Green Synthesis of Silver Nanoparticles (AgNPs), Structural Characterization, and their Antibacterial Potential

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Abstract

Background: In the field of nanotechnology, the metallic nanoparticles are of remarkable interest because of their unique electronic, magnetic, chemical, and mechanical properties.

Purpose: In the present work, silver nanoparticles (AgNPs) were synthesized using bio-reduction method.

Research Design: Silver nitrate was used as metallic precursor and the extract of *Moringa oleifera* leaves with different concentrations was used as reducing as well capping agent. The extract exhibited strong potential in rapid reduction of silver ions for the synthesis of silver nanoparticles. The synthesized silver nanoparticles were characterized by UV-visible spectroscopy, X-ray diffraction (XRD), and scanning electron microscopy (SEM) techniques.

Results: The absorption SPR peaks appeared in the range of 415 to 439 nm. SEM analysis exhibited that particles were spherical in shape with size distribution range from 10 nm to 25 nm. The synthesized silver nanoparticles were pure crystalline in nature as confirmed by the XRD spectra with average crystallite size 7 nm. *In vitro* antibacterial activity of the prepared silver nanoparticles colloidal samples as well the extract was studied using different concentrations of AgNPs (C1 = 100 μg/ml, C2 = 50 μg/ml, C3 = 25 μg/ml) by well diffusion method against Gram negative *Escherichia coli*. The antibacterial performance was assessed by measuring the zone of inhibition (ZOI).

Conclusions: The results suggested that AgNPs prepared by green approach can be considered as an alternative antibacterial agent.

Keywords

Moringa oleifera, AgNPs, antibacterial agent, *Escherichia coli*, green synthesis

Introduction

Nano-science deals with the materials manipulation and miracles at nanometer scale where materials properties behave significantly different from bulk material.[1] Nanotechnology has wide area of research, development, and industrial activity which has grown very quickly all over the world from the last decade.[2] The integration of biotechnology and nanotechnology has come up as bio-nanotechnology to grow eco-friendly and biosynthetic technology to synthesize nanomaterial. The assembly of atoms with size ranging 1–100 nm is known as nanoparticles. Metallic nanoparticles are extensively studied nowadays as they possess unique electrical, chemical, optical,

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catalytic properties and show good antibacterial activity due their large surface area to volume ratio.[3] Different kinds of nano-material like alginate[4] gold, magnesium[5] titanium, copper, zinc,[6] and silver are found. Sliver nanoparticles are reported to have strong antimicrobial activity among the most commonly used nanoparticles.[7]

Nanotechnology is one of the major applications of nano-science. Nano scale material fabrication provides the basis of integration and control on molecules as well as atoms. For instance, DNA silicon chips are wonderful application of nanotechnology. Nanotechnology can be applied in almost every field like in military defense for missile system, med-icine, astronomy, and computing.[8] There are number of latest devices and materials such as eyeliner, suntan lotion, sterile socks, and self-cleaning glass have been synthesized and fabricated with the help of nanotechnology. The injection of drug inside the human body with greater precision and ac-curacy is very strong application of the nanotechnology. According to scientists, nanofood will be effective for our stomach and prevents us from over eating.[9]

Different methods like electrochemical reduction, photo-chemical reduction, heat evaporation and biological are used for the synthesis of silver nanoparticles. As reported in literature, these methods are very expensive and also involve in the use of hazardous chemicals which may cause the biological and en-vironmental risks. Currently, silver nanoparticles synthesis from plants extract is receiving a greater attention because of environment friendly behavior.[10] The plants extract works as reducing agent as well as capping agent in synthesis of nanoparticles. Different plants extract such as *M. oleifera* (Horseradish), *A. indica* (Neeem), *T. foenum-graecum* (Fenu-greek) and *C. colocynthis* (Bitter Apple) etc. have been suc-cessfully used for the synthesis of metal nanoparticles.[11]

