ORIGINAL ARTICLE

Anti–Proliferative Activity of Green Synthesized Silver Nanoparticles (AgNPs) from a Siddha Medicinal Plant *Enicostema axillare* (Poir. ex Lam.) A.Raynal

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ABSTRACT

INTRODUCTION: Silver nanoparticles (AgNPs) have attracted more attention of the researchers due to its distinctive chemical, physical and biological properties. In the present study, a Siddha medicinal plant *Enicostema axillare* (Family: Gentianaceae, Tamil: *Vellarugu*) was used to synthesize AgNPs through green route and their anti-proliferative activity was studied in cell line model. **MATERIALS AND METHODS:** Green synthesized AgNPs using *Enicostema axillare* extract was characterized using UV, TEM, XRD and zeta potential techniques and also evaluated for their anti-proliferative activity in DLA cell line model. **RESULTS:** Among different solvents, methanol extract of *Enicostema axillare* was contained high total phenolic concentration (5412 mg GAE / L), and hence it was used to synthesize AgNPs. Formation of AgNPs was confirmed by UV absorption (443 nm) while TEM results indicated the spherical shape and diameter of AgNPs (10.93 - 13.95 nm). Results of zeta potential (-13.5 mV) showed the stability of hydrated AgNPs and the XRD and FT-IR data indicated the crystalline nature and presence of extract as capping agent, respectively. Green synthesized AgNPs showed remarkable anti-proliferative activity against the DLA cell line (74 %, IC-50 value 0.053 mg/ml). **CONCLUSION:** The Siddha plant *Enicostema axillare* could be explored to synthesis AgNPs by green route with remarkable anti-proliferative property.

KEYWORDS: *Enicostema axillare,* AgNPs, Green synthesis, DLA cells, Anti-proliferative.

1. INTRODUCTION

The advancement in the research of nanoparticles has led to its wide applications in the medicinal industry[1]. Among the many metal nanoparticles available, silver nanoparticles (AgNPs) have gained attention because of their distinctive characteristic features. They are unique in nature which has led to their swift renovations in a large number of fields ranging from biosensors, tissue engineering, chemical engineering, drug-gene delivery, food and feed, deoxyribonucleic acid (DNA) to cosmetics[2]. Currently they have been attracted attention due to their high efficiency against various forms of cancer[3]. Over the ages, AgNPs synthesis has been carried out through well-known physical, chemical and biological methods[4]. Biosynthesis of nanoparticles are having more advantages over chemical and physical methods because of the fact that biological synthesis methods use bacteria, plants, yeast and fungi[5]. The enormous energy consumption, usage of toxic chemicals, expense and its impact on environment has led to the discovery of green approach which is an eco-friendly, nontoxic

method and has better control over the size and shape of the nanoparticles[6]. In the present study, we have made an attempt to synthesize AgNPs using Siddha medicinal plant *Enicostema axillare*. *Enicostema axillare*, commonly known as Indian whitehead and *Vellarugu* in *Tamil*, belongs to the family Gentianaceae. It is a small perennial herb found throughout India and in some coastal regions[7]. It has been reported to grow up to 40 cm tall with 4 angled stems. Traditionally it is used in folk medicine for the treatment of diabetes mellitus, rheumatism, abdominal ulcers, swelling, itching and insect poisoning[8]. Recently it has been reported that this plant possess hypolipidemic[7], antioxidant [7], hepato-protective[8], anti-nociceptive[9] and antimicrobial properties[10]. In addition, *E. axillare*has also been used in the treatment of leucoderma, veterinary diseases and as anticancer drug[11]. Several secondary metabolites like iridoid glycosides wertiamarin, monoterpene alkaloid gentiocrucine, erythrocentaurin, triterpene sapogenin and flavonoids such as apigenin, genkwanin, isovitexin, swertisin, 5-o-β-D-glycoside were reported[12].In this present study, we have synthesized and characterized the AgNPs prepared through green synthesis approach using *E. axillare* extract and evaluated their anti-proliferative activity through DLA cell line model.

