Inhibitory effect of *E.conferta* mediated Silver nanoparticles against the infection causing pathogens

Rose C. Merlin* and Sherine H. Benita
PG and Research Department of Chemistry, Thanthai Periyar Government Arts and Science College (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli – 620023, Tamil Nadu, INDIA
*merlin@thanthaiperiyargasc.ac.in

Abstract
This work explores green synthesis approach of producing silver nanoparticles (AgNPs) from methanolic Elaeagnus conferta leaves extract. It emphasizes how naturally occurring biomolecules from plant extracts might get utilized as active agents for producing nanoparticles. Furthermore, the green-synthesised AgNPs' antibacterial effectiveness against microorganisms that cause infection, was assessed, highlighting their capacity to function as strong antimicrobials. The produced nanoparticles have been examined employing several methods encompassing "energy dispersive X-ray analysis (EDX), TEM (transmission electron microscopy), SEM (scanning electron microscopy), FTIR (Fourier transform infrared spectroscopy), X-ray diffraction (XRD), as well as UV-visible spectroscopy.

UV-visible spectroscopy as well as qualitative examination demonstrated the presence of phytochemicals with medicinal significance including alkaloids, flavonoids, terpenoids, phenolic compounds, as well as steroids. It was discovered that AgNPs mediated by Elaeagnus conferta had mean crystalline size 26.59nm. EDAX analysis indicated that elemental composition of nanoparticles consisted of 34.06% silver and 10.15% oxygen. EC-AgNPs were tested against infection-causing pathogens like Candida albicans, Staphylococcus aureus, Aspergillus niger, as well as Escherichia coli. Biosynthesized EC-AgNPs exhibited significant inhibitory effects against all tested pathogens. These findings conclude that EC-AgNPs have strong potential as effective antimicrobial agents.

Keywords: *Elaeagnus conferta*, infection causing pathogens, green synthesis, silver nanoparticles, characterization.

Introduction

A major reason for the increased mortality rate and morbidity rate is infection causing pathogens[8]. Severe medical complications such as diarrhoea, urinary tract infection, pneumonia, cellulitis, pharyngitis, tonsillitis, osteomyelitis etc. were caused by Gram-positive as well as Gram-negative bacteria[1]. Fungal infections (mycosis) are transmitted by fungus that can damage lungs, nails, mucous membranes, skin, hair, and other body components. Some examples of fungal infections include Aspergillosis, blastomycosis, candidiasis, chromoblastomycosis, cryptococcosis, histoplasmosis, and Jock itch[30]. Multidrug-resistant (MDR) is a global health threat that can lead to increased mortality.

As a result, need for innovative antimicrobial agents is growing, and lately, metal nanoparticles get more scholars' attention globally as a result of their high antimicrobial as well as catalytic activities[16]. Naturally silver metal has good antimicrobial properties and disinfectant effects. Reducing the size of silver metal to the nano level, increases antimicrobial property[17]. Test pathogen growth was successfully inhibited by MK-AgNPs' antibacterial activity, with zones of inhibition of different sizes.[25] AgNPs use reduced necessity of antibiotic given for the disease-causing pathogens[6]. When combined with phage ZCSE9, biofilm-AgNPs effectively inhibit along with eradicate *Salmonella enterica*.[32] Silver nanoparticles conjugated with PEG as well as Nystatin (AgNPs-PEG-NYS) exhibited superior antimicrobial effects against *S.Aureus* and *E.Coli*[13].

The green synthesized AgNPs may serve as a viable option for the development of antibacterial agents against multidrug-resistant bacterial strains.[18] The beneficial interaction of AgNPs with conventional antibiotics and antifungal agents may facilitate development of antibacterial products and antifungal medications applicable in diverse sectors such as food, agriculture, cosmetics, and medicine as well as may open future avenues for nano-medicine formulation as well as targeted drug delivery.[24]

Despite both chemical as well as physical methods, for nanoparticle synthesis, plant-mediated synthesis has emerged as promising alternative. This method offers several advantages including reduced toxicity, cost efficiency, environmental friendliness, and faster processing times[19].

Nowadays there is an increase in usage of plants and plant-based products to cure many diseases. Various ethnic therapeutic plants have been employed in formulation of bioactive components as well as diverse medicinal remedies for therapeutic purposes. *Elaeagnus conferta* was utilized for treatment of a variety of illnesses and is thought to have cytoprotective, anti-tumor, anti-inflammatory, anti-diabetic, anti-viral, anti-microbial, anti-fungal, as well as antioxidant properties.[10]

Various phytocompounds including flavonoids, polyphenols, tannins, alkaloids, sugars, as well as steroids, had been identified in *E. conferta* plant and in its different parts such as fruits, roots, leaves, along seeds. These compounds are responsible for plant's potential antioxidant, anti-inflammatory, and other medicinal properties[12]. Consequently, we employed leaves of *E. Conferta* for eco-friendly production of AgNPs.

