

Evaluation of antimicrobial effect of green synthesized silver nanoparticles from *Sapindus mukorossi* pericarp extract

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Abstract

The present study is focused on the biosynthesis of silver nanoparticles derived from *Sapindus mukorossi* pericarp extract. Silver nanoparticles were synthesized successfully by employing biological methods of synthesis. In this research, silver nitrate was used as a precursor and *Sapindus mukorossi* pericarp extract was used as a reducing agent for synthesis. The obtained silver nanoparticles were characterized by using UV-Vis spectroscopy, X-ray diffraction (XRD), Fourier transform electron microscopy (FTIR) and High-resolution transmission electron microscopy (HR-TEM). The UV-Vis spectra and visual observation showed that the color of pericarp extract of *Sapindus mukorossi* turned from golden yellow to dark brown after the addition of AgNO_3 precursor and showed the highest absorption peak at 410 nm. In addition, XRD pattern revealed the face-centered cubic structure of silver nanoparticles. The FTIR measurements confirmed the presence of different functional groups within the extract that were directly involved in the reduction and stability of biosynthesized silver nanoparticles. HR-TEM images revealed the particles to be nearly spherical with a few irregular shapes and particles size ranging from 5 to 50 nm. The study highlights the antimicrobial activity of silver nanoparticles that were tested against gram negative bacterium *viz.*, *Pseudomonas aeruginosa*, *Escherichia coli* and gram-positive bacterium *Staphylococcus aureus*, *Bacillus subtilis*. The results confirmed that the prepared silver nanoparticles showed good antimicrobial potential against all the tested microorganisms. The antioxidant potential of aqueous extract and biosynthesized silver nanoparticles was evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl) method and revealed that both aqueous extract and obtained silver nanoparticles exhibit maximum antioxidant activity.

1. Introduction

The term "silver nanoparticles" describes nanoparticles of silver with ranging from 1 nm to 100 nm in size. Out of all the metallic nanoparticles employed in biomedical applications, silver nanoparticles are the most significant and intriguing nanomaterials. Physical, chemical, and biological methods can be used to prepare silver nanoparticles [1]. Silver exhibits strong antimicrobial activity against numerous microorganisms like viruses, bacteria, and fungi [2]. The large surface area and small size of silver nanoparticles led to have significant importance in the antimicrobial research field [3].

In comparison to other methods like physical and chemical, the biological approach to creating nanoparticles is gaining a lot more attention since plant-mediated metal synthesis is less hazardous, eco-friendly, economical, and less time-consuming [4]. The hazardous compounds that demonstrate toxicity are dealt with by the chemical and physical processes used for nanoparticle manufacturing. Nanoparticles of distinct metals including gold, silver, magnesium, copper, zinc, titanium, and platinum have attracted a lot of attention in the biomedical sector due to their exceptional diagnostic properties. Silver nanoparticles among these metals show high antibacterial effectiveness [5]. In several industries, including the textile industry, engineering, and cosmetics, nanoparticle production has grown in popularity. Silver and silver-based compounds have been utilized for a long time as non-hazardous, inorganic, and antibacterial treatments in applications including wood preservation and hospital water

filtration due to their biocidal properties [6]. *Klebsiella planticola*, *Bacillus sp.*, *Pseudomonas sp.*, *S. aureus*, *Escherichia coli*, *Candida albicans*, and *Fusarium oxysporum* are a few pathogenic bacteria that are destroyed by silver nanoparticles [7].

Sapindus mukorossi is a significant medicinal plant from the Sapindaceae family, is also known as ritha, dodan, soapberry, and soapnut [8]. The current study investigates the manufacturing of silver nanoparticles utilizing an antibacterial *S. mukorossi* pericarp extract. The plant demonstrates several medicinal properties, including anti-inflammatory, anti-insect, antibacterial, anti-diabetic, anti-cancer, and anti-cardiovascular actions [9]. This plant has been used to create biologically produced medically significant silver nanoparticles due to its medicinal and pharmacological effects [10].

In the present research the biological method was adopted because of its versatile eco-friendly and non-toxic properties. Therefore, research was undertaken to synthesize and characterize the stable silver nanoparticles followed by assessing its antioxidant activity from DPPH method and antimicrobial potential against four distinct pathogens.

