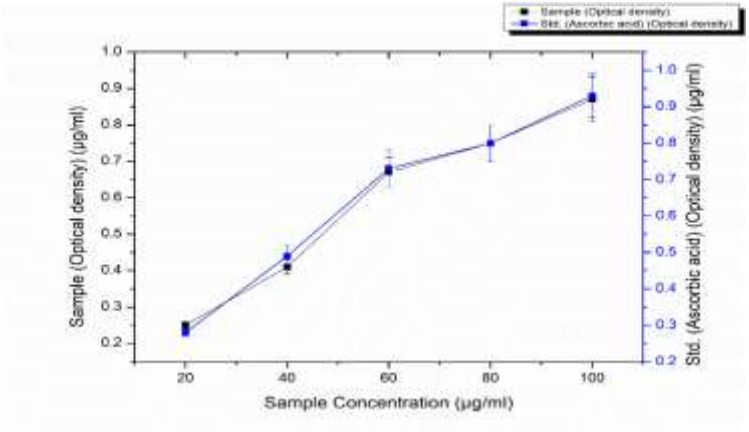


Fig.5: Reducing Power activity of sample and standard as ascorbic acid acid at different concentrations.

Free radicals in increased quantity might result in oxidative stress, leading to different kinds of biochemical and physiological lesions and metabolic impairment, even cell lysis [60]. The decreased absorbance value of the KSK indicates the reducing capacity of the KSK extract, thereby confirming the antioxidant assay. Rajalakshmi et al., [61] reported a total phenolic compound of KSK 90mgGAE/100g and IC-50: 9.29mg/L and shows no toxicity for this formulation.

CONCLUSION

The results convey that the drug KSK possesses antioxidant activity due to its ingredients' high phenolic compounds. It answers reducing power assay. These in vitro assays indicate that this combination of the polyherbal extract is a significant source of natural antioxidant, which might help prevent the progress of numerous oxidative stresses. Also, antioxidant supplementation can significantly improve specific immune responses from the immunosuppressive effects of environmental factors. Thus, antioxidant vitamins enhance immune responses that are involved in protection from infection and malignancies. The data strongly suggest that the intake needed to enhance immune responses may be many times greater than the currently recommended allowances.However, the components responsible for the antioxidant activity are currently unclear. Therefore, investigation is needed to isolate and identify the antioxidant compounds present in extract of KSK, which could be attributed to as one of the mechanisms for its activity as a potent medicine.

Source of Funding:Nil
Conflict of Interest: Nil

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Original Article

Evaluation of *in-vitro* Immunomodulatory Activity and Thrombolytic Potential of Kabasura Kudineer (KSK): An Official Siddha Polyherbal Formulation

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ABSTRACT

Aim: Coronavirus disease 2019 (COVID-19) caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), attacking mainly on the immune system of a body. This has spread mortality and morbidity all over the world. During this dreadful situation there is an urgent need for the development and rapid dissemination of COVID-19 treatment. Siddha's traditional medicine system can be used as preventive care to boost the immune system. **Materials and Methods:** The immense treasure of knowledge found in Siddha medicine can help mortality. Kabasura Kudineer (KSK) is one of the Siddha poly herbal formulation used as an immune-boosting agent against several diseases. **Results:** In the present study the KSK has been investigated for its effects of immunomodulatory and thrombolytic potential. The KSK at the concentrations of 12.5, 25, 50, and 100 μg/ml showed % immune-stimulations of 12.40 %, 20.81, 33.53, and 43.20 and for NBT showed 19.00, 25.50, 64.00, 71.00 % respectively. Moreover, similarly, the thrombolytic activity showed 50, and 100 μg/ml concentration showed 43.83 %, 71.83 % clot lysis, respectively, and the control value for the Streptokinase showed 83.78 %. **Conclusion:** Hence, it can be confirmed that KSK has immunomodulatory and thrombolytic properties *in vitro* models. Immunomodulatory and anti-thrombolytic are the steps to create a stable, safe, and efficient COVID-19 cure.

Key words: Kabasura Kudineer (KSK), Immunomodulatory, Thrombolytic, COVID-19, Siddha formulation.

INTRODUCTION

As a rule, current human healthcare services are significantly tested and challenged by the SARS-CoV-2 with its indisputable complex biochemical architecture. Its present momentum is very much persistent, making a predictable second wave.[1] The human respiratory system is very much vulnerable to different viral infections starting from coronavirus, rhinovirus, human metapneumovirus, and the human immune system is significantly affected during COVID-19 progression in the infected host.[2] Skowronski, Astell[3] had reported respiratory disease result in a cytokine-chemokine reaction resulting in severe damage to the host.

The role of immunology was the most rapidly developing scientific area and showed an evolving opportunity in the treatment and prevention of disorders, inflammatory reactions of different parts of the human body. Similarly, the infections are considered immunological diseases, whereas the neoplastic and autoimmune diseases occur in immunosuppressed state.[4] It is reported

Submission Date: 02-01-2021;
Revision Date: 03-03-2021;
Accepted Date: 02-06-2021

DOI: 10.5530/ijper.55.3.150

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that many of the synthetic, semi-synthetic and natural therapeutic agents have a suppressive and cytotoxic nature which support the immune system.[1,5-7]

In today's health wellness commerce, the role of immunomodulators is well-established as a critical component. These immunomodulators are grouped into three main classes: immunosuppressants, immunostimulants, and immunoadjuvants, and their applications in medicine and pharma industries for stimulation and suppression of the immune system. Also, these are used as both prodrugs and prophylactic drugs for the healthy populace.[8,9] Also, the plant kingdom's immunomodulators seem to be a good substitute for the synthetic chemical compounds.[10]

The World Health Organization has put a public health emergency by putting COVID-19 as a transnational threat.[11,12] There is no medicine or prophylactic treatment for this disease and has been constrained towards palliative help to the affected people. Hence, there is a dire need to produce a safe and stable COVID-19 immunisation.

The current trending strategies for the COVID-19 treatment plan have been focused on immunisation against the virus and head-on attack on the virus particle. This makes the host a vital factor in ailment's subtleties. Siddha medicine is always aimed towards a healthy routine rather than just an issue of medicine.

Immunity is termed *Vanmai* in Siddha, and it has a direct association with *Uyir thathukkal* (Vali, Azhal and Aiyam) and Seven *Udal thathukkal* (Body tissues). Natural immunity of the human body by birth is called *Iyarkai Vanmai,* its improvement with the help of intake of balanced food and medicines is called *Seyarkai Vanmai* and *Kala vanmai*, which is further defined as the change of physical state under the effects of seasons and in their affected state there might be possibilities of disease.[13-15] Human beings are the subtotal of *Uyir thathukkal* and *Udalthathukkal* forming his/her physical solid and mind results in a robust immune system. Individuals with the *Vali* trait have lesser immunity, while persons of *Aiyam* have moderate immunity, and persons are having *Aiyam* have stronger immunity compared to each other. The Siddha medicinal system has thoroughly tested the herbs and the polyherbal formulations via *in vitro* and *in vivo,* including the Urai mathirai, Saya chooranam, Nilavembu kudineer, which are very much beneficial.[16-18] The botanicals used in Kayakalpa are effective in immunomodulation and restoration of immune homeostasis.[19] Most of the Siddha medicines are found to be present in the mixture or consists of more than plant or their extracts. Some of the recent literatures have stated the importance of synergistic effects of the traditional medicines and plant extracts. Yang, Zhang[20] and his co-workers have detailed about the Chinese herbs and their synergistic effects on different biological pathways. Similarly, the potential synergistic effects of plant based biomolecules have been well studied on SARS-CoV-2 by Prasad, Muthamilarasan.[21] The synergistic combination of molecules interacts with target-disease networks which provide novel, mechanistic insights towards understanding their therapeutic potentials.[22] The docking studies carried out by us for better understanding KSK extract revealed a pathway to understanding the Siddha in a scientific manner.[23]

The COVID-19 infection cycle has two distinct phases in which the first protective phase of the adaptive immune response in a host might eliminate the virus.[24] In the current situation, hydroxychloroquine is considered a candidate for COVID-19 treatment due to its Immunomodulatory and antiviral effects.[25,26] The COVID-19 leads to blood clots in people with a severe form of the COVID-19 disease. Blood clots cause a severe problem in the blood circulatory system. Blood clots in the form of thrombus hamper blood flow in blood vessels, reducing the oxygen intake to the tissues. The fibrinolytic drug dissolves the clot trapped in coronary vessels, restoring the heart's blood, limiting the necrosis.[27] The tissue plasminogen activator, urokinase and Streptokinase are drugs prescribed as thrombolytic agent nowadays by physicians. The Indian population has been prescribed Streptokinase and urokinase due to their low cost,[25,26,] and other drugs with the hyper risk of haemorrhage.[28,29]

The COVID-19 is a trending research topic that is being researched again in every developed country. The research papers are being published in almost every branching scientific field from biotechnology, bioinformatics, physics, chemistry and many others. The traditional medicines are also involved in this research racetrack to curb the pandemic COVID-19. Siddha Medicine is a treasured healing desire that is classically used for treating viral pulmonary infections; this precept of drugs is confirmed to incorporate antiviral compounds. The Siddha medicine is prescribing KSK for the treatment of fever and as prophylactic antiviral agents.[30] At present, the Ministry of AYUSH's guidelines, Government of India, KSK, is given for boosting immunity among the ordinary people[30] but not limited to prophylaxis and so that we can take to the integrative model of therapeutics. For selecting Siddha Medicine's safety, Efficacy and availability have to be addressed. However, the immunomodulatory activity and thrombolytic activity of Kabasura Kudineer has not been reported or scientifically investigated. Therefore,

the present study focused on investigate the immunomodulatory and thrombolytic potential of KSK.

MATERIALS AND METHODS

Kabasura Kudineer Chooranam is a compound formulation consisting of fifteen ingredients which are given in Table 1. Kabasura Kudineer Chooranam was purchased from Tamil Nadu Medicinal Plant Farms and Herbal Medicine Corporation Limited (TAMPCOL). All the chemicals and solvents are of analytical grade, obtained and used in the same condition. The *Candida albicans* suspension (MTCC-183) was purchased from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India.

Extraction of the KSK and sample preparation

The dried KSK powder was weighted and was packed in Soxhlet apparatus and refluxed with distilled water. The extracts were pooled, filtered, dried, and stored below 5°C till further use. Doses such as 12.5, 25, 50, and 100 µg/ml were prepared in the isotonic solution for *in vitro* immunomodulatory activity.

In vitro immunomodulatory activity by Phagocytosis of *Candida albicans* assay

Phagocytosis of *Candida albicans* test was carried out according to method.[31-33] The Sabouraud's dextrose broth was inoculated with *C. albicans* (MTCC-183) and was incubated overnight. The *C. albicans* was then washed with Hank's balanced salt solution and was subjected to centrifugation four times, and the final cell pellet was again mixed sterile Hank's balanced salt solution and human serum ratio of 4:1. In the present experimentation, the concentration of cells used was $1x10^8$.

Table 1: Kabasura Kudineer ingredients.

S.No.	Ingredients
1	*Zingiber officinale Rosc*
2	*Piper longum L*
3	*Syzygium aromaticum*
4	*Tragia involucrata L*
5	*Anacyclus pyrethrum*
6	*Andrographis paniculata*
7	*Hygrophilla auriculata (Schum.)Heine*
8	*Terminalia chebula Retz.*
9	*Justicia adhatoda L.*
10	*Plectranthus amboinicus (Lour) Spreng*
11	*Costus speciosus*
12	*Tinospora cordifolia (Willd.) Miers ex Hook.f&Thoms*
13	*Clerodendrum serratum L.*
14	*Sida acuta Burm. f.*
15	*Cypreus rotundus L.*

Evaluation of Phagocytosis

As per Ponkshe and Indap,[31] the estimation of the phagocytosis was performed. The finger prick method was employed to assess the phagocytosis by placing a drop of blood sterile glass slide preincubated at 37°C for 25 min. Sterile saline was used to isolate the clot; care was taken not to wash away adhered neutrophils. The KSK extract was tested in 12.5, 25, 50, and 100 µg/ml concentrations and pooled serum was used a standard and were incubated at 37°C for 15 min. This step was followed by predetermined *C. albicans* suspension concentrations and was further incubated at 37°C for 60 min. After this, slides were drained, fixed using methanol and were stained using Giemsa stain. The assessment of the phagocytosed number of *C. albicans* cells by neutrophils was carried out microscopically. The number of *Candida* cells phagocytosed/engulfed by a neutrophil is Phagocytic index (P.I.), and the study was performed in triplicates. Immunostimulation was calculated in percentage using the following equation.

$$\% \text{ of Simulation} = \frac{\text{PI (samples)} - \text{PI (control)}}{\text{PI (control)}} \times 100 \quad (1)$$

Where, the Immunostimulation % = PI (samples) - PI (control) / PI (control) × 100. Were, P.I. of samples: Phagocytic index of the test sample, P.I. of control: Phagocytic index without the test sample (i.e., normally by neutrophils).

Nitroblue Tetrazolium Assay

The test was performed as described as Mali, Hatapakki[34] described with minor modification. Leucocyte suspension (5×10^6/ml) in phosphate buffer saline (PBS) was taken in all Eppendorf tubes as per Dagur and McCoy.[35] 100 µl of PBS was added into first Eppendorf tube and was used as control, second Eppendorf tube was added with 100 µl of lipopolysaccharide (10 µg/ml) was used as standard, and the remaining Eppendorf tubes were added with 100 µl of different concentration (12.5, 25, 50, and 100 µg/ml) of the Kabasura Kudineer extract. All these Eppendorf tubes were further added with 200 µl of 0.15% NBT solution and were incubated for 20 min at 37°C. After incubation, the Eppendorf tubes were centrifuged for 3-4 min at 400 g, and the supernatant was discarded. Further, the cells were treated with a small PBS solution, and a thin film was made with the drop on the clean glass slide. The slides were then dried, fixed by heating, and were countered stained with carbol-fuchsin for 15s. The percentage of NBT

positive cells with blue lumps or granules was determined by observing the stained slides for blue colour cells/ lumps/granules under 40 X objective for 200 cells. All the experiments were carried out in triplicates, and the results are expressed as mean ± S.D.

$$\% \text{ of NBT positive cells} = \frac{\text{observing blue color cells}}{200 \text{ cells}} \times 100 \quad (2)$$

In vitro thrombolytic activity of KSK

Preparation of Streptokinase (S.K.)

The lyophilised S.K. vial of 15,00,000 I.U was correctly mixed with 5 ml phosphate-buffered saline. This suspension was labelled stock from which dilutions were made to thrombolytic activity as per the *in vitro* model developed in our lab.[36,37]

Determination of thrombolytic activity

Three millilitres of venous blood were distributed in four different Eppendorf tube. The thrombolytic activity was performed by preincubating the Eppendorf tubes at 37°C for 45 mins. Subsequently, the clot formation was followed with the removal of serum without disturbing the clot. The clot weight was determined using the formula; Clot weight = weight of clot filled tube - Weight of empty tube alone. With pre-weighted clot, 100 µl of KSK extract was added to these tubes, and for the standard, 100 µl of Streptokinase and negative nonthrombolytic control - 100µl of distilled water were separately added to the control Eppendorf tubes. Incubation followed for 90 min at 3°C and was observed for clot lysis. After which, the fluid was removed, and the tubes were weighted to observe a weight difference of.[37] The difference obtained in weight taken before and after clot lysis was expressed as the percentage of clot lysis is shown below:

$$\% \text{ of clot lysis} = \left(\frac{\text{Weight of lysis clot}}{\text{Weight of clot before lysis}} \right) \times 100 \quad (3)$$

Statistical analysis

Tests were carried out in triplicate for three separate experiments. Results were expressed as Mean ± standard deviation. $P<0.05$ was considered significant and was expressed graphically.

RESULTS AND DISCUSSION

An immunomodulatory agent from the plant or animal kingdom increases the human body's immune system with the activation of non-specific immune responses. Different plants have tested for their immunostimulant and immunosuppressive properties. In support of this statement, many traditional medicine system concepts of preventive health care and the therapeutic potential have been tested and reviewed in detail.[32,38] The Ministry of AYUSH has issued guidelines for the Siddha practitioners for COVID-19 for different antiviral and immunity booster formulations, including KSK and Nilavembu Kudineer. We have reported docking studies of bioactive compounds from KSK,[23] which confirmed that this extract has excellent binding efficiency with spike protein of SARS-CoV-2. Further, in this study, we also explored the immunomodulatory and thrombolytic activity of KSK.

The *in vitro* immunomodulatory activity of the KSK extract have illustrated in Figure 1. The percentage of killed *C. albicans* have found to be near to the control sample (serum). This graph substantiates the immunomodulatory property of KSK. Similar results have been observed in *Rhododendron arboreum* leaves,[32] *Euphobia hirta*;[33] similarly, many plant isolated compounds have been reported to immunomodulating nature. The vincristine as an immunosuppressant has been employed for treating thrombotic thrombocytopenic purpura or chronic idiopathic thrombocytopenic purpura.[39] This alkaloid compound has also been utilised to treat many more diseases: idiopathic thrombocytopenia purpura, bladder cancer, cervical cancer, non-small-cell lung cancer, autoimmune haemolytic anaemia, neck cancer, and head cancer.[39,40]

Nitroblue tetrazolium test assesses the test compound's immunomodulatory activity by determining its ability to stimulate the phagocytic activity in leucocytes. Once stimulated, the membrane-permeable, water-soluble, yellow-coloured nitroblue tetrazolium is reduced to blue NBT formazan crystals by the leucocytes. The KSK extract stimulated the leucocytes' phagocytic activity in a concentration-dependent manner, as seen by the

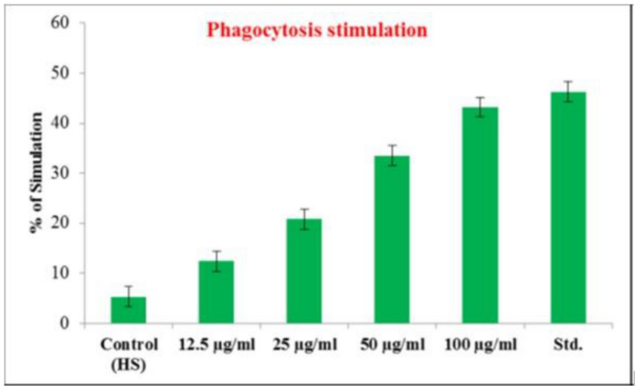

Figure 1: Percentage of killed Candida after treatment with extract by phagocytosis stimulation.

increased percentage of NBT positive cells, shown in Figure 2. The immunomodulatory effect with the aid of nitro blue assay has been observed in *Ficus glomerata* Roxb.[41] *Nelumbo nucifera* Gaertn.[42] *Pouteria cambodiana*.[43] The study's result indicates the functionality of the neutrophils in the process of phagocytosis is high, creating a proactive environment from the infection.

The COVID-19 patients have shown thrombosis as one of the symptoms.[44,45] Also, thrombus formation leads to progressive respiratory failure,[46] myocardial infarction, systemic arterial embolism in COVID-19 patients.[47] The effective thrombolytic percentages with different concentration of the KSK extract, control, 50 and 100 µg/ml and standard (S.K.) showed 22.36, 43, 71.83 and 83.75 %, respectively has been illustrated in Figure 3. From Figure 3, it is evident that the percentage of the thrombolytic activity was 71.83 % at 100 µg/ml compared to the 100 µl Streptokinase. From the different samples, the 50µg/ml showed 43% thrombolytic activity, which is higher than the distilled water (negative control). Kiran[23] have already reported the phytoconstituents of Siddha formulation KSK. These compounds have been detailed of their biological activities; some of them were found to have thrombolytic, immunomodulatory, anti-inflammatory, and fibrinolytic activity, for example, β-bisabolene,[48-51] piperine,[52-54] Squalene[55,56] Chebulagic acid,[57] Carvacrol,[58,59] Luteolin[60,61] Magnoflorine.[62-64]

*A*s per Siddha, stickiness, mucilaginous, rounded, little hard are listed as characters of *Aiyam*. A thrombus has all the qualities of increased *Aiyam*; most of the thrombolytic drugs are pungent and bitter. Kabasura Kudineer has already been screened for its anti-atherogenic property. The results also suggest the thrombolytic potential of Kabasura Kudineer owing to its fire-based elements in the ingredients. We have reported docking studies of bioactive compounds from Kabasura Kudineer,[23] which confirmed that this extract has excellent binding efficiency with spike protein of SARS-CoV-2.

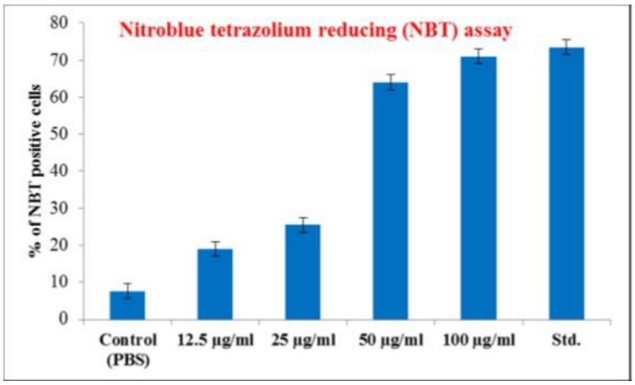

Figure 2: Percentage of NBT positive cells after treatment with extract by Nitro blue Tetrazolium Test (NBT).

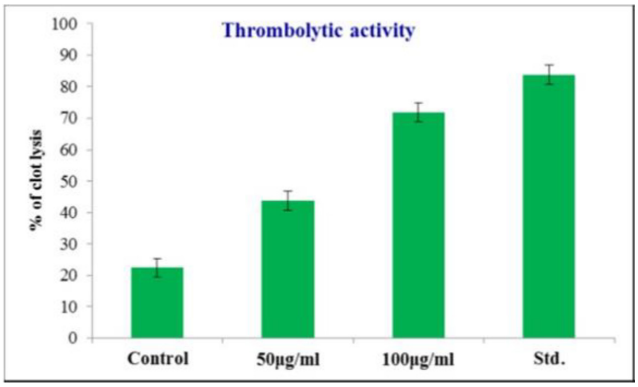

Figure 3: Thrombolytic activity (in terms of % clot lysis) of sample.

CONCLUSION

Siddha medicine is one of the best ways to control the COVID-19. The immunomodulatory and anti-thrombolytic are the stepping stones to developing a stable, safe, and working cure for COVID-19. Kabasura kudineer is a polyherbal decoction with fifteen different components, and each of them is in itself a firmly established herbal plant whose synergistic activity might probably improve human immune response and lead the human body to healthiness. The immunomodulatory property and thrombolytic activity of this miracle Siddha medicines have been studied using *in vitro* experiments but still require *in vivo* animal model experiments to understand better. This research paper has indicated and supported the notion of using the KSK extract to improve the immune response in this COVID-19 infected time.

ACKNOWLEDGEMENT

The authors thank all the institutes for their support to carry out this research and thanks to Central Council for Research in Siddha, Ministry of AYUSH, Chennai, Tamilnadu, India and Salem Microbes Private Limited Salem, Tamil Nadu, India.

CONFLICT OF INTEREST

The authors declare no Conflict of interest.

ABBREVIATIONS

KSK: Kabasura Kudineer; **MTCC:** Microbial Type Culture Collection and Gene Bank; **C. albicans:** *Candida albicans*; **P.I:** Phagocytic index; **PBS:** Phosphate buffer saline; **NBT:** Nitroblue Tetrazolium; **S.K:** Streptokinase; **I.U:** International unit.

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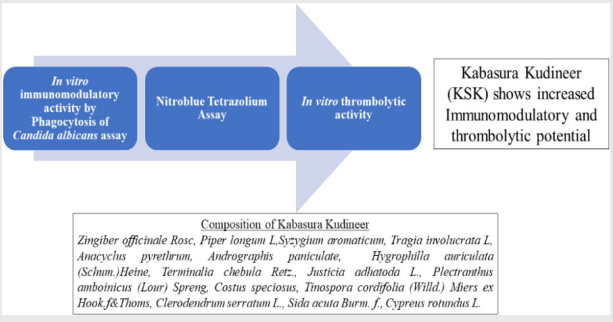
PICTORIAL ABSTRACT



SUMMARY

The present experimental paper details the utilisation and application of the KSK extract as an immunomodulatory substance for the improvement in human immune response

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Cite this article: Parameswaran S, Sampangi Ramulu SDM, Arivarasan VK, Kadarkarai K, Dhanakoti RK, Loganathan K. Evaluation of *in-vitro* Immunomodulatory Activity and Thrombolytic Potential of Kabasura Kudineer (KSK): An Official Siddha Polyherbal Formulation. Indian J of Pharmaceutical Education and Research. 2021;55(3):774-81.

European Journal of Molecular & Clinical Medicine

ISSN 2515-8260 Volume 07, Issue 10, 2020

Survival analysis to assess the length of stay of novel coronavirus (COVID-19) patients under Integrated Medicine - Zinc, Vitamin C & Kabasura Kudineer (ZVcKK)

Retrospective Study to assess the length of stay of novel coronavirus (COVID-19) patients in GMC & ESIH Coimbatore who were under- Integrated Medicine -Zinc, Vitamin C & Kabasura Kudineer (ZVcKK)

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Conflict of Interest: The authors declare that they have no conflict of interest.
Ethical approval: "For this type of study consent is obtained."
The personal identifiers such as name and address of the patient's records were removed to ensure the confidentiality and anonymity. Collection of information regarding the age, gender, address, and all other information collected from patients were also kept confidential and as this study is a data observation gone for ethical committee approval.
Ethics committee of GMC-ESI – Coimbatore – No: 20911/2020.
Informed consent: "For this type of study formal consent is obtained."

Abstract: Objective: COVID19 pandemic out of all odds has created an opportunity to offer treatment in an integrative manner. This study measures the Length of stay (LS) of patients in an integrative way as done earlier in China and Vietnam. Length of stay,

ISSN 2515-8260 Volume 07, Issue 10, 2020

Clinical presentations, and Comorbidities were analyzed among COVID19 patients in ESI Hospital, Coimbatore, Tamil Nadu, India.

Method: Retrospective cross-sectional data on 251 Positive COVID19 patients of both sexes irrespective of age admitted from 27 March 2020 and 26 April 2020 cases were included in the study. The final discharge date is taken as 5th May 2020. Kaplan Meier survival analysis was adopted.

Results: Male, female ratio were 141(56.2%): 83(33.1%), 12 (4.8%) Male Child and 15 (6.0%) were Female child. 5.2% of the patients were in the age group greater than 60, 75.3% were in the age group 20-60, and the remaining 19.5% were 0-20 age group. 84.9% of patients were Asymptomatic, while fever and cough were the main symptoms recorded in the remaining cases. CT scan was done for 7 patients. No mortality and no serious adverse events were reported. Comorbidity is 15% and does not influence hospital length of stay. The overall median length of stay is 12 days for those who were under ZVcKK (Median ST CI- 11.59-12.41).

Conclusion: This study recorded a median of 12 days in the Length of stay and 13.5 days in the Length of stay average. Comparing earlier studies, patients taking ZVcKK have savings of 7 days. i.e., the relief speed is higher while using ZVcKK.

Keywords: COVID-19, Integrated Medicine, Vit.C and Zinc tablets, Kabasura Kudineer, Kaplan Meier survival analysis, Siddha Medicine.

1. INTRODUCTION

Following the first-ever reported case in Wuhan in December 2019, PHEI (Public Health Emergency of International concern) was announced by WHO in January [1]. SARS-CoV-2 has spread widely across all continents; as of the latest situation report on August 16, 2020, by WHO, a total of 21, 294, 845 cases with mortality of 7,61, 779 have been reported [2]. There are 2 647 316 cases in India and 51,045 deaths as of August 16, 2020; these data represent the imminent risk facing the country [3]. India declared an emergency alert in March 2020. Transmission of virus spreads via physical contact with infected individuals, contaminated surfaces, and droplets [4]. COVID-19 commonly reported symptoms are fever, vomiting, chills, headache, dyspnoea, nausea, sore throat, coughing up blood, shortness of breath, myalgia, diarrhea, and malaise. The severe infection leads to pneumonia, acute respiratory distress syndrome (ARDS), and sometimes multi-organ failures such as kidney failure and even death [5]. With its strong internal and external medications, the linage of Siddha medicine has been in vogue to treat viral diseases [6]. At Present, treatment aspects are isolation and treating symptoms stands as the only option or vaccine therapy Due to the non-availability of proved therapies. Therefore, it is necessary to develop a treatment for COVID-19. Based on the Siddha system of Medicine advisory given by the Ministry of AYUSH, India for COVID-19 mentioned stages of medicines for treatment, prophylaxis, and related convalescence. Kabasura Kudineer (KSK) found its place in the advisory of the Ministry of AYUSH [7]. The Tamilnadu government advocates zinc and Vitamin c. Administration of CBE – ESI took a positive step to contain Covid19 through Integrated approaches. In this regard, the benefit offered by Integrated Medicine -Zinc – (150 mg),

ISSN 2515-8260 Volume 07, Issue 10, 2020

Vitamin C – (500 mg) & Kabasura Kudineer (ZVcKK), and the association between Average lengths of Stay in the hospital is evaluated. Siddha medicine is one among the traditional medicine originated on par with Ayurveda and recognized by WHO in India with its origin of centuries-old [8]. Efficacy of Siddha medicines was documented in earlier pandemics like HIV and epidemics like Chikungunya, Dengue, and Swine flu influenza outbreaks [9]. During Dengue outbreaks and post-mitigation Chennai floods, Nilavembu Kudineer is used extensively to prevent infection [10]. Nilavembu Kudineer has been used to mitigate Dengue, Kabasura Kudineer has been used at Swine flu epidemic. These two drugs are repurposed and found their place in the AYUSH Advisory. Kabasura Kudineer has been recommended by Tamilnadu Govt. to encounter COVID -19 under Aarogyam scheme. Kabasura Kudineer is one such concoction mentioned in the Siddha manuscript Citta Vaittiyattirattu [11], one of the books listed under the Drugs and cosmetic act [12]. It's a combination of fifteen herbs cultivated, collected, procured, and authenticated by a Pharmacognostic professional. After the purification process, this compound formulation is made into a coarse powder, which is used to prepare the concoction having a shelf life of three hours. (After three hours it is not consumable as it denatures itself, concoctions expire after three hours). Kabasura Kudineer is one of the drugs included in Advisory of Govt of India, released by the Ministry of AYUSH for symptomatic management of Covid 19 [7]. Initial studies using in silico model supported the efficacy of drug inhibiting SARS- CoV2 main protein. Govt of Tamilnadu, in its Aarogyam scheme, introduced this drug and also permitted studies involving asymptomatic and mild cases [13]. Kabasura Kudineer is a Sastric Siddha Medicine used in Slethuma Suram equated to the indicated COVID -19 like illness [11]. It is polyherbal formulation consists of fifteen Siddha Herbal drugs mixed in equal proportion. Approximately 5gms of coarse powder boiled in 240ml of water until it reduces to one-fourth of its quantity Consume 30 to 60 ml of twice or thrice daily. The ingredients in the above Siddha formulation are absorbable when administered orally [11]. COVID-19 is a new strain of coronavirus that has not been previously identified in humans. The COVID-19 is the cause of an outbreak of respiratory illness first detected in Wuhan, Hubei province, China. Coronaviruses are a large family of viruses known to cause illness ranging from the common cold to more severe diseases such as Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS).

A confirmed case was defined by epidemiological history and/or clinical features that a suspected case may contact with COVID-19 infected persons and/or had symptoms and signs of COVID-19 infection, plus positive laboratory tests including virus nucleic acid, gene sequencing, and serum antibody detection.

1.1 ETHICS

Personal identifiers such as name and address of the patient's records were removed to ensure confidentiality and anonymity. The collection of information regarding age, gender, address, and other information collected from patients was also kept confidential. This study is a data observation and gone for ethical committee approval (IHEC Approval Number: 20911). Consent obtained from the participants.

2. METHODS

Data collected from confirmed COVID-19 cases admitted in GMC & ESI Hospital Coimbatore. Collected information on personal comorbidities, Travel history, and the length of hospital stay for confirmed patients. We conducted Kaplan Meier survival analysis for result interpretation. KSK (Kabasura Kudineer) was given daily after food for Adults- 60 ml and Children – 15 ml for all participants. Tested the equality of hospital stay length is the same for different parameters using a one-way analysis of variance. And there are statistically significant differences; hence to study the equality of survival curves, the Kaplan -Meier (KM) method to estimate overall survival (OS) time is used for the analysis [14-15]. Data were analyzed using SPSS software.

2.1 STATISTICAL ANALYSIS

The relationship between patient's age, gender, the time interval from illness onset to diagnosis, hospital-grade of patients, clinical grade, and the length of hospital stay after admissions for confirmed patients were compared and analyzed by survival analysis. The event of interest is discharge status; 0 for no discharge, 1 for discharge, and death are treated as 0. The Kaplan-Meier method was used for single factor comparison. In our study, no death is reported.

2.2 SURVIVOR FUNCTION

Survivor function gives a chance for a patient to survive a specific point of time (t). ($S(t) = P(T > t)$) [16].

2.3 COMPARISON OF SURVIVOR FUNCTIONS

Wilcoxon test or Breslow test effectively compared to, or more survivor functions in survivor studies other than this log-rank test are used in large sample studies. This test compares the observations with that of the expected number of events, whereas per the null hypothesis, all the comparisons are the same. Comparisons are direct proportion with a time point and an event observation. Here each ranked item is compared with every ranked value in another group.