This investigation documented the biogenic synthesis of AgNPs utilizing methanolic extract of *E. conferta,* characterization of synthesised nanoparticle, and its evaluation of potential effect against infection-causing pathogens.

Material and Methods

The disease-free leaves had been shade-dried for 8-10 days and subsequently processed to fine powder utilizing electric blender. An unrefined plant extract was obtained using Soxhlet extraction method.[31] Approximately 50g of leaf powder had uniformly filled into thimble in addition to being extracted with 250ml of methanol solvent. Extraction process was sustained for 24 - 48 hrs or until extractor's siphon tube's solution turned colorless. Extracted solution was maintained on at 30 to 40°C on hot plate, until complete solvent evaporation occurred. Dried extracts were kept at 4°C in freezer for subsequent experiments. It is essential to find bioactive compounds present in plant extract. The standard phytochemical screening tests were performed to detect the active compounds present in *Elaeagnus conferta* methanolic extract.

Biosynthesis of silver nanoparticles: The green route technique had been utilized for manufacture of *E. conferta* mediated AgNPs (EC-AgNPs). Approximately 10mL of methanolic extract had been introduced into a 500mL conical flask containing 100mL of 1mM $AgNO_3$, maintaining ratio 1:10, and then placed on a magnetic stirrer at the ambient temperature till solution attained a reddish-brown hue. Solution was subsequently incubated in darkness for 24 hrs to ensure complete creation of AgNPs.

Characterization of silver nanoparticles: *E.conferta* mediated AgNPs was confirmed with different characterisation studies. The synthesis of *E.conferta* mediated AgNPs was seen utilizing UV-visible spectroscopy (Perklin- Elmer, Lamda 35,Germany) with the absorption range between 350-500nm. The nanoparticle synthesis was observed every 3 hrs of incubation. EC-AgNPs' FT-IR analysis and methanolic extract of *E.Conferta* had been studied employing a FT-IR spectrophotometer (Perkin-Elmer LS-55-Luminescence spectrometer) within 400 to 4000 cm^{-1} wavelength range. The surface morphology of biosynthesised EC-AgNPs had been observed by SEM-EDAX (JSM-6480 LV) and TEM analysis techniques. Mean crystalline size D of *E. conferta* mediated AgNPs had been estimated utilizing XRD approach.

Antibacterial study: Disc diffusion method[7] had been employed in order to screen for antibacterial activity. Muller Hinton Agar (MHA) was supplied by Himedia (Mumbai) which was used to test for antibacterial activity *in vitro*. Sterilized Petri plates were filled with 15 milliliters of molten medium to create MHA plates. After five minutes of plate solidification, a uniform swab of a 0.1% inoculum suspension was made, and the inoculums were left to dry for five minutes. A sterile disk measuring 6mm was filled with varying concentrations of plant-mediated AgNPs. After five minutes of allowing loaded disc to diffuse over medium's surface, plates were then placed at 37°C for 24 hrs. A measurement of inhibitory zone had been made at millimeter scale at the conclusion of incubation period.

Antifungal study: The agar well diffusion method[4] was used for antifungal study. For fungal cultures, SDA (Sabouraud's dextrose agar) has been employed. Individual fungal strains suspended within Sabouraud's dextrose broth had been administered to culture medium. Wells around 8mm diameter had been created in agar. Fluconazole, at dosage of 1mg/ml, served as positive control. Along fungal plates were grown for 72 hrs at 37°C. Sizes of inhibitory zones were measured.

Results and Discussion

Qualitative analysis: Investigation of phytochemical constituents in the methanolic extract of *Elaeagnus conferta* was done by standard phytochemical screening tests. Results indicated the existence of carbohydrates, terpenoids, flavonoids, alkaloids, phenolic compounds, and steroids. As per the literature, presence of phytochemical compounds in plant extract has very good effect of cytotoxicity [23]. Due to the presence of higher flavonoids, It may serve as an antibacterial as well as antiseptic reagent. [21] Plants that contain phenolic compounds provide antioxidant properties. Terpenoid, phenolic component, as well as flavonoids exhibit anti-helminthic properties, indicating that plant may be utilized to address gastrointestinal issues and hypertension.