2. Material and Method

2.1. Materials

The fruits of *S. mukorossi* were collected from the local village Sheglagalu, Chail Chowk, Distt. Mandi Himachal Pradesh. Deionized water served as a solvent. Silver nitrate (AgNO_3 , 99.99%) was used as a precursor for the preparation of silver nanoparticles. *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* were used in the current research as a test organism.

2.2. Preparation of plant extract

The fruit of around 30 g of *S. mukorossi* were carefully washed with deionized water and were dried in the shade for 4–5 days. After drying the shells of fruit (pericarp) were separated from the seeds. To get the fine powder of the pericarp, the dried pericarp was pulverized using pestle and mortar. The powder was then kept in an airtight container to avoid any chance of contamination. Obtained powder weighed about 10 g was mixed with 100 ml of distilled water followed by little shaking so that the powder would mix properly. To filter the obtained extract the Whatman grade No. 1 filter paper was used. After filtration the golden yellow color of the SME was visible, and the extract was stored in refrigerator at 4 °C for further use [11].

2.3. Silver nanoparticles synthesis

The preparation of silver nanoparticles was carried out by using 1 mM silver nitrate solution. 20 ml of SME was added into 80 ml of silver nitrate solution. The mixture was then kept for 2–3 hours in dark conditions at room temperature. The color changed from golden yellow to dark brown indicates the formation of silver nanoparticles. The extract was then centrifuged at 12000 rpm for 30 minutes at 4 °C. The process was repeated twice, and the supernatant was then discarded, the obtained pellets were

washed with the help of ethanol and air dried in hot air oven at 62 °C for 30 minutes [12]. The obtained dried pellets were then stored in Eppendorf tube at 4 °C temperature for further studies (Fig. 1).

2.4 Methods used for characterization of aqueous extract and silver nanoparticles of *Sapindus mukorossi* pericarp

The prepared silver nanoparticles were characterized by employing different techniques like X-ray Diffraction, UV-Vis Spectroscopy, Fourier Transform Infrared (FTIR) Spectrometer, Transmission Electron Microscopy (TEM). X-ray diffraction technique was used in the present research to identify the crystallographic nature of silver nanoparticles that are synthesized using *S. mukorossi* aqueous extract and silver nitrate solution. Using a UV-Vis spectrophotometer, the optical properties of prepared silver nanoparticles were examined. For this, an absorption measurement of a solution of silver nanoparticles in the 300–700 nm range was performed using a UV–Vis spectrophotometer. FTIR analysis was used to identify the presence of functional groups within SME and silver nanoparticles that are responsible for the reduction of Ag^+ to Ag^0 [13]. The obtained silver nanoparticles were characterized with a frequency ranging from $4000 - 400 \text{ cm}^{-1}$ using KBr pellet method. The powder form of silver nanoparticles was added with KBr and then the pellet of the mixture was created under hydraulic pressure. The pellet was then placed into the sample holder and then kept under FTIR spectroscopy to get the results. To examine the size and morphology of silver nanoparticles, High Resolution-Transmission Electron Microscopy (HR-TEM) analysis was carried out.

2.5 Antimicrobial potential of biosynthesized silver nanoparticles

Agar well diffusion technique was used to determine the antimicrobial activity of obtained silver nanoparticles at different concentrations and was checked against the gram-positive bacteria *Bacillus subtilis*, *Staphylococcus aureus* and gram-negative bacterium *Escherichia coli*, and *Pseudomonas aeruginosa* based on the guidelines of Clinical and Laboratory Standards Institute [14]. For this, 50 ml of nutrient broth containing 100 μl of the tested bacteria was incubated at 37 °C. After 18 hours of incubation, the microbial suspension was dispersed onto Muller Hinton agar plates. Four wells were then punched, and solutions containing silver nanoparticles at various concentrations were added to the wells in separate batches. Distilled water served as the negative control. The plates were then kept at 37 °C for a further 24 hours. After the incubation time, the diameter of the zone of inhibition was determined to test the antimicrobial potential of silver nanoparticles.

2.6 Antioxidant activity of aqueous extract and biosynthesized silver nanoparticles of *S. mukorossi* from DPPH assay.

To evaluate the antioxidant potential of the *S. mukorossi* crude aqueous extract and biosynthesized silver nanoparticles the DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radical assay was adopted. For this 0.004 gm DPPH was mixed in 100 ml of methanol [15]. Aqueous solution of each sample with distinct

concentrations from 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 µg/ml were prepared to evaluate the antioxidant potential. For this the prepared sample solution was dissolved with 3 ml of DPPH solution followed by periodic stirring and kept under dark condition for 15 minutes. DPPH solution was used as a control. The absorbance was recorded at 517 nm using a UV-vis spectrometer (Eppendorf Bio spectrometer). The antioxidant activity was expressed as the percentage of inhibition, which was determined using the following formula

$$\% \text{ of free radical scavenging activity} = \frac{Ac - As}{Ac} \times 100$$

where, *Ac* is the absorbance of control and *As* is the absorption of experimental samples.