3. RESULTS

Out of the 251 COVID Positive cases, 151(60.2%) had close contact with positive cases, 94(37.4%) had travel history through containment zone like Delhi, and one person 0.4% had a travel history to Kashmir and only the remaining 5(2%) were free from travel history. Duration of hospital stay varies from 1-36 days. 5.2% of the patients were in the age group greater than 60, 75.3% were in the age group 20-60, and the remaining 19.5% were 0-20 group (Table 1).

ISSN 2515-8260 Volume 07, Issue 10, 2020

Age Group	Frequency	Percent
0-10	21	8.4
11-20	28	11.2
21-30	51	20.3
31-40	62	24.7
41-50	44	17.5
51-60	32	12.7
61-70	8	3.2
71-80	3	1.2
81-90	2	0.8
Total	251	100.0

Table1: Age Group Category

During the one-month admission period, a total of 251 positive cases were admitted in the hospital is included in the study. Out of which 141(56.2%) were male, 83(33.1%) were female, 12 (4.8%) Male Child, and 15 (6.0%) were Female child (Table 2).

Gender	Frequency	Percent
M	141	56.2
F	83	33.1
MC	12	4.8
FC	15	6.0
Total	251	100.0

Table2: Gender Distribution

84.9% have admitted with no symptoms, or the symptoms are not recorded; in the remaining cases, fever and cough were the main symptoms recorded. During admission, 13 (5.2%) have a fever, 22(8.8%) have cough, 2(0.8%) have both fever & cough, 1 (0.4%) with Cough & Respiratory Distress and remaining 213(84.9%) have no such symptoms. An abdominal CT scan of 7 patients was done during the study period. 6 of them have GGO (Ground glass opacities), and one shows normal in the result. After the study period, it reduced by 3 have GGO, and all others are turned to be normal. 85 years old patient got cured of COVID 19, but showing Opacity in both CT and X-ray and is referred to CMC Hospital Vellore district. Her Length of hospital stay is 18 days. While testing the equality of hospital stay length is the same for different parameters using a one-way analysis of variance, like symptoms, travel history, gender, all variables have rejected the null hypothesis and accepted the alternate hypothesis. That is, there exists a statistically significant difference in inequality of means in all the above-mentioned parameters. Hence to study the equality of survival curves of different variables, the Kaplan -Meier (KM) method to estimate overall survival (OS) time is used for the analysis, and the results are below (Figure 1).

ISSN 2515-8260 Volume 07, Issue 10, 2020

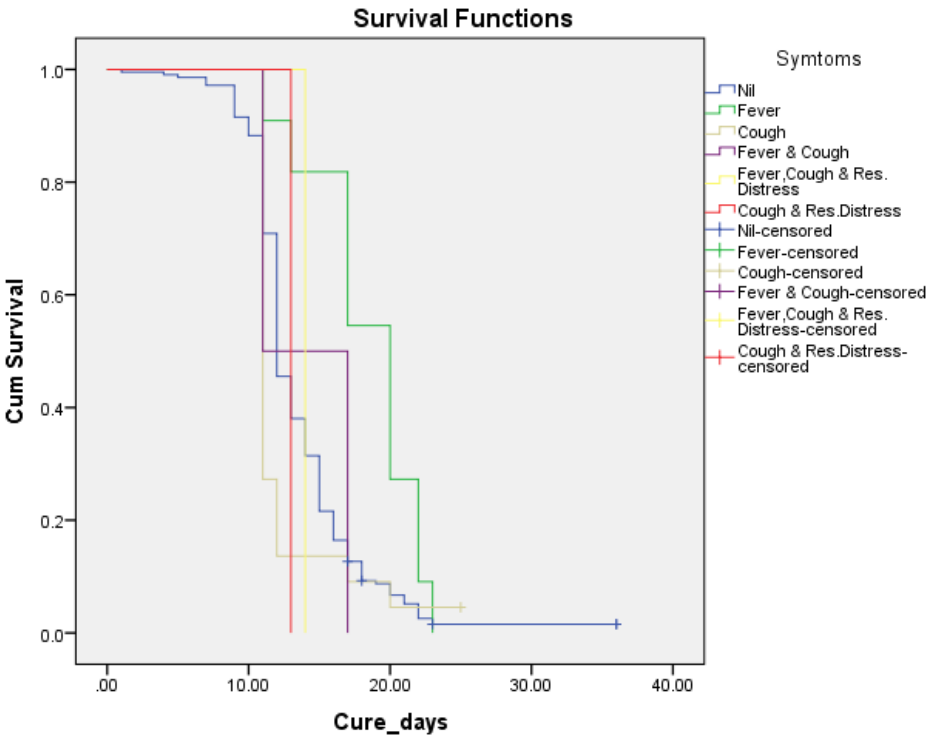

Figure1: Survival Curve of Symptoms

All positives cases became negative and discharged from the hospital are considered the events else considered censored. Out of the 251, 6 cases are censored, and 245 are events. Since the p-value of the calculated chi-square test is <0.05, reject the null hypothesis that all the compared curves are the same (Figure1). From the above chart, it is clear that all patients with Fever & Cough, Cough, Fever, and Cough & Respiratory Distress are discharged from the hospital during the study period. But patients had a fever and no symptom group; not all patients were discharged during the study period and considered censored.

In 90.9% of patients with fever recovered later - 11 days. 91.5% of patients without symptom have a length of stay in hospital is greater than 9 days. 27.3% of patients with cough take more than 11 days in a hospital. All 251 cases were cured of Coronavirus infection, but 6 cases were not discharged on the data collection's closing day.

ISSN 2515-8260 Volume 07, Issue 10, 2020

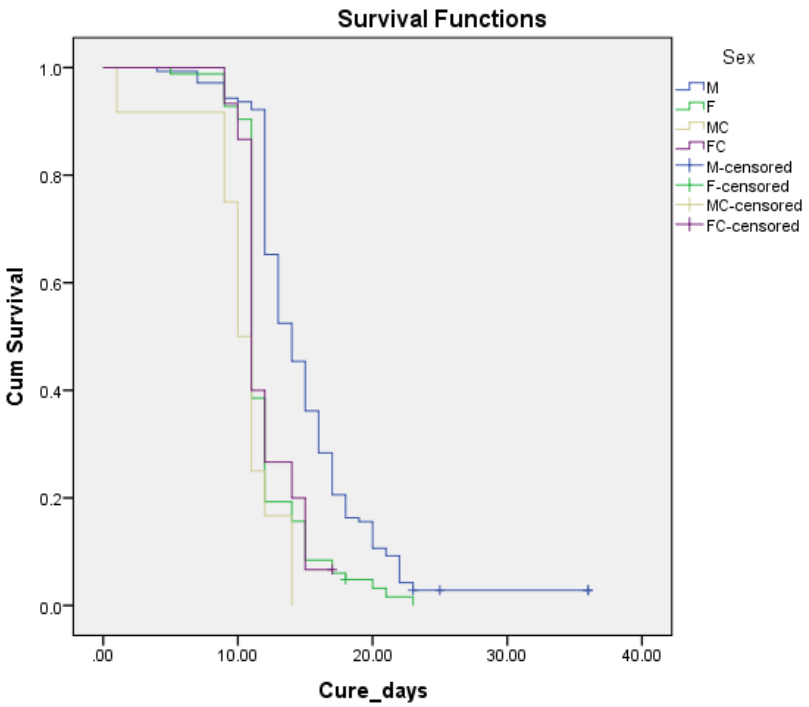

Figure2: Survival Curve of Gender

There was a significant difference in the survival curves of male(M), male child(MC), female(F), and female child(FC) patients. (Log-rank statistic =59.417, df =3, p= <0.001**).In 92.2% of Male patients are take greater than 11 days for recovery from Coronavirus infection, and 4 male patients were not discharged from the hospital till the last day of the survey. 92.8% of Female patients have a length of stay in hospital is greater than 9 days, and one female was not discharged from the hospital till the last day of the survey .75% of Male Child patients take more than 9 days in the hospital, and all MC are discharged from hospital. 86.7% of Female Child patients take more than 10 days in the hospital, and one FC was not discharged from the hospital till the last day of the survey(Figure2).

ISSN 2515-8260 Volume 07, Issue 10, 2020

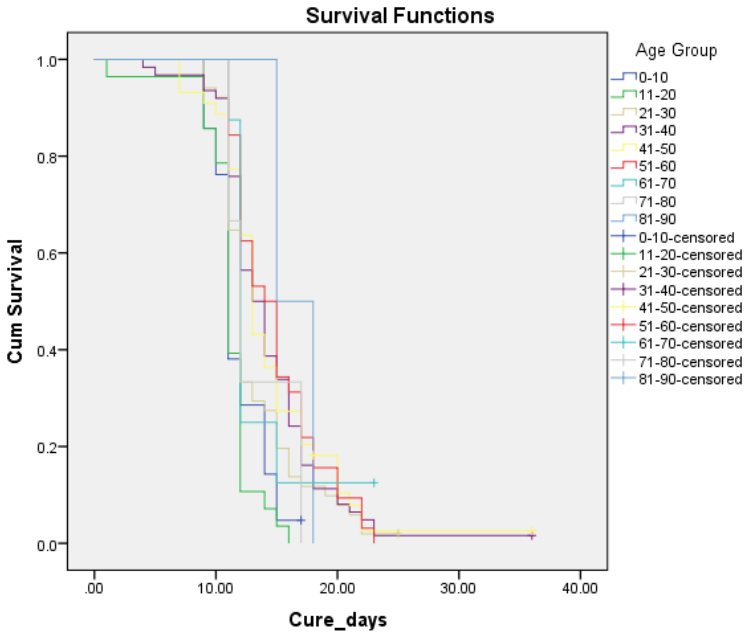

Figure3: Survival Curve of Age Group

There was a significant difference in the survival curves of different age groups of patients. (Log-rank statistic =35.716, df =8, p= <0.001**). For the age group, 0-10 length of stay varies from 9 to 17 days, 76.2% of cases LS was greater than 10 days with one censored case. In the age group 11-20, LS varies from 1 to 16 days, and 85.7% of cases LS is greater than 9 days with zero censored case. For the age group 21-30, LS varies from 9 to 25 days, and 92.2% have LS greater than 10 days with one censored case. In the category 31-40, LS varied from 4 to 36 days, and in 91.9% of the cases, LS greater than 10 days with one censored case. In the next group, 41-50 LS varies from 7 to 36 days, and for 88.6% of cases LS greater than 10 days with one censored case. For the next group, 51-60 LS was from 11 to 23 days, and for 84.4% of the cases LS greater than 11 days with complete discharges. The 61-70 group LS varies from 11 to 23 days, and for 87.5% of the cases, LS greater than 11 days with one censored case. 71-80 group LS varies from 11 to 17 days, and 66.7% of cases LS more than 11 days with all discharges. For the last group, 81-90 LS was 15 and 18 days, with all cases discharged (Figure3).

ISSN 2515-8260 Volume 07, Issue 10, 2020

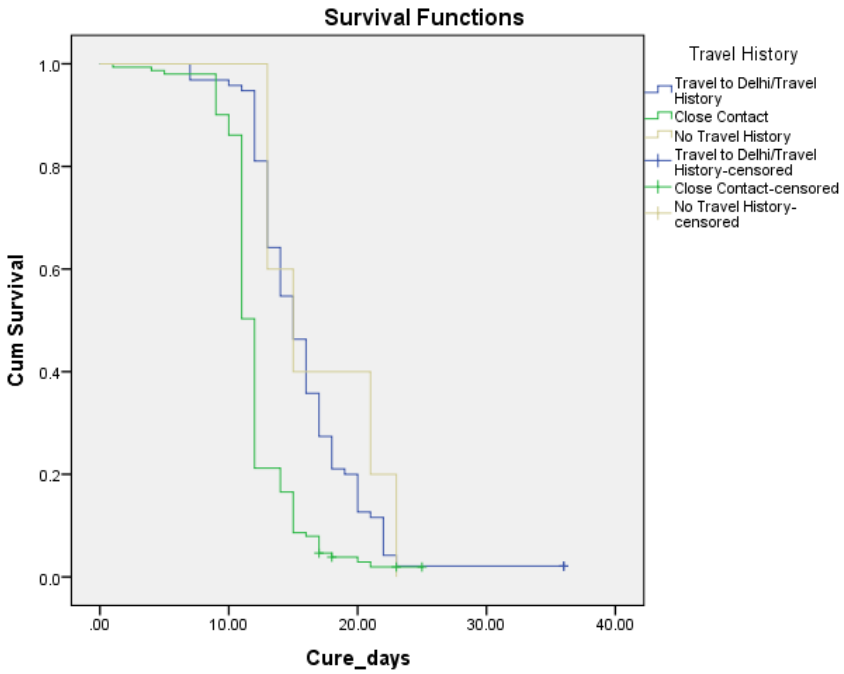

Figure4: Survival Curve of Travel History

There was a significant difference in the survival curves of the different travel histories of patients. (Log-rank statistic =54.679, df =2, p= <0.001**) (Figure4).

Patients with travel history have a length of stay varies from 7 to 36 days, 94.7% of patients have LS greater than 11 days, with 2 censored patients in the group. Patients having close contact have LS varies from 1 to 25 days; 90.1% of patients take more than 9 days of LS in the hospital with 4 censored patients in this group. Patients have no travel history LS varied from 13 to 23 days, and 60% of patients in this group takes more than 13 days of LS in the hospital with all discharges.

Table 3. Descriptive Statistics of Survival Data (SPSS Output of Kaplan-Meier Estimator)

Variables	Mean Survival Time (Days)	95% CI	Median Survival Time	95% CI	Log Rank	Df	Sig
Symptoms							
Nil	13.58	12.98-14.18	12.000	11.59-12.41	11.498	5	0.042
Fever	18.36	16.11-20.61	20.000	16.62-23.39			
Cough	12.46	10.99-13.92	11.000				
Fever & Cough	14.00	8.12-19.880	11.000				

Fever, Cough & Res.Distress	14.00	14.00-14.00	14.000				
Cough & Res.Distress	13.00	13.00-13.00	13.000				
Overall	13.74	13.17-14.31	12.000	11.59-12.41			
Gender							
M	15.14	14.31-15.96	14.000	13.15-14.85	59.417	3	<0.001**
F	12.10	11.49-12.71	11.000	10.79-11.20			
MC	10.17	8.29-12.05	10.000	8.87-11.13			
FC	12.07	10.99-13.14	11.000	10.47-11.53			
Overall	13.74	13.17-14.31	12.000	11.59-12.41			
Age Group							
0-10	11.81	10.89-12.73	11.00	10.42-11.58	34.823	8	<0.001**
11-20	11.07	10.14-12.01	11.00	10.54-11.46			
21-30	13.27	12.30-14.25	12.00	11.59-12.41			
31-40	14.29	13.14-15.45	13.00	11.60-14.40			
41-50	14.52	12.97-16.07	13.00	12.28-13.72			
51-60	14.91	13.66-16.15	14.00	12.15-15.85			
61-70	13.63	11.06-16.19	12.00	11.52-12.48			
71-80	13.33	9.69-16.97	12.00	10.40-13.60			
81-90	16.50	13.56-19.44	15.00				
Overall	13.74	13.17-14.31	12.00	11.59-12.41			
Travel History							
Travel to Delhi/Travel History	15.87	14.94-16.80	15.00	13.94-16.06			
Close Contact	12.14	11.64-	12.00	11.80-	54.679	2	<0.001**

ISSN 2515-8260 Volume 07, Issue 10, 2020

		12.63		12.20			
No Travel History	17.00	12.89-21.11	15.00	10.71-19.29			
Overall	13.74	13.17-14.31	12.00	11.59-12.41			

Patients without symptoms have, on average, takes 13.58 days (CI 12.98-14.18) of hospital stay. Patients having fever have 18.36 days (CI 16.11-20.61) is the length of hospital stay. Similarly, patients with cough have 12.45 days(CI 10.99-13.92), Fever & Cough takes 14 days(CI 8.12-19.88), Fever, Cough & Res. Distress had 14 days, Cough & Res. Distress takes 13 days is the average length of stay in hospital to discharge after having COVID 19.
Male patients' average length of stay is 15.14 days (CI 14.31-15.96) median LS was 14days. For females, the average LS was 12.1 days (CI 11.49-12.71) median LS was 11 days. In MC, the average LS was 10.17 days(CI 8.29-12.05), median LS 10 days, and FC average LS was 12.07 days (CI 10.99-13.14) with median LS 11 days.
Age Group 0-10 has average LS 11.81 days (CI 10.89-12.73) median LS 11 days. 11-20 age group have average LS 11.07 days (CI 10.14-12.01) median LS 11 days. Age group 81-90 was the highest average LS of 16.50 days (CI 13.56-19.44) with median 15 days; the second highest average LS was for the age group 51-60 with 14.91 days (CI 13.66-16.15) with median 14 days. The third highest average LS is for the category 41-50, 14.52 days(CI 12..97-16.07) days with a median of 13 days.

Patients with travel history have average LS 15.87 days (CI 14.94-16.80) median LS 15 days. Close contact has an average LS of 12.14 days (CI 11.64-12.63) median LS 12 days. No travel history group has average LS 17 days (CI 12.89-21.11) median LS 15 days. On average, we have 13.74 days (CI 13.17-14.31) was the average length of stay for the patients taking ZVcKK need to stay in hospital after having infected by Corona Virus (Table 3).

4. DISCUSSION

The dataset of patients indicates almost all the primary contacts who had a travel history are the source of infection. Invariably all the sexes including children were affected. The length of the stay of asymptomatic patients is lesser than that of the patients who are symptomatic. Among the symptoms cough, fever, and respiratory distress were reported. Even though there is a good reduction in the viral infection some patients reported ground glass opacity (GGO) of the lung which describes the long-term complication of the disease. 85-year-old women has been referred to a higher centre due to this GGO. Except this case among the 251 all the other 244 cases have been discharged and 6 patients were under treatment, none of the cases required ventilators or oxygen support. Age has an influence over the LS. Higher the age group longer the LS. Patients with primary contact travel history had an influence on LS, however the average LS among all the cases have been reported as 13.5days. Integrative therapy has its own benefit which is reflected in reduction of LS.

5. CONCLUSION:

The overall median length of stay was 12 days for those who are taking ZVcKK (Median ST CI- 11.59-12.41), and the average length of stay was 13.74 days (CI 13.17-14.31) for those having to take ZVcKK.

The result of a similar study on pneumonia patients in Wuhan shows that the median length of stay for all confirmed inpatients was 19 days [4]. On comparing with this result, patients taking ZVcKK have a saving of 7 days. i.e., the speed of relief is higher while using ZVcKK in a integrative manner.

6. STRENGTH AND LIMITATIONS OF THE STUDY

- The last admission day was 26th April 2020, and discharges were taken for analysis up-to 5th May 2020, so for the last admission, only 10 days for observation.
- Only 15.1% of them have collected with symptoms and need to concentrate on data collection. Headache, Loss of taste or smell, Rigor with chills, Sore throat, Shortness of breath, Muscle pain symptoms were not documented/reported.
- No reports from other studies in India regarding the Length of hospital stay of Covid 19 patients are available, and hence no comparison can be made with the Indian Scenario. This study could be widened by collecting a large volume of data from different Streams / Different Interventions used to compare.

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Natarajan et al. Trials (2020) 21:892
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Trials

LETTER Open Access

The efficacy of Siddha Medicine, *Kabasura Kudineer (KSK)* compared to Vitamin C & Zinc (CZ) supplementation in the management of asymptomatic COVID-19 cases: A structured summary of a study protocol for a randomised controlled trial

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Abstract

Objectives: The primary objectives of this study are to determine efficacy of Siddha medicine, *Kabasura kudineer* in reduction of SARS-CoV-2 viral load and reducing the onset of symptoms in asymptomatic COVID-19 when compared to Vitamin C and Zinc (CZ) supplementation. In addition, the trial will examine the changes in the immunological markers of the Siddha medicine against control.
The secondary objectives of the trial are to evaluate the safety of the Siddha medicine and to document clinical profile of asymptomatic COVID-19 as per principles of Siddha system of Medicine.

Trial design: A single centre, open-label, parallel group (1:1 allocation ratio), exploratory randomized controlled trial.

Participants: Cases admitted at non-hospital settings designated as COVID Care Centre and managed by the State Government Stanley Medical College, Chennai, Tamil Nadu, India will be recruited. Eligible participants will be those tested positive for COVID-19 by Reverse Transcriptase Polymerase Chain reaction (RT-PCR) aged 18 to 55 years without any symptoms and co-morbidities like diabetes mellitus, hypertension and bronchial asthma. Those pregnant or lactating, with severe respiratory disease, already participating in COVID trials and with severe illness like malignancy will be excluded.

Intervention and comparator: Adopting traditional methods, decoction of *Kabasura kudineer* will be prepared by boiling 5g of KSK powder in 240 ml water and reduced to one-fourth (60ml) and filtered. The KSK group will receive a dose of 60ml decoction, orally in the morning and evening after food for 14 days. The control group will receive Vitamin C (60000 IU) and Zinc tablets (100mg) orally in the morning and evening respectively for 14 days.

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Main outcomes: The primary outcomes are the reduction in the SARS-CoV-2 load [as measured by cyclic threshold (CT) value of RT-PCR] from the baseline to that of seventh day of the treatment, prevention of progression of asymptomatic to symptomatic state (clinical symptoms like fever, cough and breathlessness) and changes in the immunity markers [Interleukins (IL) 6, IL10, IL2, Interferon gamma (IFNγ) and Tumor Necrosis Factor (TNF) alpha]. Clinical assessment of COVID-19 as per standard Siddha system of medicine principles and the occurrence of adverse effects will be documented as secondary outcomes.

Randomisation: The assignment to the study or control group will be allocated in equal numbers through randomization using random number generation in Microsoft Excel by a statistician who is not involved in the trial. The allocation scheme will be made by an independent statistician using a sealed envelope. The participants will be allocated immediately after the eligibility assessment and informed consent procedures.

Blinding (masking): This study is unblinded. The investigators will be blinded to data analysis, which will be carried out by a statistician who is not involved in the trial.

Numbers to be randomised (sample size): Sample size could not be calculated, as there is no prior trial on KSK. This trial will be a pilot trial. Hence, we intend to recruit 60 participants in total using a 1:1 allocation ratio, with 30 participants randomised into each arm.

Trial status: Protocol version 2.0 dated 16th May 2020. Recruitment is completed. The trial started recruitment on the 25th May 2020. We anticipate study including data analysis will finish on November 2020. We also stated that protocol was submitted before the end of data collection

Trial registration: The study protocol was registered with clinical trial registry of India (CTRI) with CTRI/2020/05/025215 on 16 May 2020.

Full protocol: The full protocol is attached as an additional file, accessible from the Trials website (Additional file 1). In the interest in expediting dissemination of this material, the familiar formatting has been eliminated; this Letter serves as a summary of the key elements of the full protocol. The study protocol has been reported in accordance with the Standard Protocol Items: Recommendations for Clinical Interventional Trials (SPIRIT) guidelines (Additional file 2).

Keywords: COVID-19, Randomised controlled trial, protocol, Siddha Medicine, Herbal decoction

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13063-020-04823-z.

Additional file 1. Full Study Protocol.

Additional file 2. SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents.

Acknowledgements

The authors acknowledge significant support from the COVID Care Center and Government Stanley Medical College, Chennai

Authors' contributions

NS, AC, MP initiated the study and concept development. NS, AC, MP, SP, BP contributed to the study design. PP, PM, KK, BP supervised the project. NS, AC obtained necessary approvals. NS, AC, GA, KN and PA involved in the study conduct. All authors contributed to refinement of the study protocol and approved the final manuscript.

Funding

The research is funded by the Central Council for Research in Siddha, Ministry of AYUSH, Government of India. The funding body had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Availability of data and materials

All patient data will be kept confidential and personal identifiers of the study participants will not be disclosed to the public. Only the study investigators will have access to the study data.

Ethics approval and consent to participate

We certify that this trial has received ethical approval from the institutional human ethics committee of Government Stanley Medical College, Chennai, India on May 16, 2020. The purpose of the trial will be explained to all eligible SARS-CoV-2 confirmed patients. Informed consent will be obtained from all eligible participants willing to participate in the trial. Each participant will be informed that participation in the trial is voluntary and that s/he is free to withdraw, without justification, from the trial at any time without consequences and without affecting professional responsibilities. Informed consent will seek approval to collect blood samples and clinical data for the intended purpose of this trial.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 14 October 2020 Accepted: 16 October 2020
Published online: 27 October 2020

Publisher's Note

Srivastava et al. Trials (2021) 22:130
https://doi.org/10.1186/s13063-021-05041-x

Trials

LETTER Open Access

A double blinded placebo controlled comparative clinical trial to evaluate the effectiveness of Siddha medicines, Kaba Sura Kudineer (KSK) & Nilavembu Kudineer (NVK) along with standard Allopathy treatment in the management of symptomatic COVID 19 patients - a structured summary of a study protocol for a randomized controlled trial

Anurag Srivastava[1], Manickavasagam Rengaraju[2*], Saurabh Srivastava[1], Vimal Narayan[2], Vivek Gupta[1] and Rashmi Upadhayay[1]

Abstract

Objectives: The primary objectives of the study are to determine the effectiveness of the Kaba Sura Kudineer (KSK) & Nilavembu Kudineer (NVK) along with standard Allopathy Treatment to compared with Placebo (Decaffeinated Tea) with standard Allopathy Treatment in the management of Symptomatic COVID 19 patients and also in reduction of Hospital Stay Time & Changes in Immunological (IL6) and Bio Chemical Markers (Ferritin, CRP, D-Dimer and LDH). The secondary objectives are to evaluate the safety of the trial medicines and their effects in the reduce the risks of the disease. In addition, to document the profile of Symptomatic COVID 19 patients as per Siddha Principles.

Trial Design: A Double Blinded, Three arm, Single Centre, Placebo Controlled, Exploratory and comparative Randomized Controlled Trial

Participants: Patients who were admitted to the COVID Care Centre at Govt. Institute of Medical Sciences. Noida in India will be recruited. These will be patients with Mild and Moderate symptoms with laboratory confirmed COVID 19 (RT – PCR Tested Positive) aged 18-65, willing and consenting to participate.

(Continued on next page)

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(Continued from previous page)

Intervention and comparator:

Arm I: Decaffeinated Tea (Placebo – similar in taste and appearance to the other Two Decoctions), 60 Ml Morning and Night after Food, along with standard Allopathy Treatment for 10 days.

Arm II: Nilavembu Kudineer (The Siddha Medicines which is used as a standard Anti-Viral drug for the past Pandemics by Siddha Physicians) 60 Ml Morning and Night after Food, along with standard Allopathy Treatment for 10 days.

Arm III: Kaba Sura Kudineer (The Siddha Medicine which is proposed to be used as a Treatment for COVID 19 based on Siddha Literature) 60 Ml Morning and Night after Food, along with standard Allopathy Treatment for 10 days.

The investigational drugs are registered products under the Govt.of India and bought from GMP Certified Manufacturing Units.

Main Outcomes:

Primary outcomes:

1. Reduction in Viral load of SARS-CoV-2 at the end of treatment (10 days). 2. Time taken to convert Patient from symptomatic to Asymptomatic based on Reduction in clinical symptoms (10 days). 3. Effect of drugs inflammatory markers (IL6,) at the end of treatment (10 days). 4. Reduction in hospital stay time (20 days follow up). (Based on RT PCR CT Value 3^{rd}, 6^{th} if needed 10^{th} day). (Based on IL 6 Value needed 10^{th} day or IL6 value on turning negative. (entry level/exit level).

Secondary outcomes (10 days):

1. Reduction in use of Intensive Supportive Care. 2. Reduction in incidence of complications (Acute Respiratory Distress Syndrome, other systemic complications). 3. MuLBSTA score for viral pneumonia (multinodular infiltration, hypo-lymphocytosis, bacterial co infection, Total Leucocyte Count (TLC $\leq 0.8 \times 10^9$/L), smoking history, hypertension and age) score. 4. Laboratory markers (Haematological & Biochemical Markers). 5. Adverse events/effects Siddha-based measurements. 6. Siddha Udaliyal assessment by using Yakkai Ilakkanam (YI) Tool to diagnose body condition for covid-19 patients.

Randomisation: The assignment of the participants into 3 Groups will be allocated in 1:1:1 Ratio through randomization Blocks in Microsoft Excel by a Statistician who is not involved in the study. The allocation scheme will be made by another statistician by using a closed envelope after the assessment of eligibility and Informed consent procedures. The groups will be balanced for age and sex with 3:1 Ratio in each group for mild: severe COVID-19 symptoms.

Blinding: The Study is Double Blinded. Participants and Investigators were blinded.

Numbers to be randomized (Sample size): Sample size could not be calculated, Since there are no prior trials on KSK and NVK as a comparative trial. In addition, there are no prior trials on KSK and NVK in this region.
A total Number of 120 Patients, 40 each in 3 groups will be recruited in 1:1:1 Ratio.

Trial Status: Protocol Number : SCRUND GIMS Noida Study 1,Version: 2.0 Protocol Date : 20.08.2020
The recruitment period is completed for the trial. The Trial started its recruitment on 22.8.2020. We anticipate study including data analysis will finish in January 2021.
This is to state that it was a late submission from authors for publication of the protocol to the BMC, after enrolment in the study was over.

Trial Registration: The trial protocol was registered with CTRI (Clinical Trial Registry of India) and number is CTRI/2020/08/027286 on 21.08.2020

Full Protocol: The full Protocol is attached as an additional file, Accessible from the Trials website (Additional file 1). In the interest in expediting dissemination of this material, the familiar formatting has been eliminated. This letter serves as a summary of the key elements of the full protocol. The Study protocol has been reported in accordance with the SPIRIT guidelines.

Keywords: COVID 19, Randomised Controlled Trial, Protocol, Siddha Medicine, Herbal, CAM,

Acknowledgements
1. **Brigadier Dr. RakeshKumar Gupta,** Director, Government Institute of Medical sciences, (GIMS) Greater Noida, UP.
2. **Prof.Dr. K. Kanakavalli,** Director General, Central Council for Research in Siddha, Chennai.
3. **Prof.Dr. Jugal Kishore, Director,** HOD- Department of Community Medicine, VMMCH & Safdarjung Hospital, New Delhi.
4. **Dr.P. Sathyarajeshwaran,** Director- in-Charge, Siddha Central Research Institute, Central Council for Research in Siddha, Chennai.
RG,KK,JK and PS given their inputs to finalize the Study Protocol.

Authors' Contributions
MR, AS conceived the study. MR, AS and VN initiated the study. VG Contributed to incorporate all lab investigations. MR, AS, VN, SS and RV contributed to Protocol writing. This protocol was read and approved by all authors.

Authors' Information
AS,SS, RU and VG possess the background of Allopathy
MR and VN possess the background of Siddha (An Ancient Traditional medical system of India).

Funding
The Trial is funded by the Central Council for Research in Siddha (CCRS), Min.of AYUSH, Govt.of India and Government Institute of Medical sciences, (GIMS) Greater Noida, UP. The funding body had no role in the design of the study and collection, analysis and interpretation of data and in writing the manuscript.

Availability of Data and Materials
All participant data will be kept confidential and personal identifiers of the study participants will be disclosed to the public. Only the Investigator will have access to the trial data. All the procedures will be carried out by adhering the Good Clinical Practices (GCP). The monitor will have access to the study documents.

Ethics Approval and consent to participate
The trial received the ethical approval from the Institutional Ethical Committee of Siddha Clinical Research Unit, Safdarjung Hospital, New Delhi on 20.07.2020 and Trial Site Ethics Committee on 04.08.2020.
This is to state that the appropriate ethical committee approval was taken. Written Consent will be taken from all eligible and willing participants before their participation.

Consent for Publication
Not Applicable

Competing Interest
The authors declare that they have no competing interests.

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Received: 8 January 2021 Accepted: 11 January 2021
Published online: 11 February 2021

Publisher's Note

Srivastava et al. Trials (2021) 22:570
https://doi.org/10.1186/s13063-021-05478-0

Trials

RESEARCH Open Access

Efficacy of two siddha polyherbal decoctions, Nilavembu Kudineer and Kaba Sura Kudineer, along with standard allopathy treatment in the management of mild to moderate symptomatic COVID-19 patients—a double-blind, placebo-controlled, clinical trial

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Abstract

Background and aim: Globally, the ongoing pursuit in exploring an effective drug to combat severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) virus has not met with significant success to date. Indian traditional medicines, especially polyherbal formulations like Nilavembu Kudineer (NVK) and Kaba Sura Kudineer (KSK) of the Siddha system of medicine, have been used as public health interventions for controlling viral epidemics like dengue and Chikungunya. These traditional therapies have been found safe, effective, and widely accepted. The current study evaluates the comparative efficacy of NVK and KSK as opposed to the placebo, in the management of mild to moderate COVID-19 disease.

Methods: The study was a double-blind, placebo-controlled comparative clinical trial, with the primary objective of determining the efficacy of KSK and NVK. Patients (n=125) diagnosed with mild to moderate COVID-19 symptoms were enrolled in the study over a period of 4 months (Aug 2020—Dec 2020). Participants were randomized into 3 arms; placebo-decaffeinated tea in Arm I, NVK in Arm II, and KSK in Arm III. Each arm received 60 ml of the respective treatment twice a day, post morning and evening meals, along with standard allopathy treatment for a maximum of 10 days. The main outcome measures of the study were the reduction in SARS-CoV-2 viral load, hospital stay, and time taken by the patients to become asymptomatic from symptomatic. Efficacy assessments

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included clinical symptoms (fever, cough, and breathlessness) each day and real-time reverse transcription-polymerase chain reaction (RT-PCR), liver function test (LFT), renal function test (RFT), and electrolytes and electrocardiogram (ECG) at baseline (day 0) and days 3, 6, and 10. Post-treatment, participants were followed up for 30 days via phone for adverse effects if any. Effects of drugs on inflammatory markers (IL6) at the end of treatment were also recorded. Adverse events (AE) were monitored throughout the study.