Characterisation study of *Elaeagnus conferta* mediated silver nanoparticles: UV-Visible spectra of *E.Conferta* extract (Figure 1) showed the maximum absorbance at 354,408, 509, 534, 620 nm because of existence of conjugated double bond compounds along with hetero atoms. The absorbance at 620nm indicates chromophore (chlorophyll). In *E.conferta*, mediated AgNPs spectrum (Figure 2) shows the maximum absorbance at 405nm, validated by presence of distinct SPR (Surface Plasmon Resonance). Maximum absorption is within range of 400–500nm[3,20].

Figure 3 indicates FT-IR spectrum of *E.Conferta.* Spectrum reveals the presence of phytocompounds with a functional group like C-H, C=O, OH, C=C, as well as C≡N. FT-IR spectrum of *E.conferta* mediated AgNPs (Figure 4) reveals presence of terpenoids as well as flavonoids in leaves function as capping along with reducing agents for synthesis of AgNPs. Phenolic compounds containing hydroxyl and carboxyl responsible for metal-oxygen bond[26].

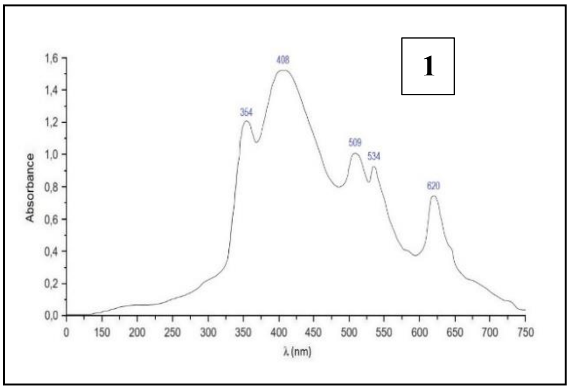

Fig. 1: UV-Visible absorption spectrum of *E. conferta*

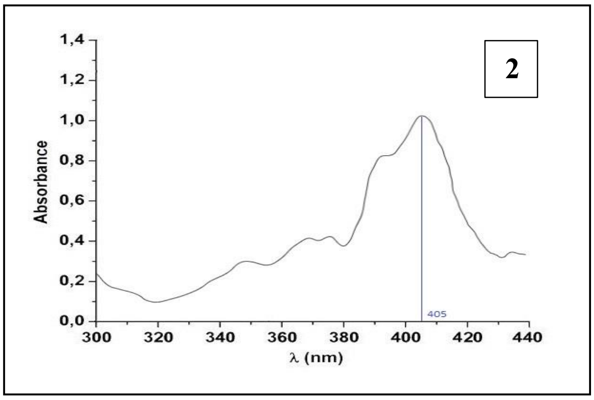

Fig. 2: UV- Visible absorption spectrum of EC mediated AgNPs

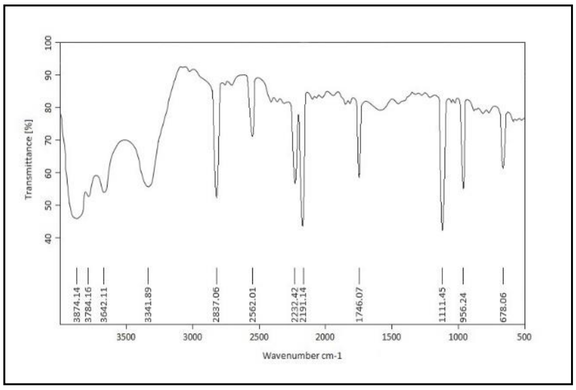

Fig. 3: FT-IR of *Elaeagnus conferta*

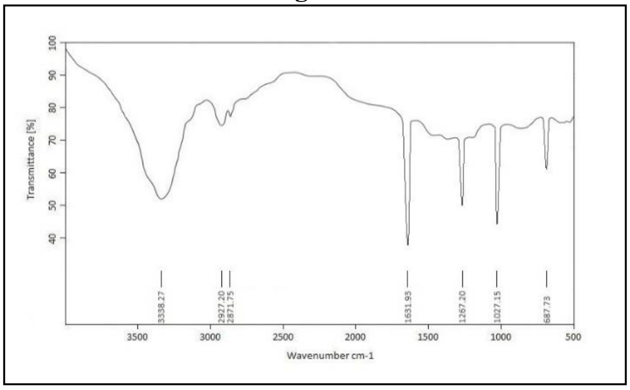

Fig. 4: FT-IR of EC mediated AgNPs

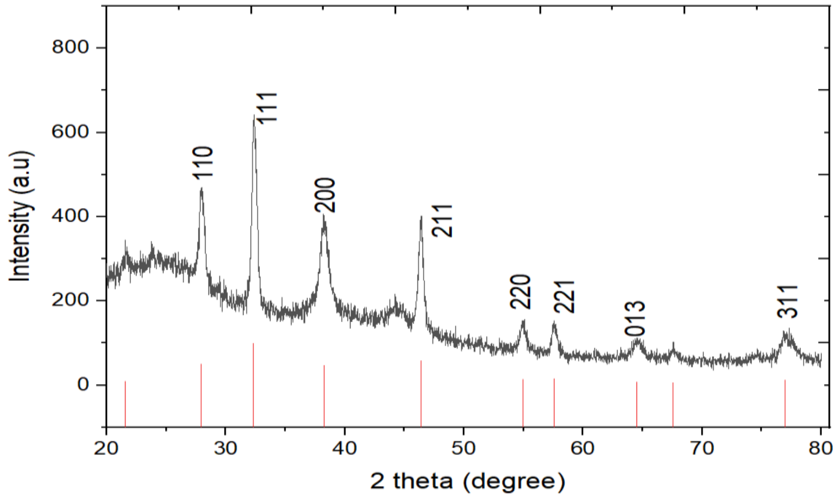

Fig. 5: XRD analysis of EC mediated AgNPs

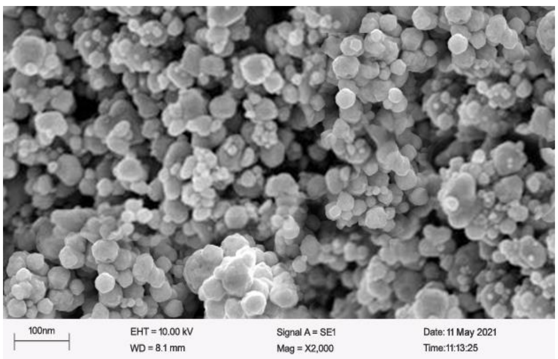

Fig. 6: SEM image of EC mediated AgNPs