3. Results and discussion

3.1 UV-Vis Spectroscopy

To evaluate the formation and stability of prepared silver nanoparticles UV-vis spectroscopy has been carried out. Different aliquots of silver nitrate solution were prepared with the help of deionized water and were analyzed under UV-Vis spectroscopy [16, 17]. The crude extract of *S. mukorossi* aqueous extract shows an intense peak of absorption at 386 nm and after addition of silver nitrate into *S. mukorossi* extract the color changed from golden yellow to dark brown and the solution shows strong peak at 410 nm on the UV-vis spectra illustrated in (Fig. 2) The optical properties of the biosynthesized silver nanoparticles were determined based on these absorption peaks which reveals that the silver nanoparticles are successfully formed and become stable after 24 h of reaction time [18].

3.2 X-ray diffraction

To determine the crystalline and structural nature of synthesized silver nanoparticles XRD analysis was carried out. The powdered sample of silver nanoparticles was placed under XRD. The X-ray diffraction (XRD) pattern of the obtained silver nanoparticle shows different peaks at $2\theta = 32.40^\circ$ (111), 42.24° (200), 63.07° (220), and 73.83° (311) which were in good agreement with the XRD spectra of pure crystalline silver structures that have already been published by the Joint Committee on Powder Diffraction Standards (file no. 04-0783). Therefore, the crystalline structure of the biosynthesized silver nanoparticles and the fcc pattern are respectively reflected based on these planes (Fig. 3). The unassigned peaks might be caused by the crystallization of bioorganic phase on the surface of silver nanoparticles [19].

3.3 FTIR

FTIR analysis was conducted to identify the presence of different functional groups within the *S. mukorossi* aqueous extract as well as in prepared nanoparticles that are responsible for reduction of Ag^+

to Ag^0 . The FTIR spectrum of plant extract that is SME shows broad band at 3408.40 cm^{-1} assigned to O-H stretching vibration that is expected because of flavanols presence. Two peaks at 2925.20 cm^{-1} and 2959.65 cm^{-1} were observed which are stretching frequencies of C-H group. The peaks at 1578.80 cm^{-1} and 1420.37 cm^{-1} are assigned to the stretching of C = O and C-O group. The peak at 1045.12 cm^{-1} is due to the presence of C-O-C bending. The presence of weak bond that is aromatic (-CH) has been observed at the peak of 924 cm^{-1} . The observed peaks of *S. mukorossi* extract confirmed the presence of flavonoids, saponins and oligosaccharides within *S. mukorossi* fruit extract that may serve as stabilizing and reducing agent. The synthesized silver nanoparticles show the absorption peaks at 3435.20 cm^{-1} , 2934.80 cm^{-1} , 1638.91 cm^{-1} , 1574.95 cm^{-1} , 1416.12 cm^{-1} , 1279.00 cm^{-1} , 1180.64 cm^{-1} , 1046.03 cm^{-1} and 1019.85 cm^{-1} . The peak at 3435.20 cm^{-1} represents the O-H stretching 2934.80 cm^{-1} represents the C-H group. The absorption peaks situated at 1638.91 cm^{-1} and 1574.95 cm^{-1} correspond to bonding vibrations of C = O of amide group and aromatic compounds C = C respectively, represents the involvement of flavonoids and saponins in the reduction process, while peak at 1416.12 cm^{-1} and 1279.00 cm^{-1} attributable to the C-F stretching vibration. The decrease in the peak at 1180.64 cm^{-1} , 1046.03 cm^{-1} and 1019.85 cm^{-1} was attributed to the formation of silver-carboxylate bonds interacting with hydrocarbon chains by means of the hydrophobic forces (Fig. 4). Thus, the functional groups present over the surface of silver nanoparticles plays an important role in its stabilization [20].