Recently, it has become a need of time to develop new bactericidal materials to resist the bacterial strains because antibiotics are not sufficient to overcome this. To handle these problems, it is very essential to fabricate these agents. Many antimicrobial agents have been created with the help of the nanotechnology although; the size of antitoxin is less than any other nanoparticle. The particles size range is from 5 to 10 nm and some particles size is in the range from 1 to 10 nm. Nanotechnology reduces and gives a compact shape which helps to increase the surface to volume ratio. Mi-croscopic scale is 50 to 5000 times greater than the nano-meter scale.[12] Metal nanoparticles have been shown their tremendous applications as antimicrobial agents and also play a vital role against various types of pathogenic viruses. So, nanomaterial provides a plate form for the invention of these antimicrobial reagents. Antimicrobial nanoparticles kill the bacteria by damaging cell membrane and in this way act as 1 of most important weapon against antibiotics resistant bacteria.[13]

Different green synthesis methods such as polyoxometalates, pollens, polysaccharide, and irradiations are used for the syn-thesis of silver nanoparticles. Green synthesis defined as the practice of biologically friendly elements like plants, bacteria

and fungi for nanoparticles synthesis. These striking green approaches are free of the short falls as per conventional syn-thetic approaches.[14,15] One of them is the bio-organism in which plant extract is being used. It is environment friendly, having no toxicity effects as compared to chemical reduction methods. The plants extract works as reducing agent as well as capping agents making steady and exact shape of the AgNPs. Bio molecules in the plant extract such as amino acids, vitamins, enzymes, and proteins work to reduce the concentration of silver ions Ag^+. Different plant extracts are used for this purpose to reduce the concentration of silver ions for instance moringa, bitter apple, fenugreek, neem, and many more.[16,17]

Food borne diseases are a major health issue in all over the world. According to WHO (2014) about 30% of the residents in industrial countries are infected by foodborne illness each year. The main source of these food borne diseases is the intake of contaminated food infected by bacteria, virus and fungal toxins in human. The food can be contaminated during pre or post harvesting due to minimal processing, trans-portation, handling procedures and during preparation. Commonly, foodborne pathogens that are found in different foods are *Escherichia coli*, *Salmonella sp.*, *Campylobacter sp.*, *Listeria sp*. and *Clostridia sp*.[18]

The prevalence of infectious diseases has increased globally as the excessive and misuse of drugs result antibiotic resistance to the pathogens known as multidrug resistant pathogens.[19] These multidrug resistant pathogens lead to numerous health issues and economic damage. The multidrug resistant pathogens may cause cross contamination of foodstuffs during transport of food. Therefore, new and natural antibacterial agents are required to condemn these multidrug resistant food-borne pathogens.[20]

M. oleifera is 1 of the magic plants that it preferably used as a medicine in traditional system. Because of its therapeutic properties, it has been used for many years. It is mostly reported as an antibacterial, antipyretic, antifungal, antiepileptic, anti-hypertensive, antiulcer, antitumor, diuretic, anti-inflammatory, antispasmodic, antidiabetic, and antioxidant. Different parts of *M. oleifera* such as leaves, seeds, peels, flowers and barks can be used for medicinal applications. Especially flowers are a rich source of phenolic acid, methionine, flavonoid, cysteine, an-tioxidant vitamin, tocopherol, potassium, calcium, β-carotene, and several different proteins.[21-23] In the present study, *M. oleifera* leave extract was used for the synthesis of AgNPs by reducing the silver ions present in the silver nitrate. The main purpose of current study was the synthesis, optimization, characterization, and evaluation of antibacterial potential of these silver nanoparticles against *E coli*.

Materials and Methods

Sample Collection

The *M. oleifera* leaves were collected from the botanical garden of university of the Punjab, Lahore, Pakistan and authenticated from the research center of the corresponding

university. The leaves were washed with tap water four to five times before detached from the shoots. Then leaves were shade dried and fine powder was made using grinder. Silver nitrate ($AgNO_3$) was obtained from chemistry department, University of the Punjab, Lahore, Pakistan.

M. oleifera Leaf Extract Preparation

For extract preparation 10 g fine powder of *M. oleifera* leaves was added into 100 mL deionized water. Then it was heated at 100°C for 20 to 30 minutes. The obtain extract was filtered using filter paper (Whatman no. 1) and stored at 4°C for further use.

Precursor Preparation

Silver nitrate ($AgNO_3$) was used as precursor for the synthesis of silver nanoparticles from the *M. oleifera* leaves extract. 1 mM solution of silver nitrate was prepared using double distilled water and stored at 4°C in refrigerator.