Morphology analysis: Biosynthesized AgNPs' crystal structure had been ascertained utilizing XRD investigation (Figure 5). 2Θ values for *E.conferta* mediated AgNPs were determined to be 27.95°, 32.32°, 46.36°, 57.54°, 67.56°, and 76.93°, correlating to XRD planes (110),(111),(211), (221),(013) and (311) depending on silver's face-centered cubic structure. Mean crystalline size of nanoparticle determined by Debye-Scherrer formula, $Dp=[0.94 \times \lambda]/[\beta_{hkl} \times Cos\Theta_{hkl}]$ was 22.17 nm. Some unassigned peaks were also noticed and the sharp peaks obtained in the spectrum indicate that the formed nanoparticles were stabilised by capping agent[14].

Morphology study of biosynthesised silver nanoparticle is displayed in figure 6 which confirms spherical shape of EC-AgNPs. Elemental composition of silver (47.47%), oxygen (10.15%) was illustrated by EDAX analysis (Figure 7). High intensity peak of silver was recorded near 3keV. Peaks of some other elements like C, O, Cl, Mg, Cu were also identified[15]. TEM image (figure 8) clearly demonstrates that biosynthesised silver nanoparticle's shape was spherical and particles are agglomerated[27]. Particle size was determined as 26.59nm.

Results of antimicrobial activity: The inhibitory potential of EC-AgNPs against infection causing pathogens is shown in figure 9 and values are recorded in table 1. It was observed that EC-AgNPs show significant potential effect against *E.coli* (Gram-negative bacteria), versus *Staphylococcus aureus* (Gram-positive bacteria). The antifungal activity" of AgNPs has been documented with effectiveness against *Candida albicans.*