3.4 HR-TEM (High Resolution Transmission Electron Microscopy)

The HR-TEM analysis was performed by preparing the aliquots of silver nanoparticle solution. The solution was deposited on a carbon-coated copper grid and allowed to dry at room temperature before TEM images were taken at different resolutions [21]. The clearly observed microscopic views of the nanoparticles were documented in different ranges of magnifications. The images of TEM were recorded and the data show that the nanoparticles are small and homogeneous, mostly spherical followed by rod, cylindrical and oval in shape. The particles ranged in size from 5 to 50 nm. This implies that the synthesis technique employed resulted in nanoparticles with a stable and restricted size distribution [22]. These results contribute to a better understanding of the physical properties and potential applications of synthesized nanoparticles (Fig. 5).

3.5 Antimicrobial potential

The antimicrobial potential of biosynthesized silver nanoparticles solution was checked against four microorganisms namely *P. aeruginosa*, *E. coli* and *B. subtilis*, *S. aureus* at distinct concentrations of prepared silver nanoparticles (60 μl , 80 μl and 100 μl) shown in (Plate 1). The presence of a defined inhibitory zone around the wells indicates that the synthesized AgNPs have antimicrobial action. The greater inhibition zone was seen against gram-negative bacteria *P. aeruginosa* and *E. coli* 18.46 ± 0.26 and 17.66 ± 0.12 respectively, at highest concentration of AgNPs that is 100 μl when compared to gram-positive bacteria *B. subtilis*, *S. aureus* showed inhibition zones of 15.01 ± 0.05 , 17.03 ± 0.05 respectively, which may be attributed to the difference in cell wall composition of gram-positive and gram-negative

bacteria [23]. Gram-positive bacteria have a stiffer and thicker peptidoglycan layer than gram-negative bacteria, which gives the cell wall structure more strength and thus better resistance (Table 1). The results of this work show that synthesized silver nanoparticles have the potential to be powerful antibacterial agents, especially against gram-negative bacteria like *P. aeruginosa* and *E. coli*. Applications in medical and healthcare contexts such as wound care, infection control, and medical device coatings are all affected by its antimicrobial effect [24]. Optimizing nanoparticle concentrations, comprehending the mechanisms of action, and investigating their potential for broader antibacterial and therapeutic uses are all possible areas for further study.

Table 1 : Antimicrobial potential of biosynthesized silver nanoparticles derived from *Sapindus mukorossi* aqueous extract against tested organisms

S. No.	Concentration of AgNPs	Zone of inhibition (mm)			
		<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>
1	Control	0	0	0	0
2	60 µl	16.26±0.14	16.23±0.41	11.83±0.16	14.83±0.44
3	80 µl	17.02±0.153	17.01±0.058	14.00±0.289	16.36±0.18
4	100 µl	18.46±0.26	17.66±0.12	15.01±0.05	17.03±0.05

Values are average of triplicates± Standard Deviation

3.6 Antioxidant activity of aqueous extract and silver nanoparticles of *Sapindus mukorossi*

The DPPH assay adopted for assessment of antioxidant activity of aqueous extract and prepared silver nanoparticles revealed that the aqueous extract and biosynthesized silver nanoparticles of *S. mukorossi* exhibited excellent antioxidant properties, where the maximum free radical scavenging rate of *S. mukorossi* extract and silver nanoparticles were found to be 91% and 88%, respectively at 1000 µg/ml of concentration due to the presence of different phytochemicals including flavonoids, polyphenols and saponin which act against free radical. Antioxidant potential of such plant extract and their derived silver nanoparticles can be used for many free radicals scavenging applications [25].

4. Conclusion

Current research investigated the preparation of silver nanoparticles by utilizing the pericarp extract of *Sapindus mukorossi*, a medicinal plant known for its wide range of therapeutic properties. The biological technique was chosen since it is non-toxic and eco-friendly, making it a better way than the risky chemical-based approaches of physical and chemical processes. We used various methods to characterize the synthesized silver nanoparticles. Their crystal structure was validated by X-ray

diffraction analysis, and UV-Vis spectroscopy revealed that silver nanoparticles with a major absorption peak at 410 nm were successfully formed. FTIR examination confirmed the presence of functional groups in both the plant extract and the nanoparticles, indicating their role in silver ion reduction. Furthermore, the aqueous extract-derived silver nanoparticles displayed excellent antimicrobial activities, notably against *P. aeruginosa*, *E. coli*, *B. subtilis* and *S. aureus*. The maximum antioxidant activity exhibited by aqueous extract and biosynthesized silver nanoparticles was found to be 91% and 88%, respectively by using DPPH assay. These findings show the possibility of employing *S. mukorossi* pericarp extract as a natural and sustainable source of preparing silver nanoparticles with increased antimicrobial and antioxidant activity. Such nanoparticles offer potential for future biological applications and help to produce environmentally friendly healthcare solutions.