Biosynthesis of Silver Nanoparticles

The green synthesis method was followed for the synthesis of silver nanoparticles. For the reduction of Ag^+ ions, 1 mL *M. oleifera* leaves extract was added drop wise into 100 mL of 1 mM aqueous solution of $AgNO_3$ and heated at 60–80°C for 1 hour. The change in color was observed from dark brown to reddish brown which indicated the formation of silver nanoparticles. Similarly, .5, 1, 2 and 2.5 mL of *M oleifera* extract was suspended for the preparation of S1, S2, S3, and S4 respectively (Table 1).

Substrate Cleaning and Sample Preparation

The glass slides were washed with double tap water using detergent. The slides were washed with acetone and IPA for 15 minutes repeatedly and dried with nitrogen gun. After cleaning the glass slides were placed on petri dishes and AgNPs solution was put on the glass slides drop by drop.

Characterization of Silver Nanoparticles

UV-VIS Absorption Spectroscopy. UV-Vis spectroscopy (Shimadzu, UV-1800, Japan) was used to measure the absorption in ultraviolet and visible range of electromagnetic spectrum. It can also confirm the quality of material. The formation of leaves extract mediated silver nanoparticles was confirmed by the spectral analysis. The UV-VIS spectra of the synthesized

silver nanoparticles were recorded using Spectrometer by continuous scanning from 200 nm to 800 nm and for baseline correction; the distilled water was used as reference.[24]

Scanning Electron Microscopy (SEM)

Scanning Electron Microscope (FEI Nova NanoSEM 450, USA) was used for the morphological analysis. The shape, size, and crystalline nature of silver nanoparticles were confirmed by the SEM.[25]

X-Ray Diffraction Analysis

For structural analysis of the synthesized silver nanoparticles X-ray powder diffractometer (D-max IIA, Rigaku, Japan) was used. The data were collected in 2θ range. The crystalline domain size was calculated using *D. Scherrer*'s equation ($D = K\lambda/\beta COS\theta$.

Where, D = average crystalline domain size, β denotes Full Width Half Maximum (FWHM);

K = .94, λ = wavelength of X-ray (1.54 A^0) and θ is the Brag's angle).[26-28]

Antibacterial Analysis. Antibacterial activity was evaluated by the method of Jain and Mehata.[29] The bacterial culture of Gram negative *E coli* strain was equipped by the standard procedures.

Growth of Bacteria

The *E coli* strain was grown in nutrient broth medium supplemented with 1 mM concentration of MLE-AgNPs, incubated at 30°C (constant pH 8.0) for 72 hours at 150 r/min to give aerobic conditions to the strain. After that interval the broth was centrifuged at 10,000 r/min for 10 minutes. OD was measured at different time intervals (6, 12, 18, 24, 30, 36, 42, 48, 54, 60 and 72 hours). Growth curves of *E coli* exposed and unexposed to MLE-AgNPs were evaluated by the absorbing value of OD_{600}.

Well Diffusion Method

M.oleifera leaf extract and three different concentrations of silver nanoparticles were sterilized by exposure to ultraviolet radiations for 1 hour to eliminate any undesirable bacterial contamination. After sterilization of petri dishes, agar was poured into the petri plates. After solidification bacterial culture was evenly

Table 1. The detailed summary of the experimental processes for the synthesis of silver nanoparticles using the green synthesis method.

Sample (AgNPs)	Precursor $AgNO_3$ (1 mM)	Reducing agent (MLE)	R, Mlatio (Precursor/Reducing agent)	Reaction Time	Stirring	Temperature
S$_1$	50 mL	0.5	1/100	60 mint	Constant	60–80°C
S$_2$	50 mL	01	1/50	60 mint	Constant	60–80°C
S$_3$	50 mL	02	1/25	60 mint	Constant	60–80°C
S$_4$	50 mL	2.5	1/20	30 mint	Constant	60–80°C

spread on nutrient agar plates with L-rod. The media was punched with 8 mm diameter holes and 20 μL aqueous samples of water, plant extract and three different concentrations of AgNPs were added in these wells by using micropipette. After 24 hrs of incubation at 37°C, the plates were observed for bacterial growth.

