Int.J.Curr.Microbiol.App.Sci (2024) *13*(02): 68-79

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 13 Number 2 (2024)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2024.1302.011

Green Synthesis of Silver Nanoparticles using Endostemon viscosus (Roth) aqueous leaf extract; Characterization; Evaluation of Antibacterial, DPPH and Anticancer efficacy

Jayachandra Nagadasari* and T. Vijaya

Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India

**Corresponding author*

Keywords

AgNPs, Characterization, Anti-bacterial, Anti-cancer, thermodynamic and chemical properties

Article Info

Received:
09 December 2023
Accepted:
20 January 2024
Available Online:
10 February 2024

A B S T R A C T

Nanoparticles are being constantly exploited through scientific community because of their multifaceted properties and applications. Production of biogenic silver nanoparticles (AgNPs) with plant material is extremely cost effective and environmentally non-hazardous. Plant extracts are wealth of various phytochemicals such as secondary metabolites and frequently act as reducing and capping agents in the synthesis of nanoparticles. The present study deals with the synthesis of silver nanoparticles by the aqueous leaf extract of *Endostemon viscosus (Roth)*. Synthesized silver nanoparticles characterized by different recent spectroscopic tools such as UV-Visible spectrophotometer, DLS-Zeta potential, FT-IR, TEM with EDS, and XRD (X-ray diffraction). UV-Vis showed that the peak at 414 nm. DLS-Zeta potential analysis revealed about stability and even size of the particles. FT-IR was studied to know what phyto-constituents are actually acting as capping and stabilization of the nanoparticles. TEM is used to find shape, size and agglomeration pattern of the nanoparticles. TEM image exhibited size range from 11.03 to 37.87 nm and average size of the EvL-AgNPs are 16.77 nm. Eventually green synthesized nanoparticles are evaluated for their efficacy on anti-bacterial, DPPH, and anti-cancer activities. Biologically synthesized EvL-AgNPs expressed excellent anti-bacterial, anti-oxidants, and anti-cancer activities. Hence, the bio-synthesized AgNPs could be considered as auspicious therapeutic option against infectious pathological situations.

Introduction

Nanomaterial improvement, especially of high quality, is a hot topic in nanoscience and technology these days. Metal techniques have sparked renewed attention due to its fascinating physical, thermodynamic and chemical properties, make great candidates for various applications such as optical electronics, catalysis, and biomedical applications (Azizi *et al.,* 2017; Mani *et al.,* 2021; Parasuraman *et al.,* 2019; Sathiyaraj *et al.,* 2021; Anju *et al.,* 2019). Nanoparticles are normally considered as particles with a maximum size of 100 nm and are produced from numerous non-metal and metal elements with exceptional topographies and widespread uses in

science and medicine due to their unique properties (Matei, 2008). Similarity in size of nanoparticles with biomolecules, i.e., proteins and poly-nucleic acids, makes them useful to interact and reduce in size during the synthesis of these nanoparticles' effects of unique physical and chemical properties of biomolecules (Yeo, 2003). Among the metal nanoparticles, silver nanoparticles (Ag-NPs) are one of the best, frequently used nanoparticles having features of very small size, high surface area and high dispersion (Mathew, 2013). They are known to have potent antioxidant and antibacterial activities (AbouEl-nour, 2010). Silver metal is highly toxic to the bacterial cells but nontoxic to animal cells in small concentration (Marambio-Jones, 2010). Nowadays, the Ag-NPs are broadly used as an active antibacterial tool against a broad spectrum of bacteria, including antibiotic-resistant strains (Percival, 2007).

Silver has various biological activities such as antimicrobial (Yugandhar and Savithramma, 2015) antihelmintic and wound healing activity (Garg *et al.,* 2014) anti-larvicidic (Sundaravadivelan *et al.,* 2013) antioxidant (Kumara *et al.,* 2015) anticancer (Vasanth *et al.,* 2014) anti-inflammatory (El-Rafie *et al.,* 2014) hepatoprotective (Bhuvaneswari *et al.,* 2014). Earlier the nanoparticles can be synthesized by using physical and chemical methods. However, the physical and chemical approaches are the most high-priced, time consuming, energy consumption, and more chemicals are needed for the production of nanoparticles and they cause threat to the environment and also inappropriateness for biological applications. Instead of these two Physical and chemical methods, researchers needed to develop another alternative method. The researchers developed the substitute biological method to reduce the abovementioned disadvantages and environmentally benign than the physical and biological methods.

This method overcame most of the problems through the synthesis of silver nanoparticles (AgNPs) by employing various biological agents like algae, bacteria, enzymes, fungi, oligosaccharides, polysaccharides, DNA, and human cell lines (Corciova *et al.,* 2018). In addition to different kinds of NPs have been prepared successfully i.e. Cadmium oxide nanoparticles through *Achillea wilhemsii* (Karimi Andeani, 2013) calcium nanoparticles from *Boswellia ovalifoliata* (Yugandhar and Savithramma, 2013). Copper Nanoparticles by the using *Magnolia Kobus* plant material (Lee, 2013) Gold nanoparticles from *Walsura trifoliata* (Venkata Subbaiah and Savithramma, 2022) Palladium nanoparticles from *Cinnamomum camphora* (Yang, 2010). Zinc oxide nanoparticles from *Catharanthus roseus* (Bhumi, 2014). Silver nanoparticles from *Adansonia digitata* (Kumar *et al.,* 2015).

Endostemon viscosus (Roth) M.R.Ashby also called as *Orthosiphon diffuses*; commonly called as Sticky Wild Basil (E) belongs to the family Lamiaceae, which is distribute in South Asian countries such as India, Thailand etc. This is a traditional medicinal plant perennial herb that is distributed in many of India especially Western and Eastern Ghats in South India. The plant is a subshrub, growing from a woody base. Branch lets are finely velvet-hairy, 4-angled, Leaves are decussate, elliptic to nearly round, up to 2 x 1.5 cm, base flat, leaf-stalk to 1 cm. Flowers are borne in whorls of 6, on a 3-chotomous raceme, flower-cluster-stalk are up to 9 cm, bracts ovate; flower-stalk 0.5 cm. Flowers are pinkish, hairy along throat. Stamens 4, didynamous, filaments 2 mm; anthers 2-celled. Disk anteriorly developed. Nut-lets are erect, enclosed in a crescent calyx (Okaiyeto *et al.,* 2018). The plant is used as treating hypertension, hepatitis/jaundice and fever by folk fore practitioners. The activity of the plant claimed by the tribal peoples in western and Eastern Ghats and was proven by different researchers too (Chin *et al.,* 2008).