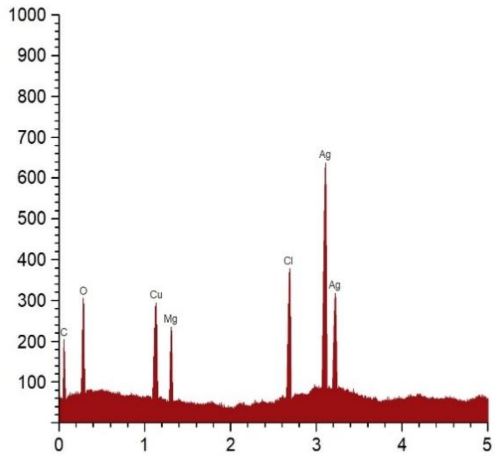

Fig. 7: EDAX spectrum of EC mediated AgNPs

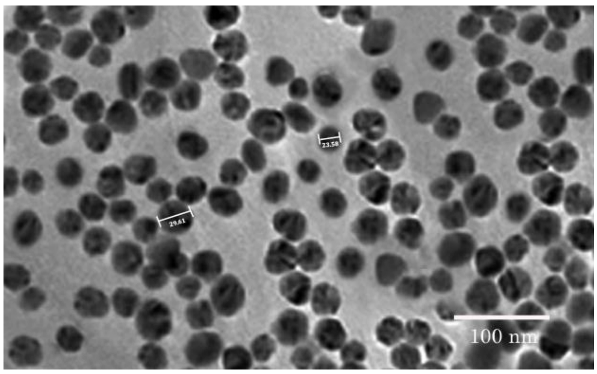

Fig. 8: TEM image of EC mediated AgNPs

Table 1
% Composition of elements

Elements	Intensity	% weight
C	0.14	6.12
O	0.27	10.15
Cu	1.13	12.83
Mg	1.31	9.35
Cl	2.67	14.08
Ag	3.09	34.06
Ag	3.21	13.41

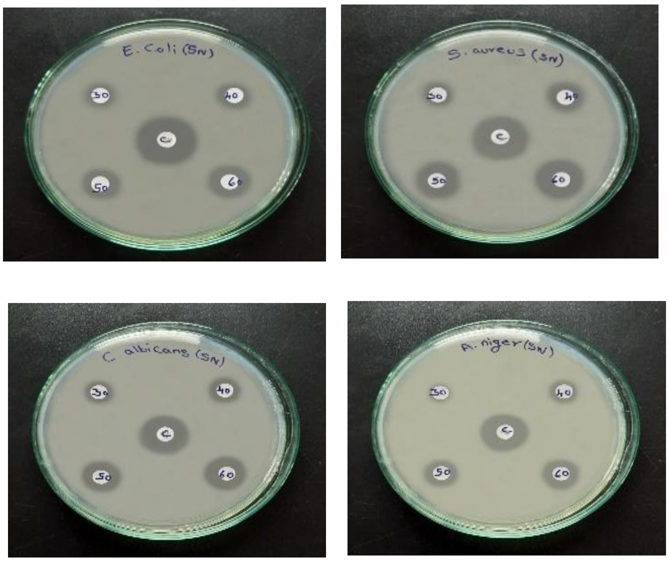

Fig. 9: Zone of inhibition of EC mediated AgNPs on *E.coli, S.aureus, C.albicans* and *A.niger*

Table 2
Antimicrobial activity analysis

Organisms	**Control**	**Concentration (µg/mL)**			
		30	40	50	60
Escherichia coli	22	9	12	14	16
Staphylococcus aureus	21	11	14	16	19
Candida albicans	19	12	13	15	17
Aspergillus niger	18	8	11	13	15