2. MATERIALS AND METHODS

2.1. Extract preparation

Leaves of *Enicostema axillare* were collected near SASTRA Deemed to be University campus, Thanjavur, India on November 2018 and the collected plant were identified and authenticated by Dr. N. Ravichandran, Botanist, Centre for Advanced Research in Indian System of Medicine, SASTRA Deemed to University, Thanjavur. Plant materials were dried for 1 week, thoroughly washed and grounded into fine powder using domestic pulveriser. Extract was prepared by taking 100 g of the dried plant powder sequentially with 500 ml of solvents with different polarity (hexane, chloroform, ethyl acetate, methanol and water) for 3 hours at room temperature with occasional shaking. This was filtered using a filter paper and the filtrate was subjected to rota-vapour (Buchi R-300) and dried in hot air oven at 50°C. The dried extract was re-dissolved in distilled water at the ratio of 10 mg/ml and stored at room temperature for future experiments.

2.2. TPC analysis

The total phenolic content (TPC) was measured using Folin-Ciacalteu reagent method[13]. The extracts (100 µl) were taken along with 250 µl of FC reagent and 1 ml of 5% sodium bicarbonate and mixed well. This was incubated in dark for 30 minutes after which the absorbance was measured at 720 nm. Gallic acid was used as standard (R^2 = 0.99) and the TPC was calculated using the formula, y = 0.0094 x – 0.0585 and the results were expressed as Gallic acid equivalents (mg GAE / L).

2.3. AgNPs synthesis

Since methanolic extract of *E. axillare* was found to possess high TPC, we have used this extract (10 ml) for the AgNPs synthesis by adding 90 ml of 1mM silver nitrate solution. The contents were mixed in a conical flask and kept in sunlight for one hour. The AgNPs synthesis was observed by the formation of dark brown coloured colloid, which was further confirmed by UV-Visible scanning (200–800) and further characterized by zeta potential, XRD (Bruker, D8 Focus) and TEM (Jeol JEM-2100F).

2.4. Anti-proliferative activity

The anti-proliferative activity was evaluated in DLA cell line model using MTT assay[14]. The cells were maintained in RPMI 1640 medium supplemented with 10% heat-

inactivated foetal bovine serum, 1% nonessential amino acids, and 1% penicillin (5000 IU/mL)-streptomycin (5000 μL/mL) solution at 37∘C under 5% CO2 atmosphere. After reaching 60% confluence, the cell was harvested and transferred to 96-well plate with a cell count of 10,000 cells per well and incubated for 6 h. Then, different concentrations (12.5, 6.25, 3.12, 1.56, 0.78 and 0.39 mg/ml) of extract and standard drug (5 Fluoro uracil) were added to the wells containing cancer cells and incubated for 6 h. Then 20 µL of 5% MTT reagent ((3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) was added in each well and incubated for 3 h at 37o C. Then 100 µL of Acidic isopropanol (0.05 M HCl in Isopropanol) was added and shaken for 30 min on a plate shaker under dark to dissolve the formazan crystals. The absorbance was measured at 590 nm and the percentage of cytotoxicity was calculated and expressed on percentage basis.