Results: The results revealed that when compared to patients in the placebo arm, those in NVK and KSK arms showed a statistically significant reduction in hospital stay time, reduction in viral load of SARS-CoV-2, and the time taken to become symptomatic from asymptomatic. Out of 125 COVID-19 patients recruited, 120 completed the study; two from the placebo group developed severe symptoms and were shifted to the intensive care unit (ICU) and three patients from Arms II and III withdrew from the study. The mean age of females (n=60) and males (n=60) enrolled was between 40.2 and 44.3 years, respectively. Results were more promising for all the patients in NVK and KSK arms as all enrolled participants (100%) under this group got discharged by day 6 as compared to only 42.5% (n=17) from the placebo group on that day. The hospital stay time for patients in Arm I was significantly longer (mean [SD]=8.4 [2.0] days) as compared to the Arms II and III (mean [SD]=4.7 [1.5] and 4.2 [1.5] days, respectively (Kruskal-Wallis test, P=0.0001). Patients in the three groups took a significantly different number of days to become asymptomatic. While Arm II and III patients took mean of 2.5 and 1.7 days, respectively, Arm I, patients took a mean of 4.2 days (Kruskal-Wallis test, P=0.0001). In all, two adverse events were recorded, one for vomiting and one for diarrhea lasting a day in Arm I and Arm II, respectively. The mean value of interleukin-6 (IL6) was significantly different in comparison to the placebo-decaffeinated tea arm (NVK=2.6 and KSK=2.2, placebo=4.0, P=0.02). The other blood biochemical parameters like C-reactive protein (CRP), lactate dehydrogenase (LDH), ferritin, and D-dimer that were analyzed at the baseline and at the time of discharge from the hospital, were not significantly different in the three arms.

Conclusion: NVK and KSK arms showed a statistically significant reduction in hospital stay time, reduction in viral load of SARS-CoV-2, and time taken for patients to become asymptomatic from symptomatic, when compared to the placebo (decaffeinated tea). The primary outcome measures of the KSK arm were significantly better than those in the NVK arm.

Keywords: Mild to moderate COVID-19, Siddha medicine, Kaba Sura Kudineer, Nilavembu Kudineer, Double-blinded RCT

Introduction

Globally, there has been an ongoing pursuit in exploring an effective treatment to combat severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). However, this quest across various treatment verticals has led to despair amongst the scientific community [1]. In India, the role of traditional treatments especially Siddha medicines in the management of various diseases is well known that has proven effective, safe, and widely accepted across all ages. During the chikungunya and dengue epidemic in the year 2015 in Tamilnadu, India, the administration of Nilavembu Kudineer (NVK) played a major role in controlling the morbidity [2]. Siddha medicine has contributed to lowering the disease burden during public health emergencies. These medicines could be repurposed for the management of COVID-19. However, there is limited evidence for the integrative treatment approach (standard of care, allopathy treatment along with Siddha medication) in the management of COVID-19.

COVID-19 is a respiratory tract infection caused by a newly emergent coronavirus, SARS-CoV-2, that was first reported in December 2019. At present, we have limited evidence from randomized clinical trials to support pharmacological treatments from conventional medicine for COVID-19 [3].

According to Siddha Medical Literature, the symptoms and signs of COVID-19 including cold, cough, and fever are analogous to Kaba Suram [4, 5].Standard Siddha medicines for tackling these conditions are Kabasurakudineer (KSK) and Nilavembu Kudineer (NVK). NVK was one of the essential medicines used as anti-viral Siddha drugs, especially in the treatment of chikungunya and dengue during the past outbreaks [6]. Recent in vitro studies have revealed that ethanolic extract of NVK has anti-viral properties against chikungunya and dengue [2, 7]. Toxicity studies utilizing NVK as per Organisation for Economic Co-operation and Development (OECD) guidelines found it to be safe for consumption. Apart from this, antipyretic, anti-microbial, anti-inflammatory, and immunostimulant activities of NVK have also been proven by phytochemical screening studies [8]. Recent clinical studies have revealed the prophylactic and antiviral activities of NVK in viral fevers [9, 10]. These

indicate the growth inhibition of viral pathogens, and the ability to effectively inhibit spill-over and transmissibility of the viruses. Therefore, in the current study, NVK was selected as one of the drugs against COVID-19.

KSK is a classical Siddha formulation comprising of 15 herbs and each herb possesses antiviral activity [11, 12]. It has been found that a few phytocomponents in KSK decoction such as Cucurbitacin B (-112.09), Cardiofoliolide (-111.5), Apigenin (−98.84), and Pyrethrin (−92.98) bind to the virus and inhibits its replication hence could be effective in the management COVID-19. In silico studies of KSK ingredients have shown to be potent against SARS-CoV-2 spike proteins [13]. Determination of organoleptic characters, preliminary phytochemical analysis, physicochemical analysis, thin layer chromatography (TLC) photodocumentation, and high-performance thin-layer chromatography (HPTLC) fingerprint studies on KSK are reported [14]. A study has shown that KSK has antipyretic, anti-inflammatory, and anti-bacterial properties and found to be safe in toxicity test [15]. KSK has also been shown as an immunomodulator and having thrombolytic activity [16]. A retrospective observational study to measure the effect of integrated therapy KSK with vitamin C and zinc on COVID-19 patients has proven that there is a reduction in length of hospital stay [17, 18].

In the absence of a systematic evaluation of integrated therapy (with the standard of care, allopathy and KSK, or NVK from Siddha system of medicine in COVID-19 management), this was proposed as a comparative study.

Materials and methods

The study was conducted at the Government Institute of Medical Sciences (GIMS), Greater Noida, Uttar Pradesh, India. Patients were enrolled from August 22, 2020, to December 31, 2020. The Ethics Committees of the participating site and Siddha Clinical Research Unit, New Delhi, Safdarjung Hospital, approved the protocol. Prior to participation in the study, each patient was informed about the nature and purpose of the study and written informed consent was obtained. All research procedures were strictly adhered to, based on AYUSH GCP and Indian Council for Medical Research (ICMR) Guidelines. The trial was registered in the Clinical Trial Registry of India (CTRI), and the registration number is CTRI/2020/08/027286 [19]. The detailed protocol of the study was already published [19].

Study design

This was a randomized, double-blind, placebo-controlled, clinical trial where mild/moderate patients were randomly assigned to study treatment in a 1:1:1 ratio, to placebo (Arm I) or NVK (Arm II) or KSK (Arm III) groups. Patient allocation to the treatment arm was performed using a simple stratified randomization method. The sample size was determined based on calculation for effect size 0.30, and the total sample size in the three groups is coming out to be 110 (Fig. 1). Considering a dropout rate of 10%, we have to recruit 125 patients for the study. Please find in Fig. 2 for more clarity.

Eligibility criteria

Eligible patients were 18–65 years of age with mild to moderate symptoms of COVID-19 and willing to consent. COVID-19 was confirmed by RT-PCR screening following the ICMR guidelines. A total of 155 confirmed COVID-19 patients with mild to moderate symptoms of COVID-19 were screened at the site. 125 patients were enrolled and admitted to the hospital, and all the guidelines laid by ICMR, Govt. of India, for COVID-19 management were followed. The participants were categorized as mild or moderate COVID-19 following WHO criteria [20]. The patients having oxygen saturation (SpO_2) of <94%>90%, respiratory rate >24 breaths per minute, and chest X-ray showing pneumonia were classified as moderate COVID-19 patients, whereas patients with SpO_2>94% and respiratory rate <24 breaths per minute were classified as mild COVID-19 patients. Patients were excluded from the study if they had a severe primary respiratory disease or other pathogenic microbial pneumonia, with uncontrolled diabetes mellitus (≥350 mg/dL fasting sugar), severe hypertension (HT) (180/120 mmHg), chronic bronchial asthma (BA) (≥5 years based on clinical history), renal dysfunction (known chronic kidney disease [CKD] ≥5 years estimated glomerular filtration rate (eGFR) stage ≥3), and pregnant and lactating mothers. Patients with other systemic malignant diseases such as malignant tumors, mental illnesses, which the researchers considered unsuitable for participation in the study, people who had a history of allergy to Siddha medicine or who were part of other COVID-19 clinical trials were excluded from the study.

Study treatment

All the patients in the three arms received standard allopathy treatment as per ICMR guidelines, which included doxycycline/hydroxychloroquine and Ivermectin/Fabiparavir. Additionally, the patients with a moderate disease also received steroids (methylprednisolone or dexamethasone, if required) and low molecular weight heparin. Participants were randomized to receive 60 ml of placebo in Arm I, 60 ml of NVK in Arm II, and 60 ml of KSK in Arm III, twice a day post morning and evening meals along with standard allopathy treatment, for a

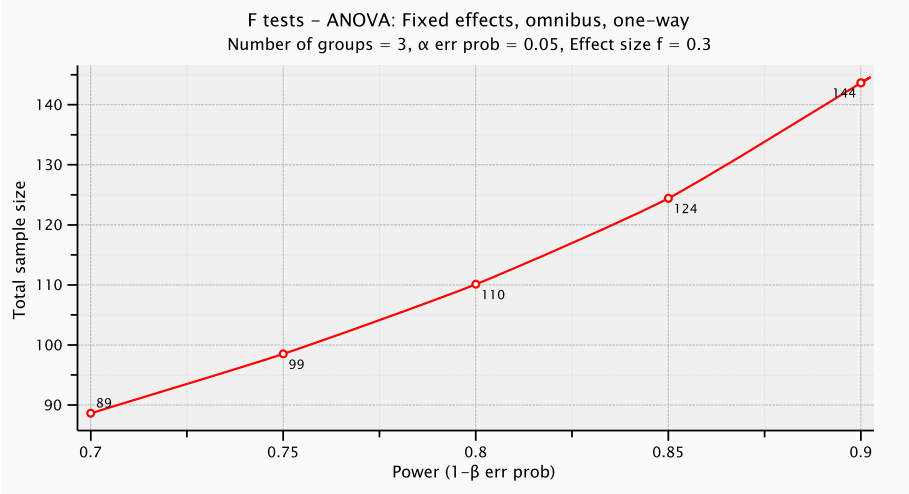

Fig. 1 Sample Size Calculation

maximum of 10 days. Post-treatment, patients were followed up for 30 days via phone for safety. The study design is displayed in Fig. 2.

Procedure to prepare a polyherbal decoction of KSK and NVK

Both the KSK and NVK decoctions were prepared as per the Siddha Formulary of India Guidelines [21]. In order to obtain NVK or KSK decoctions, a 5-mg coarse powder of NVK or KSK, obtained from the Central Pharmacy-Central Council for Research in Siddha (CCRS), Chennai, India, was boiled in 240 ml of water and reduced to one fourth (60ml), followed by filtration. The composition of polyherbal decoction ingredients of both NVK and KSK are detailed in Tables 1 and 2, respectively.

The distribution of participants included in the study (N=125) is summarized in Fig. 3. Enrolment of 120 subjects was planned. However, a total of 125 patients were enrolled (Arm 1: 42, Arm 2: 43, and Arm 3: 40) as 5 patients withdrew (Arm 1: 2 and Arm 2: 3) before the start of treatment. A total of 120 patients completed the study; 40 patients each in Arms 1, 2, and 3. In each arm, the male (n=20) and female (n=20) patients were equally distributed by stratified randomization. In each arm, the 40 patients were further divided into 3:1 ratio for mild (n=30) and moderate (n=10) cases based on ICMR, the Ministry of Health COVID-19 Criteria.

Outcome measures

The primary outcome measures were the reduction in SARS-CoV-2 viral load (RT PCR Ct value), time taken by the patient to become asymptomatic from symptomatic, and reduction in the hospital stay. The patient was discharged from the hospital if they were RT PCR negative. Immunity markers (IL-6) and other biological and hematological markers (CRP, LDH, D-dimer, and ferritin) at baseline and on the day the patient got discharged were also analyzed.

Table 1 Composition and polyherbal decoction ingredients of Nilavembu Kudineer (NVK) as per Siddha Formulary of India Guidelines [21]

S. No	Botanical name	Siddha name	Family	Part used	Parts
1	*Andrographis paniculata (Burm.f.)*	Nilavembu	Acanthaceae	Whole plant	1 part
2	*Vetiveria zizanioides* L.	Vettiver	Poaceae	Whole plant	1 part
3	*Santalum album* L.	Santhanam	Santalaceae	Wood	1 part
4	*Zingiber officinale Roscoe.*	Chukku	Zingiberaceae	Rhizome	1 part
5	*Piper nigrum L.*	Milaku	Piperaceae	Dry fruits	1 part
6	*Cyperus rotun*dus *L.*	Korai kilanku	Cyperaceae	Rhizome	1 part
7	*Hedyotis corymbosa L.Lam*	Parpadagam	Convolvulaceae	Whole plant	1 part
8	*Plectranthus vettiveroides (K.C.Jacob) N.P.Singh & B.D.Sharma*	Vilamicham ver	Lamiaceae	Root	1 part
9	*Trichochanthes cucumerina* L.	Peipudal	Cucurbitaceae	Whole plant	1 part

Table 2 Composition and polyherbal decoction ingredients of Kaba Sura Kudineer (KSK)) as per Siddha Formulary of India Guidelines [21]

S. no	Botanical name	Siddha name	Family	Part used	Parts
1	*Zingiber officinale Roscoe.*	Chukku	Zingiberaceae	Rhizome	1 part
2	*Piper longum L.*	Milagu	Piperaceae	Fruit	1 part
3	*Syzygium aromaticum (L,) &L.M Perry*	Kirambu	Myrtaceae	Flower bud	1 part
4	*Anacyclus pyrethrum L.*	Akkarakaram	Asteraceae	Rhizome	1 part
5	*Tragia involucrate L.*	Siru kanjori	Euphorbiaceae	Leaves	1 part
6	*Solanumanguivi Lam*	Karimulli	Solanaceae	Leaves	1 part
7	*Terminalia chebula (Gaertn.)*	Kadukkai	Combretaceae	Fruit rind	1 part
8	*Justicia adathoda* Linn.	Adathoda	Acanthaceae	Leaves	1 part
9	*Anisochilus carnosus (L.f) Wall, ex Benth*	Karpoora valli	Lamiaceae	Whole plant	1 part
10	*Costus speciosus (J.Koing)Sm*	Koshtam	Costaceae	Rhizome	1 part
11	*Tinospora cordifolia (Thunb.) Miers,*	Seenthil	Menispermaceae	Whole plant	1 part
12	*Clerodendrum serratum (L.)*	Siru Theku	Verbanaceae	Leaves	1 part
13	*Andrographis paniculata (Burm.f.)*	Nilavembu	Acanthaceae	Whole plant	1 part
14	*Cyperus rotundus L.*	Korai Kilanku	Cyperaceae	Rhizome	1 part
15	*Sida acuta (Burm.f.)*	Sitramutti	Malvaceae	Whole plant	1 part

Procurement and preparation: NVK and KSK were procured from the GMP certified pharmacy (Central Pharmacy – CCRS, Chennai). A 5-mg coarse powder of NVK or KSK was boiled in 240 ml of water and reduced to one fourth (60ml), followed by filtration, to obtain NVK or KSK decoctions

Efficacy evaluations

Clinical assessment for symptoms like fever (≤36.6°C or -axilla, ≤37.2°C oral or ≤37.8°C rectal or tympanic, cough), breathlessness (respiratory rate ≤24/minute on room air, oxygen saturation (SpO_2) >94% on room air, cough -mild or absent on a patient-reported scale (cough symptoms score ≤2 points, SpO_2 level ≥95 was recorded each day throughout the study. Laboratory assessments including RT-PCR, LFT, RFT, and electrolytes and ECG were performed at baseline (day 0), and day of discharge (days 3, 6, and 10).

Statistical analysis

Statistical analysis was performed using R Commander for R. The continuous variables were checked for normality tests, and those who did not follow normal distribution were transformed taking square root values. Continuous data was reported as the mean (standard deviation) while categorical data was reported as number (percentage). Three groups were compared for different biochemical parameters using Kruskal-Wallis test. Chi-square test was performed to compare proportions among groups. Values were considered significant if p value <0.05.

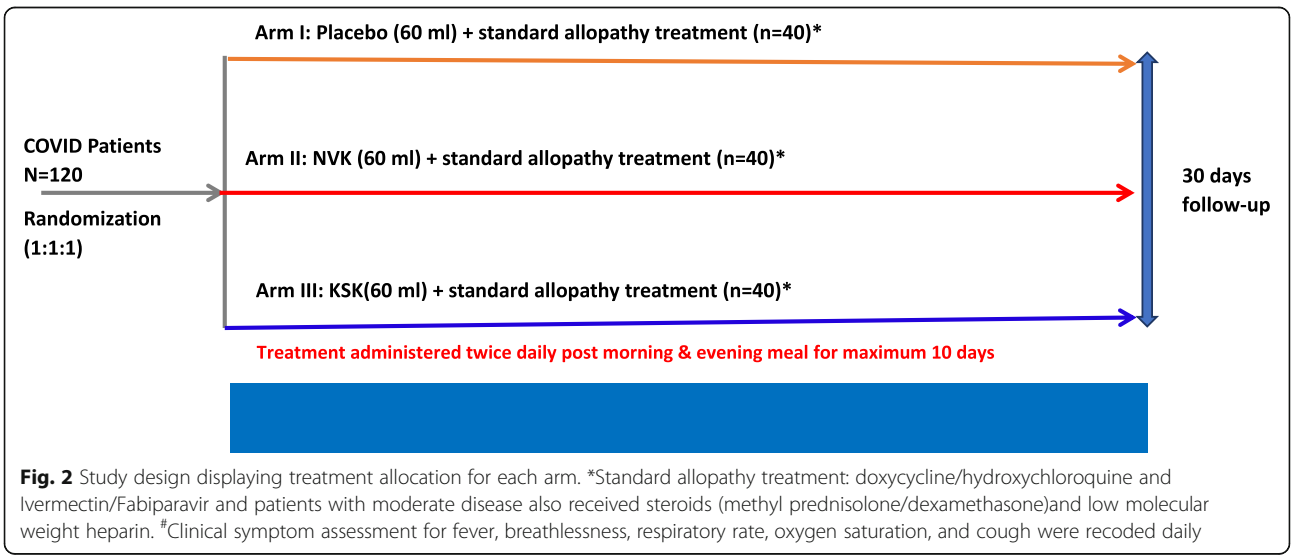

Fig. 2 Study design displaying treatment allocation for each arm. *Standard allopathy treatment: doxycycline/hydroxychloroquine and Ivermectin/Fabiparavir and patients with moderate disease also received steroids (methyl prednisolone/dexamethasone)and low molecular weight heparin. #Clinical symptom assessment for fever, breathlessness, respiratory rate, oxygen saturation, and cough were recoded daily

Blinding and randomization

Blinding and randomization were employed to avoid bias in the assignment of participants to treatment, to increase the likelihood that known and unknown subject attributes (e.g., demographics and baseline characteristics) were evenly balanced across treatment groups, and to enhance the validity of statistical comparisons across treatment groups. Participants were randomly assigned to either placebo, NVK, and KSK arm by an allocation ratio of 1:1:1. Blinded treatment was used to reduce potential bias during data collection and evaluation of clinical endpoints in the study. Measures were taken to ensure that the study patients and study staff were not unblinded. Study participants and investigators were blinded. Random allocation was done by a statistician who was not involved in the study. Siddha pharmacist who prepared herbal decoctions was not involved in the study, hence was unaware of which patient was getting which decoction. A placebo group was included to have an accurate assessment study treatment.

Results and discussion

Study subjects' demographic characteristics

The demographic and baseline characteristics were comparable across treatment groups. A total of 120 patients completed the study and comprised of equal number males 50% (n=60) and females 50% (n=60). The mean age of Arm I, Arm II, and Arm III was found out to be 44.4, 42.8, and 39.5 years, respectively, and was statistically insignificant (P=0.26).

Mean hospital stay time: reduction in hospital stay time

The number of patients discharged on days 3, 6, and 10 from Arms I, II, and III are summarized in Table 3. The patient was discharged from the hospital if the RT-PCR test was negative.

In Arm I (the placebo arm), no patients recovered from COVID-19 disease on day 3 after admission. On day 6, 65% (n=13) male and 20% (n=4) of female patients were discharged. The majority of patients, which was 35% (n=7) of male and 80% (n=16) female were discharged on day 10.

In Arm II (NVK arm), patients recovered faster in comparison to placebo hence got discharged earlier. On day 3, 50% (n=10) of male and 30% (n=6) of female patients were discharged. Remaining patients of NVK arm were discharged on day 6, i.e., 50% (n=10) of male and 70% (n=14) female, respectively. Since all patients recovered and discharged by day 6, hence patients discharged on day 10 of the study were zero.

Patients in Arm III (KSK arm) showed faster recovery even when compared to the NVK arm (Arm II). On day 3, the majority of the patients 65% (n=13) of males and 55% (n=11) of females were discharged on day 3. The remaining patients, 35% of male (n=7) and 45% (n=9) of female patients were discharged on day 6. Since all patients were discharged by day 6, there were zero discharges on day 10 from Arm III.

In patients in the placebo arm who were on decaffeinated tea along with allopathy treatment, no patients were discharged on day 3. In fact, the majority, i.e., 23 patients (57.5%) were discharged on day 10. Interestingly, all the patients taking NVK and KSK showed early recovery signs and were discharged by day 6. Further, the patients in the KSK arm recovered even faster than the NVK arm as 60% of patients of the KSK arm were discharged on day 3, as compared to only 40% in Arm II (NVK arm) on the same day.

The total number of days the patients stayed in the hospital during the treatment was recorded and compared to know if there was a reduction in hospital stay time. Patients in the placebo arm stayed significantly longer (mean [SD] = 8.4 [2.0]) as compared to the Arms II and III (mean [SD]=4.7 [1.5] and 4.2 [1.5], respectively, Kruskal-Wallis test, P=0.0001). Comparison of the Hospital Stay Time Kaplan Meier Graph for Arm I, Arm II, and Arm III is presented in Fig. 4. Hence, patients who were taking the Siddha treatment along with allopathy treatment (Arms II and III) had spent almost half the time in comparison to the placebo arm. Overall, the KSK group showed a statistically significant reduction in the hospital stay time compared to standard Siddha drug NVK and placebo which is decaffeinated tea.

Table 3 Numbers of patients discharged in Arm I, Arm II, and Arm III on days 3, 6, and 10

Time patient discharged days	Arm I: placebo n=40	Arm II: NVK n=40	Arm III: KSK n=40
Day 3	0 (0%)	16 (40%)	24 (60%)
Male (n=20)	0 (0%)	10 (50%)	13 (65%)
Female (n=20)	0 (0%)	6 (30%)	11 (55%)
Day 6	17 (42.5%)	24 (60%)	16 (40%)
Male (n=20)	13 (65%)	10 (50%)	7 (35%)
Female (n=20)	4 (20%)	14 (70%)	9 (45%)
Day 10	23 (57.5 %)	0 (0%)	0 (0%)
Male (n=20)	7 (35%)	0 (0%)	0 (0%)
Female (n=20)	16 (80%)	0 (0%)	0 (0%)

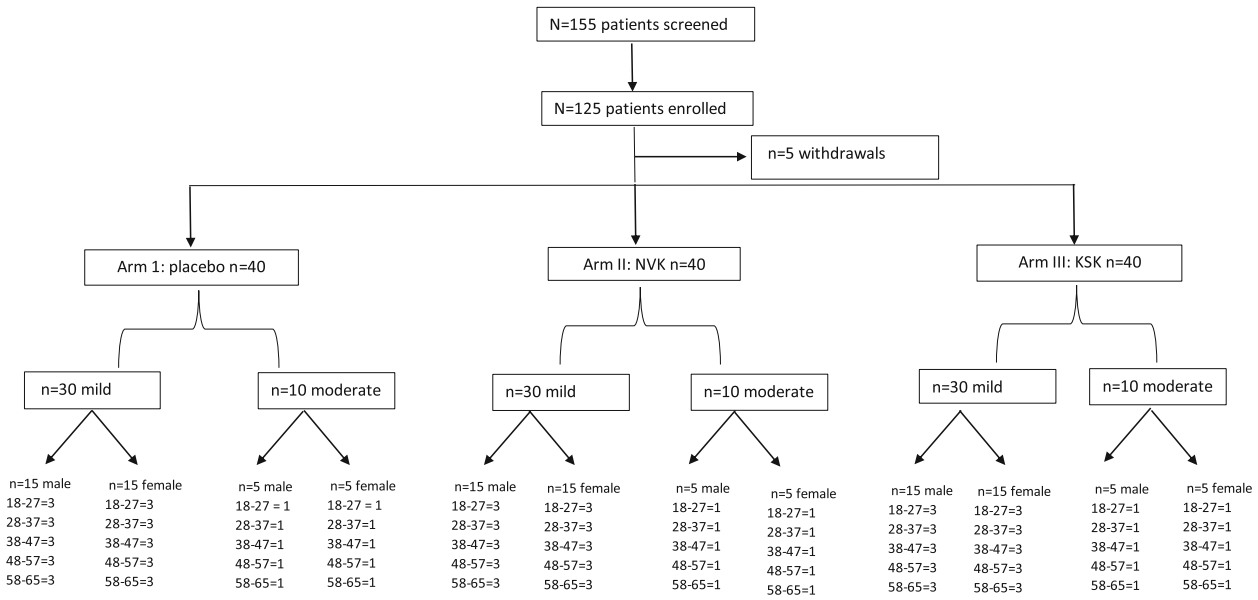

Fig. 3 Subject disposition displaying screening and treatment allocation of participants. A total 125 patients were enrolled, and 5 patients withdrew from study. Out of 120 patients, all were equally allotted into 3 groups each 40. In that 40, further divided 3:1 ratio for mild and moderate cases (based on ICMR, Min. of Health COVID-19 Criteria). Here, male/female patients were also equally distributed to all three groups. Randomization done by simple stratified randomization

Reduction in viral load of SARS-CoV-2

In order to know the viral load, RT-PCR was performed on days 3, 6, and 10. In Arm I, all patients were RT-PCR positive on day 3, whereas 38% of Arm II and 56% of Arm III got RT-PCR-negative. On day 6, patients tested RT-PCR-negative were 38% (Arm I), 62% (Arm II), and 44% (Arm III), respectively. On day 10, the remaining 61% of patients of Arm I were RT-PCR-negative whereas there were no patients on day 10 in both NVK and KSK arms, since all had got discharged by day 6. In comparison, the KSK treatment arm showed an early reduction in viral load as 56% of patients of this arm were RT-PCR-negative even on day 3 after admission.

Cycle threshold (Ct) values in RT PCR were analyzed at baseline (day 0) and day 3, and results are summarized in Table 4. At baseline, Ct values of three arms showed no significant difference (placebo=20.5, NVK= 21.2, and KSK=20.8, P=0.56). Ct values of patients in all three arms were analyzed again at the time of discharge and compared using Kruskal-Wallis test. On day 3, the mean Ct values were found to be significantly different among 3 arms (placebo=25.1, NVK=31.5, and KSK=33.1, P=0.0001). Statistically significant reduction in viral load of SARS-CoV-2 was recorded in both the Siddha treatment arms NVK and KSK compared to placebo (decaffeinated tea).

Time taken for patients to become asymptomatic

The average time taken for a patient to become asymptomatic from symptomatic in the standard Siddha treatment NVK and KSK arms was significantly less when compared to that taken by those in the placebo drug (decaffeinated tea) arm (Fig. 5). Time taken by patients to get asymptomatic from symptomatic were 2.5 mean days in the NVK arm; 1.7 in the KSK arm and 4.2 days in the placebo arm (Kruskal-Wallis test, P=0.0001). Similarly, patients in NVK arm and KSK arm took significantly lesser time (mean days) compared to placebo for both sore throat (NVK arm; 1.3, KSK arm; 1.3, placebo arm; 3.5, P value =0.0005) and short breath (NVK arm; 1.3, KSK; 1.3, placebo arm; 3.2, P value =0.0001, respectively).

Table 4 Cycle threshold (Ct) values analyzed on day 0 and day 3

Ct values	Arm I: Placebo Mean (SD)	Arm II: NVK Mean (SD)	Arm III: KSK Mean (SD)	*P* value
Day 0 (*n*=38, 37, 39)	20.5 (4.3)	21.2 (2.8)	20.8 (4.3)	0.56
Day 3 (*n*=34, 33, 35)	25.1 (3.79)	31.5 (5.11)	33.1 (4.57)	0.0001

Units: Cycle threshold
Ct values were not analyzed on days 6 and 10 as all the patients of Arm II and Arm III were discharged

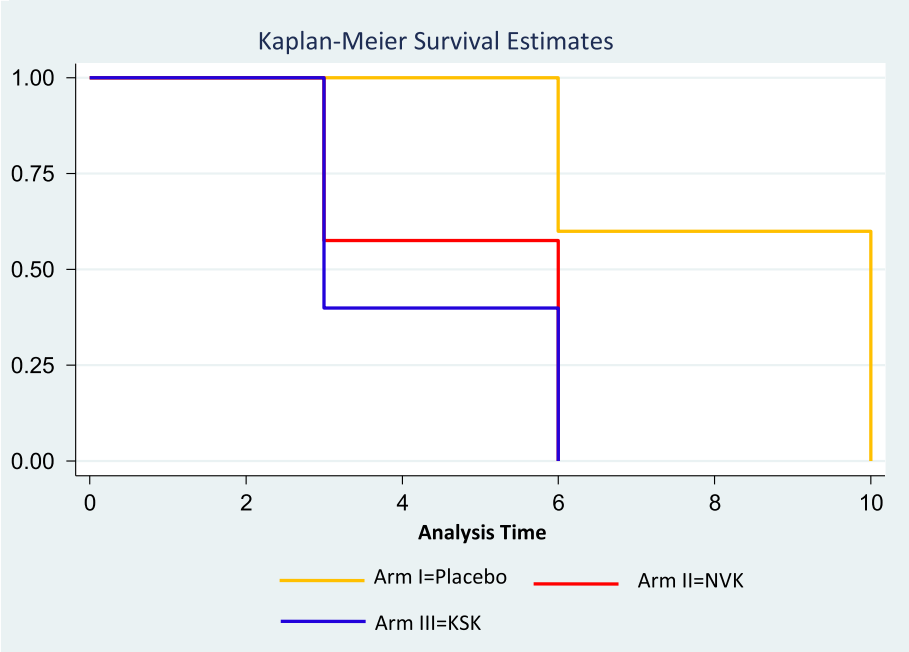

Fig. 4 Comparison of Hospital Stay Time Kaplan-Meier graph for Arm I, Arm II, and Arm III. Patients in Arm I stayed significantly longer (mean [SD]=8.4 [2.0]) as compared to the Arms II and III (mean [SD]=4.7 [1.5] and 4.2 [1.5], respectively, Kruskal-Wallis test, *P*=0.0001

Safety evaluation

In all three groups, only two adverse events (AEs) were reported. Mild episodes of AEs of vomiting and diarrhea were observed for a single day in Arms I and II. Both the episodes were reversed within a day and treatment was continued. AEs were notified to trial site IEC and DSMB (Data Safety and Monitoring Board, Ministry of AYUSH, Govt. of India), within the reporting timelines. No serious adverse events (SAEs) were reported throughout the study.

Mean variation in IL-6 value

One of the significant markers in COVID-19 disease is IL-6 which is indicative of immune response. During the study course, IL-6 values were recorded at baseline and on the day of discharge from the hospital (endpoint) as

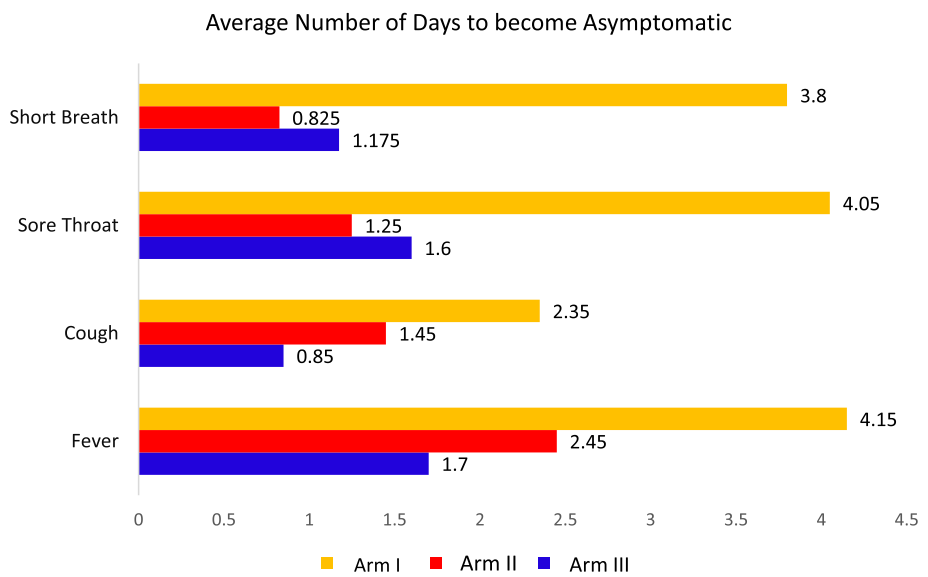

Fig. 5 Time taken to convert patients from symptomatic to asymptomatic for Arm I: placebo, Arm II: NVK and Arm III: KSK

Table 5 Biomarker parameters recorded at time of admission (baseline) and at time of discharge from hospital (endpoint)

	Arm I: Placebo Mean (SD)	Arm II: NVK Mean (SD)	Arm III: KSK Mean (SD)	*P* value
IL 6 baseline (*n*=38, 39, 34)	7.5 (4.4)	5.7 (4.2)	7.1 (4.0)	0.09
IL 6 endpoint (*n*=38, 39, 38)	**4.0 (2.9)**	**2.6 (2.5)**	**2.2 (1.3)**	**0.02**
LDH baseline (*n*=26, 25, 22)	17.2 (3.5)	16.6 (4.6)	17.3 (5.8)	0.61
LDH endpoint (*n*=24, 17, 13)	17.4 (5.6)	17.3 (3.9)	13.2 (5.6)	0.10
Ferritin baseline (*n*=26, 25, 21)	10.5 (4.5)	11.7 (5.3)	13.2 (5.8)	0.25
Ferritin endpoint (*n*=23, 16, 11)	11.4 (5.0)	11.0 (5.7)	10.6 (5.3)	0.95
D dimer baseline (*n*=26, 21, 13)	0.9 (0.7)	1.9 (3.6)	2.7 (6.8)	0.79
D dimer endpoint (*n*=16, 15, 7)	2.6 (4.8)	1.6 (2.6)	3.7 (7.5)	0.53
CRP baseline (n=24, 25, 22)	3.5 (2.8)	3.0 (2.4)	2.6 (1.5)	0.84
CRP endpoint (*n*=22, 19, 15)	3.9 (3.2)	2.5 (1.6)	3.1 (4.7)	0.26

Units: *IL 6,* picogram per milliter (pg/mL); *LDH*, units per liter (U/L); *Ferritin*, micrograms per liter (mg/L); *D-dimer*, nanograms per milliliter (ng/mL); *CRP*, milligrams per decilitre (mg/dL)

summarized in Table 5. Baselines IL-6 mean values were recorded as 7.5 of Arm I, 5.7 of Arm II, and 7.1 of Arm III. On the day of discharge (endpoint), IL6 values were showed a significant difference (Arm I 4.0, Arm II 2.6, and Arm III 2.2 and Kruskal-Wallis test, P=0.02) from baseline. This revealed an overall improvement in the IL-6 scores.