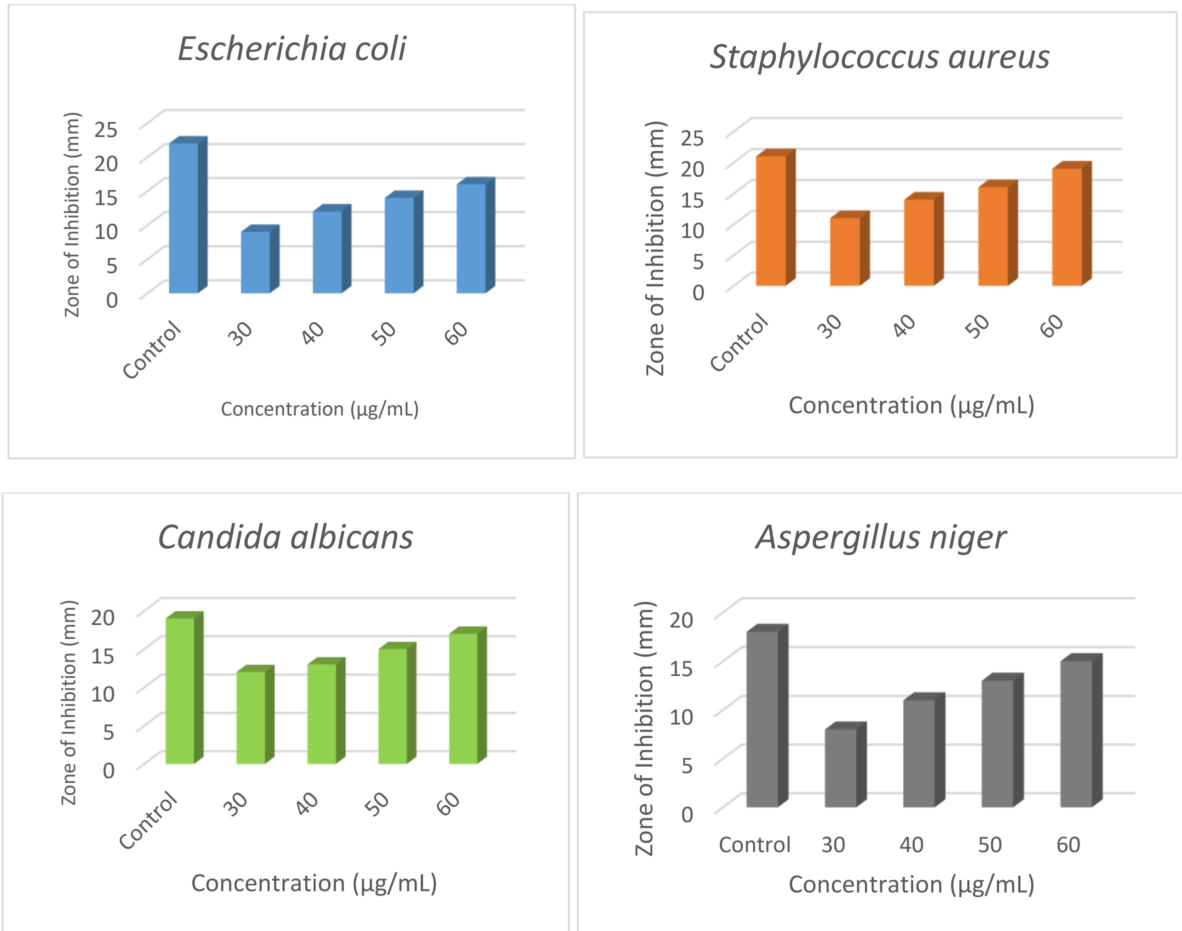

Fig. 10: Graphical Representation of Antimicrobial activity of EC mediated AgNPs against a. E.*Coli*, b. S. *aureus*, c. C. *albicans* and d. A. *niger*

A rich profile of phytocompounds in *E.Conferta* shows cytotoxic effect and antimicrobial activity[5]. The concentration of plant extract and $AgNO_3$ maintained at 1:10 ratio. Brown colour appeared during reaction clearly indicated silver nanoparticles formation[29]. Different parameters like nature of the nanoparticle formed, particle size, crystallinity and surface area were determined by the characterisation methods like UV-Visible, FT-IR, SEM-EDAX, XRD, as well as TEM analysis. Maximum absorption peak observed for biosynthesised silver nanoparticle is 405 nm. In general, AgNPs shows characteristic peaks between the range 400-480 nm[9]. The surface morphology studies revealed the spherical shape of EC-AgNPs. This is similar to previously reported results[11]. FT-IR spectrum of *E.Conferta* and EC-AgNPs confirming functional groups of phytocompounds accountable for formation of silver nanoparticle. Further, XRD analysis demonstrated the crystal planes of silver's face-centered cubic structure[22].

On antimicrobial activity, EC-AgNPs demonstrated significant efficacy against fungai (*C. albicans)* along with Gram-positive bacteria (*S.aureus*)[2]. *Candida* species are among the most prevalent pathogenic yeasts affecting humans. However, treatment options for *Candida* infections are restricted owing to advent of resistant strains, limited availability of antifungal medications, and their high costs. Thus, the use of AgNPs possesses a possibility for enhanced treatment of *Candida*-related diseases[28].

Subsequent research ought to concentrate on explaining the accurate mechanisms of action of these nanoparticles, as well as conducting in vivo assessment to understand their healing potential and safety profiles.

Conclusion

In summary, the effective production of AgNPs from *Elaeagnus conferta* highlights their efficacy as powerful antibacterial agents targeting resistant pathogens like *S. aureus* and *C. albicans*. The characterization techniques confirm structural integrity and properties regarding produced nanoparticles, indicating their appropriateness for medical uses. Current approach not only explains antimicrobial capability of EC mediated AgNPs but benefits from natural compounds present in plant extracts, offering a safer and more sustainable solution.