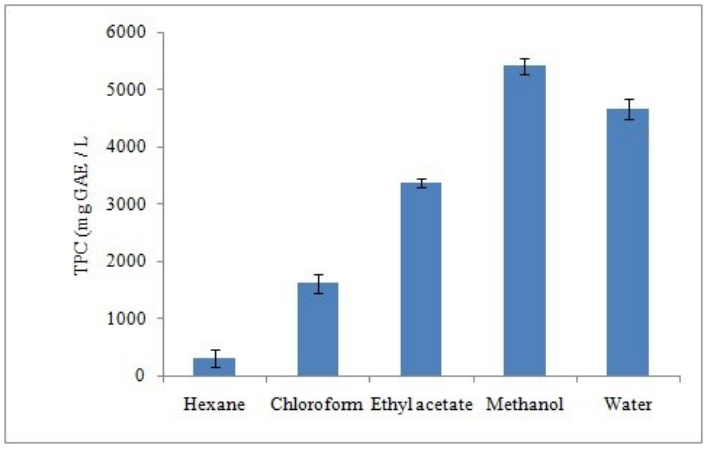
3. RESULTS AND DISCUSSION

3.1. TPC of extracts

Phenolics are a class of compound that exist naturally in an approximated number of 8000. They share the identical prevalent structure composed of an aromatic hydroxyl nucleus. So far, one of the main groups of compounds working as primary antioxidants or free radical scavengers belongs to plant phenolics. These polyphenols are effective as singlet oxygen scavengers, reducing agents as well as hydrogen atom donators. Because of this, it is logical to ascertain their total amount present in the prepared extracts *E. axillare*. There are other readily oxidized compounds in the plant materials and the heterogeneousness of natural phenolics has led to the introduction of several methods to determine total phenolics amount.

Commonly, Folin-Ciocalteu method has been found preferable. Here, a blue-coloured solution was formed when the active extracts or fractions reacted with Folin-Ciocalteau reagent in an alkaline medium due to the presence of phospho-molybdic and phosphotungstic-phenol complexes. The total phenolic content of the plant extracts showed large variations, between 198 and 5313 mg GAE/L extract (Figure 1). Based on the results, we can see that the extracts contained a mixture of phenolic compounds at different levels in the following increasing order: Hexane > Chloroform> Ethyl acetate > Water > Methanol. Methanol extract contained the highest total phenol content (5313 mg GAE/L). Phenolic groups present in plants exhibit strong antioxidant with high reducing capacity which was used in AgNPs synthesis[15]. Thus, Methanol extract has been selected for synthesizing silver Nanoparticles.

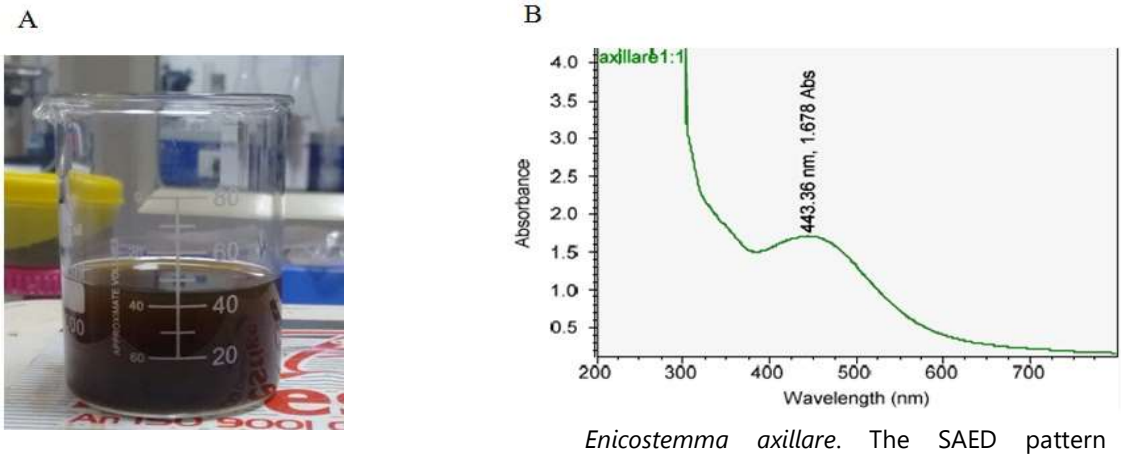
Figure 1. Total phenolic concentration of different solvent extracts of *Enicostema axillare*



