Sustainable Green Synthesis and Evaluation of Antimicrobial Potential of Silver Nanoparticles prepared from the Stem Extract of *Mangifera indica*: A Biogenic Approach

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Abstract

The present study demonstrates the production of silver nanoparticles (SNPs) utilizing an aqueous extract from the stem of Mangifera indica and their application as an antimicrobial agent against diverse pathogenic microorganisms. The presence of a dark brown colour visually supported SNP biosynthesis, which was also confirmed by UV spectra at 450 nm. FE-SEM (Field Emission Electron Scanning Electron Microscopy) examinations revealed the nanoparticles' spherical to oval shape, while EDX (Energy-Dispersive X-ray) research proved their elemental makeup. XRD research revealed that SSNPs (Stem SNPs) have a face-centred cubic crystalline structure with an average size of 10 nm. FT-IR research demonstrated that the bio-functional groups included in the plant extract stimulate the production of SNPs while also promoting the capping and stabilization of the SNPs. The phytochemical examination revealed the presence of bioactive compounds. The SSNPs had more antibacterial activity than antifungal activity. A single-factor ANOVA was used for statistical analysis and $p < 0.05$ was considered statistically significant.

The study's findings indicate that many naturally occurring bioactive chemicals in plant extracts may be usefully employed in nanoparticle-making. In contrast to traditional antibiotics, this study demonstrated an effective, practical and repeatable approach for manufacturing inexpensive, environmentally benign and long-lasting plant-mediated SNPs and their use as powerful antimicrobial agents against pathogenic microorganisms.

Keywords: Biogenic approach, Silver nanoparticles (SNPs), Stem, Characterization, Antimicrobial activity.

Introduction

The Greek word "Nanos," meaning "dwarf," is the origin of the term "nano." Nanotechnology involves creating nanoparticles of various sizes (1-100 nm), shapes and chemical compositions with controlled dispersity for human use[7]. The exceptional physical, optical and chemical properties of nanoparticles, which differ from their bulk counterparts, have sparked extensive research in fields such as materials, energy, medicine, sensors, optical fibre, antimicrobials, catalysis and agriculture[5,13]. In contemporary medicine and bioscience, nanoparticles (NPs) play a crucial role due to their distinctive physicochemical characteristics. Silver nanoparticles (SNPs) are particularly popular among metal nanoparticles because of their non-toxicity, ease of production and potential therapeutic applications[6]. Studies have shown that SNPs exhibit antifungal, anticancer, anti-inflammatory, antiplatelet and antiviral properties[3].

Physical and chemical synthesis methods for SNPs are unsuitable due to low yield and the use of toxic compounds, respectively[11]. In contrast, biosynthesis or green route synthesis approaches are environmentally friendly, cost-effective, hygienic and safe[8]. These methods utilize non-toxic solvents like plant extracts, fungi, bacteria and algae and employ safe synthesis procedures[23]. Plant extract-based biosynthesis of metal nanoparticles offers several advantages including reduced reaction time from days to hours, absence of toxic chemicals and no need for high-pressure synthesis[24]. Numerous studies have reported the use of plant extracts for SNP synthesis. This approach also facilitates large-scale production of SNPs for industrial and research purposes.

Phytocompounds provide antioxidant properties and those with antibacterial activity impart both antioxidant and antibacterial properties to the nanoparticles[16]. Mango, a widely cultivated tropical fruit in South Asian countries, belongs to the *Mangifera* species of the *Anacardiaceae* family[21]. Various parts of the mango tree including leaves, fruit, seed kernel, fruit pulp, roots, bark and stem bark, have been used for medicinal purposes in many countries since ancient times. The aqueous extract of mango stem bark has been extensively documented for its ethnomedical use in treating conditions such as cancer, diabetes, asthma, infertility, lupus, prostatitis, prostatic hyperplasia, gastric disorders, arthralgia, mouth sores and tooth pain[14].

Recent publications have reported the biosynthesis of various metal and metal oxide nanoparticles using mango leaf including silver nanoparticles (SNPs)[1,9], gold nanoparticles (AuNPs)[17], titanium dioxide nanoparticles (TiO_2 NPs)[18] and iron oxide nanoparticles (Fe_3O_4 NPs)[2]. In the current study, *Mangifera indica* SNPs were produced from the stem using a green route method, analysing its

structural and optical properties and evaluating the antimicrobial activity.

Material and Methods

Materials: The chemicals used were bought from Hi Media Pvt. Ltd. (Mumbai, India) and deionized water (DIW) was used throughout the trial. The stems were collected from the mango field of Agricultural Research Station, Borwat Farm, Banswara, Rajasthan, India (23.54 ºN, 74.433 ºE). The stems were taken in the form of branches that were hairless, pale green to greyish-brown in colour and 6-8 mm in diameter. Microbial strains were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh.

Extract Preparation: The stem underwent a week of shade drying after being properly cleaned several times with DIW to get rid of any debris. Using a pestle, mortar and grinder, the stem was crushed and ground into a fine powder before being sieved through muslin fabric. 100 ml of DIW was combined with 3 grams of stem powder. A magnetic stirrer was used to agitate the liquid for an hour after it had been in the water bath at 60°C for 30 minutes. The combination was then filtered through Whatmann filter paper no. 1 and kept at 4°C for further use.

Green Synthesis of SNPs: 50 ml of 1 mM silver nitrate and 10 ml of stem extract were mixed with deionized water. The entire reaction was carried out in dim light to lessen the photo-activation of silver nitrate. Silver nitrate has been reduced to SNPs as observed by the colour change. The reaction mixture was centrifuged at 4 °C and 3000 rpm for ten minutes. To produce SNPs, the pellets were collected and dehydrated in an oven set at 40°C. Figure 1 represents the layout for synthesizing metal nanoparticles from the stem extract of *M. indica*.

Characterization: UV-VIS analysis (Shimadzu-1900i series) was performed for the visual assessment of the stem SNPs. The substance was examined using visible and ultraviolet light with wavelengths ranging from 300 to 800 nm for proximate examination. Silver nitrate solution was used as a control. The stem extract reduced silver ions to silver nanoparticles within an hour of the reaction beginning. The type and particle size of the BSNP (Biosynthesized SNPs) were ascertained by XRD. This was done using a Bruker AXS D8 Advance X-ray diffractometer, which has a range of 40 kV for voltage and 30 mA for current. Its wavelength was 0.154 nm and Cu kα was used. Following the fine powdering of the material used for the experiment, the average bulk composition was determined. The scanning process lasted 20 to 90 seconds. The ICDD library was used to confirm the reported peaks of the SNPs for their crystalline structure.