Acknowledgement

We are grateful to Dr. M. Mekala, The Director, Microbiotek, Coimbatore for offering facilities and resources for this project. Their support facilitated the smooth execution of the research.

References

1. Agila A., Jeyaleela G.D., Vimala J.D.R., Bharathy M.S and Sheela S.A.M., Inhibitory effect of Basella alba-mediated zinc oxide nanoparticles against the infection-causing bacteria, *Biomedical and Biotechnology Research Journal (BBRJ)*, **6(3)**, 353-359 **(2022)**

2. Ali I.A.M., Ahmed A.B and Al-Ahmed H.I., Green synthesis and characterization of silver nanoparticles for reducing the damage to sperm parameters in diabetic compared to metformin, *Scientific Reports*, **13(1)**, 2256 **(2023)**

3. Ashraf J.M., Ansari M.A., Khan H.M., Alzohairy M.A and Choi I., Green synthesis of silver nanoparticles and characterization of their inhibitory effects on AGEs formation using biophysical techniques, *Scientific Reports*, **6(1)**, 1 **(2016)**

4. Ballard, Cíntia Reis and Mário Roberto Maróstica, Health benefits of flavonoids, Bioactive compounds, Woodhead Publishing, 185-201 **(2019)**

5. Begum S.R., Rao D.M and Reddy P.D.S., Role of green route synthesized silver nanoparticles in medicinal applications with special reference to cancer therapy, *Biosciences Biotechnology Research Asia*, **15(4)**, 783-790 **(2018)**

6. Bruna T., Maldonado-Bravo F., Jara P and Caro N., Silver nanoparticles and their antibacterial applications, *International Journal of Molecular Sciences*, **22(13)**, 7202 **(2021)**

7. Chowdhury S., Poddar S.K., Zaheen S., Noor F.A., Ahmed N., Haque S and Akbar N., Phytochemical screening and evaluation of cytotoxic and hypoglycemic properties of Mangifera indica peels, *Asian Pacific Journal of Tropical Biomedicine*, **7(1)**, 49-52 **(2017)**

8. Dakal T.C., Kumar A., Majumdar R.S and Yadav V., Mechanistic basis of antimicrobial actions of silver nanoparticles, *Frontiers in Mjicrobiology*, **7**, 1-17 **(2016)**

9. Elamawi R.M., Al-Harbi R.E and Hendi A.A., Biosynthesis and characterization of silver nanoparticles using Trichoderma longibrachiatum and their effect on phytopathogenic fungi, *Egyptian Journal of Biological Pest Control*, **28(1)**, 1-11 **(2018)**

10. Gill N.S and Gupta M., Elaeagnus conferta: a comprehensive review, *Research Journal of Pharmacy and Technology*, **11(6)**, 2667-2671 **(2018)**

11. Goudarzi M., Mir N., Mousavi-Kamazani M., Bagheri S and Salavati-Niasari M., Biosynthesis and characterization of silver nanoparticles prepared from two novel natural precursors by facile thermal decomposition methods, *Scientific Reports*, **6(1)**, 32539 **(2016)**

12. Guptaa M and Singhc N., Preliminary Phytochemical Screening of Elaeagnus Conferta Roxb. Seeds and Biological Evaluation, *Plant Archives*, **21(1)**, 547-551 **(2021)**

13. Ibraheem D.R., Alwas N.G., Dawood R.A., Nasser S.M., Abbood S.H., Sulaiman G.M and Ghotekar S., Inhibitory effect of silver nanoparticles–conjugated PEG-nystatin against some resistance pathogenic bacteria, *Inorganic Chemistry Communications*, **168**, 112952 **(2024)**

14. Karthik L., Kumar G., Kirthi A.V., Rahuman A.A and Bhaskara Rao K.V., Streptomyces sp. LK3 mediated synthesis of silver

nanoparticles and its biomedical application, *Bioprocess and Biosystems Engineering*, **37**, 261-267 **(2014)**

15. Khan A.R., Ahmed M., Khan H., Osman N.A.H.K., Gaafar A.R.Z and Shafique T., Beta maritima mediated silver Nanoparticles: Characterization and evaluation of Antibacterial, Antifungal, and antioxidant activities, *Journal of King Saud University-Science*, **36(6)**, 103219 **(2024)**

16. Kunkalekar R.K., Naik M.M., Dubey S.K and Salker A.V., Antibacterial activity of silver-doped manganese dioxide nanoparticles on multidrug-resistant bacteria, *Journal of Chemical Technology & Biotechnology*, **88(5)**, 873-877 **(2013)**