Declarations

Conflict of interest: None

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Data availability: The authors declare that the data supporting the findings of this study are available in the paper. If any other format of data files is required, they are available from the corresponding author upon reasonable request.

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Plate

Plate 1 is available in the Supplementary Files section.

Figures

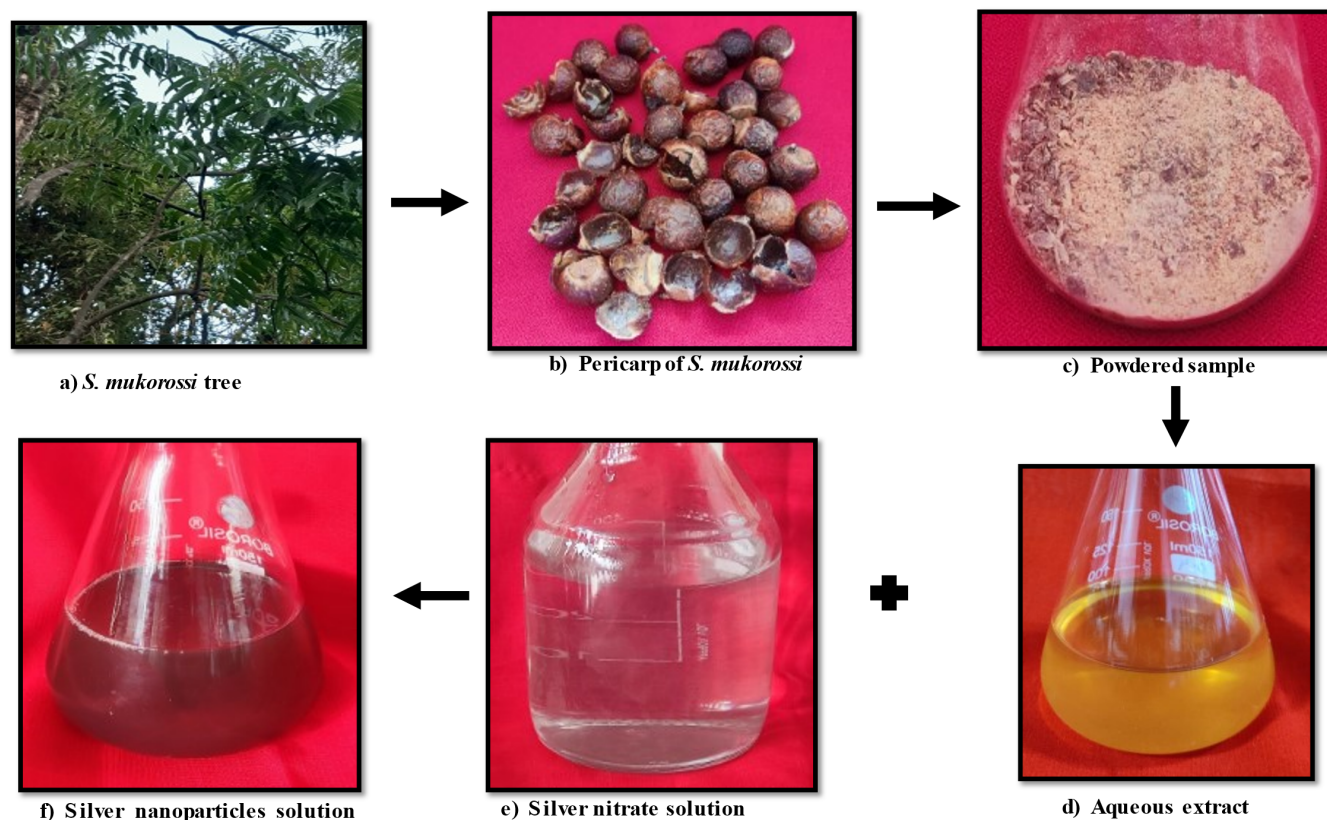


Figure 1

Figure depicting biosynthesis of silver nanoparticles from aqueous extract of *S. mukorossi* pericarp