Determination of Minimum Inhibitory Concentration (MIC) of MLE-AgNPs

Pure bacterial culture was turbid in broth, to perceive the minimum inhibitory concentration of AgNPs at which the turbidity was totally eradicated and a clear solution was found.[30] The minimum inhibitory concentration (MIC) of MLE-AgNPs against *E coli* was evaluated by exposing the bacterium with MLE-AgNPs, with different volumes of growth medium. *E coli* cells were supplemented individually to 10 mL broth culture in 20 mL volume conical flasks. The final concentration of MLE-AgNPs was .5, 1.0, 1.5, 2, and 2.5 mM and concentration of *E coli* cells was 10^6 cfu/ml. These flasks were placed for 24 hours at 37°C at 150 r/min on shaking. After 24 hours growth rate and bacterial concentration were examined by determining optical density (OD) at 600 nm. (.1 OD_{600} corresponds to108 cell/ml).

Statistical Analysis

All statistical analysis was performed using graph pad prism version 6.01. All results in this paper were presented as the mean with standard error. Significance of differences was determined by one way and two-way analysis of variance (-ANOVA) with ($P < .05$).

Results

M.oleifera leaf extract was prepared after washing, dried, and grinded leaves into powder form. The powder was mixed with deionized water to get the final extract. $AgNO_3$ was mixed with different concentrations of *M oleifera* leaf extract to form MLE-AgNPs. The main sign of silver nanoparticle formation was the change in color from light yellow to dark brown within 60 min of reaction time. MLE works as a reducing agent as well as a capping agent and controls the agglomeration. Under the necessary conditions such as constant stirring and temperature, free silver atoms agglomerated and provided the basis for the formation of colloidal silver nanoparticles. Figures 1, 2, and 3 represented the *M. oleifera* leaf extract, synthesis of silver nanoparticles (MLE-AgNPs) and different levels of MLE-AgNPs respectively.

UV-VIS Absorption Spectra Analysis

UV-VIS spectrophotometer was used for the determination of optical properties of *M. oleifera* leaves mediated AgNPs. The formation of the AgNPs was followed by measuring the SPR over the range of 300–800 nm. Prior studies displayed the silver nanoparticles (AgNPs) contributed to the absorption at around the 390–470 nm in the UV-VIS spectra. While in current study, Figure 4 shows the absorption band of the green synthesized Ag NPs. The UV-VIS spectra of AgNPs showed intense surface plasmon resonance (SPR) peak at around the 419 nm. The
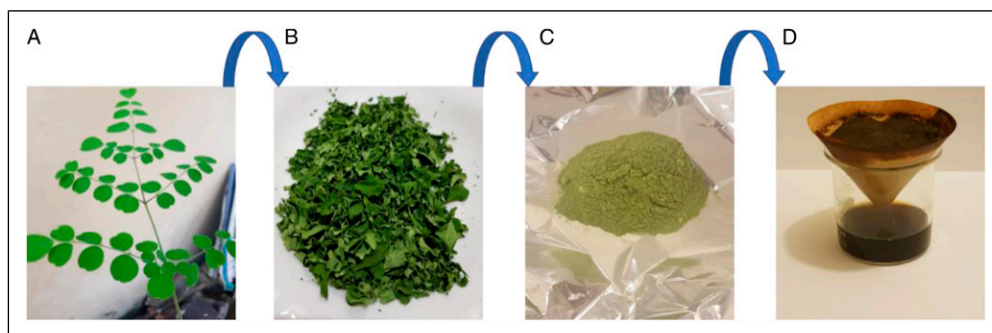

Figure 1. Extract from *M. oleifera* leaves.