Mean variation in biomarker parameters

To evaluate the overall improvement across the three arms, the other biomarker parameters considered for evaluation were LDH, ferritin, D-dimer, and CRP. These parameters were recorded at the time of admission (baseline) and at the time of discharge from the hospital (endpoint) as summarized in Table 5. The total mean value of LDH was 17 at baseline and 16.4 at the endpoint. The total mean values of ferritin, D-dimer, and CRP were 11.7, 1.6, and 3.6 at baseline showing an overall improvement at the endpoint with the mean values of 11.1, 2.4, and 3.2, respectively. The overall change in CRP, LDH, ferritin, and D-dimer was found to be non-significant (P>0.05) amongst all 3 arms.

Conclusion

This is the first randomized controlled clinical trial to study the effectiveness of two classical Siddha herbal formulations, NVK and KSK, along with the standard allopathy treatment for COVID-19. Patients of NVK (Arm II) and KSK (Arm III) recovered faster than patients of placebo (Arm I) and spent fewer days in the hospital than those in the placebo arm (Arm I). All patients of both NVK and KSK arms were discharged by day 6 whereas maximum patients of the placebo arm were discharged only on day 10. Similarly, RT-PCR test was negative by day 6 in both Arms II and III, whereas for the placebo group, 61% were RT-PCR-positive. Additionally, patients of Arms II and III took significantly less time to become asymptomatic compared to the placebo arm. Between the Siddha treatments, the KSK arm showed more promising results than the NVK arm, as over 50% patients were discharged and found RT-PCR-negative even on day 3. Patients of KSK spent the least time in the hospital among all 3 arms. IL6 markers of Siddha treatment arms showed a statistically significant difference in comparison to the placebo arm. No SAEs were recorded throughout the study. The results of this trial suggest that NVK and KSK are safe and effective drugs in the management of mild to moderate COVID-19 disease when taken along with allopathy treatment.

In spite of the limited sample size, the effects of Siddha decoctions, both NVK and KSK, along with the standard of care allopathy compared to placebo have been confirmed. The effects of these drugs were also statistically significant and proved the efficacy of an integrative approach with allopathy for COVID-19 management. This trial also complies with the National Health Policy 2017 of integrative approaches of allopathy with traditional systems of medicines especially with

Siddha medicines. The result of this trial encourages the integration of Siddha medicines with allopathy in combating pandemics like COVID-19 and also in repurposing existing Siddha drugs. A large-scale, multi-centric clinical trial can help to make it robust and reproducible.

Abbreviations

AEs: Adverse events; ARDS: Acute respiratory distress syndrome; BA: Bronchial asthma; CCRSs: Central Council for Research in Siddha; CKD: Chronic kidney disease; CRP: C-reactive protein; CTRI: Clinical Trial Registry of India; COVID 19: Coronavirus disease; CT value: Cycle threshold value; DM: Diabetes mellitus; DSMB: Data Safety and Monitoring Board; EC: Ethics committee; GCP: Good clinical practice; GIMS: Government Institute of Medical Sciences; GFR: Glomerular filtration rate; HT: Hypertension; HPTLC: High-performance thin-layer chromatography; ICU: Intensive care unit; IL6: Interleukin 6; ICMR: Indian Council for Medical Research; KSK: Kaba Sura Kudineer; LDH: Lactate dehydrogenase; LFT: Liver function test; NVK: Nilavembu Kudineer; NKA: National Kidney Association; PPVC: Peripheral Pharmacovigilance Center; RFT: Renal function test; ECG: Electrocardiogram; RT PCR: Reverse transcription-polymerase chain reaction; SD: Standard deviation; SARS COV 2 : Severe acute respiratory syndrome coronavirus 2; SAE : Serious adverse events; TLC: Thin layer chromatography; OECD: Organisation for Economic Co-Operation and Development

Acknowledgements

The authors express their sincere gratitude towards the administrative and technical support given by the following experts.

1. **Brigadier Dr. Rakesh Kumar Gupta,** Director, Government Institute of Medical Sciences, (GIMS) Greater Noida, UP.
2. **Prof. Dr. Jugal Kishore, Director,** HOD-Department of Community Medicine, VMMC & Safdarjung Hospital, New Delhi.
3. **Prof. Dr. Padma Venkat, Dean,** School of Public Health, SRM Medical University, Chennai (as a Mentor assigned by the Project Monitoring Unit – Constituted by Min. of AYUSH, Government of India).

The authors also would like to appreciate the involvement of other supporting staff in this study from both the Siddha Clinical Research Unit, New Delhi, and the Government Institute of Medical Sciences, Greater Noida, Uttar Pradesh.

Authors' contributions

MR and AS conceived the study. MR, AS, and VN initiated and conducted the study. VG contributed to incorporate all lab investigations. MR, AS, VN, SS, and RU contributed to the conduction of the trial. JK performed the statistical analysis of the trial. MR, AS, SS, VN, and JK helped in the finalization of the study report. SP and KK provided technical guidance and administrative support for the conduction of the study. AV contributed to the administrative support and provision of the study drugs. The authors read and approved the final manuscript.

Authors' information

AS, SS, RU, and VG possess the background of allopathy.
KK, SP, MR, AV, and VN possess the background of the Siddha System of Medicine.
JK possesses a background in Biostatistics and Biotechnology.

Funding

The trial is funded by the Central Council for Research in Siddha (CCRS), Min. of AYUSH, GIMS Greater Noida, Uttar Pradesh, India.

Availability of data and materials

All participants' data will be kept confidential and personal identifiers of the study participants will not be disclosed to the public. Only the investigators will have access to the trial data. All the procedures will be carried out by strictly adhering to the Good Clinical Practices (GCP). The monitors will have access to the study documents.

Declarations

Ethics approval and consent to participate

The trial received ethical approval from the Institutional Ethical Committee of Siddha Clinical Research Unit, Safdarjung Hospital, New Delhi, on July 20, 2020, and trial site Ethics Committee on August 04, 2020.
This is to state that the appropriate ethical committee approval was taken. Written consent was taken from all eligible and willing participants before their participation.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Received: 8 June 2021 Accepted: 21 July 2021
Published online: 28 August 2021

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Publisher's Note

Published online in http://ijam.co.in ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 12 (2), 347-352

An Open-Label Clinical Trial to Evaluate the Safety & Efficacy of Siddha Sastric Medicines – Fixed Regimen in COVID-19 Positive Asymptomatic, Mild or Moderate cases - A Pilot Study

Research Article

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Abstract

Background: Covid-19 is a global pandemic since 2019. SARS-CoV2 is a new virus that originated from China and is currently spread across 160 countries. Siddha medicine is one of the traditional Indian medicines, part of Ayush that tend to treat several acute and chronic diseases. Aim: The objective of this study is to observe the safety and efficacy of Siddha regimen with lab parameters like LFT, RFT, RT-PCR, LDH, FERRITIN levels, and prevention of disease complications in covid-19 positive patients on the 7th day of treatment. Experimental Procedure: A non-randomized open-label observational retrospective study was designed. Twenty patients of either sex, of age between 18 and 60 years, were selected with proper consent. The covid patients who were confirmed by positive RT-PCR test results with or without clinical features of covid-19 were selected. They were treated with Siddha Regimen for seven days. Results: Sixteen out of 20 cases turned RT-PCR negative on their 7th day of treatment. And the Ct value of RT-PCR was statistically significant. LDH and Ferritin levels were reduced after the treatment even though the before treatment values are in the normal range. The LDH level was statistically significant on the 7th day of treatment. No Remarkable changes in the safety laboratory parameters like SGOT, SGPT, Blood urea, Serum Creatinine. Conclusion: Significant changes in efficacy laboratory parameters and no changes in safety laboratory parameters have been reported in the Siddha fixed regimen for covid-positive patients. All the 20 study participants were recovered without emergency and hospitalization.

Key Words: Siddha medicine, Covid-19, Indian Traditional Medicine, Ayush System of Medicine, Anti-viral, SARS-CoV2.

Introduction

Coronavirus disease (SARS-CoV2) is a life-threatening, major infectious disease that causes a global pandemic. It can affect all age groups. Although lesser mortality when compared with the MERS virus, it is causing panic all over the world. Although most infections are self-limited, about 15% of infected adults develop severe pneumonia that requires treatment with supplemental oxygen, and an additional 5% progress to critical illness. In severe cases, COVID-19 can be complicated by acute respiratory distress syndrome (ARDS), sepsis and septic shock, multiorgan failure, including acute kidney injury and cardiac injury (1). No specific antiviral drug has been proven effective for the treatment of patients with severe coronavirus disease 2019 (2). No one can predict the complications and serious events of this disease due to unknown clear Pathophysiology and direct anti-viral therapy. Most commonly the mortality is very high in above 50 age groups with co-morbid conditions like Diabetes, Hypertension, Bronchial Asthma, Cancer, etc.

Siddha's system of medicine is well known ancient Indian medical system. It contributes the biggest role in the management of Covid-19 disease. The ancient Siddha system of medicine considers the body as a conglomeration of three doshas known as *Vatham* (*Wind*), *Pitham* (*Fire*), and *Kabam* (*Water*), corresponding to the three elements of the universe. Equilibrium between the three doshas is necessary to maintain perfect health. Any derangement in the synergic action of these doshas transforms the body as a fertile ground to pop-up any infection (3). Siddha

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medicine has already played a major role in controlling the mortality rate of chikungunya and dengue in Tamil Nadu by the administration of *Nilavembu Kudineer* during 2015 (4). Many Siddha medicines have also been used during various viral outbreaks in Tamilnadu, like *nilavembu kudineer, aadathodai kudineer*, and *Kaba sura kudineer*. The primary aim of this study is to assess the safety and effectiveness of Covid-19 positive patients for Siddha Sastric Medicines and secondary is the role of Siddha regimen in preventing the disease severity on covid patients.

Pathophysiology

The SARS-CoV-2 infection enters the host cells through the S spike protein by binding to ACE2 for internalization and aided by TMPRSS2 protease. The high infectivity of the virus is related to mutations in the receptor-binding domain and acquisition of a furan cleavage site in the S spike protein. The virus interaction with ACE2 may down-regulate the anti-inflammatory function and heightens angiotensin II effects in predisposed patients (5). The invasion of the virus to the lung cells, myocytes, and endothelial cells of the vascular system resulting in inflammatory changes including edema, degeneration, and necrotic changes. These changes are mainly related to proinflammatory cytokines including interleukin IL-6, IL-10 and tumor necrosis factor α, granulocyte colony-stimulating factor, monocyte chemoattractant protein 1, macrophage inflammatory protein 1α, and increased expression of programmed cell death 1, T-cell immunoglobulin, and mucin domain 3 (6).

Comparison of Kaba Suram and COVID-19 (7)

The symptoms of Covid-19 are analogs with the symptoms of *KAPASURAM* mentioned in Siddha textbooks. In Siddha medicine, 64 types of fever are explained. One among them is *Kaba suram*. The Siddha textbook *Agsathiyar Sura nool 300* and *Sura vagadam* both explained about the *Kaba Suram*. The symptoms include *suram* (fever), *thondai varatchi* (sore throat), *nadukkam* (rigor), *vudal sorvu* (malaise), *vudal vali* (myalgia), *thalaivali*(headache), *vayirukazhithal* (diarrhea), *erumal* (cough), *marbil kozhaikattal* (sputum production) *mochu Vida siramum* (dyspnea), *mookkuneer paithal* (running nose), etc.

Materials and Methods

Trial registration

This clinical trial is approved by CTRI with registration no: ***CTRI/2020/08/027397*** [Registered on 26/08/2020] after getting approval from IEC of GSMC, Chennai with IEC No: ***GSMC-CH-3401/ ME-2/050/2019.*** All patients were given written information about the potential risks and benefits of participation in the study. Written consent was mandatory from each patient before inclusion in the clinical study.

Study Site - TPEC Covid-19 Care Centre, Vellore, Tamilnadu, India.

Selection of Drugs

Siddha Medicines' fixed regimen includes five Siddha Sastric medicines. All the study participants have been prescribed these medicines for 7 days. *Kaba Sura Kudineer Chooranam* converted into Decoction and administered 60 ml two times daily before 30 min of meals. *Kaba sura Kudineer* was prepared by adding 5 grams of powder with 240 ml of water and boiled into 60 ml. *Adathodai manapagu* was given 10 ml twice daily with warm water after meals. *Amukkara Chooranam Mathirai,* 500 mg tablets were administered in the dosage of two tablets three times daily after meals. *Thalisathi vadagam Mathirai*, 500 mg Chewable tablets were given two tablets three times daily after meals. *Brammanandha bairavam Mathirai* 100 mg tablets one (or) two pills administered two times daily after meals with honey depending upon the physical condition. All the medicines were procured from a standard GMP-certified IMPCOPS Company, Chennai.

Inclusion Criteria

The study included patients 18 to 65 years of age with COVID -19 Positive RT-PCR confirmed. COVID – 19 Positives with or without clinical signs and symptoms such as Sneezing, Cough, Sore Throat, Throat Pain, Malaise, Tiredness, Fever, loss of smell, loss of taste, chills were also included.

Exclusion Criteria

COVID - 19 extreme signs such as Respiratory distress (Respiratory Rate >24/ minute) and reduced Oxygen saturation (Spo2) < 95% at rest were excluded from this study. Patients with uncontrolled Diabetes HbA1c > 9% or FBS >140 mg/dl and stage 3 Hypertension (BP > 160/100 mmHg) and immune-compromised conditions like HIV, Hepatitis, Tuberculosis, Cancer were also exempted. The research did not include pregnant and lactating mot hers.

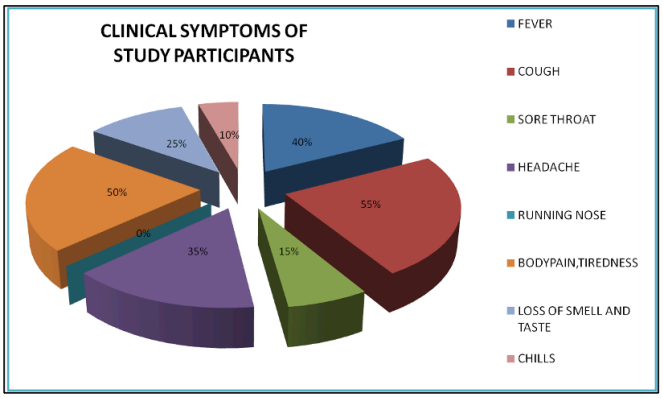
Selection of Cases and Observation of Data

From 47 positive cases, we obtained consent. 24 patients dropped according to the criteria on inclusion and exclusion out of 47 cases. In the remaining 23 cases, three have been reduced because of discontinuity. All other 20 cases were administered with Siddha-fixed regimen and the findings were evaluated by the protocol on the seventh day. Each case was documented with laboratory parameters.

Clinical Evaluation

Of the 20 subjects, 5 patients were asymptomatic during research inclusion. The remaining 13 patients showed distinct clinical symptoms and 2 were typical Influenza Like Illness (ILI). Seven cases were co-morbid in 20 cases. This study included 5 diabetic and 2 hypertensive cases.

Fig 1: Show Clinical Symptoms of Study Participants



Assessment of Haematological and Bio-chemical parameters and ADRs

The evaluation of the drug safety and efficacy was based on physical examination, vital signs, laboratory parameters like Complete Blood Count, LFT (Total, Direct, and Indirect Bilirubin, SGOT, SGPT, ALP enzyme levels), RFT (B.Urea and S.creatinine), CRP, LDH, FERRITIN, Prothrombin time and documentation of adverse effects. Participants were asked about any adverse effects daily during the rounds and the answers were recorded by the investigators. All patients were provided with the personal mobile number of the investigator for any emergency purposes. All the patients were frequently contacted through mobile phones during the study period for monitoring purposes. 7 ml of blood sample collected from all study participants on the 1st and 7th day of the intervention. The hematological and biochemical investigations of the clinical subjects before and after the clinical study were done in NABL accredited lab at GVMCH, Vellore.

Statistical Analysis

The results obtained were presented as mean+SEM. Safety and efficacy parameters were presented with a T-test to find the statistical significance. Values of $p<0.05$ were considered statistically significant, Values of $p>0.05$ were considered statistically not significant

Summary of Statistics

A paired T-Test was done on RT-PCR Results, Ct value before and after the Intervention to know the significance of Siddha intervention through statistical analysis. The negative test report is marked when RT-PCR Ct value reached 41. Most RT-PCR tests use Ct cutoffs of 35-40 cycles, so any sample with a Ct value below the cutoff, would be considered a true positive [19].The P-Value was significant (> .05) on RT-PCR, LDH, and platelet count results. It showed the efficacy of Siddha interventional medicines on covid-19 positive patients.

Table 1: Paired T-Test Results

Paired Samples Statistics					
Pair	**Mean**	**Std. Deviation**	**Std. Error Mean**	**t-value**	**P- Value**
B_urea_B	18.95	5.62	1.26	2.336	0.031
B_urea_A	16.35	3.72	0.83		
SGPT_B	27.05	13.08	2.93	-3.462	0.003
SGPT_A	51.55	34.43	7.7		
LDH_B	125.98	53.29	11.92	3.256	0.004
LDH_A	84.72	43.98	9.83		
Platelet_Count_B	273.35	61.46	13.74	-3.381	0.003
Platelet_Count_A	326.5	69.38	15.51		
RT_PCR_B	24.42	4.19	0.94	-11	<0.001**
RT_PCR_A	38.71	4.77	1.07		
SGOT_B	30.35	13.09	2.93	-1.847	0.08
SGOT_A	41.75	23.68	5.29		
Alk_Phosphatsase_B	80.45	15.98	3.57	-0.471	0.643
Alk_Phosphatsase_A	83.1	25.07	5.61		
Creat_B	0.85	0.14	0.03	0.754	0.46
Creat_A	0.83	0.2	0.05		
Ferritin_B	105.57	95.78	21.42	0.761	0.456
Ferritin_A	94.37	83.11	18.58		
Prothrombin_Time_B	13.69	1.26	0.29	1.04	0.312
Prothrombin_Time_A	13.37	1.54	0.35		

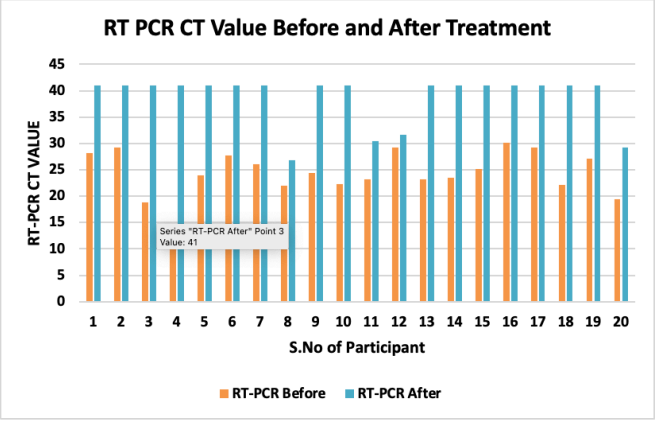
Table 1a: Significance and Non-Significance of the Results

S.NO	SIGNIFICANT	NOT SIGNIFICANT
1	B.Urea	SGOT
2	SGPT	ALP
3	LDH	S.CREATININE
4	PLATELET COUNT	FERRITIN
5	RT-PCR Ct value	PROTHROMBIN TIME

Results

Different clinical symptoms were reported in all of the 20 study patients under observation, with exception of 5 asymptomatic patients. It was observed that most of the clinical symptoms reduced on the fourth and fifth day of the intervention. 12 out of 15 symptomatic participants were entirely relieved from their symptoms like fever, cough, sore throat, fatigue, body pain, and chills on the 7 Th day of the intervention. The remaining 3 participants had mild symptoms like cough, fatigue after the intervention. There is a markable difference in the level of LDH, FERRITIN, and RT-PCR Ct value, Platelet count, Total Leukocyte count after treatment, on observation of the results (Table 1). And the safety lab parameters, like the B.Urea and S.creatinine, SGOT, SGPT, ALP enzyme levels, didn't significantly change. The level of urea in the blood before and after treatment is statistically meaningful (p-value < .05). The pre-and post-treatment level of SGPT is also statistically relevant (p-value <.05). The drug efficacy parameters LDH, Platelet count, and RT-PCR Ct value before and after treatment are also statistically significant (p-value < .05). The efficacy parameters LDH and Ferritin levels were reduced after the treatment even though the before treatment values are in the normal range. The levels of S. Creatinine, SGOT, ALP before and after treatment is (p-value > .05) statistically non-significant (Table 1 & 1a).

Fig 2: Shows RT_PCR CT Value Results before nad After Treatment



Discussion

The novel corona virus was identified as Severe Acute Respiratory Syndrome Corona virus 2 (SARS-CoV-2) which causes Corona virus Disease 2019 (COVID-19) pandemic (8). There is currently no custom-made drug for the treatment of COVID-19. The discovery of the SARSCoV-2 vaccine is ongoing, and extensive attempts are being made to create a specific drug for that too. Yet, establishing the effectiveness and safety of some new agents could take some time. Drugs such as ribavirin and corticosteroids have significant side-effects (9). It was suggested that the lower mortality rate in mainland China and the relatively rapid response in controlling the SARS 2002 outbreak could have been due to the inclusion of herbal formulations from Traditional Chinese Medicine (TCM) in the treatment protocols (10).

Traditional Siddha medicine, which is mainly practiced in Tamil Nadu (southeastern India) is quite well among Tamil-speaking people around the world. Its literature is entirely in Tamil, one of the oldest Indian languages (11). In, Siddha system, herbs are used primarily along with animal and mineral substances. The name of 'Siddha' medicine was coined by sages called *Siddhars*, and those are the origin of medicinal practices. The objective of Siddha medicine is to make the positive health, and imperishable and harmonious blending of physical, mental, social and spiritual welfare of an individual to promote longevity. The Materia Medica of Siddha system includes drugs of plants, metals/minerals, marine products and animal products. This system is mainly based on the relationship between the universe and human body by interlinking five basic elements such as air, fire, water, earth and ether (12).

For Siddha practitioners, the interventional Siddha drug regimen is no new. These medicines have long been used in southern regions of India for various ailments. For this study, *Amukkara mathirai*, which includes *Amukkara or ashwagandha (Withania somnifera),* is a natural immune enhancer and adaptogenic herb that has greatly helped to covid patients in isolation ward from anxiety and stress. *Amukkara* is well known for its anti-inflammatory property. Recent work has demonstrated that COVID-19 infections have a large immune component and can result in the development of cytokine storm, a potentially life-threatening immune reaction in which the body releases too many cytokines into the blood at a rapid rate (13). It has demonstrated that Withaferine is capable of reducing the secretion of various proinflammatory cytokines (ex. TNFα, IL-6, IL-8, and IL-18) in a metastatic model of ovarian cancer (14). *Thalisathi vadagam mathirai* is a chewable tablet comprising dry ginger (*Zingiber officinale*), pepper (*Piper nigrum*), pepper root, *thalisapatri* (*Taxus buccata*), etc.

Kaba sura kudineer contains 15 herbs which include proven anti-pyretic, anti-viral, and immunomodulatory herbs including *Nilavembu (Andrographis paniculata), Seenthil (Tinospora cordifolia)*, and proven anti-tussive, expectorant, mucolytic herbs such as *adathodai (Justicia adathoda), thippili (Piper longum), karpooravalli (Plectranthus amboinicus)*, etc. Totally 37 compounds were screened from *Kaba Sura Kudineer,* of these 9 compounds showed high binding affinity against SARS-CoV-2 spike protein in silico docking study (15). In *Adathodai manapagu, the Siddha herb Adathodai (Justicia adathoda)* has a natural mucolytic, expectorant, bronchodilator property, and it is also useful in increasing platelet count. The medication *bramanandha bairavam mathirai* is a herbo-mineral formulation that in Covid patients has tended to control fever and rigor. *Brahmanandha bairavam mathirai* and *Vishnu chakram* have been used for the treatment of pyrexic phase of Chikungunya (16).

Of the twenty cases, except five, the remaining fifteen were observed with at least any of the above-mentioned clinical symptoms. The effectiveness of the Siddha intervention clinically indicated an improvement in the symptoms of Covid patients. The platelet count, the overall number of leukocytes, has been substantially increased and this can be due to the immunomodulatory effects of the Siddha regimen. This study involved covid-positive patients with a RT-PCR Ct value of 13-30. The RT-PCR Ct value reached 41 on the 7th day in 16 patients out of 20 study participants, after the intervention and was found to be Covid-19 negative. It tends to be the Siddha Regimen's anti-viral benefit. Although correlations were revealed, viral loads determined by real -time RT -PCR assays should not be yet used to indicate COVID -19 disease severity or to monitor therapeutic response. However, low Ct values indicating high viral loads may be used as an indication of transmissibility.

Elevated LDH levels seem to reflect that the multiple organ injury and failure may play a more prominent role in this pathology in influencing the clinical outcomes in patients with COVID-19 (17). Laboratory findings in patients with severe COVID-19 showed data consistent with cytokine storm involving elevated inflammatory markers, including ferritin, which has been associated with critical and life-threatening illness (18). The anti-inflammatory effect and effectiveness of the Siddha regimen in Covid-19 patients tends to be a substantial decrease in the levels of LDH and Ferritin after treatment.

When treated with the Siddha Regimen, several of the symptoms gradually subsided within seven days. No major changes were observed in the RFT, LFT marker levels that validated the safety of the Siddha regimen in covid-positive patients. Although the study was involved, only one study case was seen with hyperbilirubinemia. Upon observation, the total level of bilirubin was also lowered in that same case after the intervention.

No patients reported major adverse events and disease complications such as reduced oxygen saturation, cytokine storm, coagulopathy, viral-induced purpura, etc., on general study observation. It thus revealed the effectiveness of Siddha interventional drugs in Covid patients and helped to minimize patient hospitalization. Consequently, in *KABASURAM* and Covid -19, the Siddha regimen contains herbs and herbo-mineral regimens which are more beneficial. On improvement in clinical symptoms and with negative RT-PCR results, the study cases were discharged.

Concomitant Medications

Of the three out of 20, ORS powder was provided for their malaise and fatigue. Just one participant in the sample was administered with *Nilavembu kudineer* to manage fever. Nearly 6 study participants were treated with Siddha drugs *Elathy chooranam mathirai and Sangu parpam mathirai* for their gastric disturbances. Of the 20, only 2 were administered with the antihypertensive drug amlodipine 2.5 mg OD or BD during the study due to the rise in blood pressure. Just one study participant was handled with Siddha medication *Triphala chooranam* externally to wash his skin rashes. Steam inhalation has been prescribed for all patients with headache.

Conclusion

The case study of 20 positive patients with Covid shows that the formulation of Siddha Regimen has shown a strong response to the symptoms of Covid. On the 7th day of their treatment, 16 out of 20 turned negative. Out of 20, 15 were effectively treated for their symptoms. The effectiveness of the Siddha regimen on covid-positive patients was shown. No major adverse drug reaction (ADR) was triggered by the drug. In adolescents and geriatric age groups without any ADR, this combination is a safe and successful traditional symptomatic intervention to eliminate any covid complication. The administration of the preferred drug thus avoided hospitalization, thus minimising the expense of human productivity. To treat this devastating disease and manage the pandemic, India can use the abundance of comprehension existing in the Indian Systems of Medicine. This is also a valuable way to recognize the effectiveness of the Siddha system of medicine. But a limitation of this study is the 20 sample scale. In a wider population, this can also be accomplished.

Conflicts of interest

We announce that there is no conflict of interest in this study

Funding

This study earned no particular grant from public, private, or non-profit funding organizations.

Declaration of competing interest

The authors do not report any conflicts of interest. The authors themselves are responsible for the outcome and drafting of the paper.

Acknowledgement

Mr. N.P.Vinoth, Statistician, SCRI, Chennai is acknowledged for statistical report.

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Current Traditional Medicine

Title: Survival Analysis based on the Siddha Body Constitution of Asymptomatic SARS-CoV-2 Patients under Integrative Management of Hydroxychloroquine (HCQ) and Kaba Sura Kudineer (KSK): A Retrospective Cross-sectional Case Series from Tirupati

VOLUME: 7

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Keywords: Body constitution (https://www.eurekaselect.com/search/aws_search.php?searchvalue=Body constitution), COVID-19 (https://www.eurekaselect.com/search/aws_search.php?searchvalue= COVID-19), hydroxychloroquine (https://www.eurekaselect.com/search/aws_search.php?searchvalue= hydroxychloroquine), length of stay (https://www.eurekaselect.com/search/aws_search.php?searchvalue= length of stay), novel coronavirus (https://www.eurekaselect.com/search/aws_search.php?searchvalue= novel coronavirus), siddha medicine (https://www.eurekaselect.com/search/aws_search.php?searchvalue= siddha medicine), yakkai ilakkanam. (https://www.eurekaselect.com/search/aws_search.php?searchvalue= yakkai ilakkanam.)

Abstract: Background: The present study analysed the impact of the integrated medical care of hydroxychloroquine (HCQ) and Siddha herbal preparation KSK on asymptomatic COVID-19 patients based on their body constitution.

Objective: The present study aimed to analyse the duration of the hospital stay of asymptomatic COVID-19 patients treated with the integrated medical care of hydroxychloroquine (HCQ) and herbal decoction of Kaba Sura Kudineer (KSK).

Design: The study included a retrospective case series of 19 asymptomatic confirmed SARS-Cov- -2 patients from District COVID Care Centre, Tirupati, India, between 23rd May to 7th June 2020.

Methods: Clinical data were collected using a standardised case report form containing demographic information, length of hospital stays, and Siddha Yakkai Ilakkanam (body constitution) from the records. The association between the length of hospital stay, age, gender, and Siddha YI for the confirmed patients after admission was analysed by the Kaplan Meier survival analysis method.

Results: Patients belonging to the Aiyam group stayed for at least nine days in the hospital, and 80% took ten or more days to cure the disease. About 71.4% took more than four days and three days of hospital stay in the Azhal and Vali groups, respectively. It was observed that 75% of females and 73.3% of males took nine days or more of hospital stay, respectively. The range of hospital stay was between 2-15 days for patients aged between 19 – 40 years.

Conclusion: The present study explored the significance of integrating Siddha medicine with Western medicine in the management of SARS Cov-2 infection. An overall median of 9 days in the length of stay and 8.5 days in the overall mean survival time was documented. The patients of the present study on integrative treatment recovered about nine days earlier in comparison to the patients studied in Vietnam and China.

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Siddha and Biomedicine Integrative Management of Novel Corona Virus Disease - A Case Report

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Abstract:

In the wake of Covid-19 pandemic, Traditional Siddha Medicine has gained wide popularity in Tamilnadu. A 39 year old female Covid positive patient is presented here under who was introduced with the combined therapy of Siddha and biomedicine along with dietary advice and standard quarantine care. The subject had all the peculiar clinical features of Covid 19. *Nilavembu kudineer* (NVK) was administered twice daily at the dose of 60 ml along with the prescribed biomedicine. Qualitative SARS-COV-2 RT-PCR test was used as the confirmatory test for Covid 19 along with basic haematological and biochemical parameters. After the initiation of integrative medicine, the subject showed improvement symptomatically and gradual disappearance of symptoms without adverse effects. RT-PCR after 14 days of therapy reported negative and the subject was greatly relieved. This case report suggests the importance of safer and effective integrative drug therapy using Traditional Siddha Medicine and biomedicine.