Materials and Methods

Collection of Plant Material

The healthy leaves were gathered from Tirumala hills, Tirupati, Andhrapradesh, India. The collected material was cleaned thrice using with running tap water, as well as with distilled water for one time for the removal of admixtures on leaves surface and shade dried for 10-15 days and ground to a fine powder with the help of electrical blender.

Plant Extract Preparation

25 g of weighed plant material was taken into 250 ml conical flask 100 ml of milli-Q water added to this shaken well for 5 min. Then this is kept on water bath for 20 min at 100 ^{0} C and this was cooled at room temperature. Later extraction is done by the using Whatman No 1 filter paper. The extraction is stored in amber colour bottle until the synthesis at room temperature.

Preparation of Ag $(No_3)_2$ Solution

10 gram of silver nitrate purchased from Sigma Aldrich and prepared 1 mM silver nitrate solution with using 100 ml of milli-Q water in sterile Erlenmeyer conical flask. Then the solution was stored in amber colour bottle.

Synthesis of Silver Nanoparticles (AgNPs)

The leaves aqueous extract was added to prepare 1 mM AgNo3 solution. It was heated on water bath at 60-80^{0}C. The colour change of the solution from light yellow to thick brown indicated the silver nanoparticles were synthesized from the leaf extract, and then this is further used for the characterization and antibacterial, antioxidant and anticancer efficacy.

UV- Vis Spectra Analysis

The bio-reduction of silver ions was analysed by UV-Vis spectrophotometer Nano drop range from190-750 nm. The absorbance peak was recorded before 24 hours. It is the primary method to confirm the formation of bio-synthesized AgNPs in the prepared reaction mixture.

FT-IR Spectral analysis

Fourier Transform Infrared (FTIR) spectral analysis was carried out by using dried powder of silver nanoparticles (AgNPs). For the getting dry powder of AgNPs following protocol was applied. When mixing the plant extract to the 1 mM AgNo3 solution 1:9 ratio kept on water bath at 80^0 C for 15 minutes, the solution is changed into brown coloured AgNPs formed.

It was centrifuged at 20000 rpm for 15 minutes. Formed pellets were dispersed in distilled water. Again it was centrifuged and this process was repeated thrice. Then the pellet of AgNPs was dried and this was utilised for FTIR analysis.

DLS Particle Size and Zeta Potential

To determine the size of the particle and size distribution in aqueous AgNPs solution performed by the advanced equipment Dynamic Light Scattering (DLS) Malvern-Zeta analyzer. This is an advanced tool; it depends on the interaction of Brownian motion of spherical particles with the light passing by a colloidal solution (Saxena *et al.,* 2010).

Transmission Electron Microscopy (TEM)

TEM analysis is done by the using HF-3300 advanced 300 kV TEM from Hitachi. It is the valuable, frequently used and important technique for the characterization of nanoparticles, used to procure particle quantitative measure and /grain size, size distribution and morphology of nanoparticles. TEM has two advantages than SEM: it can be provide better spatial resolution and the capability for additional analytical measurements. The images were taken by coating the suspension of AgNPs on carbon- coated copper grid. The average size of the Bio-synthesized AgNPs was calculated with the help of Image J software through TEM images.

XRD Studies

X-ray diffraction (Shimazdu XRD-6000) was analysed to confirm the crystalline nature and average size, and measure the degree of crystallinity of the PmL-AgNPs. XRD is the powerful characterization tool for both qualitative and quantitative analysis of nanoparticles. Diffraction intensities were compared with the standard JCPDS files. The average crystalline size of the AgNPs was calculated by the Debye-Scherer equation.

$$D=k\,\lambda/\beta\cos\theta$$

Where D is diameter of NPs, k is the Scherrer constant, λ is the wave length of X-ray radiation source, β is full width half maximum value of XRD diffraction lines and θ is the half diffraction angle-Bragg angle.

X-ray diffraction patterns of EV-AgNPs peaks shows at

Anti-Bacterial Studies of Fm-F AgNPs

The anti- bacterial activity analysed by the bio-synthesized Ev-L AgNPs against two gram positive bacteria like *Bacillus subtilis* (MTCC-441), *Staphylococcus aureus* (MTCC-731) and two gram negative bacteria species like *Escheria coli* (MTCC-443), *Klebsiella pneumonia* (MTCC-741). Disc diffusion method was executed by using standard protocol Anonymous (1996). 20 µl of $Ag(No_3)_2$, plant extract, AgNPs and Streptomycin was applied on separated sterile filter paper discs (Whatman No. 1 filter paper discs with 6 mm diameter), and allowed to dry before being placed on nutrient agar medium. The activity is examined by triplicate of each solution of above

mentioned and incubated at 37^0 C for 24 h in inoculation chamber. Diameter of zone of inhibition (ZOI) was measured with the help of scale and the results were tabulated.

Antioxidants Activity

Antioxidant activity of Ev-L -AgNPs was assessed by DPPH- (2, 2-Diphenyl-1-picry Hydrazyl radical Scavenging activity). For the assay, 1mM DPPH stock solution was prepared by adding 4 mg DPPH in 100 mL of methanol. 2 mL of DPPH stock solution was added to 1 mL of methanolic solution of Ev-L -AgNPs consisting vary concentrations of Ev-L -AgNPs (25, 50, 75 and 100 µg). The reaction solution was incubated for 45 min in the dark room at room temperature. Later incubation absorbance values were recorded at 518 nm. DPPH activity of the Ev-L -AgNPs was calculated using with the following formula % of inhibition= ((Absorbance of control- Absorbance sample)/ Absorbance of control) X 100. And the concentration of inhibition of 50% free radicals (IC_{50}) was calculated by regression coefficient (R_2=0.9).