3.2. UV-Visible spectroscopy

The formation of silver nanoparticles can be visualized after the addition of methanolic extract of *E. axillare* in 1 mM silver nitrate solution, which is evident of change in colour light yellow to dark brown due to reduction of silver ion by extract which is characterized by using UV-Visible spectroscopy owing to the surface plasmon resonance (SPR), which is the interaction between electromagnetic radiation and the electrons in the conduction band around the nanoparticles. Silver nanoparticles were noted strongly in the range of 400–450 nm in the visible region. In present work, $\boldsymbol{\lambda}_{max}$ appeared at443.36 nm and is depicted in Figure 2. The rapid formation of AgNPs is evident from visual observation which may be due to the ionization of the phenolic group present in the extract.

Figure 2. Synthesis of AgNPs from *Enicostema axillare*(A) and its UV-Vis spectrum (B)



3.3. Zeta potential

The surface charge potential or zeta potential is one of the parameters which determines stability of nanoparticles in aqueous solution. Zeta potential of E. axillare was shown in Figure 3, in present study synthesized AgNPS show -13.5 mV value which indicate negative charge on synthesized nanoparticles. Chahar et al.[16] also observed -15.93 mV of zeta potential in Rheum austral.

3.4. Transmission electron Microscopy (TEM)

The application of TEM in nanoscience is significant in respect to the viewing of particles in nanoscale. The TEM images of synthesized EA- AgNPs and the SAED pattern obtained are depicted in Figure 4. This gives us a clear idea as to the size, shape and size distribution of the obtained nanoparticles. It can be seen from the images that the AgNPs are capped with phyto-constituents of *Enicostemma axillare*. The SAED pattern obtained reveals its crystalline nature. The approximate size of the synthesised EA-AgNPs ranges from 12-18 nm.

3.5. Anti-proliferative activity

Green synthesized spheroid shape silver nanoparticle from *Enicostema axillare* methanolic extract exhibited remarkable anti-proliferative activity on DLA cell line (Figure 5). It has shown the better activity than the methanolic extract of the selected plant. At 100 µg/ml dose EA-AgNp have maximum cell death of 74.79%, which is comparable to that of standard drug 5-Fluoro uracil (76.97%) and higher than the anti-proliferative activity of *Enicostema axillare* extract (35.07%) (Figure 5). Similarly, Sarkar et al.[17] also prepared silver nanoparticle from an Indian anticancer plant *Madhuca longifolia* and noticed higher anti-proliferative activity of AgNPs (85%) than the extract (64%) in 4T1 breast cancer cells. Thus,

encapsulating the AgNPs with extracts of Indian traditional anticancer plant like Enicostema axillare might increases the anti-proliferative efficacy of the herbal constituents because of easy passage of nanometric AgNPs into the cancer cells and induction of apoptosis.

Figure 3. Zeta potential result of AgNps synthesized from *Enicostema axillare*

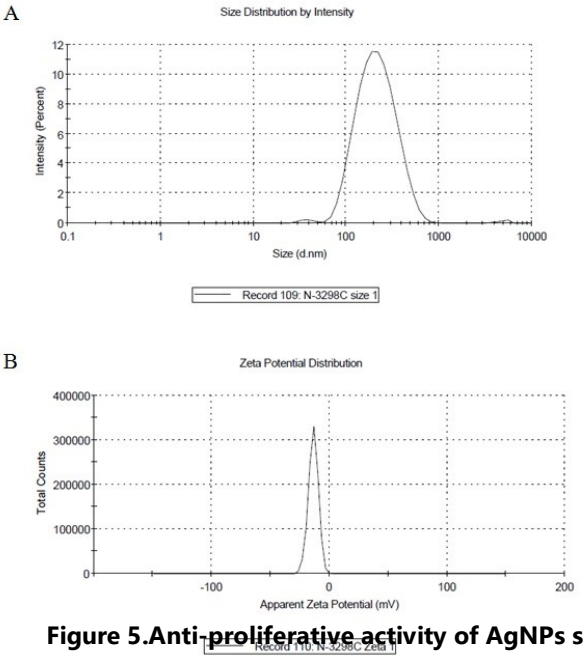


Figure 4. Transmission Electron Microscopy (TEM) of AgNPs synthesized from *Enicostemma axillare*: (A) Morphology, (B) Size, (C) Capping and (D) SAED pattern