The FTIR spectroscopy was done with a Bruker FTIR Alpha spectrophotometer. The FTIR spectra were obtained in the transmission mode between 4000 and 400 cm^{-1}, with a spatial resolution of 4 cm^{-1}. The samples were generated using the KBr pellet process and the surface chemistry of the reduced silver ion was determined along with the existence of any bio-functional groups in the samples under study. The Carl Zeiss Gemini SEM300 FE-SEM was utilized for Energy-dispersive X-ray (EDX) analysis and Scanning electron microscopy. The powder sample was deposited directly onto an aluminium stub with carbon tape on top and then stored inside the equipment for inspection.

Phytochemical Screening: The stem's aqueous extract was subjected to qualitative phytochemical analyses using standard procedures[22].

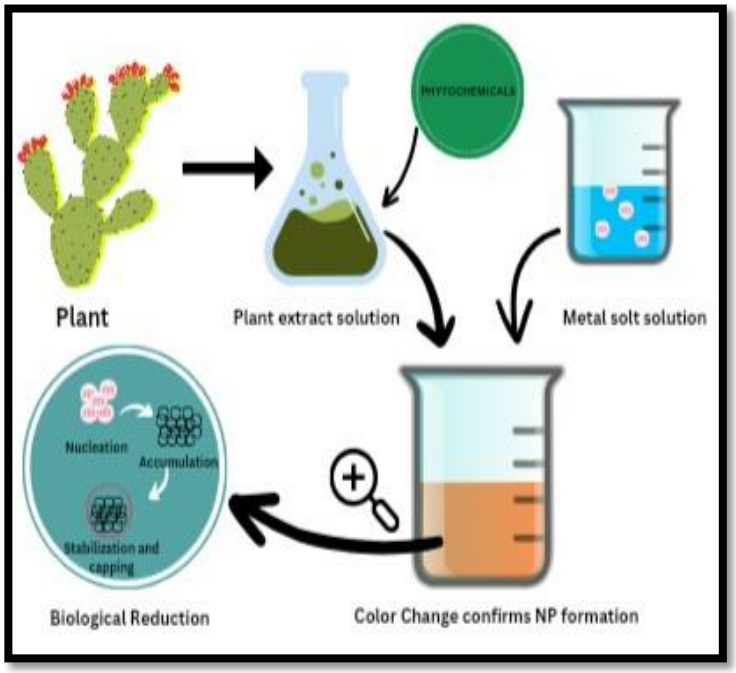

Figure 1: General layout for biosynthesis of Stem SNP

Antibacterial Efficiency of Stem SNPs: Using the conventional disc-diffusion method (Kirby-Bauer technique), the antibacterial properties of the biosynthesized silver nanoparticles were assessed against Gram-positive (SA and SP) and Gram-negative (EC and PA). The initial bacterial culture was produced on nutrient broth (NB) at an incubation of 24 hrs to get a suspension of 1.5 X 10^6 CFU/ml. 20 ml of autoclaved Mueller–Hinton Agar (MHA) medium was transferred to the sterile Petri plates and allowed to solidify. These plates were infected using the obtained bacterial suspension with a sterile cotton swab. The sterile paper discs of 5 mm size were utilized and inoculated with 1 mM silver nitrate, 50 µl of SSNP1 (Stem SNP), 100 µl of SSNP2 and the crude extract of the stem.

The paper discs were left to air dry before being uniformly distributed throughout the inoculated plates. The antibacterial activity of the corresponding discs was assessed by measuring the diameter of the clear zone of inhibition (ZOI) surrounding them after 24 to 36 hours of incubation. Ampicillin was used as the positive control for antibacterial activity. Three separate runs of the experiments were conducted

Antifungal Activity: *Candida. glabrata* and *Candida. albicans* were cultivated in PDA (Potato Dextrose Agar) fluid media for two days at 35ºC to assess the antifungal effect of the stem silver nanoparticles using the Kirby-Bauer disc-diffusion technique. They were subsequently swabbed on fresh SDA (Sabouraud Dextrose Agar) media where 5 mm discs of SSNPs (50 µl and 100 µl), silver nitrate and stem crude extract were placed. The plates were incubated for 24-48 hours at 35ºC and the test sample's antifungal activity was assessed by measuring the diameter of the clear zone of inhibition (ZOI) in millimetres. In this study, fluconazole was used as a positive control. The antifungal activity was performed in triplicate.

Results and Discussion

Synthesis of Stem SNP: The addition of the stem extract of *M. indica* to an aqueous silver nitrate solution resulted in colour change from pale yellow to deep brown indicating silver nanoparticle formation (Figure 2).

UV-Vis Spectroscopy: The colour change of the reaction mixture was confirmed by a UV-Vis spectrum taken at intervals of 0, 15, 30, 45 and 60 minutes. The peak intensity kept on increasing with the increasing time at 450 nm (Figure 3). An absorption peak around 450 nm associated with the photoexcitation of electrons indicates the surface plasmon resonance (SPR) of SNPs leading to a band shift. The presence of these peaks was due to the stem extract containing the organic compounds responsible for reducing silver ions and stabilizing the resulting nanoparticles[19].

X-Ray Diffraction: Figure 4 displays the SNPs made from the stem's dry powder XRD pattern. The arrangement of atoms in a crystalline substance can be determined using XRD. When compared to the standard powder diffraction card of the ICDD (International Centre for Diffraction Data) card No. 04-0783, all the diffraction peaks matched the distinctive crystalline face-centred cubic (FCC) silver lines. The four different planes that correspond to the 2θ of the silver facets (111), (200), (220) and (311) have strong Bragg peaks that are 38.24º, 44.44º, 64.54º and 77.5º respectively depicted in table 1.

The arrangement found in this investigation verified that silver was the primary component of the nanoparticles[15] and the particles have a crystal structure. The peaks' strength demonstrated the SNPs' high degree of crystallinity. The XRD patterns are consistent with earlier reports[20].