Keywords: Alternative medicine, Covid-19, Integrative and Complementary Medicine, *Nilavembu kudineer*, SARS Cov-2, *Siddha*

Received: 18.09.2020 Revised: 25.09.2020 Accepted: 28.09.2020

Quick Response code

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Introduction:

The burden of Covid 19 pandemic is increasing in a faster pace. As of now the total active Covid cases in Tamilnadu is 5, 69,370 with reported 9148 deaths. [1-2] In the current status, the entire health care system along with the indigenous medicines is focussed for a positive outcome either for its prevention or its management. Traditional Siddha medicine (TSM) is popular in Tamilnadu as the native medical system of treatment that has a long history of customary, culinary and medical practice bounded with the Tamil tradition.[3] The medical system composes numerous potent formulations for treating simple ailments to dreadful diseases. The long history of practice has proved its exquisite role in managing epidemics through the systematic approach consisting of drug, diet, therapies and life style modifications.[4] The preventive concept of Siddha medicine not only covers the general community prevention protocols, but also stresses on the case specific dietary and lifestyle modifications based on the subjects *Nadi* (Pulse diagnosis) and *Dehi* (Body temperament). Few of the classical medicines are supported as a combination, aimed for improving the general health and immunity and as a remedy for both upper and lower respiratory illness.[5] In this case report, a Covid positive individual was subjected to combined therapy with Siddha and biomedicine for documenting its beneficial role in integration. One of the Siddha classical decoctions *Nilavembu Kudineer* (NVK) along with standard care and diet was found effective in this case. [6]

Case Report:

A 39 years old healthy woman, a bank employee in Chennai, Tamil Nadu was suffering with the following complaints namely loss of smell, tastelessness, sensation of heat and body pain for 4 days. It started apparently on one day morning as a one sided head ache, which was continuous and progressed throughout the day. The headache was gnawing in nature without any relation to food, stress or sleep. There was no associated nausea, vomiting or vision disturbances but the subject felt some gastric disturbances, bitter taste in tongue, tastelessness while having food, loss of appetite, mild skin rashes and frequent loose stools on the same day. She started feeling pain in all the joints, heaviness of body from the 2nd day onwards along with a feverish feeling inside. On the 3rd day she observed the progressive loss of smell from morning itself. Initially she was able to appreciate local odours then she became unable to sense food articles and pungent food substance, finally to perfumes. Furthermore, adding to her suffering she got a mild sore throat and dry cough. Throughout the period she was not having any complaints of cold, rhinorrhoea, sneezing or nasal congestion. On examination, the subject was weak and pale. There were neither rashes nor petechiae. She had tenderness over all the joints without swelling.

She was euthyroid, non-diabetic and did not report any history of hospitalization, pneumonia, tuberculosis, influenza, bronchial asthma and allergy. None of her family members reported any symptoms or been diagnosed for Covid 19. She did not report any travel history but however she

indicated that four colleagues working in the same bank were affected by COVID. So accordingly, she was advised to isolate herself due to the current pandemic and screen for a qualitative SARS-COV-2 (COVID 19) RT-PCR test (Oropharyngeal swab specimen) along with basic haematological and biochemical parameters. She was confirmed with a positive Covid 19 test report. Her haematological investigation reported a low profile in almost all the parameters. It was inferred that she may have acquired the infection from the bank where she was working regularly with exposure to colleagues and multiple clients.

Therapeutic Intervention:

Nilavembu Kudineer was advised for a period of 2 weeks according to the advisory of Ministry of AYUSH and guidelines for Siddha practitioners published by the Ministry of AYUSH.[10] Standard prescription from an allopathic doctor for 2 weeks was scheduled as LDH and ALT was elevated indicating cytokine storm. The medicines were separately taken by following a time gap (Table 1). Counselling was done to alleviate fear and instil confidence to the subject. She was free to follow a normal diet, to include plenty of fruits with some restrictions to avoid chilled food and drinks till the recovery period. Chukku kanji (dry ginger gruel) was also prescribed to take regularly as a healthy recipe. Follow up was done telephonically every day for monitoring the prognosis, or for any untoward effects. She was advised to strictly follow home isolation and quarantine schedule as per the Ministry of Health and Family Welfare (MOHFW) guidelines.[2]. In addition, she was advised to measure her oxygen saturation (Spo2) levels using a pulse oximeter three times a day and her SpO_2 levels remained between 98-99 and pulse rate ranging between 72-76/min.

Table- 1: Oral medicine Schedule:

Intervention	Drug Name	Dose	Timings
Siddha Medicine	*Nilavembu kudineer*	60 ml (Day -1 -14)	Twice daily before food
	Chukku kanji (Gruel prepared with dry ginger): 5 g powder of dry ginger boiled well with rice gruel	1 bowl (Day -1 -14)	Thrice daily before food
Modern medicine	Acyclovir	800 mg (Day -1 -7)	5 times a day (6 a.m, 10 a.m, 2 p.m, 6 p.m and 10 p.m) after food
	Methyl Prednisolone	8 mg (Day -1 -7)	Thrice daily after food
		4 mg (Day -8-9)	Thrice daily after food
		2 mg (Day -10 -11)	Thrice daily after food
		2 mg (Day -12-13)	Twice daily after food
		2 mg (Day -14)	once daily after food

Table -2: Results – Haematological and Biochemical Investigations:

Parameters	Day 0	Day 14
Erythrocyte (RBC) Count	4.55 mill/cu.mm	4.62 mill/cu.mm
Haemoglobin (Hb)	10.6 gm/dL	10.8 gm/dL
PCV (Packed Cell Volume)	34.3 %	37.0 %
MCV (Mean Corpuscular Volume)	75.4 fL	80.1 fl
MCH (Mean Corpuscular Hb)	23.3 pg	23.4pg
MCHC (Mean Corpuscular Hb Concn.) 32-36	30.9 g/dL	29.2 g/dL
RDW (Red Cell Distribution Width)	17.1 %	16.1 %
Total Leucocytes (WBC) count	3800 cells/cu.mm	15130 cells/cu.mm
Neutrophils	38 %	67.20%
Lymphocytes	54 %	29.50%
Monocytes	3 %	1.90%
Eosinophils	4 %	0.40%
Basophils	1 %	0.30 %
Platelet count	365 10^3 / µl	5.61 Lakhs/cmm
SGPT (ALT)	134 U/L	22.1 U/L
SGOT (AST)	Not done	8.2 U/L
ESR	30	Not done
D-dimer	0.50 FEU	0.35 FEU
CRP	0.71 mg/L	0.4 mg/ L
Serum ferritin	16.4 ng/ml	8.86 ng/ml
LDH	304 U/L	Not done
Fibrinogen	Not done	340 mg/dl

Outcome of the intervention:

The outcome was assessed based on the improvement of symptoms and a repeated Covid test supported by basic haematological and biochemical investigations on day 14 of the intervention (Table 2). It was observed that with the integrated therapy, the subject was improving and the well-being of the subject was maintained with no downfall after the initiation of integrative therapy. She regained her appetite and was mentally fit and confident than before. She did not report any form of adverse effects or discomfort with the combined therapy. Her Covid-19 (SARS-CoV-2) Qualitative RT-PCR test reported negative with overall improvement in haematological and biochemical profiles.

It was observed that the total leukocyte count (TLC) was reduced to 3800 cells / cu.mm indicating mild leucopenia on day zero and was elevated to 15130 cells / cu.mm indicating leucocytosis on day 14 whereas Alanine Amino transferase (ALT) - SGPT was raised to 134U/L on day zero which later was found to be within normal limits (22.1 U/L) on day 14. The subjected exhibited neutropenia, and mild increase of ESR on day 0. The other important prognostic markers classic of Covid-19 such as serum ferritin, C – reactive protein (CRP), and D-dimer was within limits on day zero except increased LDH to 304 U/L, a sign of cytokine storm.

The reason for the higher leukocyte count on day 14 could be a possible urinary tract infection with increased pus cells and epithelial cells in complete urine examination. Moreover, the patient complained of occasional burning micturition and passing of yellowish urine. In this direction, the next day early morning sample was subjected to urine culture and sensitivity examination but reported as no growth in the urine culture with subsequent relief of aforesaid urinary symptoms with an advice to consume two and half litres of water daily.

Discussion:

Covid 19 is a viral infection of pandemic in nature caused by a novel corona virus or Beta CoVs. It is transmitted through droplets from cough or sneezing from an infected subject, and recently aerosol transmission also has been reported. [7] In TSM, the symptoms of Covid 19 could be more or less correlated with the Siddha terminology '*Iyya suram' or Kaba suram*.[8] *Iyyam* typically denotes phlegm humour as one of the main manifestation and *suram* indicating febrile conditions. It is a generic diagnostic terminology used by the Siddha physicians to denote febrile conditions with or without infections origin, in which there is either vitiation, aggravation of *iyyam* pertaining commonly to respiratory system as well as affecting other systems .[8,9] These group of phlegmatic fevers shows a replica of clinical symptoms as that of Covid like respiratory illnesses namely fever, myalgia, malaise, sore throat, cough, dyspnoea or shortness of breath.[8] TSM advocates a holistic approach for the prevention and management of Covid 19 primarily aimed for elimination or correction of the phlegmatic aggravation and its clinical association through the administration of herbal, herbo-mineral medications and other therapies.

The medications depend on the stage of the illness, and in cases useful as preventive and in palliative care for Covid. Ministry of AYUSH in coordination with the stake holders of TSM had published the guidelines for Siddha Practitioners for managing Covid illness through Siddha medicine.[10] The guidelines incorporate several immune enhancing dietary advice, internal medications and *Varmam* (Pressure therapy) in a stage wise and systematic order.

One of the important concoctions of the guideline as well as advisory dated 6th March 2020 from Ministry of AYUSH is *Nilavembu kudineer* (NVK) was administered to the subject along with the allopathic intervention. NVK, a classical polyherbal formulation which is prepared as decoction is very popular for its efficacy in managing different manifestations of fever, particularly of viral origin.[11] It is composed of 9 herbs in course powder form, in which *nilavembu* (*Andrographis paniculata* Burm. f.Nees) is the key ingredient.[6] It is reported in one of the studies that the ingredients of NVK and its potential lead molecules possess anti-viral, anti-plasmodial, anti-inflammatory, hepato-protective, anti-oxidant and immune-modulatory properties in general.[12,13] One in-silico study, reported that the bioactive compounds of aqueous extract of NVK (Benzene 123 Triol) has shown its binding and blockade effects against the ACE2 receptor, the prime drug target of Covid 19 virus. [14]

The dengue outbreak in Tamilnadu in the year 2012 witnessed the clinical potential of NVK, when the government and the health department (Tamilnadu) highly recommended it to the public for its broad spectrum role in prevention of outbreak and managing the burden associated with it. [14] Initiatives thus were taken to promote its usage in all the government clinical establishments. The effect of NVK is further justified through its fundamental six taste theory referred in Siddha medicine. [3] The primary taste of NVK is bitter and the associate taste pungency would impart a greater role in determining its hot potency to mitigate *kabam* or *iyyam*. The bitter taste suggests that NVK possess germicide, anti-microbial, blood purifying and anti-pyretic properties whereas pungency eliminates stagnated phlegm which is considered as one of the underlying causes for advancement of any ailment particular to lung or other systemic illness. [3]

In this particular case, NVK was administered twice a day before food for a period of 14 days. The subject was asked to prepare the concoction by herself, by boiling 5 grams of NVK powder in 240 ml potable water and reducing it to 60 ml i.e., ¼ th of the volume which has to filtered and consumed before it cools. Based on anecdotal evidences, the authors infer that her improvement with the symptoms is specific to the NVK decoction. The improvement in haematological parameters like TLC and LFT may be attributed to its anti-viral and hepato-protective effects. Its mitigating effect on Covid 19 virus could be explained on the basis of six taste theory, its potential lead molecules with ACE2 inhibitor effect and its specific action which have an overall support in Covid care.

Conclusion:

The case report throws light on the importance of including TSM along biomedicine in the fight against Covid 19. TSM offers a better means of its time tested safer formulations in treating epidemics, or to reduce the health burden of Covid 19 victims.

Limitation of study:

This is single case study it needs systematic clinical studies on large samples that are better appreciated for validating remedies mentioned under TSM for Covid 19.

Written consent:

The study was verbally explained to the patient and written consent was obtained. For the ethical purpose, if any poor prognosis were suspected then the patient would have been referred to a higher centre as per government norms.

Acknowledgements:

The authors acknowledge the guidance given by Dr. V. Balamurugan, Siddha Physician and Consultant, Amrutha Centre for Siddha Medicine, Chitlapakkam, Chennai – 600 064, Tamil Nadu. Moreover, the authors thank the panel of allopath doctors and especially Dr. L. Mugunthan, Former Research Officer (Modern Medicine), CCRS for their guidance in providing advice wherever necessary. In addition, the authors thank the technical section of CCRS for their assistance and timely help to accomplish this paper.

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Conflict of interest: Author declares that there is no conflict of interest.

Guarantor: Corresponding author is guarantor of this article and its contents.

Source of support: None

How to cite this article:

Vinayak S, Eugene W, Kanakavalli K. Siddha and Biomedicine Integrative Management of Novel Corona Virus Disease – A Case Report. Int. J. AYUSH CaRe. 2020; 4(3):154-160.

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OPEN ACCESS Freely available online

Journal of Clinical Trials

Commentary

Commentary on COVID-19 Clinical Trials in India Based on CTRI (Clinical Trials Registry-India)

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STUDY DESCRIPTION

Convergent effect of multiple efforts made by Indian scientist and physicians to find effective drugs for Covid-19 is reflected in CTRI (Clinical Trials Registry- India) [1]. Allopathic and AYUSH systems share the total number of registered Clinical trials (n=233 as on from 1st March 2020 to 22nd June 2020). Out of these, 146 were Interventional trials, 84 Observational trials, and three Post-marketing surveillance. In most of the trials, the interventional agent is either multiple drug combinations or compound drug formulations compared to single drug administration. Among the trials, 46 Allopathic interventions, 41 Ayurveda interventions, 14 Homeopathy interventions, 11 Siddha Interventions, one in Unani, and 2 in yoga and Naturopathy.

Preparedness of various Indian health stakeholders controlled by two Ministries, one dealing with the conventional therapy (Ministry of Health and Family welfare) and the other with Indian traditional medicine (Ministry of AYUSH) could be tracked from CTRI. Clinical trial registries also reported newer ideas and newer interventions. The kinesis of conducting a Clinical trial during a pandemic is different from the normal situation affirmed by MEURI guidelines [2,3]. The decision of Govt. of India to register every clinical trial in CTRI has created accountability and responsibility during pandemics. Ministry of AYUSH has released a particular GO, regulating the clinical trial during COVID -19 [4].

Randomized, non-randomized, observational studies, including Questionnaire, were the frequent Study designs. Global trials were very minimal, indicating distancing also in research. This pandemic has raised traditional medicine status in the country, which is evident from equal sharing of the total registered clinical trials. This may also be a reason for lesser mortality in a thickly populated country [5,6]

Allopathy and AYUSH's health sectors have attempted rapidity, which shows their surge in acting against COVID -19. Revalidated interventions that have been used earlier in Dengue, Malaria, Immunomodulators and Earlier anti-virals and drugs used in Cancer and HIV are among the selected repurposed interventions. Prophylactic interventions equate therapeutic studies, and AYUSH interventions have a major role. Integrative clinical studies are higher in number during Covid–19. Therapeutic exploratory and therapeutic confirmatory precedes safety trials; however, some studies using a repurposing technique checked the effects of the lozenges available in the market. Even though there are quite a few blinded studies (Double, triple, quadruple), the blinding tendency is minimal (14%) due to non-conformity in therapeutic interventions. However, research institutions and medical colleges allowed the country to make effective participation. In a particular time, sub-continent had 107 trials more than China, the epicentre of Covid -19 [7]. The outcome objectives were symptom reduction, getting negative in RT-PCR, reduction in hospitalization, minimal use of ICU, ventilators, and reduced mortality were the objectives observed. Post COVID-19 management clinical trials were not registered during the said time. However, there exists a lot of scope and necessity to do trials in Post Covid prospectively. These clinical trials confirm the preparedness of the subcontinent in pandemic mitigation.

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Received date: December 24, 2020; **Accepted date:** January 08, 2021; **Published date:** January 15, 2021

Citation: Devi MSS, Sathiyarajeswaran P (2021) Commentary on COVID-19 Clinical Trials in India Based on CTRI (Clinical Trials Registry-India). J Clin Trials. 11:446.

Characteristics of COVID-19 Clinical Trials in India Based on the Registration Data on CTRI (Clinical Trials Registry- India): a cross-sectional analysis

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Abstract:

Objectives:

The 2019 pandemic of coronavirus disease (COVID-19) has prompted several efforts to find safe and effective drugs, but little is understood as to where early efforts were centered. Several clinical trials, both Allopathy and AYUSH medicines have been registered in the Clinical Trial Registry of India (CTRI). We aimed to characterize and extract relevant data registered under CTRI for COVID -19.

Materials and Methods:

A cross-sectional analysis was performed of clinical trials for the treatment of COVID-19 that were registered in the Clinical Trial Registry of India (CTRI) from 1st March 2020 to 22nd June 2020. Relevant trial records were downloaded, deduplicated, and independently analyzed by three reviewers.

Main outcomes:

Trial intervention, design, sponsorship, phase of the trial, and indicated outcomes.

Results:

233 COVID-19 clinical trials, was registered from India in CTRI. Out of these, 146 were Interventional trials, 84 Observational trials, and three Post-marketing surveillance. Questionnaire and survey-based intervention occupy a significant portion. Randomized control trials are large in number 37.8% than non-randomized. 20% of the trials were recruiting patients, and the Research institution (34%) sponsored more than half of the trials. Global trials are minimal, occupying 3% of total trials and Indian trials were 97%. In most of the trials, the interventional agent is either multiple drug combinations or compound drug formulations compared to single drug administration. Among the trials, 46 Allopathic interventions, 41 Ayurveda interventions, 14 Homeopathy interventions, one in Unani, and 2 in yoga and Naturopathy.

Conclusion:

This study will provide a background of COVID-19 clinical trials registered in CTRI and provide specific issues observed related to clinical trial designs, which offer information to perform clinical trials on COVID-19

Keywords: COVID-19, CTRI, Clinical Trial Registry of India, Ministry of AYUSH, Clinical trial, Interventional, Randomized, Blinding.

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Introduction:

Since Coronavirus Disease 2019 (COVID-19) [1] emerged from Wuhan, China, in December 2019, has become pandemic and rapidly crossed all countries by reporting cases by April 2020[2]. There are 4 10 461 cases and 13 254 deaths in India as of 30th June 2020. These data correspond to the imminent risk facing the country. Transmission of the virus spreads via droplets, physical contact with infected individuals, contaminated surfaces [3]. Fever, Cough, Headache, and Throat pain are the most common symptoms [4]. The severe infection leads to pneumonia, acute respiratory distress syndrome (ARDS), and sometimes multi-organ failures such as kidney failure and even death [5]. Coronaviruses (CoVs) are the family of Coronaviridae with four gene era (alpha, beta, gamma, and delta), and only the alpha and beta- strains identified to be pathogenic to human [6, 7]. There are no specific anti-viral drugs to treat COVID-19. As of now, symptomatic supportive therapy will be the treatment protocol. Govt of India has released guidelines via the Ministry of Health and via the AYUSH Ministry for mitigation of COVID-19. However, medical researchers on both the ends are rigorously working for solutions to combat COVID-19. Ethical issues involved in

these attempts should guide by existing Meuri guidelines that evolved during the Ebola outbreak.

In this context, it becomes mandatory to analyze the conduct, creditability, ethical issues, willingness, consensual integration; intervention used out of pocket expenses, availability of the drugs, and drugs that emerged out of Indian origin. Above all, the characters were extracted from the trials registered in Clinical Trials Registry—India (CTRI).

The Clinical Trials Registry—India (CTRI) is a free and online public record domain for registration of clinical trials conducted in India from 20th July 2007. Drug Controller General of India has made it mandatory to register clinical trials at CTRI since 15th June 2009. Registering clinical trials is a contemporary issue in present health researchers [8]. The analysis performed using the data available online with CTRI helped us look for the characters mentioned above in the purview of COVID-19, which may help more in future pandemics. In the present study, the content from clinical trials registered in India was summarized and analyzed from various perspectives and design of clinical trials for COVID-19.

Materials and Methods

Search Strategy

'COVID-19' and 'INDIA' 'Coronavirus' or 'COVID-19' or 'COVID19' or '2019 novel coronavirus' or '2019-nCoV' or 'SARS-CoV-2' (were used as the keywords to search for all COVID-19 clinical trials registered from India on CTRI (Clinical Trials Registry – India) [9] from 1st March 2020 to 22nd June 2020. All retrieved records were downloaded. The following data were collected: study title, study type, study design, primary outcome measures, condition, Number of groups, Intervention, blinding, phase of trials, and sample size. A standard Microsoft-Excel database was created for the analysis.

Trial selection

We included all studies conducted on patients diagnosed with COVID-19. First, we selected clinical trials based on the 'study type' variable. The variable contains interventional and observational studies and others. We included all AYUSH system trials, and our study does not have any limits based on the outcome.

Statistical Analyses

All continuous values were expressed as a median and interquartile range, and categorical variables are expressed as percentages. All statistical procedures were performed using the SPSS software (Version 26).

Data extraction and management

We extracted the following information from each trial: CTRI trial number, registration date, recruitment status (recruiting, not yet recruiting, withdrawn or canceled), recruitment country, phase (0, 1, 2, 3, 4, 1–2, 2–3, not applicable and missing), sponsor, health condition, an intervention model (single-arm, parallel, cross-over, factorial, platform trial or sequential), type of study, study design, sample size, trial duration, allocation, blinding (open, single, double, triple or quadruple), primary outcomes.

Results

The Number of COVID-19 Clinical Trials Registered in India, 233 COVID-19 clinical trials, was registered from India in CTRI. After the general analysis, 146 Interventional trials, 84 Observational trials, and 3 Post-marketing surveillance were registered. The rates of trial registration are incoherence, the incidence, and the intensity of the disease. There is an ascending pattern in registration from March reaching its peak in May and slowing down in June. India had registered 107 more trials than China in a set period of three months (n- India (233) - n-China (126) = n=107) (Table – 1)(Figure – 1,2)

The recruitment status has been calculated here at least fifteen days after the trial is registered in CTRI. At this point, not less than 50 % of the trials have not started recruiting. COVID-19 is a peculiar condition wherein prophylaxis plays an important role, which is evident from 32% of the people either in the survey or as a prophylactic intervention have been registered under the category as healthy volunteers. However, it is also noteworthy that 62% of the trials are COVID-19 affected individuals, which may benefit human society by and large (Figure -3)

The intervention of various Medical systems are described in descending order; surveys are dealt with separately. Allopathy and Ayurveda have a significant share of (25% each) followed by Homeopathy 5.6%, Siddha 4.3%, Unani 0.5%, Yoga 1.3% (Table – 1) (Figure -4). Questionnaire and survey-based intervention occupy a significant portion. There is a demand for analyzing the nature of people's disease, psychological, social, and

lifestyle adaptation toward the new regular life pattern in lockdown. In certain hospitals, integrated treatment is available for Ayurveda and Homeopathy, Ayurveda, and Yoga & Naturopathy. There are database observations reported in certain hospitals that portrayed integrated treatment between Allopathy and Siddha in Tamil Nadu. Clinical trials with integrated approaches of Allopathy and Siddha are happening at Noida. New Delhi.

As of the current date, more than 46 Allopathic interventions from Anti-viral, Antimalarial, oncologic drugs, Immunotherapies, Vaccines, and Plasma therapy are in testing. At the same time, Siddha has 10 Medicines in the clinical trial platform. Ayurveda has used 41 medicines, 14 are used in Homeopathy Medicine, and Unani had one, and 2 in Yoga and Naturopathy were tried (Table -2).

Study design

Randomized control trials are large in number 37.8% than non-randomized, 11.2%, and Single-arm trials are next to RCT sharing 20.2%. Cohort studies are lesser 0.5% alone. Among the global trials except one study checking the efficacy of remdesiver and all other studies are observations and surveys. However, in the domestic trial scenario is a little different. Nearly 20% of the trials were recruiting patients, and more than half of the trials were sponsored by Research institution (34%) followed by others (29%), colleges (10%), Pharmaceutical industry – Indian (10%), and private colleges/ clinic (10%) (Table – 1) (Figure -5). The Number of COVID-19 clinical trials registered in CTRI is considerably high over the past three months.

Sponsors

As in other scenarios, Research Institution occupies Trials' significant share (33%), followed by Pharmaceutical Industries and Medical colleges. Private hospitals and medical colleges register 25 %of trials (Table – 1).

Percentage of Global trials

Intercontinental of the inter-country collaborated global trial is minimal in Number, indicating the complexity of the disease. Global trials are minimal, occupying 3% of total trials and Indian trials were 97% (Table – 1) (Figure -6).

Sample size

The sample size varies between n=1 to n=5000 and above. 43% of trials have a sample size within 100 and followed by 32% of cases with a sample size within 500. This is followed by 11% of trials having a Sample size between 1 and 5000. 7% of trials have a large sample size above 5000, and 6% recruit within 1000 (Table – 1) (Figure -7).

Randomization

Certain trials have adopted Adaptive Randomization, which is 3%, which has a chance of allocating patients following the already existing treatment groups. Stratified and Block Randomization occupies equal share (4% each). Stratified randomization helps in small trials, Permuted block randomization balances between the two intervention groups. However, many of the COVID 19 trials have been used—computer-generated randomization to prevent bias and human error. Computer Randomization occupies a significant share of 22% (Table – 1).

Blinding

While 86% of Trials are not blinded, Double-blind trials are 5.5%, followed by Single blinded 5.15%. 1.72% of trials are triple blinded, and two trials are quadruple blinded (Table – 1).

Trial Phases Involved

From the applicable 48%, below 4% evaluate the Safety of Drugs, Therapeutic exploratory is 24%, and Therapeutic confirmatory occupies 16%. Some trials have been listed as Phase IV supporting repurposing phenomena like Lozenges.

Trial Duration

Five % of trials only showed trial duration greater than one year. 35% of both category with 1-3 month and 3 to 6 months falls in this duration (Figure -8).

Discussion:

This study aims to measure the preparedness of various Indian health stakeholders controlled by two Ministries, one dealing with the conventional therapy (Ministry of Health and Family welfare) and the other with Indian traditional medicine (Ministry of AYUSH).

Clinical trial registries are depots of newer ideas and newer interventions. The kinesis of conducting a Clinical trial during a pandemic is different from the normal situation. Understanding this scenario, Govt. of India has directed every clinical trial registered in CTRI. Ministry of AYUSH has released a particular GO, regulating the clinical trial during COVID -19. A bird-eye view and the Interventions used to support an ideology of repurposing existing drugs for novel coronavirus. PM of India has emphasized using immunomodulators from the AYUSH sector in his famous Mann Ke Baat talk. This study revealed that large-scale prophylactic prevention trials have been registered and used as a weapon to contain the disease to its might. Encouragingly, many medical colleges have collaborated with the AYUSH sector to integrate with the public's interventions or compare the ordinary person's benefits. It is evident that both healthy volunteers (32%) and affected individuals (68%) have also been subjected to trial in this pandemic situation, and many have consented to participate. It shows the tendency of humankind to find a solution to hunting problems and returning to normalcy.

Allopathic versus AYUSH interventions are equal in which Allopathy and Ayurveda occupy 49%, followed by Homeopathy, Siddha, Unani, and Yoga and Naturopathy in descending order.

As this a newer pandemic, after 100 years, a country like India and there exists a particular need for a keen understanding of the disease condition and its epidemiological aspects. This is reflected in the CTRI having a sizeable number of survey questionaries. Almost all the systems Ayurveda and Siddha, in particular, have integrated with Allopathy. Siddha, as integrated with Yoga and Ayurveda, has integrated with Homeopathy in certain hospitals.

At this pandemic, health stakeholders have taken a significant step to stick with standards reflected in 38% of the RCTs in CTRI. Next occupies open-ended single-arm trial followed by Non-randomized trial. It is tougher to follow a group in a pandemic, evident from only 0.5 % of cohort studies.

Research institutions and Medical colleges occupy a significant part of the trial, but only 10% of the pharmaceutical industry is taking up this issue. There exist much scope for

pharmaceutical industries to invest more in real research. Global integrated trials are very minimal, which once again is an alarming sign in research.

Therapeutic exploratory and therapeutic confirmatory occupies a significant share while a sizeable number of trials have searched for Safety; based on the repurposing idea, some trials are designed as Phase IV, which are like measuring the benefit of lozenges among COVID -19 patients. The disease's complexity has affected blinding in the trials, where only 14% of the trials are blinded. Among them, double-blinding occupies many, and some are triple and quadruple blinding.

At a given point in time, India has 107 trials than China, which expresses the country preparedness following the incidence and intensity of the disease.

Conclusion:

Despite the lockdown, the front line workers and health stakeholders of this country are serious about their business finding a solution for the problem stated, i.e., COVID -19. Allopathy and AYUSH's health sectors have attempted in rapidity, which shows their surge to act against COVID -19. Revalidated interventions that have been used earlier in Dengue, Malaria, Immunomodulators and Earlier anti-virals and drugs used in Cancer and HIV are among the selected interventions. The outcome objectives were symptom reduction, getting negative in RT-PCR, reduction in hospitalization, minimal use of ICU, ventilators, and reduced mortality were the objectives observed. Post COVID-19 management clinical trials were not registered during the said time. However, there exists a lot of scope and necessity to do trials in Post Covid prospectively.

Limitations:

In this, the analysis was limited to a cross-sectional study of trials registered in the COVID -19 pandemic concerning AYUSH and Allopathy stream on CTRI. Like study type, study design, sponsorship, and relevant details on trial characteristics presented and analyzed from 1st March 2020 to 22nd June 2020. We did not include trials from the WHO database, US Canada, China, and other Clinical Trials Database. This analysis will give useful data based on CTRI by gathering information to improve quality research opportunities. In

the future, follow-up is necessary to update results based on the information available to work further inconsistency.

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Table 1: General characteristic of registered trials

Number of new trials registered from 1st March 2020 to 22nd June 2020	
Variable	N (%)
Type of Trial	
Interventional	146(62.66)

Number of new trials registered from 1st March 2020 to 22nd June 2020	
Variable	N (%)
Observational	84(36.05)
PMS	3(1.29)
Total	233(100)
Month of Registration	
March	1(0.43)
April	38(16.31)
May	130(55.79)
June	64(27.47)
Total	233(100)
Recruitment Status	
Not Applicable	70(30.00)
Not Applicable Indian: Not Yet Recruiting	136(58.37)
Not Applicable Indian: Open to Recruitment	19(8.15)
Not Yet Recruiting Indian: Not Applicable	1(0.43)
Not Yet Recruiting Indian: Not Yet Recruiting	2(0.86)
Open to Recruitment Indian: Not Applicable	1(0.43)
Open to Recruitment Indian: Not Yet Recruiting	3(1.29)
Open to Recruitment Indian: Open to Recruitment	1(0.43)
Total	233(100)
Health condition	
Healthy Human Volunteers	90(38.63)
Patients	143(61.37)
Total	233(100)
Intervention - Type of Study	
Allopathy	58(24.89)
Ayurveda	58(24.89)
Homeopathy	13(5.58)
Siddha	10(4.29)
Unani	1(0.43)
Yoga	3(1.29)
Not applicable	90(38.63)
Total	233(100)
Study Design	
Cohort Study	1(0.43)
Non-randomized	26(11.16)
Other	71(30.47)
Randomized	88(37.77)
Single Arm Trial	47(20.17)
Total	233(100)
Type of Sponsor	
Contract research organization	1(0.43)

Number of new trials registered from 1st March 2020 to 22nd June 2020	
Variable	N (%)
Government funding agency	12(5.15)
Government medical college	22(9.44)
Ministry of AYUSH	3(1.29)
Other	66(28.33)
Pharmaceutical industry-Global	3(1.29)
Pharmaceutical industry-Indian	22(9.44)
Private hospital/clinic	13(5.58)
Private medical college	12(5.15)
Research institution	78(33.48)
Self-funded	1(0.43)
Total	233(100)
Sample Size	
Global	7(3)
Indian	226(97)
Total	233(100)
Sample Size Group	
1-100	101(43.35)
101-500	74(31.76)
501-1000	15(6.44)
1001-5000	26(11.16)
>5000	17(7.3)
Total	233(100)
Trial Duration	
<=1 Month	24(10.3)
1 - 3 Month	79(33.91)
3 - 6 Month	80(34.33)
6 Month - 1 Year	36(15.45)
> 1 Year	12(5.15)
Missing	2(0.86)
Total	233(100)
Method of Generating Random Sequence	
Adaptive randomization, such as minimization	7(3)
Coin toss, Lottery, toss of dice, shuffling cards etc.	6(2.58)
Computer generated randomization	53(22.75)
N/A	135(57.94)
Other	14(6.01)
Permuted block randomization	9(3.86)
Stratified randomization	9(3.86)
Total	233(100)

Number of new trials registered from 1st March 2020 to 22nd June 2020	
Variable	N (%)
Method of Concealment	
Alternation	2(0.86)
An Open list of random numbers	19(8.15)
Case Record Numbers	9(3.86)
Centralized	14(6.01)
Not Applicable	157(67.39)
On-site computer system	9(3.86)
Pharmacy-controlled Randomization	4(1.72)
Pre-numbered or coded identical Containers	3(1.29)
Sequentially numbered, sealed, opaque envelopes	16(6.87)
Total	233(100)
Blinding/Masking	
Case Record Numbers	1(0.43)
Double Blinded	13(5.58)
Not Applicable	135(57.94)
Open Label	66(28.33)
Quadruple Blinded	2(0.86)
Single Blinded	12(5.15)
Triple Blinded	4(1.72)
Total	233(100)
Phase of Trial	
N/A	120(51.50)
Phase 1	4(1.72)
Phase 1/ Phase 2	6(2.58)
Phase 2	40(17.17)
Phase 2/ Phase 3	18(7.73)
Phase 2/ Phase3	1(0.43)
Phase 3	28(12.02)
Phase 3/ Phase 4	9(3.86)
Phase 4	4(1.72)
Post Marketing Surveillance	3(1.29)
Total	233(100)

Table : 2 Products being assessed in Clinical Trial Registry of India (CTRI) for COVID -19 infection.