Anticancer Studies (Cell proliferation Assay using SRB (Sulforhodomine-B)

Different human cancer Cell lines (MDA-MB-231,SK-OV-3,PC3,PANC-1, and HeLa) purchased from the American Type Culture Collection. For this SRB assay is a quantitative colorimetric method used for determination of cell survival and proliferation based on the measurement of cellular protein content. Cell were grown in Dulbecco's modified Eagle's medium (containing non-essential amino acids and 10% FBS). PC-3 was grown in RPMI with glutamine containing non-essential amino acids. Cell lines were maintained in humidified atmosphere of 5% CO_2 at 37°C.Cells were trypsinized when sub-confluent from 90mm dishes and seeded in 96 well plates at a concentration of 1×10^4 cells/mL in complete medium a day before treatments. Cells were incubated with different concentrations of Ag NPs (12.5-100µg/ml) for 48h for potent compounds; entire process was executed in triplicates.

Results and Discussion

The reduction biologically synthesized silver nanoparticles (AgNPs) was primarily observed by visualization method of colour change pattern of the reaction mixture and it was changed brown into deep brown colour, after process of synthesis; then this solution was performed by the using of UV-Vis Spectroscopy range from 190 to 750 nm Nano drop. The absorbance peak was recorded at 414 nm which was further confirmation the reduction nanoparticles are silver (AgNPs) (Fig.1). This respective peak shown because of the Surface Plasmon Resonance (SPR) of electrons present on the surface of the synthesized nanoparticles. Similar type of results was observed in the AgNPs synthesized from the leaf source aqueous extract of *Odontonema strictum* (Luhata *et al.,* 2022). For this Fourier transform infra-red (FT-IR) spectral analysis with scan range of 4000-500 cm^{-1}(ALPHA interfero meter ECO-ATR, Bruker Ettlingen, Karlsruhe Germany) was used. Here the FT-IR revealed broad peaks 3300.20 assigned for O-H bond phenols, 1587.42 belongs to the strong C=O amide, 1382.96 assigned for medium stretch CH3 bend aliphatic group, 1261.45 indicates strong stretching C-O alkyl aryl ether, 1114.86 assigned strong C-O starching ester, 1047.35 belongs to strong broad CO-O-CO stretching anhydride, 810.10 assigned for strong C-H bending 1, 4-disubstituted, 597.93 and 520.78 assigned to strong C-I stretching halo compound.

All these constituents interacting with silver nanoparticles (AgNPs) as reducing agents (Fig.2). The DLS method revealed average size 5.3 nm and the zeta potential negatively charged with -3.5 mV, which proved that the particles are dispersed in the reaction mixture (Fig.3a & b). A higher magnification analysis was carried out through TEM to find out the size, shape and agglomeration pattern of AgNPs. TEM analysis shows higher magnification due to these green synthesized nanoparticles and exhibited interface between two lattice fringes with small-sized nanoparticles belonging to attachment of AgNPs between the surface sheets of the TEM micrographs. TEM micrographs disclosed about size range between 11.03- 37.87 nm at 50 nm scale bar, Average size of the AgNPs 16.77 nm.

At 5 nm scale bar revealed 0.24 nm as well as no seen any agglomeration between the particles (Fig.4). Such type of results observed in synthesized AgNPs by the using Leaf extract of the *Uvaria narum* (Ajaykumar *et al.,* 2023). The peaks obtained at 2θ of X-axis 38.11^0,44.27^0, 64.42^0, 77.47^0 and 81.53^0corresponds to 222,111, 200, 220 and 311 Bragg reflections of Y- axis respectively which may be indexed based on the end-centred monocrystalline structure of AgNPs. Based on the X-ray diffraction reports clearly indicates that the formed particles are AgNPs (Fig.5).

Table.1 Antimicrobial activity of *Endostemon viscous* aqueous leaf extract biologically synthesized EvL-AgNPs, Ag $(No_3)_2$ solution and antibiotic Streptomycin.

Bacteria	Extract	$AgNo_3$	EVAgNP	Streptomycin
Bacillus subtilis	8.71±0.21	8.19±0.44	18.28±0.47	25.14±0.36
Staphylococcus aureus	8.24±0.17	7.90±0.40	18.22±0.23	25.40±0.39
Klebsiella pneumoniae	9.5414±0.64	9.85±0.18	19.12±0.38	24.31±0.19
Escherchia coli	9.69±0.51	9.92±0.42	19.53±0.65	23.72±0.78

Figure.1 UV-Vis spectra analysis spectrum using aqueous leaf extract of *Endostemon viscous* AgNPs

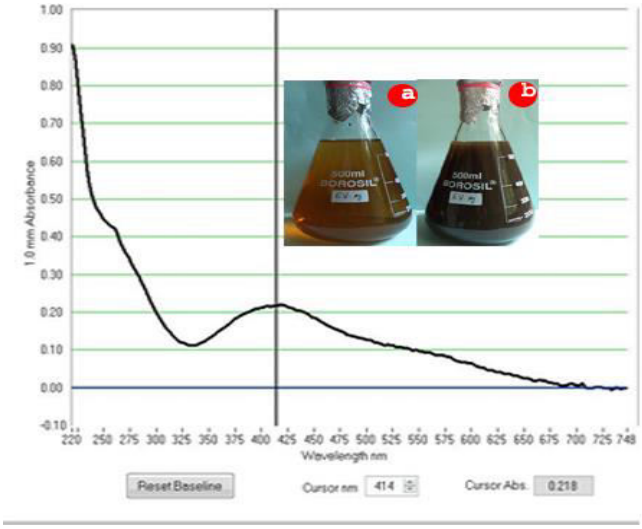


Figure.2 Fourier- Transform Infra-Red (FT-IR) spectra of bio-synthesized AgNPs from *Endostemon viscous*