Figure 2: Stem SNP (Before and after)

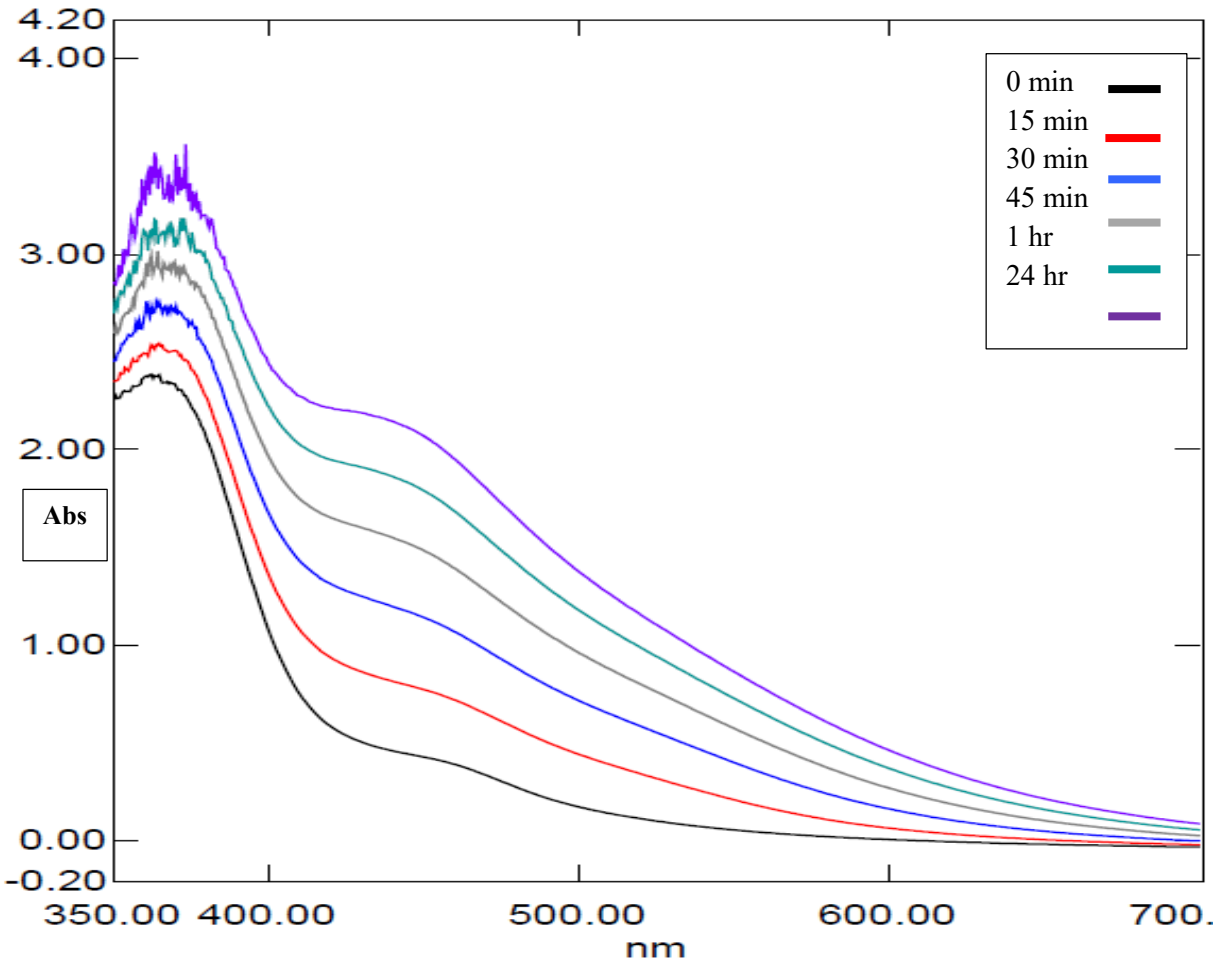

Figure 3: UV-vis spectrum of Stem SNP

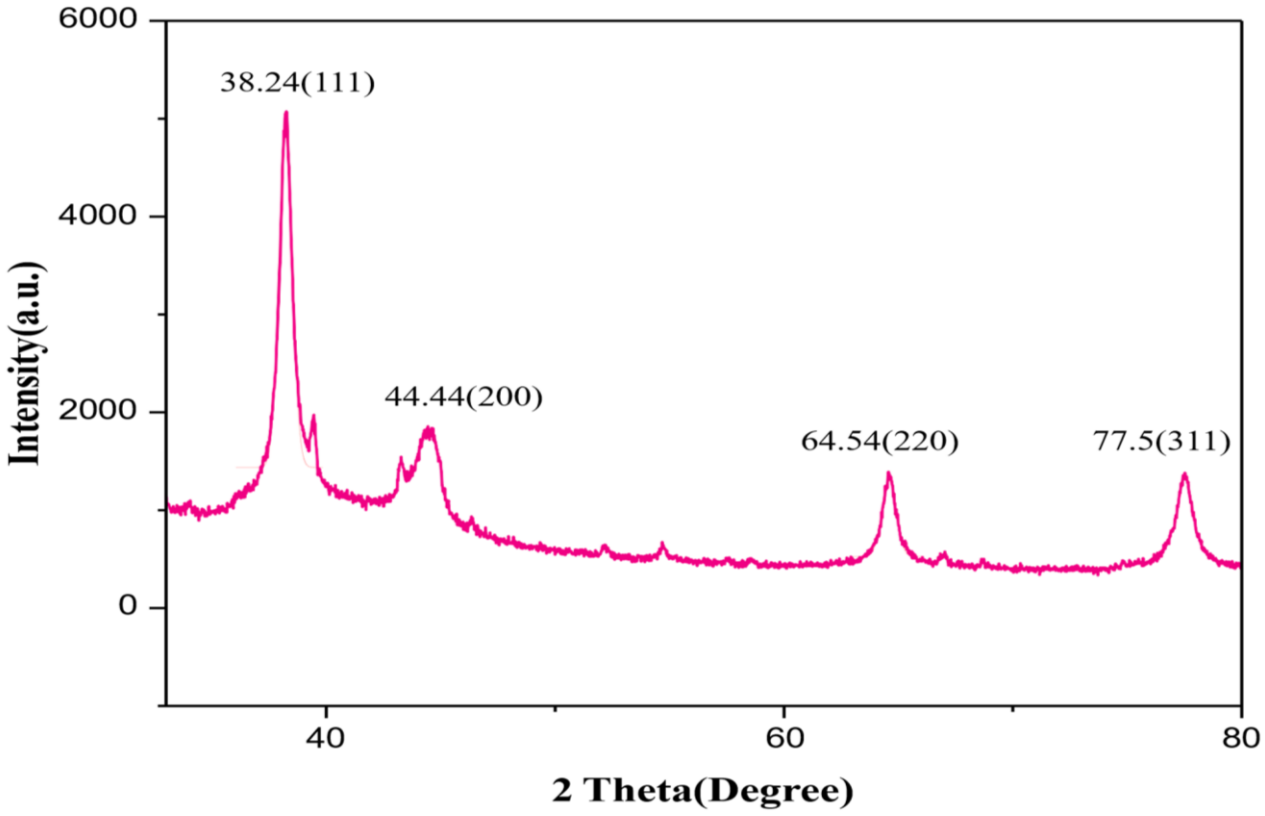

Figure 4: X-ray diffraction of synthesized Stem SNP

Table 1
XRD analysis of Stem SNP

S.N.	Peak Value (2 Theta)	FWHM	hkl Value (MI)	Crystal Size nm (D)	Average Size (D)
1	38.24	0.96617	111	9.1	9.75nm
2	44.44	0.9441	200	9.5	
3	64.54	0.97801	311	10.05	
4	77.5	0.9742	222	10.43	