Drugs	Drug class	Registered

		trials, N*
Anti- virals - 6		
sofosbuvir	Anti- virals	1
Favipiravir	Anti- virals	1
Ribavirin	Anti- virals	1
Lopinavir	Anti- virals	1
Ritonavir	Anti- virals	1
Remdesivir	Anti- virals	1
Anti-malarials -7		
Hydroxychloroquine	Anti-malarials	7
Glucocorticoid - 2		
Ciclesonide	Glucocorticoid	1
Tocilizumab	Glucocorticoid	1
Anthelmintics -7		
Ivermectin	Anthelmintics	5
Nitazoxanide	Anthelmintics	1
Niclosamide	Anthelmintics	1
Anti-cancer -2		
Imatinib	Anti-cancer	1
Thymoquinone	Anti-cancer	1
Immunotherapy -2		
Inj. Sepsivac	Immunotherapy	1
Cytokine cocktail therapy	Immunotherapy	1
Plasma therapy -3		
Convalescent Plasma	Plasma therapy	3
Therapy procedure - 9		
Intubation / Others	Therapy procedure	9
Vaccines -3		
BCG	Vaccines	3
Supplements - 3		
Resveratrol	Supplements	1
Copper	Supplements	1
Chlorophyllin	Supplements	1
angiotensin receptor blockers - 1		
Losartan	angiotensin receptor blockers	1
Anticoagulants -1		
Heparin	Anticoagulants	1
Siddha Medicines - 10		

Kabasura kudineer		4
1.Kabasura kudineer 2.Vasanthakusumakaram Mathirai 3.ThippiliRasayanam 4.Adathodai Manapagu		1
Kabasura kudineer and Nilavembu kudineer		1
Kabasura kudineer, Amukkara churnam and Nellikai ilagam		1
Kabasura kudineer, Amukra churnam, Thalisadhi churnam, Adathodai Manappagu		1
Kabasura kudineer; Amukkara choornam; Nelikai legiyum		1
Kabasurakudineer Nilavembukudineer		1
Ayurveda Medicines		41
Homoeopathy Medicines		14
Unani Medicines		1
Yoga and Naturopathy		2
Survey		4
Not applicable		118

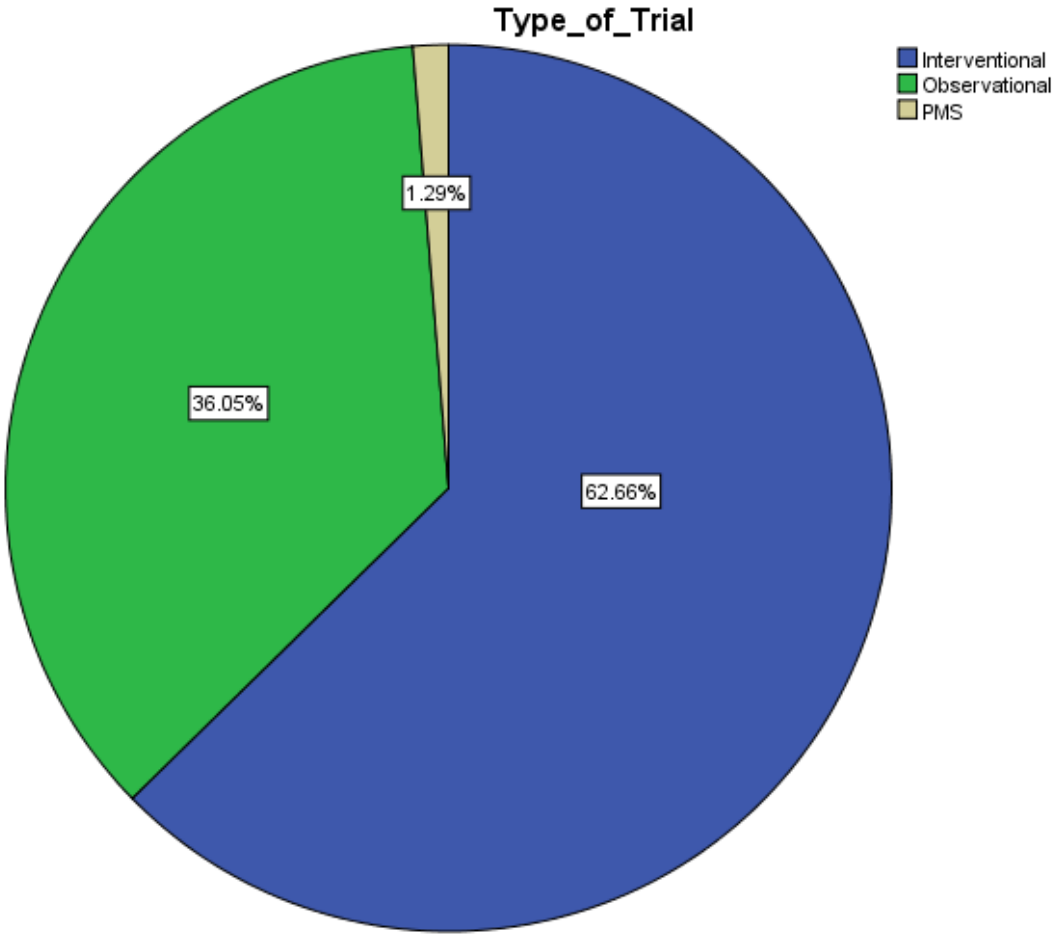

Figure: 1 Type of Trial

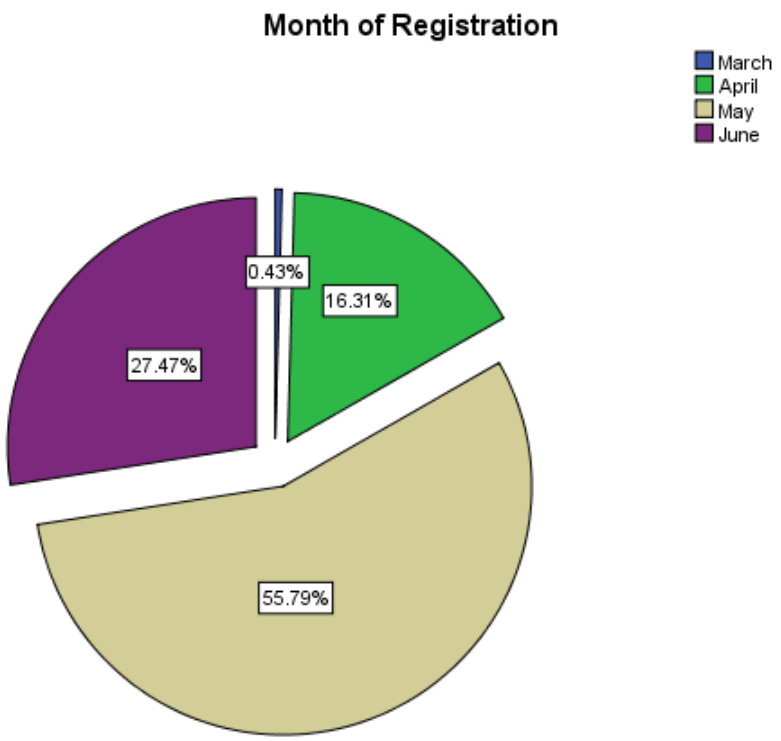

Figure: 2 Month of Registration

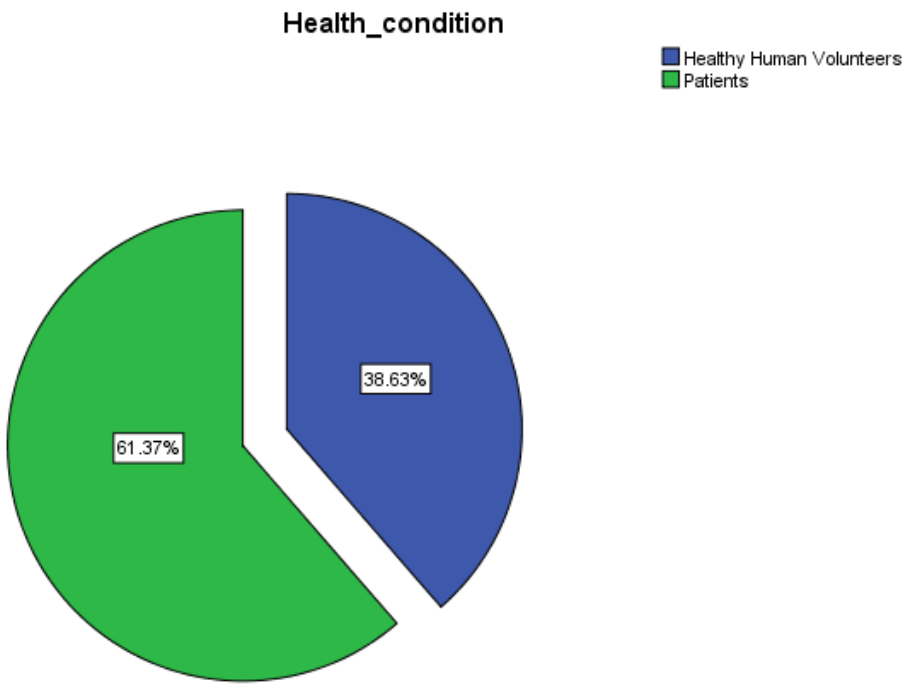

Figure: 3 Health Condition

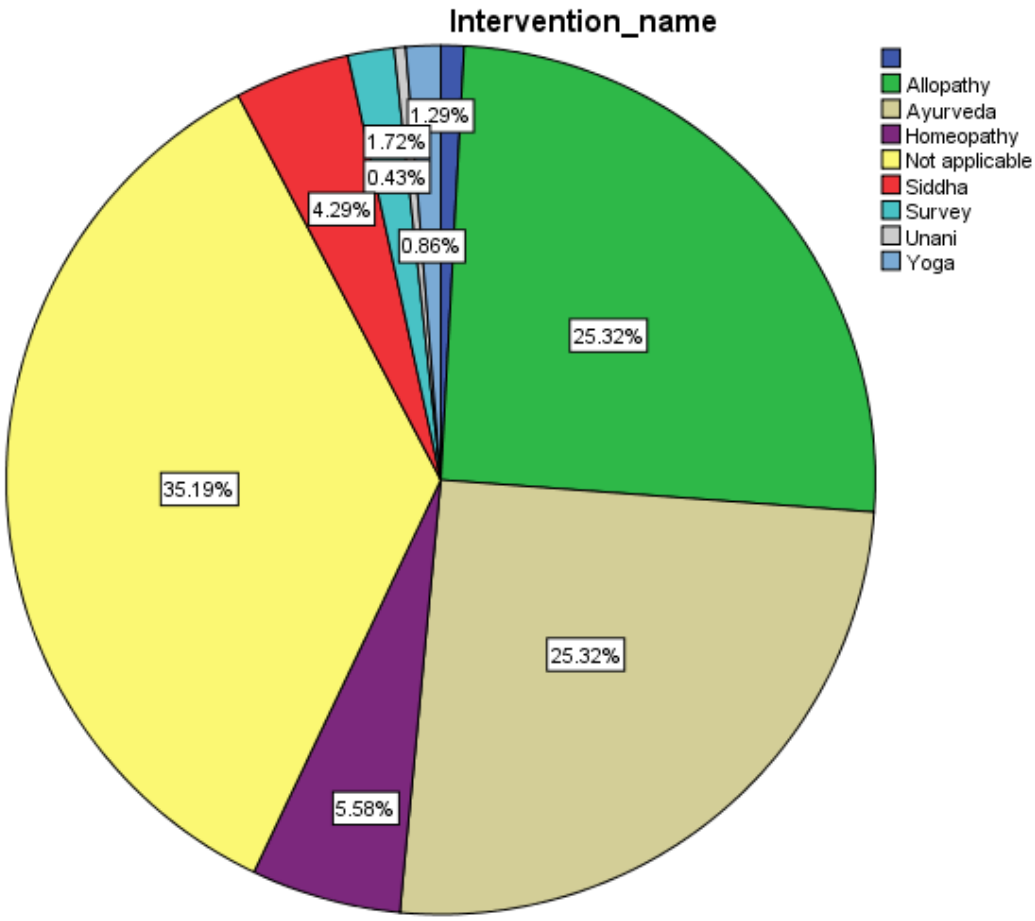

Figure: 4 Intervention - Type of Study

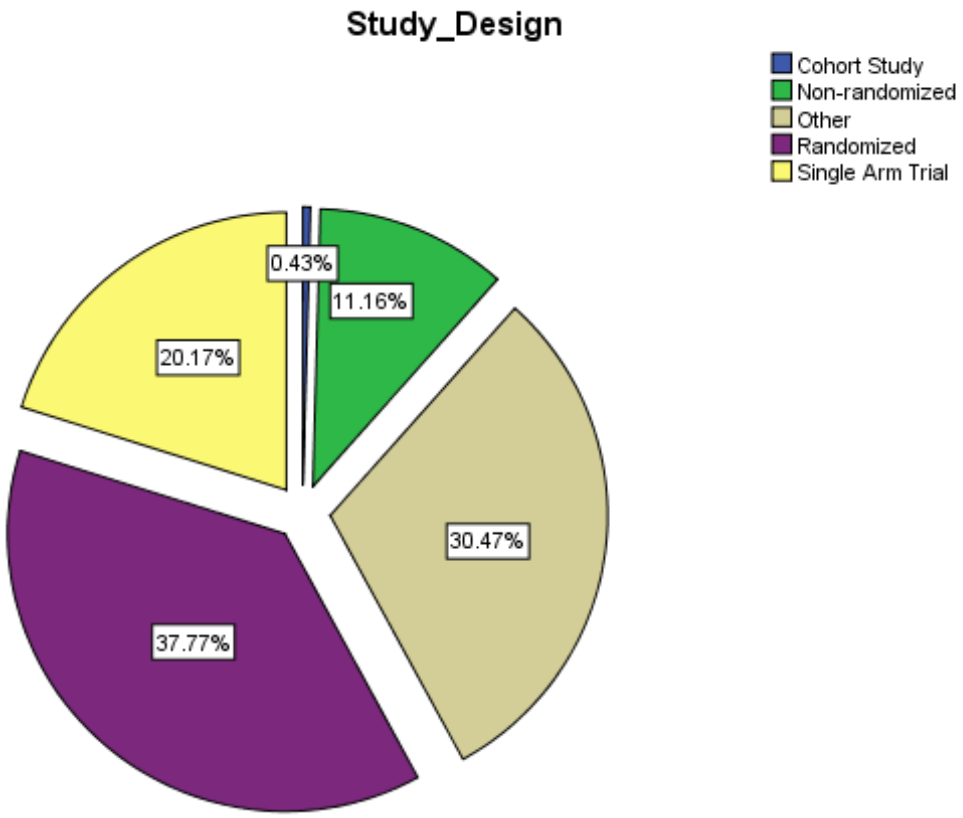

Figure: 5 Study Design

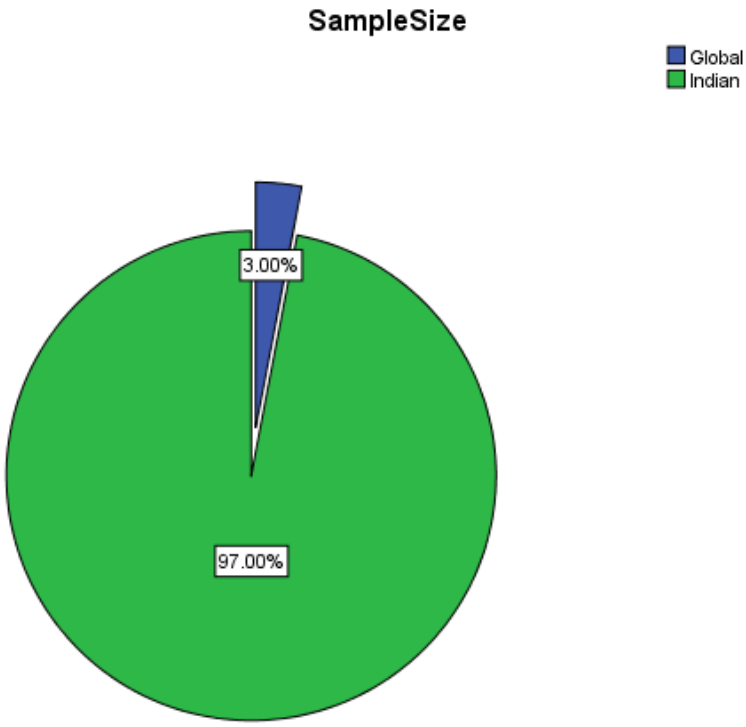

Figure: 6 Sample Size

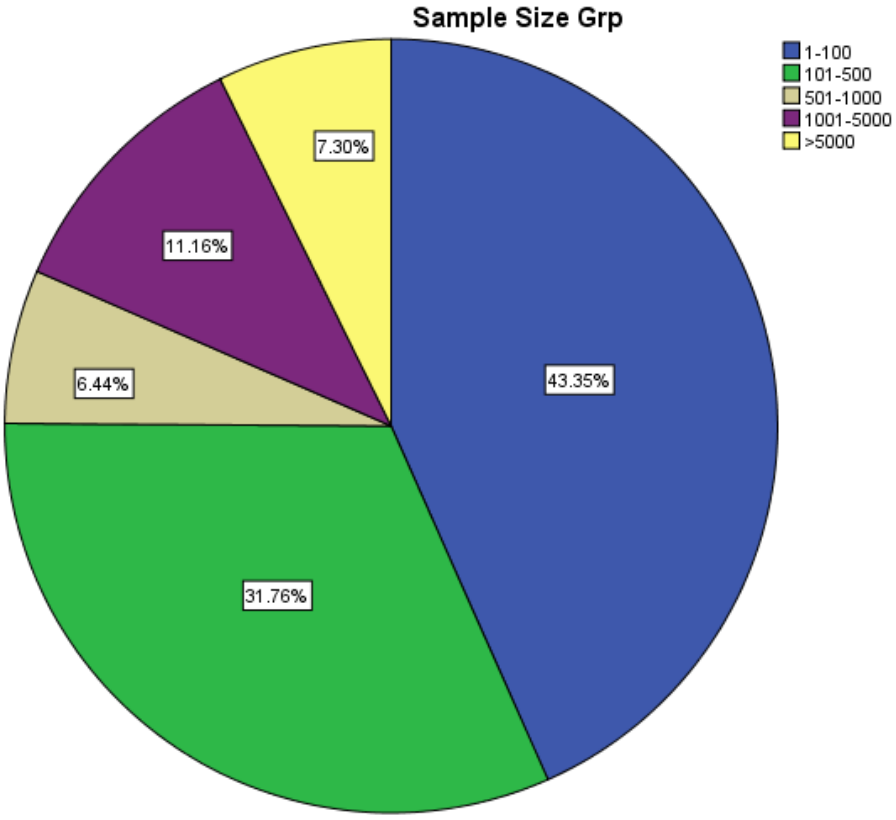

Figure: 7 Sample Size Group

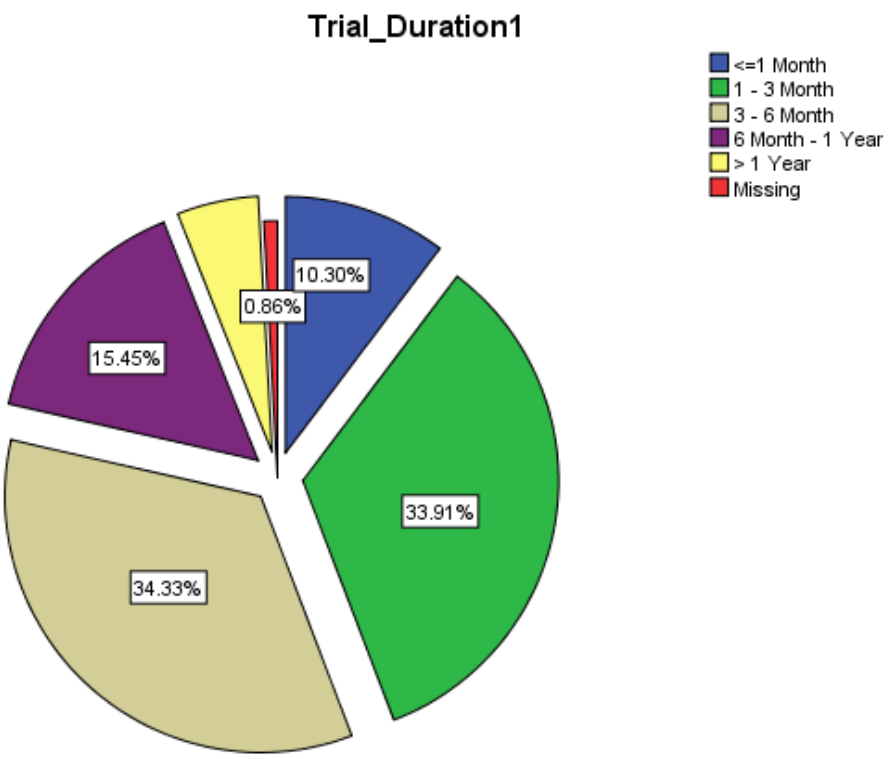

Figure: 8 Trial Duration

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Brief Communication

Preparedness of Siddha system of medicine in practitioner perspective during a pandemic outbreak with special reference to COVID-19

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ABSTRACT

COVID-19 (Corona Virus Disease-2019) is an infectious respiratory disease caused by the most recently discovered coronavirus, SARS-CoV-2 (Severe Acute Respiratory Syndrome Corona virus-2). This new viral disease was unknown before the outbreak began in Wuhan, China, in December 2019. As of November 16th 2020, it affects about 54.3 million populations, death troll increased to 1.32 million cases in worldwide. Whereas in India 8.85 cases are infected with COVID-19, of which 1, 30, 112 cases were died. Till now there has been no specific anti-virus drug or vaccines are available for the treatment of this disease, the supportive care and non-specific treatment to the symptoms of the patient are the only options in Biomedicine, the entire world turns its attention towards alternative medicine or Traditional medicine. Siddha medicine is one of the primordial systems of medicine practiced in the southern part of India, it dealt a lot about pandemic, and its management. This review provides an insight into Pandemic in Siddha system and its management in both ancient history and modern history, National and state level Government policies related to current pandemic, World Health Organization (WHO) guidelines on usage of unproven drug during infectious disease outbreak, Preparedness of Siddha system during a pandemic outbreak Challenges and Recommendations.

Keywords Herbal medicine, Alternative medicine, Traditional medicine, Quarantine, Isolation, Corona virus

1. INTRODUCTION

A pandemic is a disease outbreak that spreads across countries or continents, affects more people and takes more lives than an epidemic. The World Health Organization (WHO) affirmed COVID-19 to be a pandemic when the illness was become severe and spreading quickly over a wide area (Web MD, n.d.). Human population has suffered from many pandemics throughout history like smallpox, tuberculosis, HIV/AIDS, H1N1. It has created terrible damage in many different forms. The following table shows a list of pandemic events occurred Worldwide (Samal J, 2014) (Table 1).

Table 1. Pandemic Events Occurred Worldwide

Name of the pandemic	Year of the event
Plague of Athens (Typhoid Fever)	430 BC
Antonine Plague (Small pox)	165-180
Plague of Cyprian	252-256
Plague of Justinian (Bubonic Plague)	541-750
Black Death	14th Century
Third Pandemic (Plague)	19th Century
Smallpox	1518
Smallpox	1520
Smallpox	1618-1619
Smallpox	1770
Smallpox	1780-1782 & 1837-1838
1st Cholera Pandemic	1816–1826
2nd Cholera Pandemic	1829–1851
3rd Cholera Pandemic	1852–1860
4th Cholera Pandemic	1863–1875
An Outbreak of Cholera	1866
5th Cholera Pandemic	1881–1896
6th Cholera Pandemic	1899–1923
7th Cholera Pandemic	1962–66
Russian Flu	1889-1890
Spanish Flue	1918-1919
Asian Flue	1957-1958
Hong Kong Flue	1968-1969
H1N1	2009

2020 is a year of pandemic, the whole world suffers from a pandemic disease called COVID-19, and it was started in Wuhan city, china, in December 2019. It has emerged as an outbreak of unexplained pneumonia, later on Feb 2020, the World Health Organization (WHO) named this pneumonia disease as Corona virus Disease-2019 (COVID-19). This novel corona virus disease shows symptoms such as fever, fatigue, dyspnea, myalgia, severe cough, diarrhea etc. This disease spreads through nasal discharge and salivary droplets. This infection is usually mild in Children and young adults, whereas in old people and those with co-morbidities like diabetes, blood pressure, cardiovascular diseases develop severe illness, even death. Unfortunately specific antiviral drugs or vaccines are

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Received Aug 12, 2020; **Accepted** Nov 4, 2020; **Published** Nov 30, 2020
doi: http://dx.doi.org/10.5667/CellMed.2020.0029

currently not available. The drugs used for COVID-19 management is categorized in four classes Antiviral, Anti inflammatory drugs, Anti-malaria drugs, Traditional Medicine. The researches are going on in both Biomedicine and Traditional medicine at both Clinical and Pre-clinical level, to prove its efficacy and safety. Hence, the lack of potential drug against COVID-19 in conventional medicine turns the world attention towards traditional medicine in various countries like China, Iran, India, etc.

Siddha system of medicine is one of the primordial system of medicine, has its origin in southern part of India. Siddhars are spiritual scientist, who wrote many medicine preparations using herbs, metals, minerals and some animal products too. They have mentioned 32 types of internal and 32 types of external medicine not only for curative health, but also for preventive and promotive health. Siddhars have mentioned 4455 diseases, definitions, types and its management, initially it was written in palm leaf, later it is converted into printed books. This manuscript critically review the pandemic/ epidemic mentioned in Siddha system, its management mentioned in Siddha system, previous pandemic/ epidemic management through Siddha system, and current pandemic (COVID-19) management through Siddha System.

2. PANDEMIC OR INFECTIOUS DISEASES IN SIDDHA SYSTEM (*KOLLAI NOI/OTTU NOI*)

In Siddha system of medicine, pandemic is termed as *kollainoi.* It is defined as, the disease which occurs spontaneously, spread rapidly, affects abundant humans in a district or state or even whole country. This may occur during climatic changes, from February to May months. Another term used in Siddha system for infectious diseases is *Ottunoi*. The diseases which are caused by any germ are called as *Ottunoi*. Some important pandemic or infectious diseases, in Siddha are small pox (*ammai*) and Cholera (*Vulinoi*).

2.1. Siddha Mode of transmission of Infectious diseases (*Ottunoi*)

1. The disease will spread via infected person excretions such as respiratory droplets, sputum, urine, feces, etc.
2. It may also spread by touching the objects handled by an infected person.

2.1.2 Small pox (*Ammainoi*)

The disease which starts with fever, later causing blister in some parts of the body or all over the body is called as Small pox (*Ammainoi*). There are 14 types of small pox in Siddha system.

2.1.3 Cholera (*VuliNoi*)

Cholera is disease causing vomiting, diarrhoea, indigestion, dehydration. It will occur as a pandemic between the month of August and October. The other name of the *vulinoi* is *natpunoi*, which indicates, it is an infectious and contagious disease.

2.2. Pandemic management in Siddha

In Siddha, the pandemic is managed with 4 steps (Durairasan. ko, 1993) they are,

2.2.1 Notification

When a person is identified with the diseases, neem leaves (*Azadirachtaindica*) and turmeric (*Curcuma longa*) are inserted in the entrance of the house to notify that 'the person inside the house is infected'. In current COVID-19 pandemic, people in home quarantine are noticed with a paper notice sticked at the house entrance, in ancient days it was noticed with neem leaves and turmeric.

2.2.2 House isolation

When a person is infected, the person is isolated from others, to prevent others from infection.

2.2.3 Quarantine

Quarantine is an evidence of ignorance. As soon as the true cause of any disease is recognized, attention is directed to its prevention in the first instance rather than to its suppression after the disease has appeared. To control any disease by means of quarantine we must restrain all infected individuals during their entire period of infection (Bagde S, Shukla P, Srivastava RK, Mondal R &Anupam R., 2019). It was practiced especially in small pox.

I. When the outbreak incidence starts, no persons are permitted to go outside and come inside the town. Even the visitors are not allowed to go to their home town.
II. The infected person is isolated in a separate room and lay down in neem leaves spread clean cloth. The cloth and neem (*Azadirachtaindica*) leaves should be changed every day. Neem possess potent anti-septic, anti-viral properties against different viruses such as Coxsackie B virus, variola, Chikungunya, dengue, polio, measles (Ruchta .T, Amit Kumar verma, Sandip Chakraborty, KuldeepDhama, ShoorVir.S, 2014).
III. The isolated room is disinfected with turmeric water, blockade with turmeric dipped cloth. Curcumin isolated from curcuma long has Anti-viral, anti-microbial, anti-bacterial activity (Moghadamtousi SZ *et al.*, 2014).
IV. The family members who stayed with the infected person will not go to other houses, won't share their things with others.

2.2.4 Disinfection

Neem leaves (*Azadirachtaindica*), turmeric water (Curcuma longa) and Cow dung can be used as a disinfectant. The floor of the house and streets are molded thoroughly with cow dung. Fumigation of *sambirani* (*Styraxbenzoi*), *kungiliyam* (*Shorearobusta*) were also mentioned as disinfected.

Cow dung has antiseptic, anti-radioactive and anti-thermal activities. The traditional fumigation with herbs such as garlic (*Allium sativum*) peel, turmeric (*Curcuma longa*) powder, Carom (*Trachyspermumammi*) seeds and *Loban* (resin of *Styraxbenzoin* and *Boswellia* species) is effective in reducing air-borne bacteria, disinfect inert surfaces and also improves the quality of air (Surajpasalwad, 2017).

2.3 Modern history of the epidemic or pandemic management through Siddha system of medicine

Dengue infection is one of the rapid spreading mosquitos borne viral disease in the world which accounts for nearly 50 million cases, annually (Sahanaa C, Mishra AK, Bazroy J, 2018). At the time of dengue epidemic 2012, the Tamil Nadu government distributed a Siddha herbal decoction, *Nilavembu kudineer* (NVK) at free of cost. Health and Family Welfare Department Letter No. 41459/IM1(2)/2012, dated 21.11.2012 stated that "*Nilavembu decoction*, traditional Siddha drug is effective in

the treatment of viral fevers like dengue". The *Nilavembukudineer* has nine ingredients they are *Nilavembu* (*Andrographispaniculata*), *VilamichaiVer* (*Plectranthusvettiveroides*), vetiver (*Vetiveriazizanioides*), *Cukku* (*Zingiberofficinale*), *Milagu* (*Pipernigrum*), *Koraikizhangu* (*Cyperusrotundus*), *Santanam* (*Trichosanthescucumerina*), *Parpadagam* (*Mollugocerviana*). This is not only to treat diseases, but also to prevent diseases. As a result, there is a reduction in morbidity and mortality of dengue fever. The antiviral activity of *nilavembukudineer* was estimated by Jaspreet jain*et al*., shows, protection against CHIKV and DENV-2 during active infection and also help to prevent virus infection in the cells (Rajalakshmi S, Sathiyarajeswaran P, Samraj K &Kanagavalli K, 2020).

3. INDIAN GOVERNMENT POLICIES SUPPORTING SIDDHA SYSTEM OF MEDICINE

3.1 Central Government

Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy systems are the five indigenous systems of medicine practiced in India. Although homoeopathy is of German origin, the system is being practiced in India together with the indigenous forms of medicine. A department called the Department of Indian System of Medicine and Homoeopathy was created in March 1995 and renamed AYUSH in November 2003. Its aim was to give greater attention to the development of these systems of medicine. AYUSH ministry has also released the guidelines to manage COVID-19 pandemic through AYUSH systems of medicine.

3.1.1 Yoga campaign

The Ministry of AYUSH started Yoga at home campaign. In this campaign, they released the Yoga Posture, to counter stress and promote physical and mental well being during lockdown in this pandemic.

3.1.2 AYUSH web portal for COVID-19

Prime Minister Shri Narendra Modi met with representatives of the AYUSH sector, on 28th March 2020, discussed about the COVID-19 outbreak. PM underlined the importance of unproven claims of AYUSH having a cure for the disease. He said scientists from AYUSH, the Indian Council of Medical Research (ICMR), the Council of Scientific and Industrial Research (CSIR), and other research organisations must come together for evidence-based research. As a follow-up of that meeting, the Ministry of AYUSH started a COVID19 input portal on its website. On this portal, any registered AYUSH practitioner can submit a suggestion, concept or proposal about COVID-19. The suggestions received so far, across the country have been made public as advisory from Ministry of AYUSH (T. C. Jame, 2020).