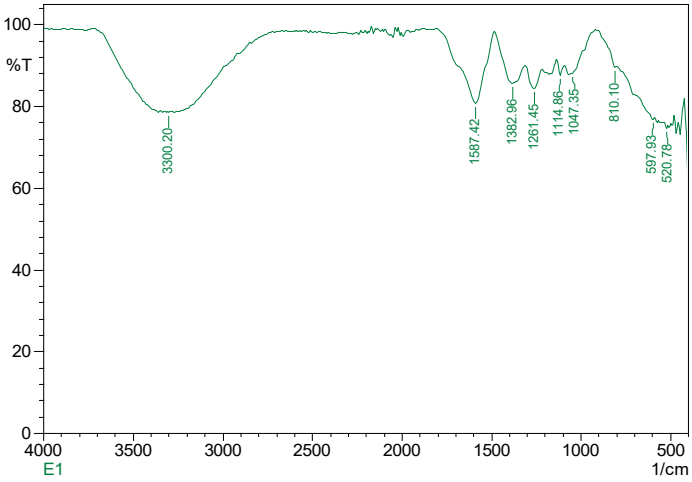


Figure.3 a). Particle sizeand b). Zetapotential *studies of Endostemon viscous* AgNPs

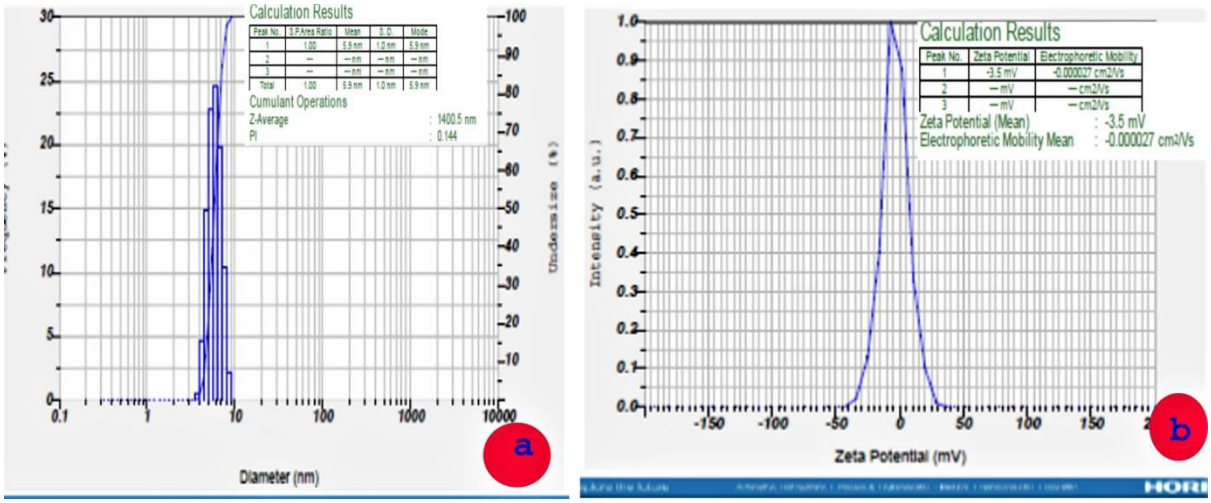


Figure.4 TEM images of *Endostemon viscous AgNPs* a). At 5 nm 0.24 nm, b). At 50 nm AgNPs are spherical in shape and Average size of the AgNPs 16.77 nm. c). At 51 nm beam of TEM d). EDAX analysis

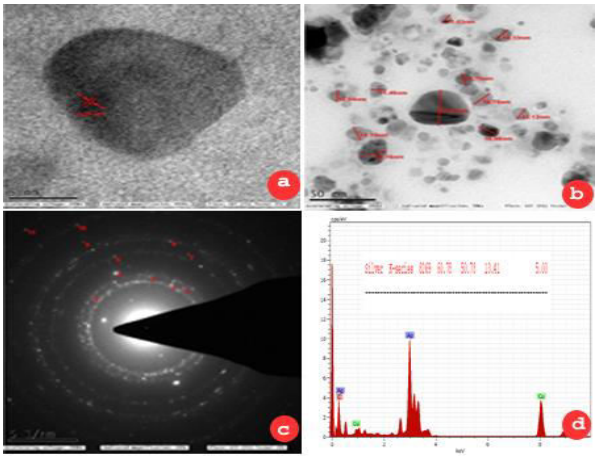


Figure.5 XRD pattern analysis of leaf sourced biologically synthesized AgNPs of *Endostemon viscous*

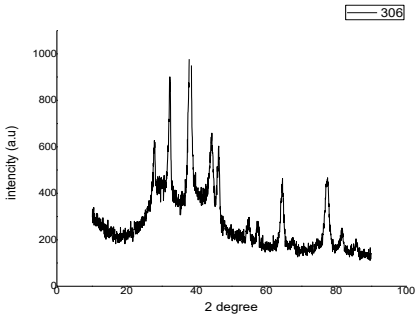


Figure.6 Antimicrobial activity of biologically synthesized EvL-Ag NPs against Two gram gram positive and two negative bacteria. 1. Plant extract 2. Ag $(No_3)_2$ solution 3.ZnO NPs 4.Streptomycin.

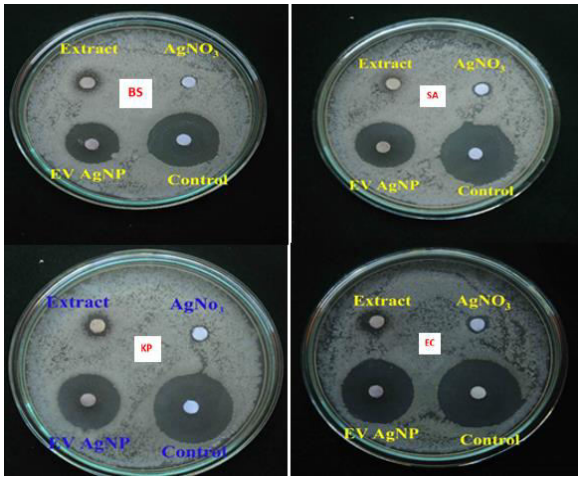


Figure.7 Graphical representation of anti-bacterial activity using bio-synthesized EvL-AgNPs