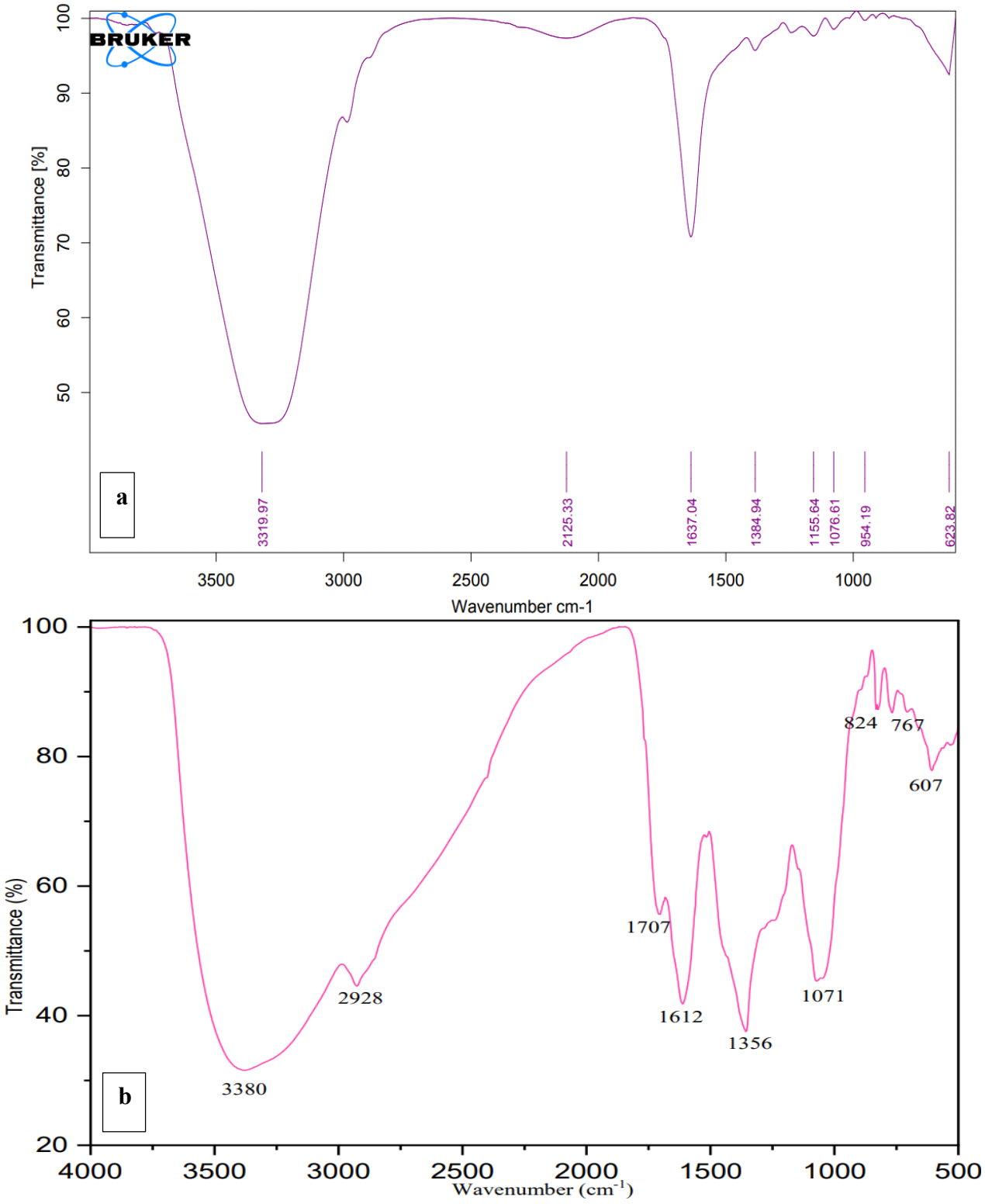

Figure 5: FTIR spectra (a) Stem Extract (b) SSNP after reduction

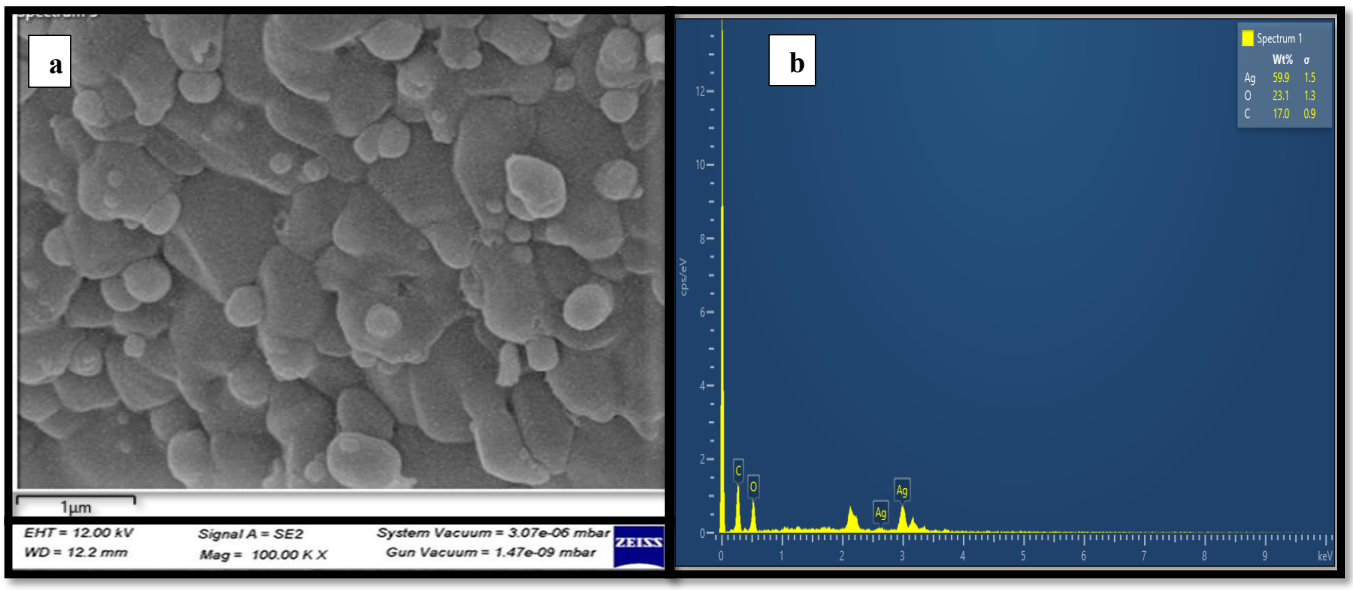

Figure 6: (a) Scanning Electron Microscopy micrograph and (b) Energy Dispersive X-ray spectrum of elemental composition of Stem SNP

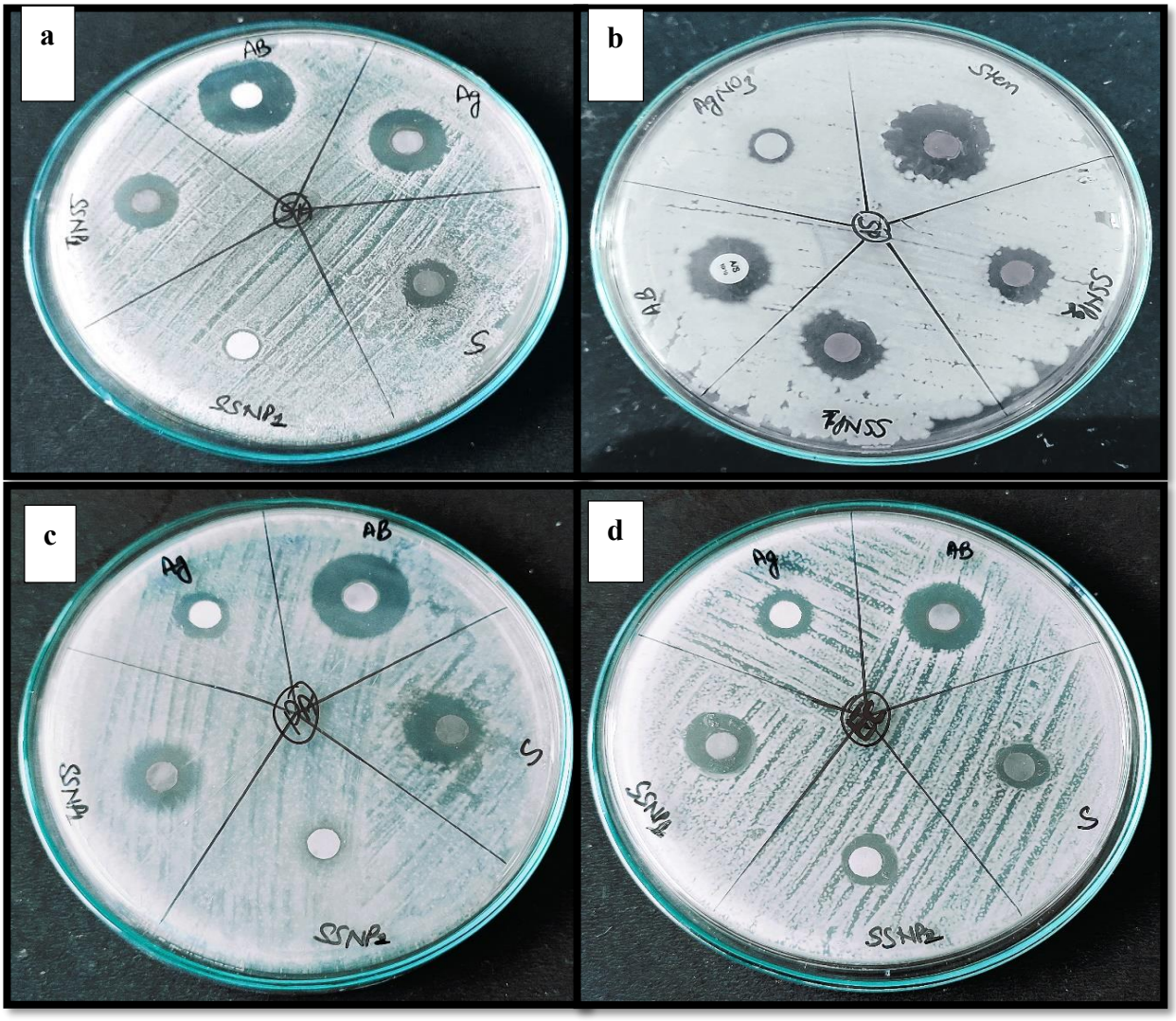

Figure 7: Antibacterial Activity of Stem against Gram-Positive (a: SA [*Staphylococcus aureus*] and b: SP [*Streptococcus pyogenes*]) and Gram-Negative (c: PA [*Pseudomonas aeruginosa*] and d: EC [*Escherichia coli*]) bacteria [(SSNP1=100µl, SSNP2=50µl, Ag=1mM, S= Stem Extract crude, AB=Ampicillin (10µg disc)]