3.1.3 Interdisciplinary AYUSH Research and Development task force

The ministry of AYUSH, government of India No. 17020/1/2020-E.I Dated 2nd April, 2020 established a task force with 17 members of AYUSH experts. This was constituted for initiating, coordinating and monitoring the Research and development (R & D) activities in the AYUSH sector related to COVID-19 disease. Based on this task force AYUSH ministry called for Extra Mural Research (EMR) projects {No.Z-28015/48/2020-HPC (EMR) AYUSH Dated: 21/4/2020}, to support short-term research projects for evaluating the impact of AYUSH interventions/medicines in the prophylaxis and clinical management of COVID-19 (State Principal Secretaries [Health/AYUSH], 2020, April 21)

3.1.4 Insurance coverage for AYUSH practitioners

Ministry of Health & Family Welfare launched an Insurance scheme for health workers fighting COVID-19 for the period of 90 days {D.O No. Z.18016/1/2020-PMGKP/NHM-II (Pt File), Dated 10.04.2020}. The Scheme cover all the healthcare providers who have been drafted for COVID-19 related duties, which also includes AYUSH practitioners (Insurance-coverage, Ministry of Health & Family Welfare, Government of India, 2020, April 10).

3.1.5 AYUSH Sanjivani Mobile application

The ministry of AYUSH has developed "AYUSH Sanjivani" mobile application, for analyzing the impact of AYUSH advisories on immunity enhancement. The app will create data on acceptance and usage of AYUSH advocacies and measures among the population and its impact in prevention of COVID-19 (Harsh vardhan, 2020).

3.1.6 AYUSH for immunity campaign

The ministry of AYUSH launched a three month campaign on August 2020 aimed at increasing awareness about easy and affordable practices for enhancing immunity and preventing diseases especially COVID-19. The objective of this campaign is to provide valuable, simple, widely available day to day cooking, as food to prevent diseases (AYUSH for immunity, 2020, August).

3.2 State Government of Tamil nadu

3.2.1 Aarokyam scheme

Health and family welfare {G.o. (Ms). No.201 dated 23.04.2020} implemented "Aarokyam" Special programme with AYUSH Interventions for COVID-19, for immune enhancement. This scheme insist the use of Siddha medicines (KabasuraKudineer/Nilavembukudineer) for mild COVID-19 cases along with the allopathy treatment. Further insisit the use of Siddha medicines (AmukkaraChooranamMathirai, Nellikailegium) in convalescence period too. Along with the above Siddha medicines, some Ayurveda, Unani, homeopathy medicines, yoga postures, immunity enhancing fresh juices and hot drinks are also recommended in this scheme (Aarokyam, Ministry of health & Family welfare, Government of India, 2020, April 23).

3.2.2 Siddha medicine for Frontline workers

Health and family welfare Department (letter no. 1714/P1/2020-1, dated 25.04.2020), recommended Siddha medicines (KabasuraKudineer/Nilavembukudineer) to the police personnel, Healthcare workers, other persons who are in COVID-19 duty, with high risk and low risk in containment area (Ministry of Health & Family Welfare, Government of India, 2020, April 25).

3.2.3 Stand alone Siddha COVID Care Centers in Tamilnadu

Tamilnadu government has established Siddha care centers for treating COVID-19 patients at 29 places with 100-1000 beds. So far 75,000 COVID-19 patients have been treated under Siddha system of medicine as of 1st August 2020. Traditional food items and herbal concoctions were being given as a part of treatment. Siddha medicines such as Kabasurakudineer, Amukkarachooranam, Nellikailegium, Adathodaimanapagu are used along with herbal steam inhalation, gargling, millet snacks,

turmeric milk, and herbal tea. Siddha yogam, meditation, Varmam (Physical manipulation) techniques were taught to the Covid-19 patients. Herbal mask, Herbal water spray, used as a environmental sanitation are other significant practices in Siddha Covid care center. Asymptomatic COVID-19 positive patients and those with mild symptoms are provided treatment at Siddha care center with Siddha medicines alone. Also in other biomedicine hospitals in tamilnadu, integrated treatment was given for COVID-19 patients (Health minister, C. Vijayabaskar, The Hindu,2020, August 1).

4. EMERGENCY USE OF UNPROVEN INTERVENTIONS OUTSIDE OF RESEARCH, WHO GUIDELINES FOR RESEARCH DURING THE OUTBREAK

World Health Organization (WHO) released a guidance document on ethical issues that arise specifically in the context of infectious disease outbreaks at the time of the Ebola outbreak in West Africa (2014–2016). In that, WHO recommended the use of unproven intervention at the time of infectious disease outbreak, provided,

I. No proven effective treatment exists.
II. It is not possible to initiate clinical studies immediately.
III. Data providing preliminary support of the intervention's efficacy and safety are available, at least from the laboratory or animal studies, and use of the intervention outside clinical trials has been suggested by an appropriately qualified scientific advisory committee on the basis of a favorable risk–benefit analysis.
IV. The appropriate country authorities, as well as an appropriately qualified ethics committee, have approved such use.
V. Adequate resources are available to ensure that risk can be minimized.
VI. The patient's informed consent is obtained.
VII. The emergency use of the intervention is monitored, the results are documented and shared in a timely manner with the wider medical and scientific community.

The application of experimental interventions under these circumstances is referred to as "monitored emergency use of unregistered and experimental interventions" (MEURI) (World Health Organization, 2016)

5. Preparedness in pandemic outbreak- Challenges and Recommendations- Siddha Practitioner perspective

Although WHO recommended the use of unproven intervention at the time of infectious disease outbreak. In order to build confidence and trust of the public and also from the government policy makers, the preparedness is imperative and they are listed below (Table 2).

Table 2. Preparedness of Siddha system of medicine in pandemic outbreak- Challenges and Recommendations

S.no	Challenges	Recommendations
1.	Safety Evaluation	Strengthening Pharmacovigilance
2.	Efficacy Evaluation	Conducting Randomized controlled clinical trials and Meta analysis
3.	Cross-references, cross-learning and collaborations between the Biomedicine and Siddha medicine	Siddha medicine as a part of the M.B.B.S curriculum
4.	"Solidarity" clinical trial in Siddha medicine	Establishment of international policy for Siddha medicine utilization

5.1 Evaluation of safety

For any drug, the safety is evaluated through the pre-clinical toxicity studies (In-vivo acute and sub-acute toxicity studies, In-vitro studies) and clinical studies. According to WHO guidelines for traditional medicine, evidence obtained from the clinical trials is considered as Grade-A proof (World Health Organization, Geneva; 2000). In a study of David NziokaMutua *et al.* revealed that, the evaluation of side effects in large clinical trials for herbal extracts is absent and in adequate (Mutua ND, Juma KK, Munene M, Njagi ENJ, 2016). In the same way the research in pre-clinical toxicity studies in Siddha medicines are more, whereas the clinical safety studies, Adverse Drug Reaction (ADR) reporting publications are not enough. This may be because, the misconception of Siddha practitioners is that the particular drug will be banned in the future when it is reported against ADR.

Strengthening Pharmacovigilance

Publications, documentation of side-effects (recorded according to established principles of Pharmacovigilance) of a single herb or compound herbal preparations or herbo-mineral preparation is needed. Reporting, publishing case report and case series related to ADR will built up a trust to biomedical practitioners and also ease the policy making. Such reports should be attached in Under Graduate (UG) Siddha curriculum itself to give confidence to budding Siddha practitioners. ADR related awareness classes should be conducted to avoid the misapprehension in Siddha practitioners as well as in students.

5.2. Evaluation of Efficacy

The limited evidence for efficacy remains a challenge to use of Siddha medicines. One of the concerns of healthcare administrators and policy makers is absence of clinical trial in Siddha system of medicines.

Conducting Randomized controlled Trials and Meta analysis

Though the Tamilnadu government recommended Nilavembukudineer at the time of the epidemic, there is no evidence of randomized clinical trials. As per WHO guidelines Evidence obtained from meta-analysis of randomized controlled Trials is considered as grade-A proof. Such studies facilitate the acceptance of Siddha medicines in different regions.

5.3. Cross-referencing, cross-learning and collaborations between the Biomedicine and Siddha medicine

In an assessment of the mainstreaming of AYUSH in Rajasthan, India [Society for Economic Development and Environmental Management (SEDEM) n.d.], revealed that AYUSH is popular and regularly accessed by the community. Approximately half the allopathic doctors studied reported never referring patients to AYUSH doctors; there is a sharp status gap between modern medicine and AYUSH (Boovaragasamy C, Narayanan S., 2019).

The allopathic alleges that standards of medical care would be diluted after the integration.

Siddha medicine as a part of curriculum

AYUSH medicine should be included in the Bachelor of Medicine and Bachelor of Surgery (MBBS) curriculum itself, it should include, varmam, yoga, preventive measures in Siddha, strengths of Siddha medicine (for example excellent in treating skin diseases, liver disorder, renal stone, peptic ulcer, musculoskeletal disorders etc.), the science of Siddha, ongoing research in Siddha. Sulochanabhat *et al*. conducted a study on 202 Biomedicine practitioners; the study revealed that all the respondents were aware of basic principles and strengths of Ayurveda. They also opined that, integration is helpful to strengthen the overall health care delivery in India. Further, they uttered that the communication barrier between practitioners of Ayurveda and Biomedicine, research work with respect to safety and efficacy of Ayurvedic medicines and therapies, inadequate policy initiatives are the important obstacles in implementing integration between Ayurveda and Biomedicine (Sulochana Bhat, Saketh Ram Thrigulla, N Srikanth, M.M. Padhi, Kartar Singh Dhiman, 2015). The integration of education, research and practice of both systems at all levels will happen only if both Siddha and biomedicine practitioners should appreciate the relative strengths, weaknesses, and role of each system.

5.4. "Solidarity" clinical trial in Siddha medicine

"Solidarity" is an international clinical trial to help discover an effective treatment. This was launched by the World Health Organization and partners during COVID-19 pandemic. The Solidarity trial is one in which four treatment options is compared against the standard care, to assess their relative efficacy against COVID-19. By enrolling patients in various countries, the Solidarity trial aims to rapidly discover whether any of the drugs slow disease progression or improve survival.

Establishment of international policy for Siddha medicine utilization

A solidarity clinical trial in Siddha system is possible by the cooperation of other countries like China with strong traditional medicine systems. The relevant protocols will have to be got approved by the World Health Organization. That would require considerable diplomatic effort and documentation.

6. CONCLUSION

Siddha system of medicine has rich knowledge about various pandemic diseases and its preventive measures and management. Pandemic is not new to Siddha system it is dealt 2000 years before by siddhars. Prevention and management of pandemic through Traditional medicine is vital in this era. The well established research works in the safety and efficacy of Siddha medicine, Cross referral, cross learning between Siddha and Biomedicine practitioners, establishment of international polices for Siddha medicine are the measures to be taken in the future. This has to be supplemented by active research, development, standardization of Siddha medicine. The preparedness helps the government to initiate an immediate response and fulfils the expectations from the public and builds their confidence and trust. With that we can tackle the outbreak, prevent their spread and save human lives.

AUTHOR'S CONTRIBUTION

Samraj. K made the outline of the article, Rajalakshmi. S wrote the whole article, Sathyarajeswaran. P validated and gave inputs to the article, Kanagavalli. K did overall supervision.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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COVID-19-specific clinical research using traditional medicine: lessons from traditional Chinese medicine for India's AYUSH systems

S. Natarajan, C. Anbarasi, M. Sendhilkumar and P. Manickam

Challenges of COVID-19

The global pandemic due to the novel coronavirus (SARS-CoV-2) leading to coronavirus disease 2019 (COVID-19) originated in China and was reported to the World Health Organization (WHO) on 3 January 2020 (refs 1, 2). The WHO declared this SARS-CoV-2 as a Public Health Emergency of International Concern (PHEIC) on 30 January 2020, and subsequently, as a pandemic on 11 March 2020, as it affected more than 100 countries[3]. In view of the significant morbidity and mortality associated with this pandemic, many research activities are ongoing globally to explore possible therapeutic regimens or prophylactic agents. Further, the scientific community has started repurposing existing drugs for SARS-CoV-2 (ref. 4). Also, options available from the whole group of traditional/complementary and alternative medicine are being explored worldwide. While leading the world with the battle against the virus, the People's Republic of China (PRC) also leads in the search for potential traditional medical systems through traditional Chinese medicine (TCM)[5].

After China, the Indian subcontinent has a rich heritage of traditional medicine. The Indian traditional medical systems are collectively referred to as AYUSH (as an acronym earlier it meant the medical systems of Ayurveda, Yoga, Naturopathy, Unani, Siddha and Sowa-Rigpa, and Homoeopathy). These systems are well-recognized and well-supported by the Government of India (GoI) through various plans and policies. The systems are governed by a Ministry of AYUSH, GoI[6]. During the pandemic due to the lack of any therapeutic or prophylactic modalities, India turned towards the AYUSH systems. In the past individually the AYUSH systems have reported to have contributed to outbreak conditions. For COVID-19, the Ministry of AYUSH, GoI, released several advisories/guidelines[7]. However, the research potential remains untapped.

Given the complexity of crisis and paucity of related research from AYUSH at this stage, we reviewed TCM's COVID-19 specific reactions and responses available from public domains (Government websites, medical research databases and media) to draw relevant lessons for India.

TCM response to COVID-19

Building on its long-historical tradition, the engagement of TCM for COVID-19 response was from the very beginning of the pandemic (Table 1). The Government of China initially developed a framework to combine the TCM and Western medicine to jointly respond to the pandemic[8]. The TCM physicians observed the clinical signs and symptoms, and its progression. Based on traditional principles, the TCM experts developed guidelines to treat the patients. The TCM treatment plan included multiple herbal prescriptions targeting fever, heavy coughing, loss of appetite, nausea, vomiting, diarrhoea, shortness of breath and tiredness[9].

A specific chapter detailing TCM treatment during a patient's medical observation, clinical treatment and recovery has been included in the latest version of the COVID-19 diagnosis and treatment scheme released by the National Health Commission of PRC. TCM contributed in improving a patient's physical condition and immune function, whereas bio modern medicine concentrated on the respiratory and circulatory life-saving assistance. This fetched good results in managing COVID-19 patients. Mild symptoms showed obvious improvement after TCM treatment, and for critical patients, TCM decreased their lung exudation, stabilized blood oxygen saturation and reduced respiratory support and antibiotic use. Wuhan's coronavirus control headquarters ordered integrated treatment of TCM and Western medicine,

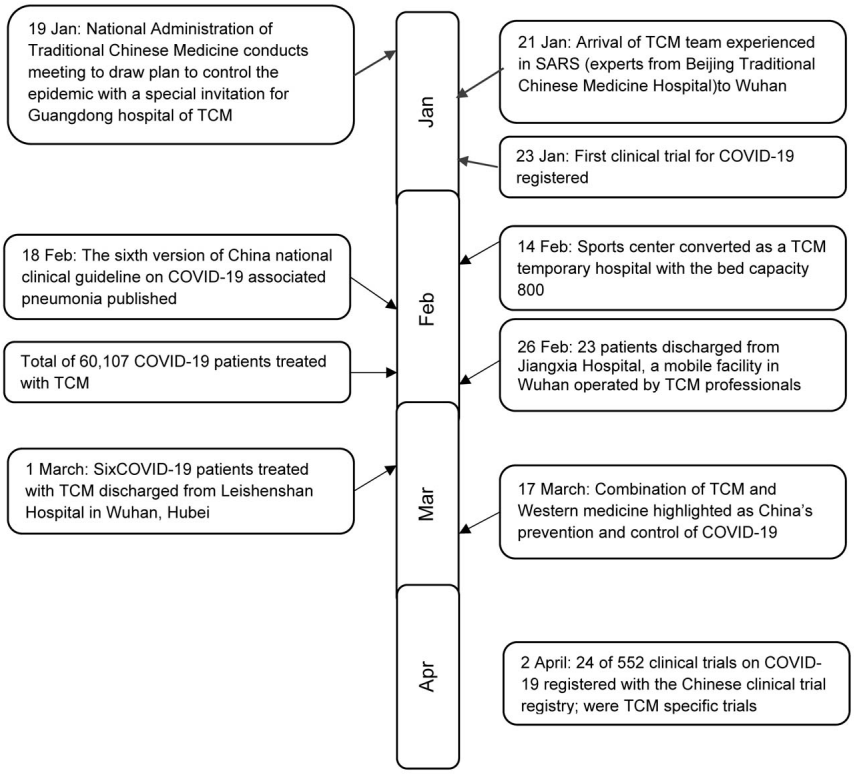

Table 1. Timeline of Traditional Chinese Medicine activities during COVID-19.

especially among non-critical patients, and observation of the curative effects of TCM at designated hospitals. TCM offered individualized treatment to the patients at different stages of the disease. More than 2220 TCM professionals from across the country were deputed to Wuhan to combat the epidemic. TCM was used in the treatment regimen of nearly 95% of the admitted patients. Among those discharged, over 90% underwent integrated treatment of TCM and Western medicine[10].

Apart from clinical care services to COVID-19 patients, government institutions, physicians and scientists initiated scientific research amidst the epidemic. The first clinical trial for COVID-19 was registered on 23 January 2020. In fact, one-third of the registered clinical trials on COVID-19 in the registry were that of TCM. As of 2 April 2020, 552 clinical trials were registered in the Chinese registry on COVID-19. Among them, 24 trials were registered under TCM[11].

In 2003, TCM played an important role in treating patients during the severe acute respiratory syndrome (SARS) outbreak[12]. Of the total SARS cases in the world, 58% were from the Chinese mainland. China extensively involved TCM during SARS. Building on such experience, PRC could engage TCM and formulate a plan in the immediate aftermath of the COVID-19 pandemic. In fact, historically, TCM has played a significant role in the control of communicable diseases in China. In 2016, PRC accorded equal status to TCM on par with Western medicine. This was in terms of ideological, legal, academic and practical applications. TCM has been included in the Chinese National Health Policy 2030 (ref. 13). China has successfully integrated TCM in the modern hospitals. Efforts were made to improve the system of administration related to TCM, increased financial input, formulation of specific policies, laws and regulations suited to the unique features of TCM, promotion of coordinated development of TCM and Western medicine. A platform was set up for TCM and Western medicine to complement each other[13].

In the COVID-19 context, amidst efforts to contain the pandemic, TCM has steered scientific research with rigour. Almost 30% of the clinical trials registered during January–March 2020 in the Chinese clinical trial registry were under standalone TCM interventions for COVID-19. Another 15% of the clinical trials were registered with TCM and Western medicine as integrative treatment for COVID-19 (ref. 11). The dividend sharing of TCM in registering clinical trials was high. The spirit of taking TCM to the scientific platform by conducting trials is a good lesson to all the countries with a rich heritage of traditional medical systems as a part of their healthcare. Sixteen national clinical research bases have been set up as part of the clinical research system for preventing and treating infectious diseases and chronic non-infectious diseases with TCM.

TCM has distinctive training schemes for professionals. Training has been designed as multi-dimensional education comprising integrative medicine, community health, conservation of traditional pharmacological skills and master apprenticeship[13].

Lessons for the AYUSH sector in India

Overall, the national status of AYUSH is increasingly becoming prominent, but not treated on par with that of Western medicine. The National Health Policy accords such status; however; ground-level realities are far from satisfactory. TCM status in PRC is something worth emulating in India.

The Indian policy recognizes medical pluralism and supports 'integration' of medical systems. However, in terms of practical implementation, except for 'co-location' in public sector facilities, 'integration' is yet to be fully realized[14]. Similar to TCM, AYUSH has been striving to achieve functional integration for a long time. Integrative medicine, as proposed in the policy documents, will help in appropriately distributing resources and addressing priority health conditions meaningfully.

In the context of research, AYUSH research has been limited to few clinical entities. The quality and quantity of such studies are insufficient[15]. India's AYUSH infrastructure for research and education has been well conceived and well supported. However, research output has to be commensurate with such investment. Interdisciplinary approach is the key for empowering AYUSH research. Collaborating with national-level medical, science and technology bodies is crucial towards achieving the same.

Initiatives such as the International Yoga Day highlighted the role of AYUSH in non-communicable diseases/ lifestyle disorders[16]. The COVID-19 pandemic has brought the focus back on AYUSH. As such the capacity of AYUSH in public health response has been limited and not fully explored, except in some settings. GoI has issued a number of advisories for the same. The Indian Prime Minister has called for evidence-based research in AYUSH for COVID-19. GoI has established 'interdisciplinary AYUSH Research and Development Task Force' towards COVID-19 response and to take the research forward[17]. We learn that more than 1000 research proposals on AYUSH interventions have been submitted in response to a call by this Task Force. TCM has shown the way right from SARS to COVID-19 in addressing public health challenges posed by any pandemic. It is time to convert the threat into an opportunity to collaborate and undertake interdisciplinary research with scientific rigour. Taking up AYUSH research in progressive way is the only option to take it to the international scientific community.

In the context of outbreak response, it is imperative that AYUSH professionals need clinical and public health-specific orientation and training around outbreak conditions. In fact, the Ministry of AYUSH, GoI has trained more than 3000 health professionals for COVID-19 surveillance and response. Taking clue from PRC's framework and early engagement of TCM is a model for AYUSH systems. AYUSH should aim to empower their professionals by incorporating integrative medicine, public health and professional development in their curriculum.

Beyond the current situation, these are ways and means to increase the contribution of AYUSH by making sure integration works, research delivers and public benefits at large. Such efforts can bring AYUSH closer to reality and thus create opportunities at times of public health emergency such as the current pandemic and therefore sustain during 'peaceful' times as well.

Finally, undertaking any interdisciplinary AYUSH research will be translated into good clinical practice as a positive outcome. AYUSH research outcomes will not only provide solution to the current COVID-19 problem, but also create an opportunity for managing any

potential outbreaks in the future. Even though, it is considered difficult to address the pandemic with AYUSH interventions at present, the efforts taken are worth in gaining knowledge. As the conduct of integrative clinical trials with AYUSH intervention is still uncertain, the Ministry of AYUSH, GoI has to make efforts to contribute in the management of COVID-19 through thoroughly scrutinized research proposals received by it.

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Connecting the unconnected: the way forward for public health to reach the unreached tribal communities in India

Nishant Saxena, Dinesh Kumar and Aparup Das

India is home to more than 10.43 crore Scheduled Tribe (ST) people accounting for 8.6% of the country's total population. Among the 705 STs, 75 groups are categorized as particularly vulnerable tribal groups. The Government of India has a number of programmes and schemes for holistic development of the tribes. In spite of this, tribal health continues to be a major concern. Invariably, in every tribe there are traditional healer(s) who provide primary healthcare and also a medium to connect man with nature and the divine. However, till date there are no policies to recognize and acknowledge the services being provided by these healers for the community at large. This note envisages to draw attention to this critical policy gap which, if filled, could help in achieving universal health coverage for all, especially in tribal areas.

India is home to more than 104 million tribal people who comprise 705 different ethnic groups classified as Scheduled Tribes (STs) according to Article 342 of the Constitution of India[1]. The tribal population is in fact not a homogenous group[2]. Recent studies have shown that as a group STs fare much worse compared to non-STs in the country with respect to health outcome indicators[3]. The Government of India introduced affirmative provisions for the overall socio-economic development of this marginalized section of the society about seven decades ago soon after the independence. Even so, the concept of Tribal Sub Plan (TSP) was introduced about five decades ago to ensure that there is no dearth of funds for the holistic development of the tribes. In spite of all these efforts, tribal health remains a major concern even now.

Studies from across the globe, and not just in India, have shown that tribal groups have their own unique ways of defining health and disease, and also dealing with health issues[4]. Almost all these communities have traditional healers who provide the connect between man, and nature and the divine. There is no formal system of learning these practices and all these healers are carrying forward the traditional knowledge gathered over generations of habitation close to nature. Most importantly, it has been seen that the tribal people in India approach this traditional medicine man first when facing health issues[5], irrespective of their education level. However, when we evaluate the connect between traditional healers and the public health system, we find that it simply does not exist. In a country with such a huge tribal population, the public health system has so far failed to acknowledge the presence of this age-old system of healing which is still in vogue, especially in areas inhabited by these ethnic groups. The need of the hour is to integrate the traditional healers, known as *Gunia*, *Guni* or *Bhumka* in Baiga, Bhil and Bharia tribes

Brain, Behavior, & Immunity - Health 11 (2021) 100200

Contents lists available at ScienceDirect

Brain, Behavior, & Immunity - Health

journal homepage: www.editorialmanager.com/bbih/default.aspx

Commentary

The current situation of COVID-19 in India

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ARTICLE INFO

Keywords
COVID-19
India
Clinical management
AYUSH
COVID-19 vaccines

ABSTRACT

The COVID-19 pandemic has now risen to a global health crisis across the globe. This novel virus outbreak has challenged India's economic, medical and public health infrastructure. Health care professionals and researchers around the world are looking for an effective treatment regime for COVID-19. The number of people infected by COVID-19 in India crossed 9.74 million; nearly eleven - months after the country reported its first case. The Ministry of Health and Family Welfare of India (MOHFW) has taken numerous measures to raise awareness on COVID-19 and the necessary actions to control the spread of the virus. The central and state governments are formulating several wartime protocols to achieve this goal. The MOHFW has implemented the new clinical management protocol to treat COVID-19. Besides, the Ministry of AYUSH has also provided guidelines to use conventional preventive and treatment strategies to enhance immunity. The national recovery rate has increased to 94.66% and the reported fatality rate is down to 1.45, due to "test, track and treat". MOHFW and Ministry of AYUSH are the two pillars of health care to prevent and manage the current pandemic outbreak in India. Since, there is no specific drug or vaccine effective against COVID-19 infection, exploring every possible option for prevention and treatment is of great importance.

1. Introduction

COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) first emerged in late 2019 in Wuhan, China and the number of cases rose quickly across the world. Approximately 67 780 361 COVID-19 cases and 1 551 214 deaths were reported by the World Health Organization (WHO) as of December 09, 2020 with cases reported in more than 220 countries or territories (COVID-19 update, COVID-19 update. WHO, 2020). The number of people infected by the coronavirus in India crossed 9.74 million; nearly eleven - months after the country reported its first case in the state of Kerala on January 30, 2020 (Kumar et al., 2020). Subsequently, the country witnessed drastic rise in the number of cases across all states or union territories. Relative to the population, India's numbers are still low, but the steep rise in absolute numbers risks overwhelming the healthcare system. The pandemic has so far claimed more than 141 360 lives in India. The national recovery rate has reached 94.66% and the case fatality rate is down to 1.45%, due to "increasing of test, tracking, timely and effective clinical management of the patients in critical care" according to Ministry of Health and Family Welfare (MOHFW) on December 08, 2020 (COVID-19 update, COVID-19 India, 2020). India tested 149 836 767 cumulative samples by December 07 and 1 022 712 samples were tested on December 08, 2020. Current status of reported positive coronavirus disease cases in India (State-wise) are presented in Fig. 1. This novel corona virus outbreak has burdened India's economic, medical and public health infrastructure. The Gross Domestic Product (GDP) shrank by the steepest pace ever, 23.9% in the April–June period when the coronavirus brought the country to a standstill. Apart from the health-related consequences caused by COVID-19, the pandemic is likely to cost the world $90 trillion for the combined global public health and economic crises (Global Economic Effects of COVID19, 2020), after COVID-19 was declared as a world health emergency in January 2020 by WHO.

2. Clinical management

In the current, pandemic situation, a myriad of strategies would be extremely critical to battle the rapid virus spread and to treat the infection. The MOHFW, Government of India has taken several steps to spread awareness about the intensity and effects of the pandemic outbreak and has employed various measures to control the spread of COVID-19. The

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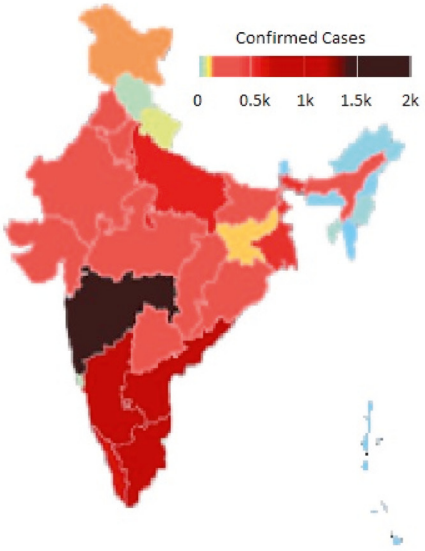
https://doi.org/10.1016/j.bbih.2021.100200
Received 28 September 2020; Received in revised form 10 December 2020; Accepted 15 December 2020
Available online 6 January 2021

Confirmed cases
9 735 975 (↑32061)
Recovered
9 215 581 (↑36635)
Deaths
141360 (↑402)
Active cases
378909



Sample tested
Cumulative total
Up to December 7
148 814 055

on December 8
1 022 712

State / UT	Confirmed	Active	Recovered	Death	Recovery Ratio	Case Fatality Ratio
Maharashtra	1859367↑4026	73374	1737080↑3635	47827 ↑53	93.4%	2.6%
Karnataka	895284 ↑1280	25015	858370 ↑1019	11880 ↑13	95.9%	1.3%
Andhra Pradesh	872839 ↑551	5429	860368 ↑744	7042 ↑4	98.6%	0.8%
Tamil Nadu	792788 ↑1236	10588	770378 ↑1330	11822 ↑13	97.2%	1.5%
Kerala	644697 ↑5032	59748	582351 ↑4735	2473 ↑31	90.3%	0.4%
Delhi	597112 ↑3188	22310	565039 ↑3307	9763 ↑57	94.6%	1.6%
Uttar Pradesh	558173 ↑1776	21374	528832 ↑2111	7967 ↑23	94.7%	1.4%
West Bengal	507995 ↑2941	23750	475425 ↑2971	8820 ↑49	93.6%	1.7%
Odisha	321913 ↑349	3106	316970 ↑523	1837 ↑6	98.5%	0.6%
Rajasthan	284116 ↑1604	20875	260773 ↑2380	2468 ↑20	91.8%	0.9%
Telangana	274540 ↑682	7696	265367 ↑761	1477 ↑3	96.7%	0.5%
Chhattisgarh	249699 ↑1467	191516	227158 ↑1525	3025 ↑15	91%	1.2%
Haryana	246679 ↑1391	11947	232108 ↑1557	2624 ↑13	94.1%	1.1%
Bihar	240249 ↑684	5157	233791 ↑ 674	1300 ↑3	97.3%	0.5%
Gujarat	221493 ↑1325	14172	203211 ↑1531	4110 ↑15	91.7%	1.9%
Madhya Pradesh	217302 ↑1345	13280	200664 ↑1497	3358 ↑11	92.3%	1.5%
Assam	214019 ↑94	3575	209444 ↑102	997 ↑2	97.9%	0.5%
Punjab	157331 ↑492	7274	145093 ↑792	4964 ↑30	92.2%	3.2%
Jammu and Kashmir	114038 ↑470	4995	107282 ↑524	1761 ↑6	94.1%	1.5%
Jharkhand	110639 ↑182	1753	107898 ↑188	988	97.5%	0.9%
Uttarakhand	79141 ↑632	5399	71541 ↑436	1307 ↑12	90.4%	1.7%
Goa	48935 ↑159	1310	46924 ↑146	701	95.9%	1.4%
Himachal Pradesh	46201 ↑504	7577	37837 ↑808	743 ↑14	81.9%	1.6%
Puducherry	37311 ↑41	388	36308 ↑45	615	97.3%	1.6%
Tripura	32922 ↑28	427	32102 ↑15	370	97.5%	1.1%
Manipur	26396 ↑171	2919	23166 ↑169	311 ↑2	87.8%	1.2%
Chandigarh	18239 ↑126	962	16981 ↑82	296 ↑3	93.1%	1.6%
Arunachal Pradesh	16395	735	15605	55	95.2%	0.3%
Meghalaya	124160 ↑96	602	11686 ↑113	122 ↑2	94.2%	1%
Nagaland	11479 ↑61	628	10666 ↑8	67 ↑1	92.9%	0.6%
Ladakh	8969 ↑73	793	8054 ↑42	122 ↑1	89.8%	1.4%
Sikkim	5213 ↑13	364	4639 ↑20	117	89%	2.2%
Andaman and Nicobar Islands	4778 ↑5	70	4647 ↑16	61	97.3%	1.3%
Mizoram	3968 ↑32	198	3764 ↑8	6	94.9%	0.2%
Dadra and Nagar Haveli and Daman and Diu	3345 ↑5	21	3293 ↑2	2	98.4%	0.1%

Fig. 1. State-wise distribution of Covid-19 cases in India (Sources: MoHFW; https://www.covid19india.org).

Government of India is encouraging and rigorously enforcing the practice of isolation, contact tracing, social distancing and wearing of mask and had implemented a complete nationwide lockdown to prevent the spread of the virus. The MOHFW and Government of India has implemented the new protocol for the clinical management of COVID-19 and the protocol also mentions the directions for investigational therapies such as the use of remdesivir, tocilizumab, convalescent plasma therapy and prophylactic dose of low-molecular weight heparin such as enoxaparin. Dexamethasone, a corticosteroid, has also been included in the treatment protocols for COVID19 patients in moderate to severe stages of illness among other therapeutic measures (Clinical management protocol: COVID-19 India, 2020). The use of azithromycin in combination with hydroxychloroquine (HCQ) to treat patients with severe coronavirus infections has been rolled back.