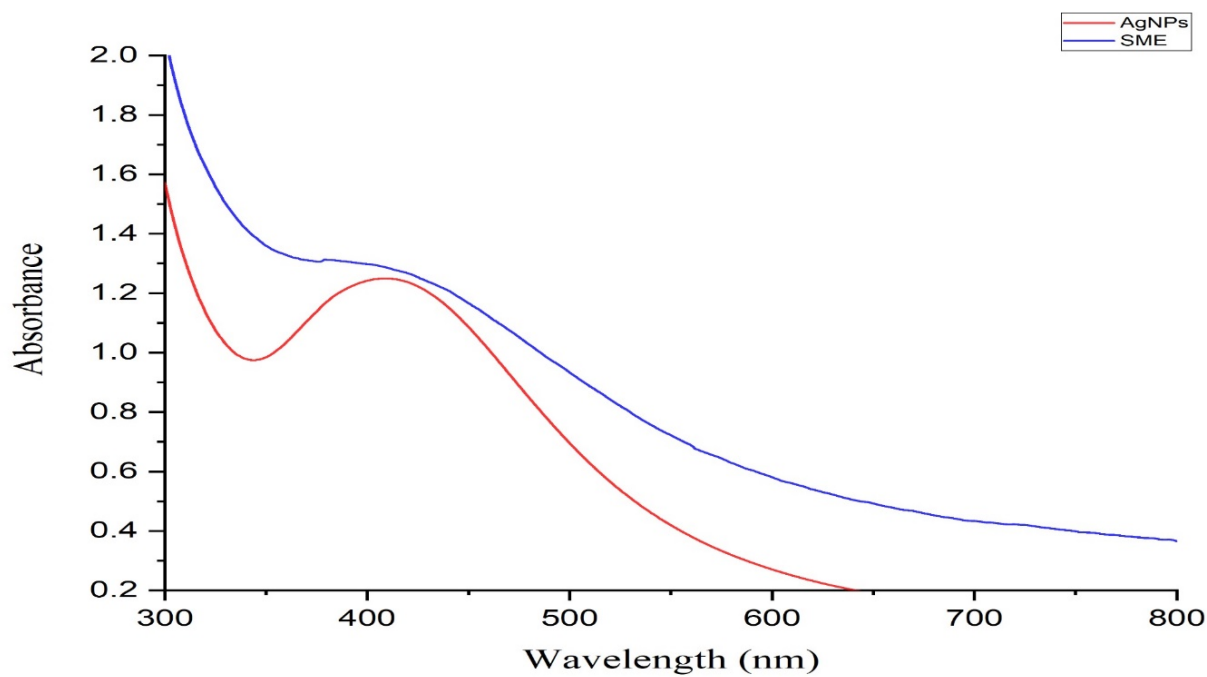


Figure 2

UV- Vis spectrum of *S. mukorossi* aqueous extract and prepared silver nanoparticles using *S. mukorossi* aqueous extract