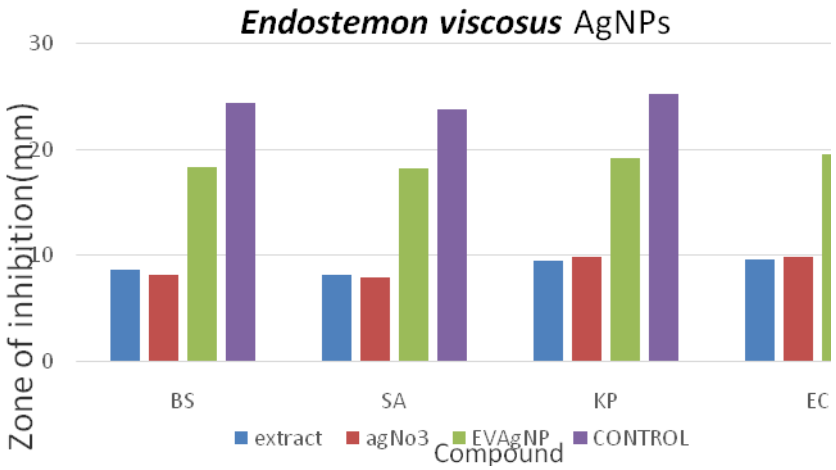


Figure.8 Graphical representation of anti-oxidants activity by synthesized EvL-AgNPs

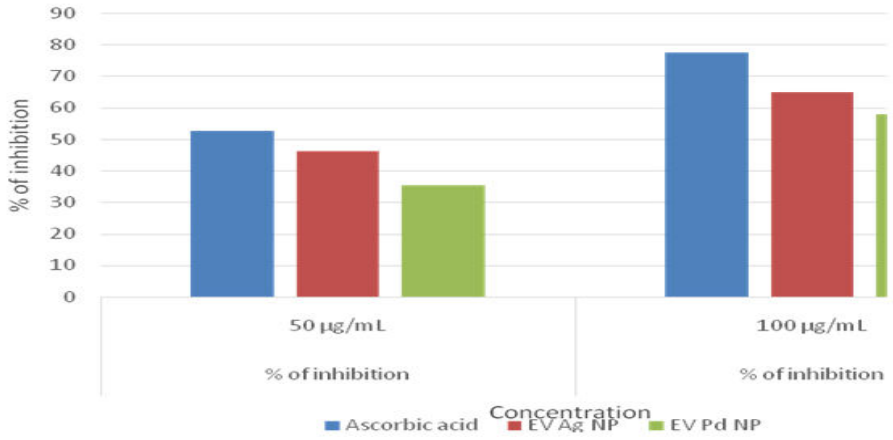


Figure.9 Anti-cancer activity on selected human cancer cell lines by synthesized EvL-AgNPs 1). PC-3 2). MDA-MB-231 3). SK-OV-3 4). PANC-1 5). HeLa
a) Cell control b). Standard control c). 12.5 µg/ml d). 25 µg/ml e). 500 µg/ml and f). 100 µg/ml

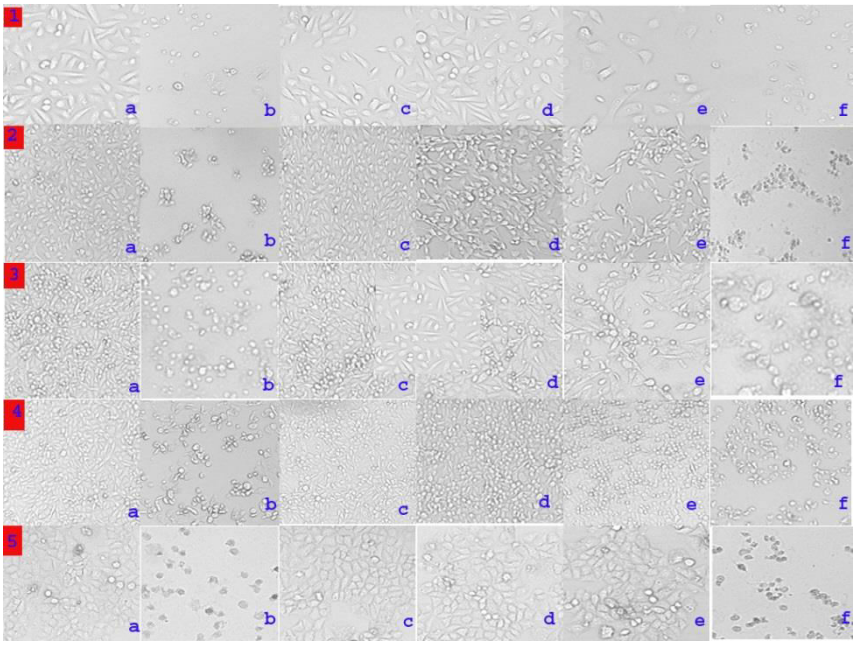
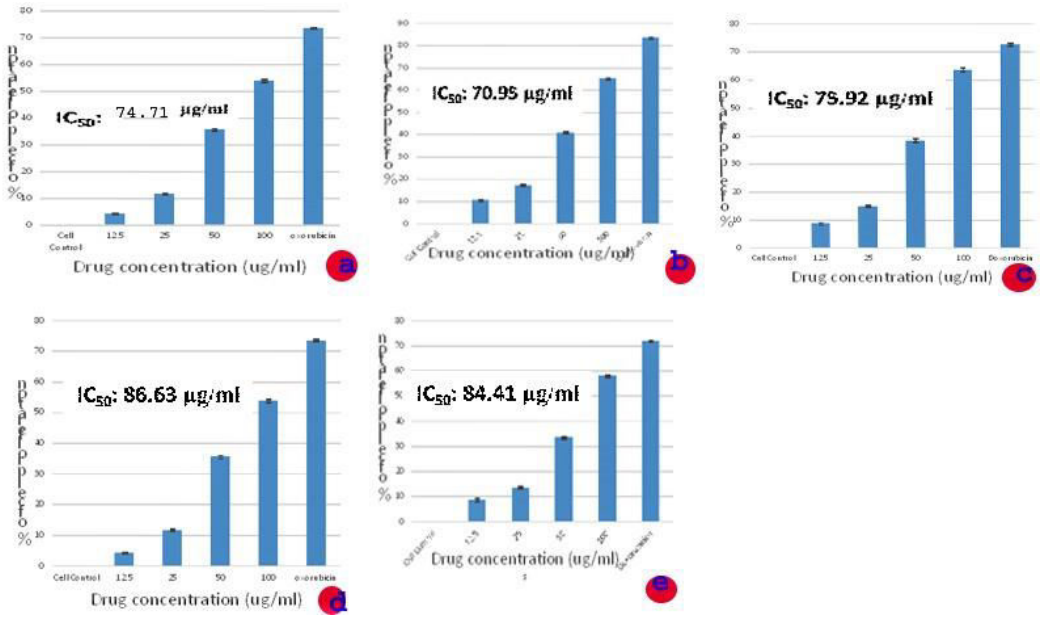