3. COVID-19 scenario

India is eagerly awaiting a COVID-19 vaccine to prevent COVID-19 and thereby prevent the complications and deaths resulting from the disease. The nation is the worst-hit country in Asia; it just surpassed Brazil as the country with the second-highest number of cases, after the United States. India recorded its highest single-day spike with 97 894 cases on September 16, 2020. The spike is also the highest daily cases of any country in the world since the pandemic outbreak. Maharashtra has been the worst affected state in the country, Karnataka came in second place, Andhra Pradesh is the third, and Tamil Nadu has the fourth highest number of infections. With the opening up of more activities due to a variety of region-specific reasons such as poverty, labour migrations and economic slowdown the state and central government were forced to provide relaxations (Ranga et al., 2020) from September 1, 2020. With unlock 4.0, more than one lakh cases per day were expected. However, a gradual drop is being seen in the number of daily cases and since mid-September this has been consistent. The daily cases have dropped from about one lakh to nearly 60 000 in October 31, 2020 and 31 179 in November 30, 2020 despite ramping up testing capacity. After five months of suspension, metro rail services resumed in selected parts of the country. With facemasks and social distancing protocols being mandatory, only asymptomatic people will be allowed to board the trains. Even today, citizens of India continue to be frightened into compliance and are "afraid to restart their lives normally". Though many states of India have flattened their COVID-19 infection curve, authorities across the nation are now fearing the onset of a "second wave of infection" due to festival crowding as well as monsoon. Govt. Of India has advised the citizens to take precautionary measures like social distancing and wearing of mask during public gathering. Further, few states like Maharashtra, Rajasthan, Gujarat, etc. Have introduced new restrictions such as travel restrictions and night curfew to battle a second wave.

4. COVID-19 related clinical trials

Seven Indian pharmaceutical companies namely Bharat Biotech, Serum Institute, Zydus Cadila, Panacea Biotec, Indian Immunologicals, Mynvax and Biological E have initiated the development of COVID-19 vaccine in India. The Drug Controller General of India (DCGI) has granted permission to start phase I and II human clinical trials of the most advanced vaccines of Bharat Biotech and Zydus Cadila, named Covaxin (Clinical Trials Registry-India (CTRI): CTRI/2020/07/026 300) and ZyCov-D (CTRI/2020/07/026 352), respectively. The Indian Council of Medical Research (ICMR) has developed the indigenous COVID-19 vaccine (BBV152 COVID-19 vaccine or Covaxin) partnered with Bharat Biotech International Limited (BBIL). The Phase-III human trials of indigenous COVID-19 vaccine Covaxin has already begun at All India Institute of Medical Science (AIIMS) in New Delhi. The Council of Scientific and Industrial Research (CSIR), India is working towards the development of activated vaccines such as RNA vaccines and recombinant DNA vaccine. Serum Institute of India, which is handling the clinical trials in India, has already received a nod from India's top drug regulatory body for conducting phase-II and III clinical trials on 'Covishield' vaccine (CTRI/2020/08/027 170) and has an agreement with AstraZeneca to manufacture and market the vaccine in India. Unfortunately, Covishield trials were stopped as a precautionary measure after one of the volunteers in the UK trials was diagnosed with transverse myelitis. Twelve weeks ago, Serum Institute of India restarted the clinical trial after a shot pause. Moreover, the clinical trials of favipiravir indicate that early treatment with favipiravir may improve clinical outcomes for patients with mild to moderate COVID-19 infection and could potentially prevent patients from progressing to ARDS and mortality. Furthermore, Zydus Cadila has received approval from the DCGI to start the phase 3 clinical trials of its biological therapy Pegylated Interferon alpha-2b or PegiHep in COVID-19 patients. Very recently, Indian drug maker Dr Reddy's Laboratories (DRL) has signed the agreements with Russian Direct Investment Fund (RDIF) for conducting final-stage human trials of Russia's Sputnik V Covid-19 vaccine in India, with an aim to distribute 100 million doses to Indians beginning in the late 2020, DCGI has also granted permission for conducting trials. Short while ago, Pfizer India has sought permission from the DCGI to import the vaccine (Pfizer/BioNTech vaccine against COVID-19) for sale and distribution in the country. But the challenge of logistical issues linked to the distribution of this vaccine to smaller towns and rural areas remains as the vaccine needs to be stored at a temperature of −70 °C. Serum Institute of India has also sought government approval for emergency use of the coronavirus vaccine (The Indian Express, 2020). Pharmaceutical companies and government agencies worldwide are working round the clock to find a vaccine against the virus. There are more than 163 candidate vaccines in development worldwide, of these 51 vaccines are in clinical evaluation with 13 candidates moving into the final phase of testing (Mullard, 2020; COVID-19 Candidate Vaccines, COVID-19 update. WHO, 2020). Moreover, around 172 countries are engaging with the COVAX facility designed to ensure equitable access to Covid-19 vaccines. The WHO is also coordinating global efforts to develop a vaccine, with an eye toward delivering two billion doses by the end of 2021 (Coronavirus coverage, 2020).

5. AYUSH

Traditional, complementary and alternative medicine systems have a long history and also play an important role in providing primary healthcare to populations. In India, the published data provide fruitful evidence of the antiviral properties of the traditional formulations of the AYUSH systems of medicine (Muthappan and Ponnaiah, 2020). Indian Systems of Medicine (ISM) is defined as systems of medicine which are considered to be of Indian origin or the medicine systems which have adapted to the Indian culture. India has unmatched alternative systems of medicine in the form of Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homeopathy, which are now jointly referred to as AYUSH, recognized by the Government of India (Rudra et al., 2017). Ministry of AYUSH, Govt. Of India has also issued an "Advisory on Coronavirus" to manage this outbreak and this broadly comprises of preventive and prophylactic symptom management of COVID-19 like illnesses and also insights to interventions based on AYUSH systems of medicine through evidences for immunity boosting as well as relieving the respiratory symptoms. Since the beginning of the pandemic in India, the sale of local immunity boosting products has increased drastically. Several states have started including AYUSH systems of medicine in their strategy to fight COVID-19, and also certain food ingredients to naturally boost one's immunity. The world has seen how certain traditional Indian ingredients and homemade products have benefited mankind. The advisory has also suggested AYUSH medicines as add on interventions to the conventional care. These are presented in Table 1 (Ministry of AYUSH, Government of India, 2020). In total 125 studies were registered in CTRI as of 11th July in which 87 (69.6%) were trials exploring Ayurvedic interventions followed by Homeopathy (12%) and Siddha (11.2%). (Charan et al., 2020).

Table 1
AYUSH approach to manage the outbreak of pandemic.

Medical system Name	Preventive and prophylactic	Symptom management of COVID-19 like illnesses	Add on Interventions to the conventional care
Ayurveda	Samshamani Vati 500 mg: twice a day with warm water for 15 days. The medicine contains aqueous extract of Tinospora cordifolia.	AYUSH-64: 2 tablets twice a day. Agasthya Hareetaki: 5 gm twice a day with warm water. Anuthaila/Sesame oil: 2 drops in each nostril daily in the morning.	AYUSH-64: 2 tablets twice a day. Agastya Hareetaki: 5 gm twice a day with warm water.
Siddha	Nilavembu Kudineer decoction 60 ml: twice a day for 14 days. The medicine contains aqueous extract of Andrographis paniculata & others.	Nilavembu Kudineer/Kaba Sura Kudineer decoction: 60 m1 twice a day. Adathodai Manapagu syrup: 10 ml twice a day.	Vishasura Kudineer: decoction: 60 m1 twice a day. Kaba Sura Kudineer decoction: 60 m1 twice a day.
Unani	Preparation of decoction by boiling Behidana (Cydonia oblonga) 3 gm, Unnab (Zizyphus jujube) 5 in number. Sapistan (Cordia myxa) 9 in number in water. This decoction may be taken twice a day for 14 days.		
Homoeopathy	Arsenicum album 30: daily once in empty stomach for three days. The dose should be repeated after one month by following the same schedule till corona virus infections prevalent in the community.	Various medicine which found to be effective in treating flu like illness are Arsenicum album, Btyonia alba, Rhus toxico dendron, Belladonna Gelsemium Eupatorium perfolia tum. All these medicines should be taken in consultation with qualified physicians of respective AYUSH systems.	Medicine mentioned symptom management of COVlD-19 like illnesses.

Most were sponsored by the government and various stakeholders associated with the Ministry of AYUSH. Currently, several prophylactic, observational and interventional clinical trials are in progress at a good pace to evaluate the safe and effective use of AYUSH medicines in participants with COVID-19 infection. Further, more data continue to emerge, stressing on the beneficial effects of AYUSH medicines through controlled clinical trials which might be potent to treat novel coronavirus.

6. Post COVID management protocol

Although, the mortality rates for COVID-19 cases in India remain less than 2%, long term complications among survivors of the infection having clinically significant disease are not yet available. The post-infection complications are rising and this is the major concern for upcoming years. Most of the patients are managing well in isolation during the first 10 days, but many are also developing breathing difficulties after turning COVID-19 negative. Only follow-up studies will clarify the extent of the sequelae on organ functions, such as respiratory, renal, cardiovascular, as well as psychological or psychiatric disorders and related chronic pain. MOHFW, Government of India has published the post COVID-19 management protocol which includes drinking of adequate amount of warm water, immunity promoting AYUSH medicines, personal hygiene, practice of Yogasana, Pranayama and meditation, balanced nutritious diet, avoid smoking and consumption of alcohol and self-health monitoring at home. Moreover, the protocol also emphasized that the recovered individuals should be encouraged to share their positive experiences with their friends and relatives for creating awareness. The first follow up visit (physical/telephonic) should be within 7 days after discharge, preferably at the hospital where he/she underwent treatment. Subsequent treatment/follow up visits may be with the nearest qualified allopathic/AYUSH practitioner/medical facility of these systems of medicine (Post COVID management protocol, 2020). In the present scenario lack of vaccine and drugs to prevent or treat coronavirus patients is a challenge for healthcare professionals. Hence, exploring every possible treatment strategy is essential and might prove beneficial for containing COVID-19 infection. MOHFW and Ministry of AYUSH are the two pillars of the Indian health care system to manage the current pandemic outbreak. Data from on-going clinical trials of vaccines for COVID-19 and AYUSH medicines for prophylaxis, and symptomatic relief in COVID-19 infected patients are awaited with much interest.

Funding

No funding Source.

Ethical approval

Not required.

Declaration of interests

We declare no competing interests.

Declaration of competing interest

The author declares no conflict of interest.

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OPEN ACCESS Freely available online

Journal of Bioequivalence & Bioavailability

Short Communication

Compliance to WHO'S Strategic Preparedness and Response Plan 2019 (n-CoV): Success Story of Department of Indian Medicine, Tamilnadu

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INTRODUCTION

The World Health Organization declared COVID-19 as "public health emergency of international concern "on January 30, 2020 and called for collaborative efforts of all countries to prevent the rapid spread of COVID-19 [1]. This Operational planning guideline was developed to accompany the SPRP 2021, to provide countries with practical, high-level actions under each of ten preparedness and response pillars that can be adapted as appropriate and implemented at national and subnational levels [2].

The Strategic response plan towards mitigation of COVID-19 by WHO is a capsule of the activities to be done by the authorities in administration right from the head of the nation and to all the stake holders involved in the mitigation. The strategic plan has ten pillars and understanding the effectiveness of these action points, Commissionerate of Indian medicine had followed every single action point in collaboration with the mainstream health stake holders, district magistrates and administrators of disaster management.

The key actions outlined in this document are quantitative and qualitative evaluation of response on par with ISM Department of Tamilnadu are as follows:

KEY ACTIVITIES

Activate multi-sectorial, multi-partner coordination mechanisms to support preparedness and response

A Multi sectoral team involving Taskforce of Tamilnadu and Various officials a team has been formed for Mitigation by ISM. All the district level officers have been instructed to act in prevention, containment, and treatment. The guidelines issued by Ministry of AYUSH [3] are followed and a separate G.O. (no. 201/ 23/04/2020) has been issued. Based on this G.O.19000 cases were treated in Siddha Covid Care Centres which reduced the burden on IP Beds in Govt. hospitals by admitting the needy only in the first wave. Siddha based Interventions are used is a big support to AYUSH system. 1,78,43,773 members (44%) have taken Kabasura Kudineer as Prophylaxis in Chennai alone. 11,08,875 in home quarantine 20,52,892 people in containment zone were benefitted with Kabasura Kudineer and Nilavembu Kudineer – Siddha Formulary drugs [4,5]. In this process 1050 Siddha manpower has been utilised.

Risk communication and community engagement

Directorate of Indian medicine has worked in collaboration with Disaster team and periodically communicated the benefit and limitations of Siddha intervention during the Camps and community meetings. All the containment zones were provided with Kabasura Kudineer as prevention.

Surveillance, rapid response teams and case investigation

Certain cases returned from a single cluster point has been quarantined and given medication. People who turned positive has been treated in Integrative manner. People who have been isolated, who were in Quarantine centres (Melapalayam) have been taken proper care by giving Siddha Interventions and watching them for symptoms and contained properly.

Points of entry, international travel and transport, and mass gatherings

Contact cases identified *via* tracing arising from the clusters have been isolated and treated successfully. As an example a single case (Female) belonging to Krishnapuram who has been a contact of particular cluster has been traced, quarantined and treated and parallelly entire village have been treated with prophylactic intervention and observed for recurrence of disease.

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Received: August 06, 2021; **Accepted**: August 20, 2021; **Published**: August 27, 2021

Citation: Ganesh S, Parthiban P, Kanakavalli K, Sathiyarajeswaran P, Shree Devi MS (2021) Compliance to WHO'S Strategic Preparedness and Response Plan 2019 (n-CoV): Success Story of Department of Indian Medicine, Tamilnadu. J Bioequiv Availab. 13: 439.

Laboratories and diagnostics

Directorate of Indian medicine has established contacts with the Covid-19 testing centers (Govt. and Govt. Approved) and was utilizing their services to admit patients with RTPCR positivity who are debounded with in Asymptomatic and mild category. The standardization of interventions (Kabasura Kudineer) used have been tested in State and Central laboratories for their standards.

Infection prevention and control, and protection of the health workforce

Almost all the front-line workers including police personnel had the opportunity to take Kabasura Kudineer intervention. Aarokyam Android Mobile application has documented 4443 records of the Front-line workers, who feels better in health status.

Case management

Cases were treated in three different category, Home quarantine, Isolation wards and Covid Care Centres. 29 Covid centres have been opened exclusively for Siddha. All home quarantined individuals received Kabasura Kudineer along with regular Siddha Standard of Care. Certain Medical college Covid Care distributed Kabasura Kudineer along with Allopathic Standard of care. Around 28,000 cases have been treated in Siddha Covid Care Centre and discharged.

Operational support and logistics, and supply chains

Interventions for all the Siddha Covid Care Centres were supplied by Tamil Nadu Medicinal Plant Farms and Herbal Medicine Corporation Limited (TAMPCOL) and a strategy of bulk production is planned which is periodically monitored by Honorable Health Minister of Tamilnadu [6].

CONCLUSION

Trials were conducted following ethical guidelines including MEURI to check the efficacy of the drugs in Prevention therapeutics and as integrated medicine. Introduction of simple but effective Managements like Mooligai thiri pugai (Herbal Fumigation), Oma pottanam (Ajwain/Carom Seeds Medicated Pouch/Packs), and herbal masks are some of the innovations happened. They have also adopted international R&D blueprint guidance and WHO protocols for special studies (companionate use, Monitored Emergency Use of Unregistered and Investigational Interventions) to investigate additional epidemiological, virologic, and clinical characteristics; designate a clinical trial or study sponsor.

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Brief Communication

Is Mucormycosis an inevitable complication of Covid-19 in India?

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ARTICLE INFO

Article history:
Received 11 June 2021
Accepted 14 June 2021
Available online 5 July 2021

Keywords:
Mucormycosis
Covid-19
Steroidal drugs
Diabetes mellitus
Immunity

ABSTRACT

Mucormycosis or black fungus infection is a less common disease but highly fatal infection, infecting the immunocompromised individuals. The site of predilection of the fungus is found to be lungs and brain in addition to its sequestration in sinusoidal spaces. Presently with the ongoing COVID 19 pandemic, the prevalence of this infection is found to be high in the Indian population. The fungus establishes itself by affecting the compromised immune system of an individual and thereby making the individual susceptible to other diseases/ infection. The reasons attributed to the sudden upsurge are steroidal therapeutics abuse, tocilizumab therapy and diabetes mellitus.To avert the cytokine storm, the medical health workers are necessitated to include steroid drugs in COVID 19 treatment protocol however inclusion of these drugs in patients who do essentially require steroids can have their immune system debilitated and permit the invasion of this fungus. According to International Diabetes Federation (IDF), 77 million Indians are known to be diabetic, cautioning the physicians to be vigilante of the impending black fungus infection in the event of COVID19 affliction in such individuals. There is causal relationship between anti-hyperglycemic drugs and weakened immune system and opportunity for the fungus invasion. This review attempts to explain the inter-relatedness of COVID19 infection, its treatment and eventual black fungus infection risk.

Human Corona Viruses (HCoV) are the type of viruses that cause infections mainly in gastrointestinal and respiratory systems. The first discovered human corona viruses were HCoV−229E and HCoV−OC43 from the nasal cavities of human patients who were suffering from common cold, in 1960s.[1] Other human coronaviruses include SARS−CoV (in 2003), HCoV NL63 (in 2004), HKU1 (in 2005), MERS−CoV (in 2012), and the latest one SARS−CoV−2 (in 2019) resulting in coronavirus disease (COVID-19).[2] Globally, there have been 173,674,509 confirmed cases of COVID-19, including 3,744,408 deaths reported as on June 09, 2021. As of June 10, 2021 there were 1,167,952 active cases in India while 27,655,493 people had recovered from the disease.[3] While the national COVID-19 recovery rate dropped to 94.77%, the case fatality rate was down to 1.23% according to the MoHFW.

In the year 2021, multiple challenges have been faced while treating Covid-19 infections, including Mucormycosis

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https://doi.org/10.1016/j.bjid.2021.101597

(black fungus). Mucormycosis is an opportunistic fungal infection that belongs to the zygomycete family and is ubiquitous in the environment. The major route of infection is via inhalations of spores of Rhizopus oryzae a common cause of rhino-orbital-cerebral form of infections, which spread to the paranasal sinuses and lungs. These moulds are generally found in soil, plants, manure, decaying fruits and vegetables. Mucormycosis is a rare but severe and eventually fatal fungal infection that usually affects patients with altered immunity. In general, these fungi are non-pathogenic in immune competent patients but in the case of patients immune compromised by either steroids or any other co-morbidities like diabetes, solid cancers, malignant and hematologic disorders may become life-threatening. Other risk factors including organ transplantation and high levels of iron in serum may increase the risk of black fungus mortality.[4]

In covid-19 patients, especially those who need oxygen support, the immune system may become weak due to inflammatory storm or usage of steroids. This may pave the way for opportunistic infections including mucormycosis. The common symptoms of this infection include discoloration of the nose and tongue, redness in the eyes, blurred vision, difficulty in breathing, chest pain, cough, fever, headache, black lesion inside the mouth, and vomiting. Diagnosis is usually made by clinical suspicion and histopathological examination. As per recent reports in India, the number of people dying from black fungus is steadily increasing and experts are referring to it as 'pandemic within COVID-19 pandemic'.

In India more cases were noticed in the second wave of Covid-19 than in the first wave with increased mortality rate. The major complications faced with mucormycosis in the second wave were reduced oxygen levels, difficulty in breath and other respiratory tract associated problems like pneumonia and Acute Respiratory Distress Syndrome. At present in India, the patient who underwent long oxygen supportive therapy are more prone to be infected with mucormycosis.

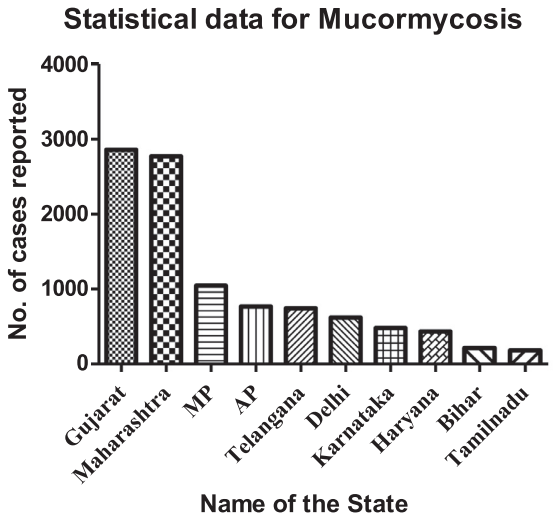

Fig. 1 – Mucormycosis cases across India. *MP= Madhya Pradesh, AP= Andhra Pradesh.*

During the second wave of Covid-19 pandemic the very first case of mucormycosis or black fungus infection was reported at Gujarat in a 15-year-old boy who recovered successfully from Covid-19 and got discharged. Later on the cases were reported in more than 18 states of India.[5] Auraiya district in Uttar Pradesh has reported the first death caused by *mucormycosis*. The highest death rate was recorded in Rajasthan. India has recorded more than 12,000 cases of *mucormycosis*, and over 300 deaths were recorded in the past few months.[5] Most of the cases are reported from Gujarat, Maharashtra, Rajasthan, Karnataka, Andhra Pradesh, Haryana and Telangana as per the latest government reoprts.[6] Ten states in India reported large number of *mucormycosis* cases; those are graphically represented in Fig. 1.

According to literature, it is clear that animals/humans with decreased number of phagocytes or having impaired phagocytic function are at higher risk of black fungus infection. Therefore, patients with severe neutropenia are at increased risk of developing the infection.[7] In contrast, as HIV/AIDS patients do not seem to be at increased risk for developing black fungus infection, it suggests that neutrophils play a key role in the inhibition of fungal spore proliferation, but not necessarily T lymphocytes. In the condition of diabetic ketoacidosis, phagocytes are dysfunctional and have impaired chemotaxis and defective intracellular killing by both oxidative and non-oxidative mechanisms. The pulmonary alveolar macrophages harvested from lungs of immunocompetent mice are able to inhibit the germination of fungal spores. But the same pulmonary alveolar macrophages isolated from immunosuppressed mice are unable to inhibit the fungal spore germination in *in-vitro* and *in-vivo* experiments.[8] The mechanisms by which phagocytes turn dysfunctional by diabetes mellitus and corticosteroids need to be determined.[8] One of the essential elements for cell growth and development is iron. Therefore, pathogens use some processes for obtaining iron from the host. Transferrin, ferritin, and lactoferrin are the carrier proteins to which the excess iron is bound in hosts and avoids toxic effect of free iron by reducing the excess free iron levels in serum. This is the unique host defense mechanism against bacteria, virus and even fungus especially mucorales because they cannot grow in normal serum unless iron is added exogenously.

To treat severe *mucormycosis* infection, the patient should be strictly monitored by a team of health specialists including microbiologists, internal medicine specialists, neurologists, ENT specialists, ophthalmologists, dentists, surgeons, etc. In India the first line drug being used for treating *mucormycosis* is liposomal amphotericin-B and approximately 20 vials of this antifungal injection may be required to treat a single infected person. In India, the current price for each vial is around Rs.5000-6000, according to reports. The second line drug is Posaconazole. Doctors are warning that all these medicines should only be taken under strict medical supervision.

In few cases surgical removal of infected tissues may be required. At the hospital MGM Health care located at Chennai, Tamilnadu a person diagnosed with black fungus in the right naso-orbital region involving sinuses was

saved by a surgery performed with the help of multiple angled high-definition endoscopes. Of a total of 700 cases reported in Jaipur over 200 patients underwent jaw removal surgery.

Abuse of steroids

WHO strongly recommends corticosteroids to be given orally or intravenously for the treatment of patients with severe and critical COVID-19 once daily for 7-10 days and advised to not to be given for the patients with non-severe COVID-19. Steroids are an admirable treatment, if used judiciously and in the appropriate condition. Experts believe that the improper use of corticosteroids (i.e. dexamethasone, hydrocortisone or prednisone) in Covid-19 patients is the primary reason for black fungus infection. Even though steroids are very good agents to reduce many types of inflammations like rheumatoid arthritis and pulmonary diseases such as asthma and chronic obstructive pulmonary disease, the chronic usage or over dose of steroids will suppress the body's immune response and make the patient more prone to other infections like *mucormycosis* or black fungus infection in India.

Improper usage of oxygen cylinders

Another probable reason for the surge in post-COVID *mucormycosis* is the unhygienic delivery of oxygen or low-quality tubing system to these patients at the hospital ICUs, the oxygen cylinders with unclean masks or using contaminated/tap water in humidifiers and prolonged usage of same mask for more than two patients.[9]

Worsening of diabetes

Diabetes mellitus is a chronic life treating metabolic disorder and is the 6th leading cause of death in India. The incidence of diabetes in India has increased dramatically over the past 40 years. Around 77 million people have been diagnosed with diabetes in India, over 90% type-2 diabetes mellitus (T2DM). T2DM is caused by insufficient insulin secretion by pancreatic β-cells and/or the inability of insulin-sensitive tissues to respond appropriately to insulin. T2DM may increase risk of developing black fungus infection in post-COVID-19 patients with more than 8 in 10 black fungus cases diagnosed in patients with diabetes. Diabetics usually have lower immune responsiveness which is further impaired by hypoglycemic agents. Extensive care should be taken in Covid-19 patients with the history of diabetes mellitus.

Tocilizumab therapy

COVID-19 infection induces a dose-dependent production of IL-6 pro-inflammatory cytokine from bronchial epithelial cells. To control the levels of IL-6 in these particular patients, tocilizumab, an FDA approved drug, has been prescribed along with dexamethasone. Tocilizumab is a recombinant humanized anti-IL-6 receptor monoclonal antibody. Chronic usage of this therapy to control inflamation triggered by severe acute respiratory syndrome-associated corona virus (SARS-CoV) weakens the patient's immune response. In this way tocilizumab increases the risk of *mucormycosis* in post-COVID-19 patients.

Conclusion

COVID-19 disease is shaking the world as did the Spanish flu in 1918 (caused by the H1N1 influenza-A virus). It had very fast dissemination over the globe, but with an appreciable recovery rate. In 70–80% of COVID-19 patients the severity of the infection is mild-moderate and recover without any other complications. However, in the remaining patients the infection is rather severe and the respiratory system may collapse. Those patients require external oxygen supply, corticosteroid administration and regular monitoring of blood glucose levels. The improper use or chronic usage of this treatment measures may lead to the development of other infections like black fungus infection and even white and yellow fungal infections.

Conflicts of Interest

All authors are declaring that they have no conflicts of interest.

Financial support and sponsorship

Nil.

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Perspective

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Kabasura Kudineer, a Siddha medicine against COVID-19 infection: scope and future perspective

Abstract

Indian Systems of Medicine (ISM) is defined as medicines which are considered to be of Indian origin or the medicine system which has adapted in to Indian culture. India has unmatched alternative system of medicine in the form of Ayurveda, Yoga and Naturopathy, Unani, Siddha, Homeopathy, which is now jointly referred to as AYUSH, recognized by the Government of India. Siddha medicine is one of the oldest and well documented medical systems of India. Retrospective studies revealed that Siddha medicines are used for prophylaxis and to treat viral diseases such as chicken pox, mumps, influenza, dengue etc. No specific drug is available till date to combat the rapid spread of COVID19. Further, exploring every possible treatment option will be of greater significance. Siddha medicine such as Kabasura Kudineer is recommended officially by the government for clinical management of viral infections. Data from on-going clinical trials of Kabasura Kudineer for improving the symptoms of COVID-19 are awaited with much interest. Kabasura Kudineer might be potent adjunct therapeutic option in the management of COVID-19.

Keywords: Indian Systems of Medicine, Siddha medicine, Kabasura Kudineer, COVID-19

Volume 14 Issue 3 - 2021

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Received: June 15, 2021 | **Published:** June 30, 2021

Indian Systems of Medicine (ISM) is defined as medicines which are considered to be Indian origin or the medicine system which has adapted in to Indian culture. India has unmatched alternative system of medicine in the form of Ayurveda, Yoga and Naturopathy, Unani, Siddha, Homeopathy, which is now jointly referred to as AYUSH, recognized by the Government of India . In addition, ISM comprising of Ayurveda, Yoga, Unani and Siddha were practiced since time immemorial and much before the recognized health system came into vogue.[1] Siddha medicine is one of the oldest (5000 years old) and well-documented medical systems of India and is practiced mainly in South India especially in Tamil Nadu,[2] but also in Sri Lanka, Malaysia, Singapore and Mauritius on account of migration of people native to Tamil Nadu. The Government of India through its Ministry of AYUSH holds responsible for policy formulation, development and implementation of programs for the growth, development and propagation of Siddha System of Medicine.

In the current, pandemic situation, a myriad of strategies would be highly critical to combat the rapid virus spread and to treat the infection. The Ministry of AYUSH, Government of India has taken numerous initiatives and setup an interdisciplinary AYUSH research and has implemented various measures to control the spread of COVID-19 including a Task Force involving experts from diverse fields to formulate and develop strategies to control the spread of virus and infection. The Ministry of AYUSH, Govt. of India has issued an 'Advisory on Corona virus' to manage the outbreak which broadly comprises of preventive and prophylactic symptom management of COVID-19 like illnesses and also insights to interventions based on AYUSH systems of medicine through evidences for immunity boosting as well as help in improving the respiratory symptoms. Kabasura Kudineer (KK) is one of the drugs included in advisory of Govt. of India, released by the Ministry of AYUSH for symptomatic management of COVID-19.[3,4] KK, is a classical Siddha formulation which comprises 15 herbal ingredients such as Zingiber officinale, Piper longum, Syzygium aromaticum, Tragia involucrata, Anacyclus pyrethrum, Andrographis paniculata, Hygrophilla auriculata, Terminalia chebula, Justicia adhatoda, Plectranthus amboinicus, Costus speciosus, Tinospora cordifolia, Clerodendrum serratum, Sida acuta and Cypreus rotundus.[5] It has been recommended to boil approximately 5gms of KK coarse powder in 240ml of water until it reduces to one-fourth of its quantity. Consumption of the concentrated 30 to 60ml of KK decoction twice or thrice daily can alleviate the symptoms of COVID-19.[6] According to Siddha medicinal practice, 'Kaba' or 'Kabam' represents one of the three subtle physiological principles existing in our body (Vatham, Pitham, Kabam), which should be maintained in equilibrium for one's physical and mental well-being. Imbalance of 'Kabam' generally correlates with respiratory tract ailments. 'Sura' or 'Suram' means fever. 'Kudineer' represents a therapeutic recipe in the form of decoction.

The anti-viral properties of Siddha traditional formulations are backed with strong scientific evidences.[7–9] In 2015, the KK formulation was used and found to be effective in swine flu influenza outbreaks.[10] The state government/research institutes have distributed Siddha medicines to millions in the Indian population with an intent to boost the immunity against COVID-19 with a special focus to the containment zones in Tamil Nadu, India. Short while ago, the Union Ministry of AYUSH has launched a massive nationwide campaign to distribute its proven poly herbal Siddha drug Kabasura Kudineer, aiming to strengthen its position in fight against COVID19 pandemic. In addition, state authorities have also opened exclusive Covid-19 treatment centre with Siddha System of Medicine in Tamil Nadu. It is also believed that traditional system of medicine improves general psychological quality of people thereby reducing infection risk.[11]

Recently, Kiran et al. provided in-silico methods to narrow down on nine phytoconstituents of KK such as Magnoflorine, 5-Hydroxy-7,8-dimethoxyflavanone, Tinosponone, Cirsimaritin, Chrysoeriol, 6- Methoxygenkwanin, Vasicinone, Quercetin and Luteolin that may have high binding affinity and good binding interactions with SARS-CoV-2 spike protein. Additionally the phyto-constituents were free from carcinogenic and tumorigenic properties.[5] Further, Acetoside,

Luteolin 7 –rutinoside, Rutin, Chebulagic acid, Syrigaresinol, Acanthoside, Violanthin, Andrographidine C, Myricetin, Gingerenone -A, Tinosporinone, Geraniol, Nootkatone, Asarianin, and Gamma sitosterol are the primary compounds from KK formulation which may inhibit COVID-19 by acting on the main protease (Mpro) with better energy.[12] Moreover, many of the herbs which are present in the KK decoction had demonstrated anti-viral activity against dengue virus serotype 1 (DENV1), influenza A virus, and HIV, antiplatelet aggregation activity, immuno-modulatory and anti-inflammatory activities.[13] Recently, Shree Devi et al. provided in-vitro anti-viral activity of water extract of KK was able to reduce viral RNA of E and N of novel Corona virus (SARS-CoV-2).[14] Further, these Siddha medicines might be enough potent to prevent and also be an adjunct treatment option against novel corona virus. Therefore, it is essential to elucidate the underlying molecular mechanisms of these medicines. In addition, patients with COVID-19 positive who were treated with integrated medicine –Zinc (150mg), Vitamin C (500mg) and Kabasura Kudineer (ZVcKK) benefited with shorter hospitalization and amelioration of symptoms. Furthermore, patients treated with ZVcKK had reduced the hospital stay by 7days. During the conduct of the trial, there was no report of serious adverse events and mortality in the subjects.[6] Currently, several clinical trials involving Siddha medicine either as stand-alone or adjunct with allopathic medicine that are taken up; such as a double blinded placebo controlled comparative clinical trial to evaluate the effectiveness of Siddha medicines, KabaSura Kudineer & Nilavembu Kudineer along with standard allopathy treatment in the management of symptomatic COVID 19 patients (Clinical Trials Registry- India (CTRI) registry: CTRI/2020/ 08/027286). The efficacy of Siddha medicine, Kabasura Kudineer compared to Vitamin C & Zinc (CZ) supplementation in the management of asymptomatic COVID-19 cases (CTRI/2020/05/025215). Also the efficacy and safety of Siddha treatment in COVID-19 patients (CTRI/2020/06/025625). The efficacy of Kabasura Kudineer and Vitamin C & Zinc supplementation in asymptomatic COVID-19 patients is progressing which is a prospective, single centre, randomized open labelled clinical study (CTRI registry: CTRI/2020/05/025215) etc., are in various stages of completion/yet to be published. Further, more data continue to emerge, stressing on the beneficial effects of Siddha medicines through controlled clinical trials which might be potent to treat novel corona virus.

Acknowledgments

None.

Conflicts of interest

The author declares no conflicts of interest

Funding

No funding sources.

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