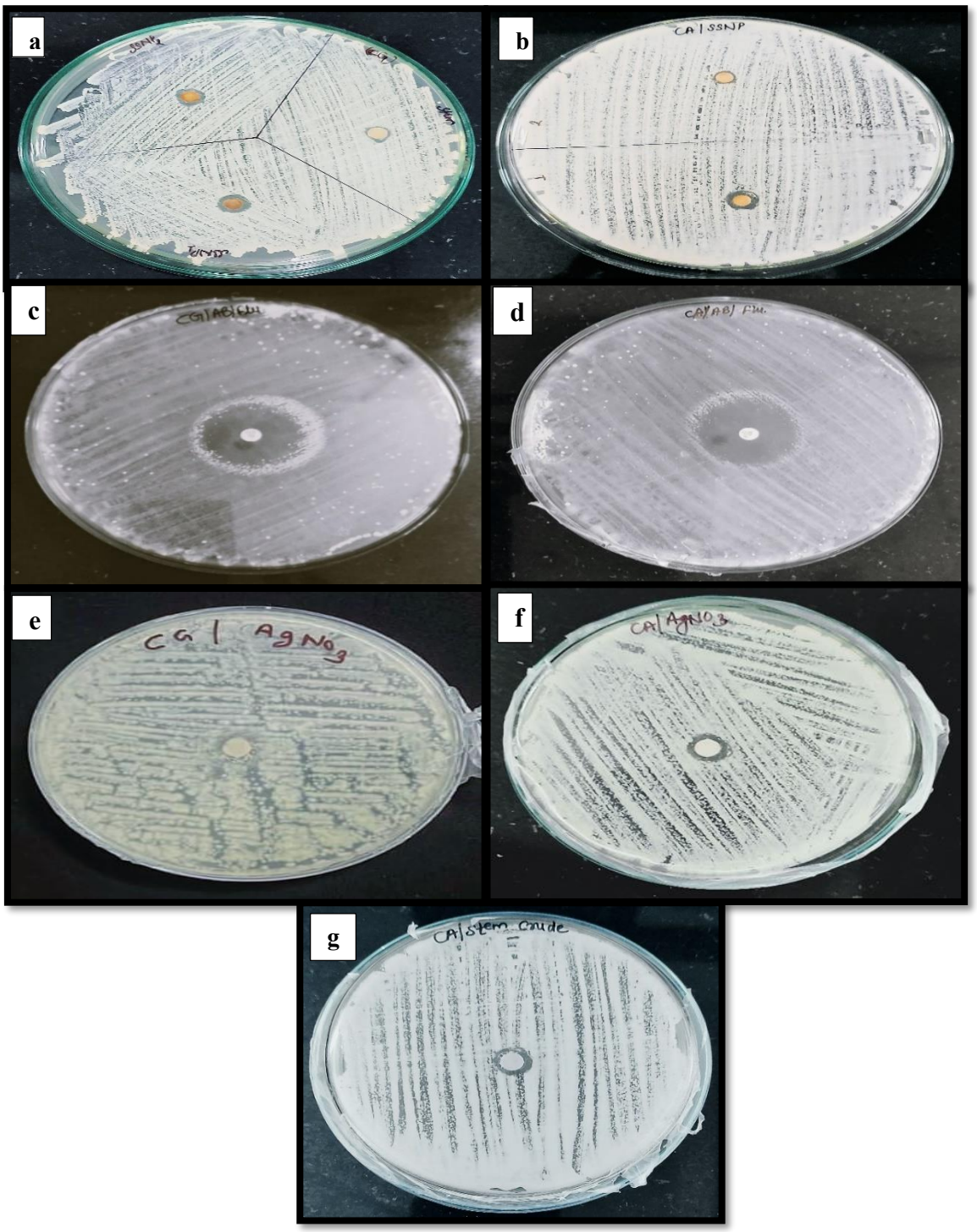

Figure 8: Antifungal Activity of Stem SNP against *C. glabrata* (CG=a, c, e) and *C. albicans* (CA=b, d, f, g) [SSNP1=100µl, SSNP2=50µl, $AgNO_3$=1mM, S=Stem extract crude, AB=Fluconazole (10µg/disc)]

FT-IR Spectroscopy: FTIR analysis is an important tool for identifying the bio-functional groups and their interactions with the metals. The FTIR spectra for stem extract were observed at 3319, 2125, 1637, 1384, 1155, 1076, 954 and 623 cm^{-1} (Figure 5a). The FTIR peaks for the Stem SNP were found at 3380, 2928, 1707, 1612, 1356, 1071, 824, 767 and 607 cm^{-1} (Figure 5b). The strong-broad peaks at 3380, 3319 and 2928 cm^{-1} indicated the O-H stretching of alcohol and carboxylic acid and the N-H stretching of amine salt. The medium band at 2125 cm^{-1} corresponds to the C-N stretching of alkyne. The bands at 1707, 1637 and 1612 cm^{-1} suggested the presence of C=O stretching of carboxylic acid, N-H bending of amines and C=C stretching of alkene.

Bands at 1384, 1356, 1155, 1076 and 1071 cm^{-1} correspond to N-O stretching of the nitro compound and C-O stretching of primary and tertiary alcohol. The weak peaks at 954, 824, 767, 623 and 607 cm^{-1} were attributed to the C=C bending of alkene, C-H and C-Cl/C-I stretching of halo compounds. It is evident from the FTIR spectra that the metallic silver

ions were reduced by the functional groups present in the stem extract and are responsible for the capping and stabilization of the synthesized SNPs[4,10].

SEM Analysis: The morphology of the synthesized SNPs was revealed by the SEM analysis which was spherical with a range of 10–50 nm (Figure 6a). EDX analysis was used to determine the elemental silver present in the SNPs. According to Lee et al[12], the EDX analysis primarily displayed significant signals at 3 keV and validated the existence of SNPs. Silver was 60% present in the EDX quantitative analysis, along with trace amounts of carbon and oxygen (Figure 6b).

Phytochemical Screening: The phytochemical analysis of the *M. indica* stem extract is shown in table 2, which shows the presence of a number of phytochemicals including proteins, alkaloids, phenolic compounds, saponins, flavonoids, carbohydrates and flavonoids. Flavonoids include hydroxyl (OH) groups, which can reduce silver to silver nanoparticles and can act as a stabilizing or capping agent for the nanoparticles.

Antibacterial Activity: The antibacterial efficiency of the stem silver nanoparticle was analysed with the help of microbial species like *Staphylococcus aureus* [SA], *Streptococcus pyogenes* [SP], *Escherichia coli* [EC] and *Pseudomonas aeruginosa* [PA] as shown in figure 7. The clear zone of inhibition was observed for each strain for the different concentrations of SSNPs (100 and 50 µl), crude extract of the stem, along with $AgNO_3$ and ampicillin as depicted in table 3. It was observed that the higher concentrations of SSNPs were more effective than the lower concentrations and were compatible with the positive control. The highest ZOI was shown by SP (13.73 mm) and the least by PA (12 mm) for SSNP1 and SSNP2 as shown in table 3.