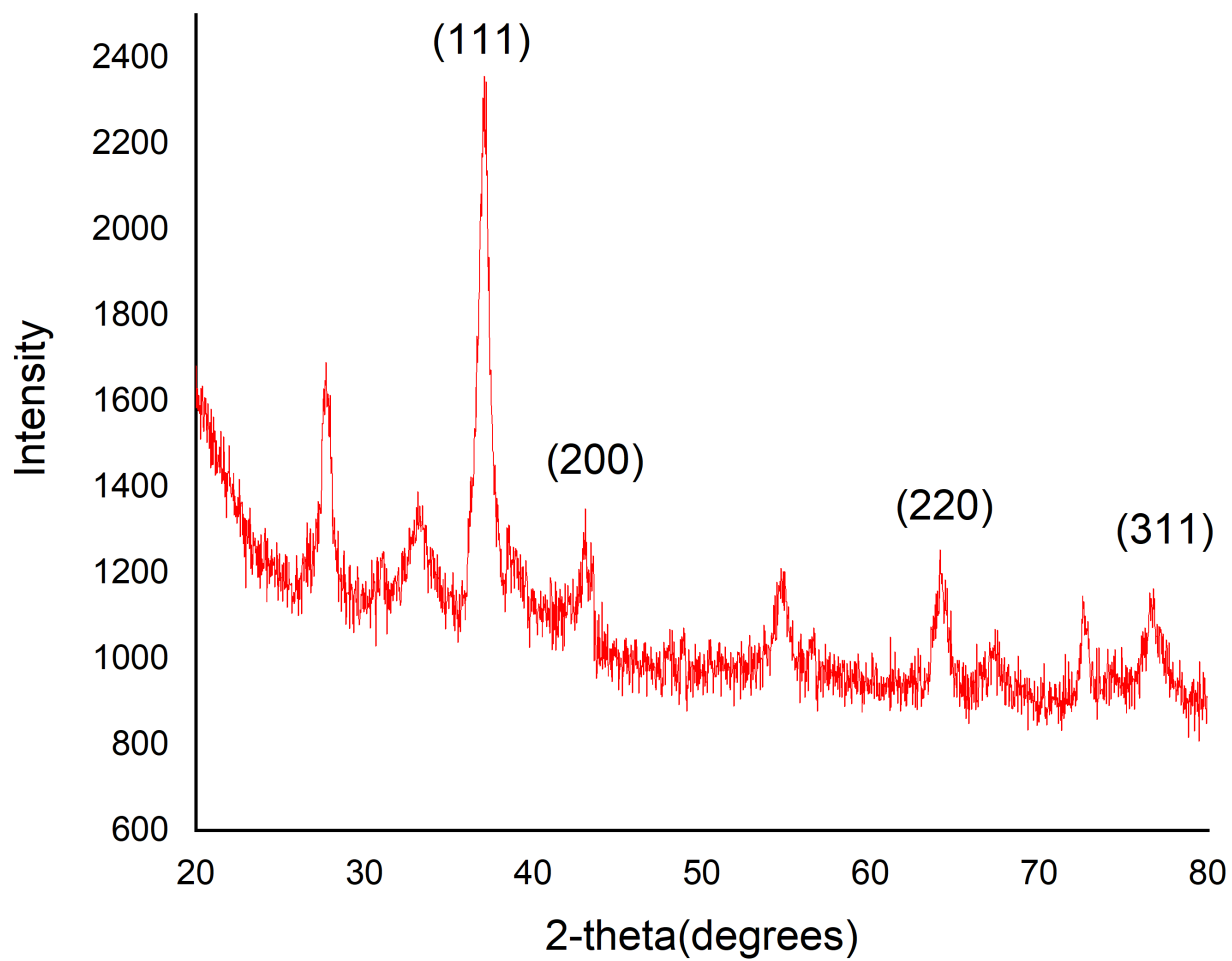


Figure 3

XRD pattern of biosynthesized silver nanoparticles from *S. mukorossi* aqueous extract