Figure.10 Graphical representation of anticancer activity by using EvL-AgNPs against five selected human cancer cell lines a). PC-3 b). MDA-MB-231 c). SK-OV-3 d). PANC-1 e). HeLa



Biologically synthesized AgNPs showed excellent bacterial activity on selected two gram positive (*B. subtilis* and *S. aureus*) and two gram negative (*K.pneumoniae* and *E.coli*). Synthesized EvL- AgNPs exhibited a maximum zone of inhibition on two gram negative bacteria than positive bacteria. This is due to Gram positive bacteria contain a thick layer that is made up of peptidoglycan when compared to Gram negative

bacteria and penetration of AgNPs by the cell membrane is easy in case of Gram negative bacteria but also inhibition of growth is possible. Among the activity suggesting that the clearly highest activity was seen on *E.coli* (Fig.6 & 7 Graph; Table.1). These types results find in bio-fabrication of AgNPs with leaf aqueous source of *Walsura trifoliolata* (Venkata Subbaiah and Savithramma, 2022). Biosynthesized EvL- AgNPs were tested with DPPH method. The activity depends on the reduction of DPPH radical from DPPH to DPPH-H, where anti-oxidant donated by hydrogen. Among the activity exhibited concentration depends on the dependant scavenging activity against DPPH scavenging. In which concentration of EvL- AgNPs is increased from 50 µg/ml -100 µg/ml. Therefore, the activity was exhibited by increased from 46.07 ± 0.34µg/ml - 64.83±0.31 µg/ml. IC_{50} values 77.54 ± 0.4 clearly suggested that EvL- AgNPs are magnificently showed anti-oxidants activity at 100 µg/ml. The DPPH activity showed excellent activity with WT-AgNPs than a plant extract and 1mM Ag $(NO_3)_2$ solution. (Fig. 8 Table). These kind of results observed in AgNPs synthesized from leaves extract of *Sauropus androgynous* (L.) Merr (Anu Abhimannue and Ashwathi Menon, 2021). Green synthesized EvL-AgNPs furtherly evaluated on various selected five human cancer cell lines such as MDA-MB-231, SK-OV-3, PC3, PANC-1, and HeLa. Experiment was carried out as cell proliferation assessment using SRB (Sulforhodomine-B) colorimetric method.

Diverse concentrations of EvL- AgNPs (12.5 µg/ml, 25.0 µg/ml, 50 µg/ml, and 100 µg/ml) and Doxorubicin as standard control applied to the growing cell lines like dose dependant process. The cytotoxic results demonstrated that the small sized nanoparticles were easily penetrates the cell membrane damaging the DNA, ultimately causing cell death with the reactive oxygen species (ROS) mechanism in cell (Alvur *et al.,* 2022; Ko *et al.,* 2022). EvL-AgNPs exhibited excellent activity on five selected human cancer cell lines. The activity was manifested magnificently on five selected human cancer cell lines, highest activity was seen in PANC-1 73.43 percent at 100 µg/ml, and higher IC50 was seen in PANC-1 (86.63 percent) when comparing the remaining selected human cell lines (Fig. 9 & 10). These kinds of results accordance with *Cucumis prophetarum* leaf extract AgNPs evaluated on different human cancer cell lines (Hemalata *et al.,* 2020).

The present work on synthesis of EvL-NPs leaf aqueous extract of *Endostemon viscous* was utilised as a capping agent for thr stabilization of the biological synthesized nanoparticles. For the preparation of the AgNPs we followed facile, cost-effective, eco-friendly method. Here, less chemicals were used and environmentally safer approach. This is a most effective way of synthesis which involves non-toxic and very conventional method which leads to find out for further directions of environmentally amiable nanoparticles. UV-Vis (scan range from 190 to 750 nm.) spectra analysis is an initial technique to notice of formation of EvL-AgNPs in the synthesized solution, through this instrument at 414 nm we found peak. FT-IR results suggested about phenols, amide, aliphatic group, alkyl aryl ether, ester, anhydride, and halo compound were interfered to form of nanoparticles and these compounds act as capping and stabilization agents to prevent agglomeration in the reaction mixture.

The crystallinity of the synthesized nanoparticles was proven from XRD analysis. The morphology, shape, size and agglomeration pattern of the EvL-AgNPs was performed by HR-TEM. The average size of the bio-synthesized AgNPs was 16.77 nm, sherical shaped, without any agglometation was observed and EDAX was results exhibited about 60.78 % weight percentage in the reaction mixture. The green synthesized EvL-Ag NPs was showed remarkable antimicrobial activity against both gram negative (*K.pneumoniae and E.coli*) and gram positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*). Hence the highest bacterial growth zone of inhibition (ZOI) was recorded towards gram negative bacteria when compare the gram positive bacteria by the small sized bio-synthesized nanoparticles. Different concentrations of aqueous leaf extract of EvL-AgNPs exhibited strong anti-oxidant activity. Anti-cancer activity was expressed notable with the using diverse human cell lines such as MDA-MB-231, SK-OV-3, PC3, PANC-1, and HeLa. The bio-synthesized AgNPs showed promising Anti-bacterial, anti-oxidants and anti-cancer activities, suggesting their capability utilize in different bio-medical applications.

Acknowledgements

Practical design and manuscript preparation was carried out by our research supervisor Prof. T. Vijaya, Department of Botany, Srivenkateswara University, Tirupati. The first author N. Jayachandra has done field work and practical work performance and assisted to our research supervisor Prof. T. Vijaya. The First Author highly Thanks to DST-PURSE Sri Venkateswara University, Tirupati providing characterization facility.