Figure 2. Synthesis of silver nanoparticles using *M. oleifera* leaves extract.
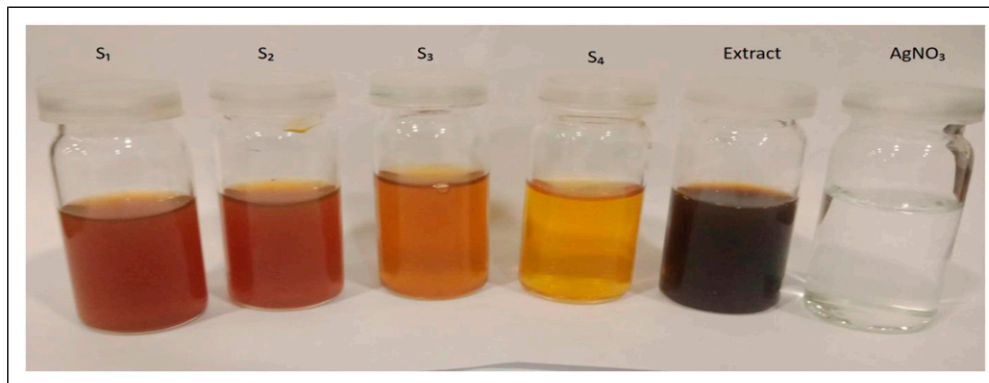
Figure 3. Four different types of AgNPs shown different colors along with extract and AgNO3.
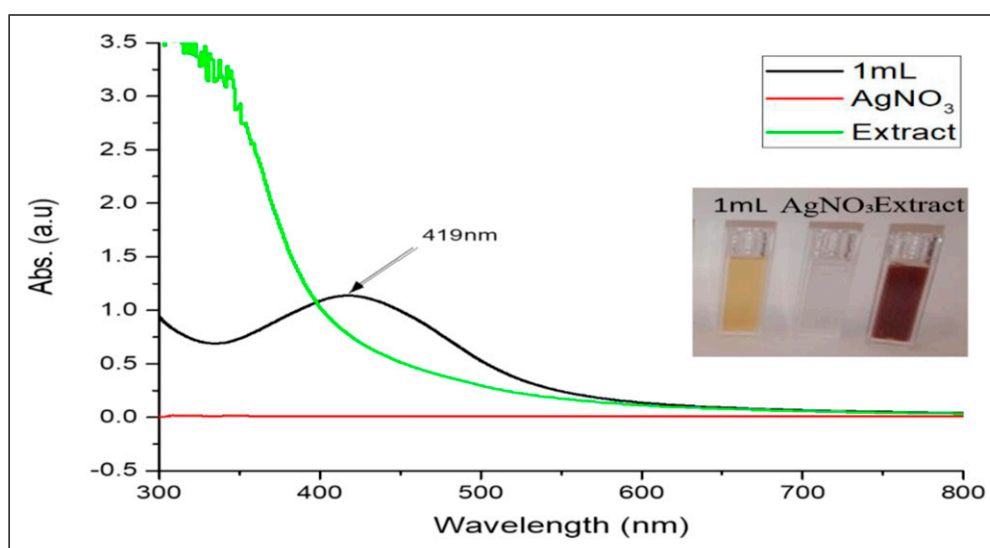

Figure 4. UV-Vis absorption spectra of silver nanoparticles $AgNO_3$ and extract.

shape, size, morphology, and composition of the synthesized silver nanoparticles directly influenced the SPR bands.

Effect of Extract Concentration

Concentration effect of MLE was also an interesting observation on the synthesis of silver nanoparticles. The concentration of the MLE on the preparation of the AgNPs was inspected by the UV-VIS spectroscopy. A significant effect was observed by the concentration on the morphology during UV-VIS analysis. The concentration of MLE varied from 1 mL to 10 mL in 90 mL $AgNO_3$ (1 mM) other condition keeping constant. The shape and position of the SPR vary with concentration variation.

X-Ray Diffraction (XRD) Analysis

X-ray diffraction method was used for the investigation of the crystalline structure along with lattice characteristics of the synthesized AgNPs. The angle 2θ values ranging from $20°$ to $100°$ using Cu Kα whose wavelength range 1.5406 A°.

Four sharp peaks can be seen clearly in the Figure 5 that appeared at 2θ values $38.4°$, $44.5°$, $64.90°$, and $77.4°$. These 2θ values are the representation of the following diffraction planes (111), (200), (220), and (311) of the FCC structure of the synthesized silver nanoparticles. The maximum intensity peak found at (111) plane that is the ideal orientation of the structure. There are some extra star peaks observed in the graph because of the biological components in extract. Crystallite size is 8.05 nm (Table 2) that can be calculated by using *Debye Scherrer*'s formula