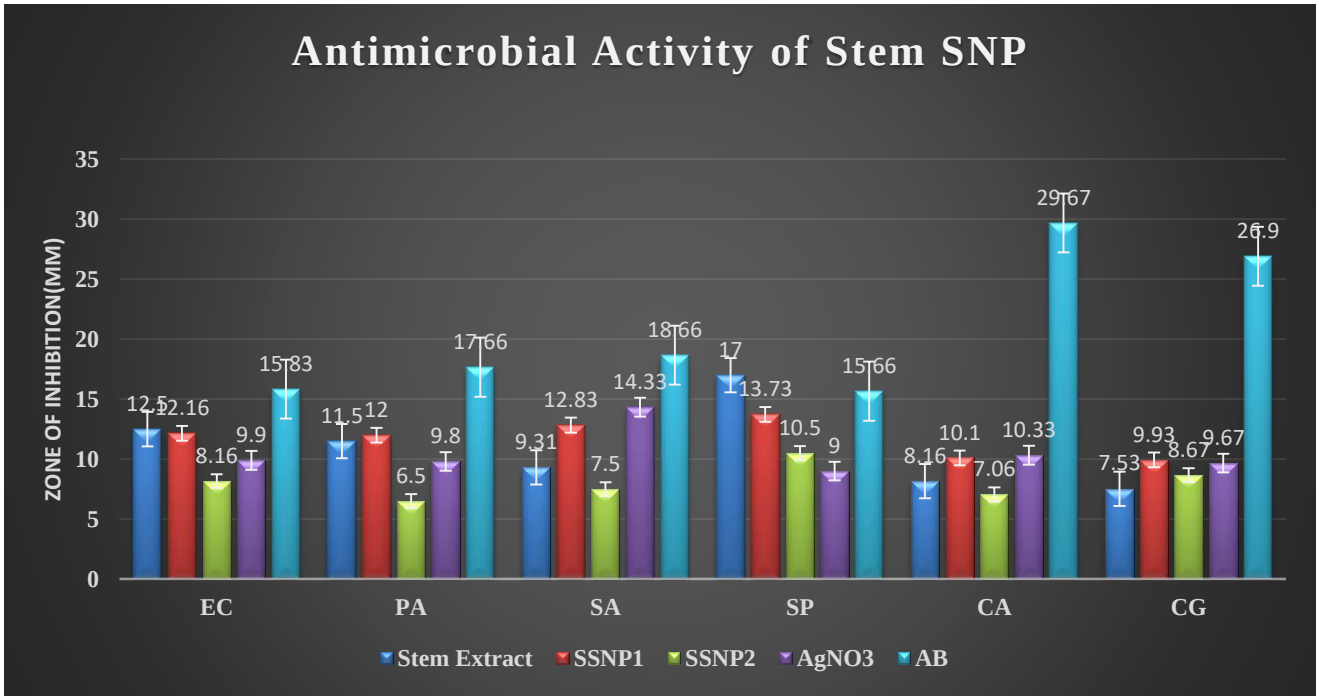

Figure 9: Antimicrobial action of Stem SNP

Table 2
Phytochemical analysis of the crude aqueous extract of Stem

S.N.	Phytochemicals	Test	End Result	Presence / Absence
1	Alkaloids	Mayer's reagent	Reddish-brown ppt. or colouration	+
2	Flavonoids	Alkaline reagent	Yellow colour changes to colourless	+
3	Glycosides	Keller-Killani test	Green-blue colour	-
4	Phenolics	Ferric chloride test	Colour change observation after adding base	+
5	Proteins	Xanthoproteic test	Yellow to Deep orange colour	+
6	Saponins	Foam Test	Froth formation at the surface	+
7	Starch	Starch Test	Bluish- black ppt	-
8	Steroids	Hesse's Test	Reddish-brown colour	-
9	Sugars	Fehling's test	Greenish colour changing to red	+
10	Tannins	Braymer's test	Blue or greenish colour	-

Table 3
Antimicrobial activity of Stem SNPs represented by Zone of inhibition (mm)

Microbes	Stem Extract	SSNP1	SSNP2	$AgNO_3$	AB
EC	12.5	12.16	8.16	9.9	15.83
PA	11.5	12	6.5	9.8	17.66
SA	9.31	12.83	7.5	14.33	18.66
SP	17	13.73	10.5	9	15.66
CA	8.16	10.1	7.06	10.33	29.67
CG	7.53	9.93	8.67	9.67	26.9

Hence it can be said that Gram-positive bacteria were better inhibited by the stem SNPs as compared to the Gram-negative bacteria, yet the antibacterial efficacy of the SSNPs was equally effective against both the Gram-negative as well as Gram-positive bacteria.

Antifungal Activity: Figure 8 and table 3 show the antifungal activity of stem SNPs against *C. albicans* and *C. glabrata*. The stem SNPs equally inhibited both the fungal strains. At higher concentrations of stem SNPs, CA was inhibited more whereas at lower concentrations, CG was effectively inhibited. Fluconazole was used as the positive control. Hence it is evident that the green synthesized stem SNPs showed better antibacterial activity as compared to the antifungal activity against the microbial strains chosen. Silver nanoparticles' ability to impede cell wall function has been linked to cytoplasmic leakage, cell death and subsequent cell wall attack[3].

Conclusion

Silver nanoparticles have been successfully synthesized using *M. indica* stem in a less expensive and environmentally friendly manner. Characterization of the synthesized silver nanoparticles was done using UV, FTIR, XRD, SEM and EDX. SNPs that are biosynthesized, are harmless and possess antifungal and antibacterial properties. At higher concentration, stem SNPS showed a good zone of inhibition against strains of both bacteria and fungi. These extracts acted as reducing agents, assisting in the production of nanoparticles and the reduction of metal ions. The natural bioactive substances involved in the reduction and stabilization process included sugars, alkaloids, phenols, flavonoids and others.