Abbreviation

mM - Milli molar, nm - Nano meter, AgNPs - Silver nanoparticles, Ev - *Endostemon viscous*

Author Contribution

Jayachandra Nagadasari: Investigation, formal analysis, writing—original draft. T. Vijaya: Validation, methodology, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval: Not applicable.

Consent to Participate: Not applicable.

Consent to Publish: Not applicable.

Conflict of Interest: The authors declare no competing interests.

References

AbouEl-nour K M, Eftaiha A, Al-Warthan A, Ammar R A. Synth Appl Silver Nanopart*Arab J Chem*. 2010;3:135–40. https://doi.org/10.1016/j.arabjc.2010.04.008

Ajaykumar, A. P.; Mathew, A.; Chandni, A. P.; Varma, S. R.; Jayaraj, K. N.; Sabira, O.; Rasheed, V. A.; Binitha, V. S.; Swaminathan, T. R.; Basheer, V. S.; *et al.,* Green Synthesis of Silver Nanoparticles Using the Leaf Extract of the Medicinal Plant, *Uvaria narum* and Its Antibacterial, Antiangiogenic, Anticancer and Catalytic Properties. *Antibiotics* 2023, 12, 564. https://doi.org/10.3390/antibiotics12030564

Alvur O, Kucuksayan H, Baygu Y *et al.,* (2022). The dicyano compound induces autophagic or apoptotic cell death via Twist/c-Myc axis depending on metastatic characteristics of breast cancer cells. *MolBiol Rep* 49:39–50. https://doi.org/10.1007/s11033-021-06817-9

Anju, V. T., Paramanantham, P., Sruthil Lal, S. B., Sharan, A., Syed, A., Bahkali, N. A., Alsaedi, M. H., Kaviyarasu, K., Busi, S., 2019. Antimicrobial photodynamic activity of toluidine blue-carbon nanotube conjugate against *Pseudomonas aeruginosa* and *Staphylococcus aureus*-understanding the mechanism of action. *Photodiagn.Photodyn.Ther*. 27, 305–316. https://doi.org/10.1016/j.pdpdt.2019.06.014

Anonymous, Pharmacopiea of India (the Indian Pharmacopiea) 3rd ed. Delhi: Ministry of Health and Family Welfare, 1996.

Anu P. Abhimannue and Ashwathi Menon (2021). Green synthesis of silver nanoparticles using *Sauropus androgynous* leaf extract with potential biological properties; IJPSR, Vol. 12(8): 4267-4274. E-ISSN: 0975-8232; P-ISSN: 2320-5148; *International Journal of Pharmaceutical Sciences and Research 4267 IJPSR* (2021), Volume 12, Issue 8. https://doi.org/10.13040/IJPSR.0975-8232.12(8).4267-74

Azizi, S., Mahdavi Shahri, M., Rahman, H. S., Rahim, R. A., Rasedee, A., Mohamad, R., 2017. Green synthesis PdNPs mediated by white tea (*Camellia sinensis*) extract with antioxidant, antibacterial, and antiproliferative activities toward the human leukemia (MOLT-4) cell line. *Int. J. Nanomed*. 12, 8841–8853. https://doi.org/10.2147/IJN.S149371

Bhumi G and Savithramma N. Biological Synthesis of Zinc oxide Nanoparticles from *Catharanthus roseus* (L.) G. Don. Leaf extract and validation for antibacterial activity. Int J Drug Dev Res. 2014; 6(1):208-14.

Bhuvaneswari R, Chidambaranathan N, Jegatheesan K. Hepatoprotective effect of *Embilica officinalis* and its silver nanoparticles against ccl4 induced hepatotoxicity in Wistar albino rats. *Dig J NanomaterBiostruct*. 2014;9(1):223-35.

Chin J H, Abas H H, Sabariah I. Toxicity study of *Orthosiphon stamineus* benth (*misai kucing*) on Sprague dawley rats. Trop Biomed 2008; 25:9-16.

Corciova A and Ivanescu B. Biosynthesis, characterisation and therapeutic applications of plant mediated silver nanoparticles. *J Serbian Chem Soc*. 2018; 83(5):515-38. https://doi.org/10.2298/JSC170731021C

El-Rafie H M and Hamed M A. Antioxidant and anti-inflammatory activities of silver nanoparticles biosynthesized from aqueous leaves extracts of

four *Terminalia species*. *Adv Nat SciNanosciNanotechnol.* 2014; 5(3):035008. https://doi.org/10.1088/2043-6262/5/3/035008

Garg S, Chandra A, Mazumder A, Mazumder R. Green synthesis of silver nanoparticles using *Arnebia nobilis* root extract and wound healing potential of its hydrogel. *Asian J Pharm*. 2014;8(2):95-101. https://doi.org/10.4103/0973-8398.134925

Hemlata, Prem Raj Meena, Arvind Pratap Singh, and Kiran Kumar Tejavath (2020). Biosynthesis of Silver Nanoparticles Using *Cucumis prophetarum* Aqueous Leaf Extract and Their Antibacterial and Antiproliferative Activity against Cancer Cell Lines; *ACS Omega* 2020, 5, 5520−5528. https://doi.org/10.1021/acsomega.0c00155

Karimi Andeani J and Mohsenzadeh S. Phytosynthesis of cadmium oxide nanoparticles from *Achillea wilhelmsii* flowers. *J Chem.*; 2013:1-4. https://doi.org/10.1155/2013/147613

Ko H-J, Chiou S-J, Tsai C-Y *et al.,* (2022). BMX, a specifc HDAC8 inhibitor, with TMZ for advanced CRC therapy: a novel synergic effect to elicit p53-, β-catenin- and MGMT-dependent apoptotic cell death. *Cell Commun Signal* 20:200. https://doi.org/10.1186/s12964-022-01007-x

Kumar C M, Yugandhar P, Suhrulatha D, Savithramma N. Synthesis, characterization and antimicrobial studies of stem bark mediated synthesis of silver nanoparticles from *Adansonia digitata* (L.). J Pharm Sci Res. 2015; 7(2):76-82.