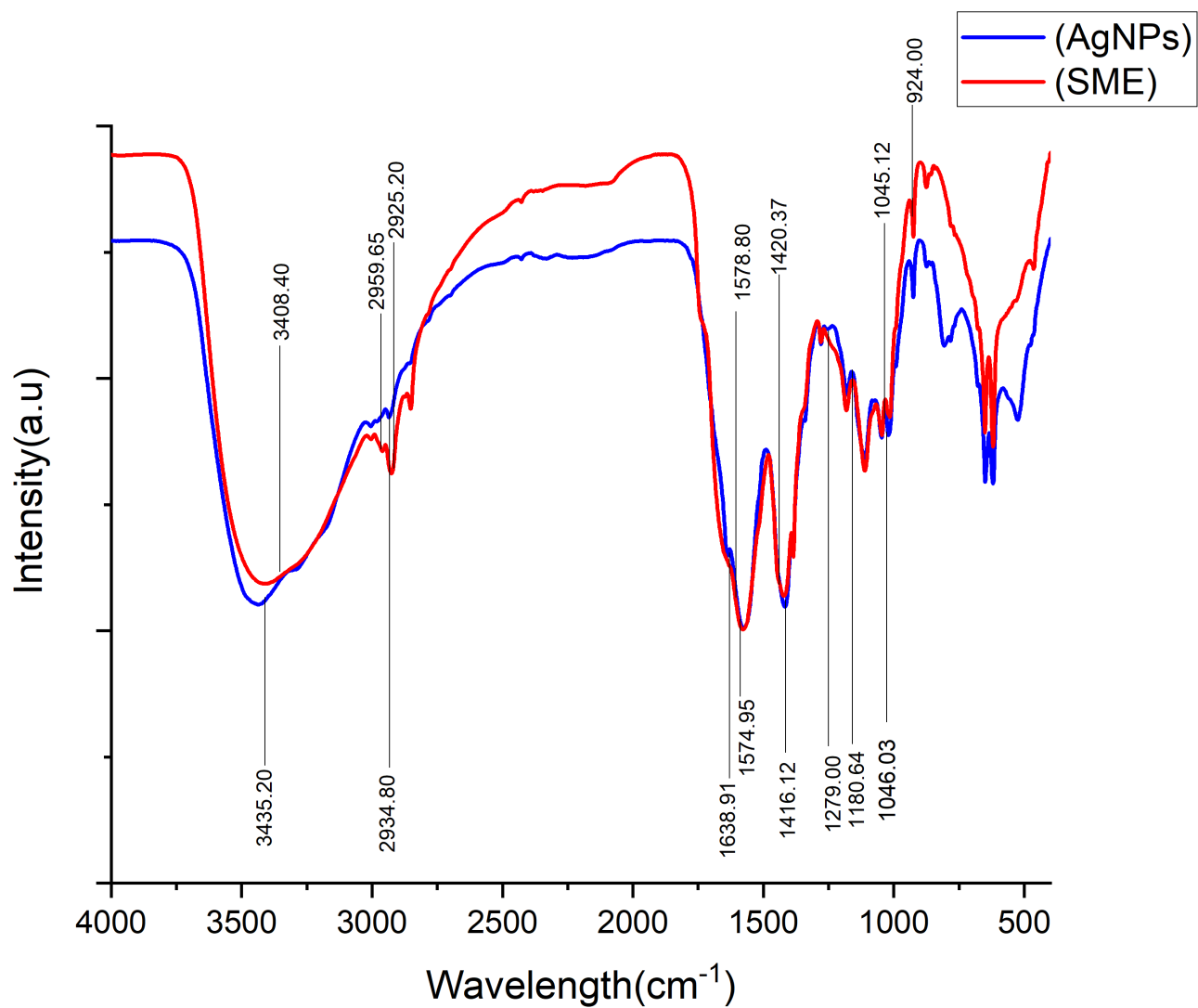


Figure 4

FTIR spectrum of *S. mukorossi* aqueous extract and prepared silver nanoparticles using *S. mukorossi* aqueous extract

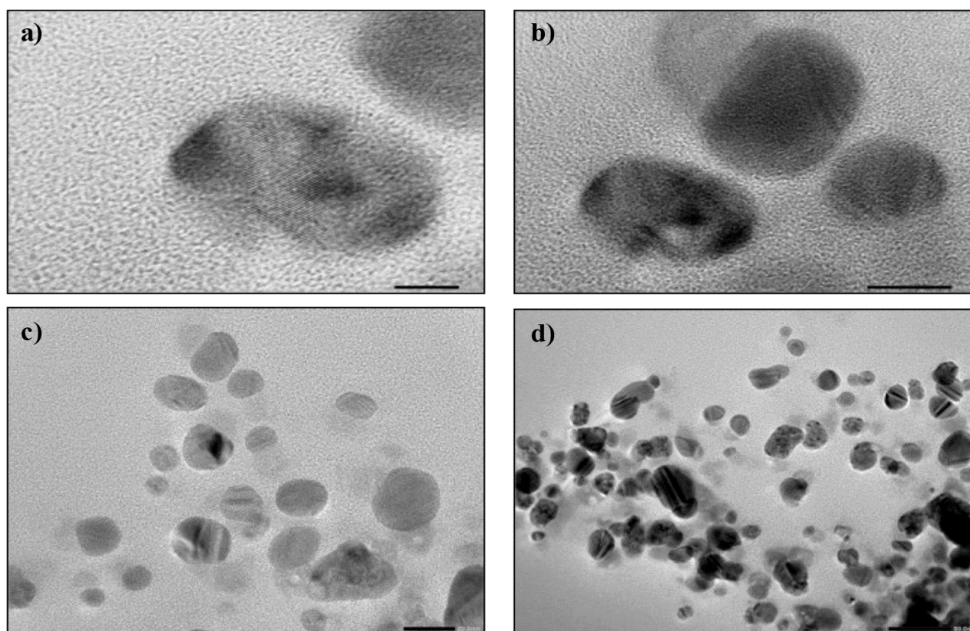


Figure 5

HR-TEM micrographs of prepared silver nanoparticles depicting shape and size at different magnifications- a: 5 nm, b: 10 nm c: 20 nm, d: 50 nm

Supplementary Files

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