This green approach to SNP synthesis has several benefits for bulk production including 1) ease of use, low energy consumption, eco-friendliness and cost-effectiveness; 2) the absence of hazardous reagents and chemicals for reduction and processing and 3) the absence of the need for heating or sophisticated synthesis equipment. The powder XRD data on silver nanoparticles is compatible with standard ICDD cards, according to the researchers. The XRD pattern indicated that the sample consisted of crystalline face-centred cubic (FCC) lattice structures of elemental silver. UV-Visible spectroscopy research indicated that SNP has a distinctive absrption spectrum. The results are very encouraging and show how plant-based products can be used to create a number of therapeutically significant byproducts through the use of nanotechnology. In order to further explore the new frontiers of medical science for healthy living, it may be inferred that a variety of unknown plant components can be used for the creation of metal NPs.

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References

1. Ahmad B., Shireen F., Bashir S., Khan I. and Azam S., Green synthesis, characterization and biological evaluation of AgNPs using *Agave americana*, *Mentha spicata* and *Mangifera indica* aqueous leaves extract, *IET Nanobiotechnology*, **10(5)**, 281-287 **(2016)**

2. Al-Ruqeishi M.S., Mohiuddin T. and Al-Saadi L.K., Green synthesis of iron oxide nanorods from deciduous Omani mango tree leaves for heavy oil viscosity treatment, *Arabian Journal of Chemistry*, **12(8)**, 4084-4090 **(2019)**

3. Ameen F., Srinivasan P., Selvankumar T., Kamala-Kannan S., Al Nadhari S., Almansob A., Dawoud T. and Govarthanan M., Phytosynthesis of silver nanoparticles using *Mangifera indica* flower extract as bioreductant and their broad-spectrum antibacterial activity, *Bioorganic Chemistry*, **88**, 102970 **(2019)**

4. Aniqa and Rizvi Z.F., Physio-chemical analysis and antioxidative response of Ag NPs synthesized by sufaid chonsa mango pulp extracts of different ripening stages, *Scientific Reports*, **14(1)**, 28514 **(2024)**

5. Annavaram V., Posa V. R., Uppara V. G., Jorepalli S. and Somala A.R., Facile green synthesis of silver nanoparticles using *Limonia acidissima* leaf extract and its antibacterial activity, *Bionanoscience*, **5**, 97-103 **(2015)**

6. Benakashani F., Allafchian A.R. and Jalali, S.A.H., Biosynthesis of silver nanoparticles using *Capparis spinosa* L. leaf extract and their antibacterial activity, *Karbala International Journal of Modern Science*, **2(4)**, 251-258 **(2016)**

7. Bharathi V., Firdous J., Muhamad N. and Mona R., Green synthesis of *Mangifera indica* silver nanoparticles and its analysis using Fourier transform infrared and scanning electron

microscopy, *National Journal of Physiology, Pharmacy and Pharmacology*, **7(12)**, 1364-1367 **(2017)**

8. Das A.K., Marwal A. and Verma R., Preparation and characterization of nano-bio hybrid-based magneto liposome, *International Journal of Pharmaceutical Sciences and Research*, **6(1)**, 367-375 **(2015)**

9. Dubey S.P., Lahtinen M. and Sillanpää M., Tansy fruit mediated greener synthesis of silver and gold nanoparticles, *Process Biochemistry*, **45(7)**, 1065-1071 **(2010)**

10. Guan Q., Xia C. and Li W., Bio-friendly controllable synthesis of silver nanoparticles and their enhanced antibacterial property, *Catalysis Today*, **327**, 196-202 **(2019)**

11. Keshari A.K., Srivastava R., Singh P., Yadav V.B. and Nath G., Antioxidant and antibacterial activity of silver nanoparticles synthesized by *Cestrum nocturnum*, *Journal of Ayurveda and Integrative Medicine*, **11(1)**, 37-44 **(2020)**

12. Lee K.J. et al, Synthesis of silver nanoparticles using cow milk and their antifungal activity against phytopathogens, *Materials Letters*, **105**, 128-131 **(2013)**

13. Marwal A. and Gaur R.K., Nanophytovirology: an emerging field for disease management, Plant Diseases-Current Threats and Management Trends, *IntechOpen*, Chapter 2, doi: 10.5772/ intechopen.86653 **(2019)**

14. Okwu D.E. and Ezenagu V.I.T.U.S., Evaluation of the phytochemical composition of mango (*Mangifera Indica Linn*) stem bark and leaves, *Int J Chem Sci*, **6(2)**, 705-16 **(2008)**

15. Otunola G.A., Afolayan A.J., Ajayi E.O. and Odeyemi S.W., Characterization, antibacterial and antioxidant properties of silver nanoparticles synthesized from aqueous extracts of *Allium sativum*, *Zingiber officinale* and *Capsicum frutescens*, *Pharmacognosy Magazine*, **13(Suppl 2)**, S201 **(2017)**

16. Pasupuleti V.R. et al, Biogenic silver nanoparticles using *Rhinacanthus nasutus* leaf extract: synthesis, spectral analysis and antimicrobial studies, *International Journal of Nanomedicine*, **8**, 3355-3364 **(2013)**

17. Philip D., Rapid green synthesis of spherical gold nanoparticles using *Mangifera indica* leaf, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **77(4)**, 807-810 **(2010)**

18. Rajakumar G., Rahuman A.A., Roopan S.M., Chung I.M., Anbarasan K. and Karthikeyan V., Efficacy of larvicidal activity of green synthesized titanium dioxide nanoparticles using *Mangifera indica* extract against blood-feeding parasites, *Parasitology Research*, **114**, 571-581 **(2015)**

19. 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)**, 1-10 **(2019)**

20. Safa M.A.T. and Koohestani H., Green synthesis of silver nanoparticles with green tea extract from silver recycling of radiographic films, *Results in Engineering*, **21**, 101808 **(2024)**

21. Salati S., Doudi M. and Madani M., The Biological Synthesis of Silver Nanoparticles by Mango Plant Extract and Its Anti-*Candida* Effects, *Journal of Applied Biotechnology Reports*, **5(4)**, 157-161 **(2018)**

22. Shaikh J.R. and Patil M., Qualitative tests for preliminary phytochemical screening: An overview, *Int. J. Chem. Stud*., **8(2)**, 603-608 **(2020)**

23. Tailor S., Jain K., Marwal A., Meena M., Anbarasu K. and Gaur R.K., Outlooks of nanotechnology in organic farming management, *Def Life Sci J*, **7(1)**, 52-60 **(2022)**

24. Yang N., Wei Hong L. and Hao L., Biosynthesis of Au nanoparticles using agricultural waste mango peel extract and its in-vitro cytotoxic effect on two normal cells, *Materials Letters*, **134**, 67-70 **(2014)**.

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