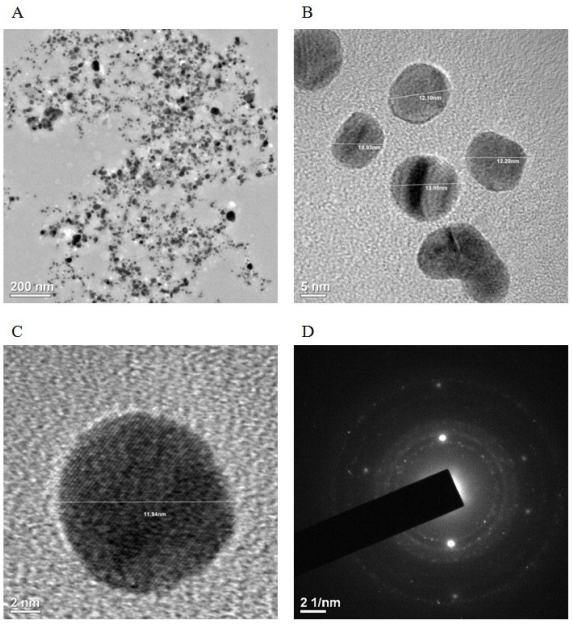
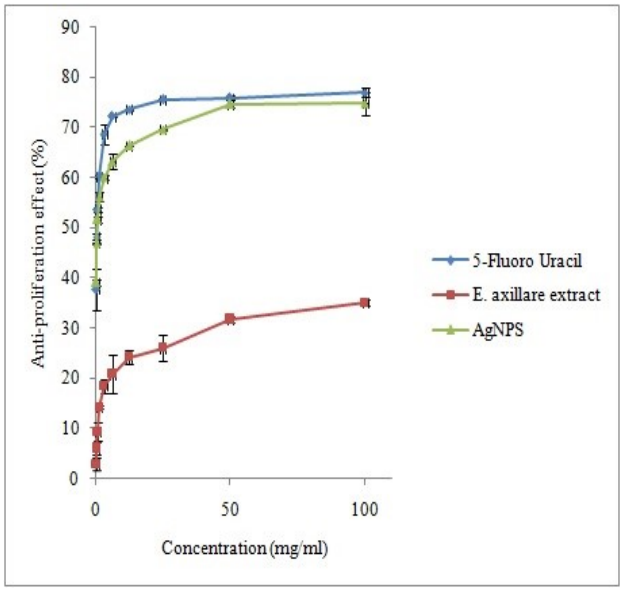


Figure 5.Anti-proliferative activity of AgNPs synthesized from *Enicostema axillare* in DLA cell line model



4. CONCLUSIONS

This study has demonstrated the use of methanol extract of *Enicostema axillare* in the reduction of metallic silver into AgNPs through a green approach. The phyto-constituents present in *Enicostema axillare* extract can act as reducing and capping agents for the production of AgNPs. The synthesized AgNPs were found to have a crystalline structure with nanometric size. The TEM images had shown that the synthesized AgNPs are having the size around 17 nm (11-18 nm). The results obtained from zeta potential (-13.5 mv) helps us to understand the stability of AgNPs, which might be due to encapsulation of the metal nanoparticles by *Enicostema axillare* extract as capping agent. Hence, the extract of Siddha plant *Enicostema axillare* could be effectively used to synthesis AgNPs with efficient anticancer activity, which opens new vistas for the development of novel anti-cancer agents from traditional medicine.