Kumara Swamy M, Sudipta K M, Jayanta K, Balasubramanya S. The green synthesis, characterization, and evaluation of the biological activities of silver nanoparticles synthesized from *Leptadenia reticulata* leaf extract. *ApplNanosci*. 2015; 5(1):73-81. https://doi.org/10.1007/s13204-014-0293-6

Lee H J, Song J Y, Kim B S. Biological synthesis of copper nanoparticles using *Magnolia kobus* leaf extract and their antibacterial activity. *J ChemTechnolBiotechnol*. 2013;88(11):1971-7. https://doi.org/10.1002/jctb.4052

Luhata L. P, Chick C. N, Mori N, Tanaka K, Uchida H, Hayashita T, Usuki T: Synthesis and Antioxidant Activity of Silver Nanoparticles Using the *Odontonema strictum* Leaf Extract. *Molecules* 2022, 27, 3210. https://doi.org/10.3390/molecules27103210

Mani, M., Harikrishnan, R., Purushothaman, P., Pavithra, S., Rajkumar, P., Kumaresan, S., Al Farraj, D. A., Elshikh, M. S., Balasubramanian, B., Kaviyarasu, K., 2021. Systematic green synthesis of silver oxide nanoparticles for antimicrobial activity.*Environ. Res*. 202:111627 https://doi.org/10.1016/j.envres.2021.111627

Marambio-Jones C, Hoek E M V. A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. *J Nanopart Res*. 2010; 12(5):1531–51. https://doi.org/10.1007/s11051-010-9900-y

Matei A, Cernica I, Cadar O, Roman C, Schiopu V. Synthesis and characterization of ZnO-polymer nanocomposites. *Int J Mater Form*. 2008;1:767–70. https://doi.org/10.1007/s12289-008-0288-5

Mathew T V, Kuriakose S. Studies on the antimicrobial properties of colloidal silver nanoparticles stabilized by bovine serum albumin. *Colloids Surf B*. 2013;101:14–18. https://doi.org/10.1016/j.colsurfb.2012.05.017

Okaiyeto K, Nwodo U, Mabinya L, *et al.,* A review on some medicinal plants with hepatoprotective effects. Pharmacogn Rev 2018; 12:186-199. https://doi.org/10.4103/phrev.phrev_52_17

Parasuraman, P., Anju, V.T., Sruthil Lal, S. B., Sharan, A., Busi, S., Kaviyarasu, K., Arshad, M., Dawoud, T. M. S., Syed, A., 2019. Synthesis and antimicrobial photodynamic effect of methylene blue conjugated carbon nanotubes on *E. coli* and *S. aureus*. *Photochem.Photobiol*. Sci. 18 (2), 563–576. https://doi.org/10.1039/C8PP00369F

Percival S L, Bowler P G, Dolman J. Antimicrobial activity of silver containing dressings on wound microorganisms using an in vitro biofilm model. *Int Wound J*. 2007;4:186–91. https://doi.org/10.1111/j.1742-481X.2007.00296.x

Sathiyaraj, S., Suriyakala, G., Dhanesh Gandhi, A., Babujanarthanam, R., Almaary, K. S., Chen, T.-W., Kaviyarasu, K., 2021. Biosynthesis, characterization, and antibacterial activity of gold nanoparticles. *J. Infect. Public Health* 14 (12), 1842–1847. https://doi.org/10.1016/j.jiph.2021.10.007

Saxena, A., *et al.,* (2010). Biological synthesis of silver nanoparticles by using onion (*Allium cepa*) extract and their antibacterial activity. Journal of Digestive Nanomaterials Biostratigraphie, 5(2), 427–432. https://www.chalcogen.ro/427_Tripathi.pdf.

Subbaiah K V, Ankanna S, Savithramma N (2022). Bio-

fabrication of Silver Nanoparticles from *Walsura trifoliata* (A. Juss.) Harms Characterization, Antibacterial, and Anti-oxidant Efficacy. *Int J Pharm Phytopharmacol Res.* 2022;12(3):1-9. https://doi.org/10.51847/ZA11uxzcJw

Sundaravadivelan C, Nalini Padmanabhan M, Sivaprasath P, Kishmu L. Biosynthesized silver nanoparticles from *Pedilanthus tithymaloides* leaf extract with anti-developmental activity against larval instars of *Aedes aegypti* L. (Diptera; Culicidae). *Parasitol Res*. 2013; 112(1):303-11. https://doi.org/10.1007/s00436-012-3138-9

Vasanth K, Ilango K, Mohan Kumar R, Agrawal A, Dubey G P. Anticancer activity of *Moringa oleifera* mediated silver nanoparticles on human cervical carcinoma cells by apoptosis induction. *Colloids Surf B Biointerfaces*. 2014; 117:354-9. https://doi.org/10.1016/j.colsurfb.2014.02.052

Yang X, Li Q, Wang H, Huang J, Lin L, Wang W, *et al.,* Green synthesis of palladium nanoparticles using broth of *Cinnamomum camphora* leaf. *J Nanopart Res*. 2010; 12(5):1589-98. https://doi.org/10.1007/s11051-009-9675-1

Yeo S Y, Lee H J, Jeong S H. Preparation of Nanocomposite fibres for permanent antibacterial effect. *J Mater Sci*. 2003;38:2143–7. https://doi.org/10.1023/A:1023767828656

Yugandhar P and Savithramma N. Green synthesis of calcium carbonate nanoparticles and their effects on seed germination and seedling growth of *Vigna mungo* (L.) Hepper. *Int J Adv Res.* 2013; 1(8):89-103.

Yugandhar P, Savithramma N. Leaf Assisted Green Synthesis of Silver Nanoparticles from *Syzygium alternifolium* (Wt.) Walp. Characterization and Antimicrobial Studies. Nano Biomed Eng. 2015;7(2):29-37. https://doi.org/10.5101/nbe.v7i2.p29-37

How to cite this article:

Jayachandra Nagadasari and Vijaya, T. 2024. Green synthesis of Silver Nanoparticles using *Endostemon viscosus (Roth)* aqueous leaf extract; Characterization; Evaluation of Antibacterial, DPPH and Anticancer efficacy. *Int.J.Curr.Microbiol.App.Sci.* 13(2): 68-79. **doi:** https://doi.org/10.20546/ijcmas.2024.1302.011