17. Liu X., Chen J.L., Yang W.Y., Qian Y.C., Pan J.Y., Zhu C.N and Zhang D.P., Biosynthesis of silver nanoparticles with antimicrobial and anticancer properties using two novel yeasts, *Scientific Reports*, **11(1)**, 15795 **(2021)**

18. Loo Y.Y., Rukayadi Y., Nor-Khaizura M.A.R., Kuan C.H., Chieng B.W., Nishibuchi M and Radu S., *In vitro* antimicrobial activity of green synthesized silver nanoparticles against selected gram-negative foodborne pathogens, *Frontiers in Microbiology*, **9**, 1-7 **(2018)**

19. Meena P.R., Singh A.P and Tejavath K.K., Biosynthesis of silver nanoparticles using Cucumis prophetarum aqueous leaf extract and their antibacterial and antiproliferative activity against cancer cell lines, *ACS Omega*, **5(10)**, 5520-5528 **(2020)**

20. Melkamu W.W and Bitew L.T., Green synthesis of silver nanoparticles using Hagenia abyssinica (Bruce) JF Gmel plant leaf extract and their antibacterial and anti-oxidant activities, *Heliyon*, **7(11)**, 1-11 **(2021)**

21. Nath P and Yadav A.K., Anthelmintic activity of a standardized extract from the rhizomes of Acorus calamus Linn. (Acoraceae) against experimentally induced cestodiasis in rats, *Journal of Intercultural Ethnopharmacology*, **5(4)**, 390-395 **(2016)**

22. Ohiduzzaman M., Khan M.N.I., Khan K.A and Paul B., Biosynthesis of silver nanoparticles by banana pulp extract: Characterizations, antibacterial activity, and bioelectricity generation, *Heliyon*, **10(3)**, 1-12 **(2024)**

23. Patil S.R., Patil R.S and Godghate A.G., Mentha piperita Linn: Phytochemical, antibacterial and dipterian adulticidal approach, *Int. J. Pharm. Pharm. Sci*, **8(3)**, 352-355 **(2016)**

24. Patra J.K and Baek K.H., Antibacterial activity and synergistic antibacterial potential of biosynthesized silver nanoparticles against foodborne pathogenic bacteria along with its anticandidal and antioxidant effects, *Frontiers in Microbiology*, **8**, 1-14 **(2017)**

25. Pethannan Rajarajan, V. Shashank, Kuppuswamy Amudha Chinnaiah, Acharya Vaishnavi Ganapati and Petkar Sanjota K., Adaptive Resilience: Unraveling the Path of Heavy Metal Tolerance in Multidrug Resistant Pathogens, *Res. J. Biotech.*, **19(6)**, 105-115 **(2024)**

26. Raja K., Saravanakumar A and Vijayakumar R., Efficient synthesis of silver nanoparticles from Prosopis juliflora leaf extract and its antimicrobial activity using sewage, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **97**, 490-494 **(2012)**

27. Rautela A and Rani J., Green synthesis of silver nanoparticles from Tectona grandis seeds extract: characterization and mechanism of antimicrobial action on different microorganisms, *Journal of Analytical Science and Technology*, **10(1)**, 5 **(2019)**

28. Renganathan S., Subramaniyan S., Karunanithi N., Vasanthakumar P., Kutzner A., Kim P.S and Heese K., Antibacterial, antifungal, and antioxidant activities of silver nanoparticles biosynthesized from Bauhinia Tomentosa Linn., *Antioxidants*, **10(12)**, 1959 **(2021)**

29. Sandhu S.S., Shukla H and Shukla S., Biosynthesis of silver nanoparticles by endophytic fungi: Its mechanism, characterization techniques and antimicrobial potential, *African Journal of Biotechnology*, **16(14)**, 683-698 **(2017)**

30. Williams C., Ranjendran R and Ramage G., Pathogenesis of fungal infections in cystic fibrosis, *Current Fungal Infection Reports*, **10**, 163-169 **(2016)**

31. Yadav R.N.S and Agarwala M., Phytochemical analysis of some medicinal plants, *Journal of Phytology*, **3(12)**, 10-14 **(2011)**

32. Yakoup A.Y., Kamel A.G., Elbermawy Y., Abdelsattar A.S and El-Shibiny A., Characterization, antibacterial, and cytotoxic activities of silver nanoparticles using the whole biofilm layer as a macromolecule in biosynthesis, *Scientific Reports*, **14(1)**, 364 **(2024)**.

(Received 20th January 2025, accepted 22nd March 2025)