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REFERENCES

1. Tolaymat TM, El Badawy AM, Genaidy A, Scheckel KG, Luxton TP, Suidan M. An evidence-based environmental perspective of manufactured silver nanoparticle in syntheses and applications: A systematic review and critical appraisal of peer-reviewed scientific papers. Sci Total Environ 2010; 408 (5): 999-1006.
2. Tolaymat TM, El Badawy AM, Genaidy A, Scheckel KG, Luxton TP, Suidan M. An evidence-based environmental perspective of manufactured silver nanoparticle in syntheses and applications: A systematic review and critical appraisal of peer-reviewed scientific papers. Sci Total Environ 2010; 408 (5): 999-1006.
3. Rai M, Ingle AP, Gupta I, Brandelli A. Bioactivity of noble metal nanoparticles decorated with biopolymers and their application in drug delivery. Int J Pharm 2015; 496 (2): 159-172.
4. Sharma H, Mishra PK, Talegaonkar S, Vaidya B. Metal nanoparticles: A theranosticnano tool against cancer. Drug Discov Today 2015; 20 (9): 1143-1151.
5. Merga G, Wilson R, Lynn G, Milosavljevic BH, Meisel D. Redox catalysis on "naked" silver nanoparticles. J PhysChem C 2007; 111 (33): 12220-12226.
6. Yilmaz M, Turkdemir H, Kilic MA, Bayram E, Cicek A, Mete A, Ulug B. Biosynthesis of silver nanoparticles using leaves of Stevia rebaudiana. Mater ChemPhys 2011; 130 (3): 1195-1202.
7. Oliveira MM, Ugarte D, Zanchet D, Zarbin AJ. Influence of synthetic parameters on the size, structure, and stability of dodecanethiol-stabilized silver nanoparticles. J Colloid Interface Sci 2005; 292 (2): 429-435.
8. Thirumalai T, Therasa V, Elumalai EK, David E. Hypolipidaemic and antioxidant effect of EnicostemmalittoraleBlume. Asian Pac J Trop Biomed 2011; 1 (5): 381-385.
9. Jaishree V, Badami S. Antioxidant and hepatoprotective effect of swertiamarin from Enicostemaaxillare against D-galactosamine induced acute liver damage in rats. J Ethnopharmacol 2010; 130 (1): 103-106.
10. Jaishree V, Badami S, Kumar MR, Tamizhmani T. Antinociceptive activity of swertiamarin isolated from Enicostemaaxillare .Phytomed 2009; 16 (2): 227-232.
11. Deore SL, Khadabadi SS, Bhagure L, Ghorpade DS. In vitro antimicrobial and antioxidant studies on Enicostemaaxillare (Lam.) Raynal. leaves. Nat Prod Rad 2008; 7 (5): 409-412.
12. Kavimani S, Manisenthlkumar KT. Effect of methanolic extract of Enicostemmalittorale on Dalton's Ascitic Lymphoma. J Ethnopharmacol 2000; 71 (1): 349-352.
13. Desai PD. Chemical investigation of some Indian medicinal plants: Part II. Indian J Chem 1966; 4: 457-459.
14. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Method Enzymol 1999; 299: 152-178.
15. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Method 1983; 65: 55-63.
16. Pietta PG. Flavonoids as antioxidants. J Nat Prod 2000; 63 (7): 1035-1042.
17. Chahar V, Sharma B, Shukla G, Srivastava A, Bhatnagar A. Study of antimicrobial activity of silver nanoparticles synthesized using green and chemical approach. Colloids Surf A PhysicochemEng Asp 2018; 554: 149-155.
18. Sarkar MK, Vadivel V, Raja MRC, Mahapatra SK. Potential anti-proliferative activity of AgNPs synthesized using M. longifolia in 4T1 cell line through ROS generation and cell membrane damage. J PhotochemPhotobiol B: Biol 2018; 186: 180-186.