$$D = \frac{\delta\lambda}{\beta\cos\theta}$$

Scanning Electron Microscopy Analysis

SEM (scanning electron microscope) was used to determine the size and morphology of the synthesized AgNPs. Structural studies done by SEM showed the presence of phenolic
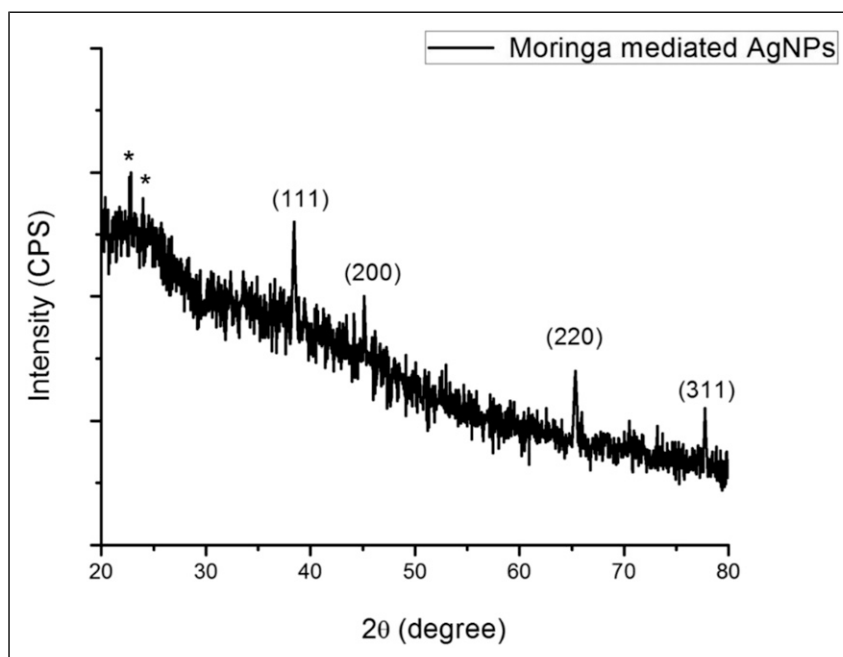
Figure 5. X-ray diffraction spectra of *M. oleifera* leaf extract.

Table 2. A brief summary of silver nanoparticle obtained from XRD results.

Sample	Peak Position (2θ)	Diffraction Plane (hkl)	FWHM, (β) radians	Lattice Parameter (a), nm	Crystalline Size (D),nm
S	38.4°	111	.0221	.404	8.05

compounds, amino acids, waxes, antioxidants vitamins, and flavonoids. SEM image and its particular magnified image along with histogram are shown in Figure 6. The magnified SEM image of sample S showed the agglomeration of the silver nanoparticles. Observation shown that mostly particles are of spherical in shape, the particles size distribution is in the range of 10 to 25 nm and their average size is 18 nm revealed by the histogram (Table 3).

Antibacterial Activity

Agar well diffusion method was used to investigate the antibacterial activity of the synthesized silver nanoparticles against the *E coli* bacterium. Different concentrations of the silver nanoparticles of sample 's' along with leaves extract and pure water as negative control were tested to diminish *E coli*. The zone of inhibition around the wells was the representation of antibacterial activity of silver nanoparticles (Figure 7). The zone of inhibition were 19 mm, 17 mm, and 15 mm for the concentration C_1 (100 µg/ml), C_2 (50 µg/ml), and C_3 (25 µg/ml), no zone of inhibition appeared for pure water and MOF extract. The antibacterial activity of the synthesized AgNPs increases steadily as the stack solution increases.

Minimum Inhibitory Concentration

The growth curve of *E coli* by measuring the OD_{600} with different concentrations of MLE-AgNPs is shown in Figure 8. The concentrations .5, 1.0, 1.5, 2 and 2.5 mM of MLE-AgNPs, the growth curve of *E coli* includes three phases: lag, exponential, and stabilization phase. Though, decline phase could not be exposed since we evaluated the total number of *E coli*, (live and dead) based on OD_{600}. *E coli* reached exponential phase promptly when there was no MLE-AgNPs. *E coli* growth with different time intervals was also measured and maximum inhibition was observed in silver nanoparticles compared to without silver nanoparticles as shown in Figure 9. While increasing the concentration of MLE-AgNPs more delay takes place in growth and vice versa. When concentration was 5 mM, there was no growth of *E coli* within one week, representing the minimum inhibitory concentration (MIC) of MLE-AgNPs to *E coli* was 5 mM.

Discussion

Previously, numerous studies were performed for synthesize AgNPs with significant antimicrobial activities from plant
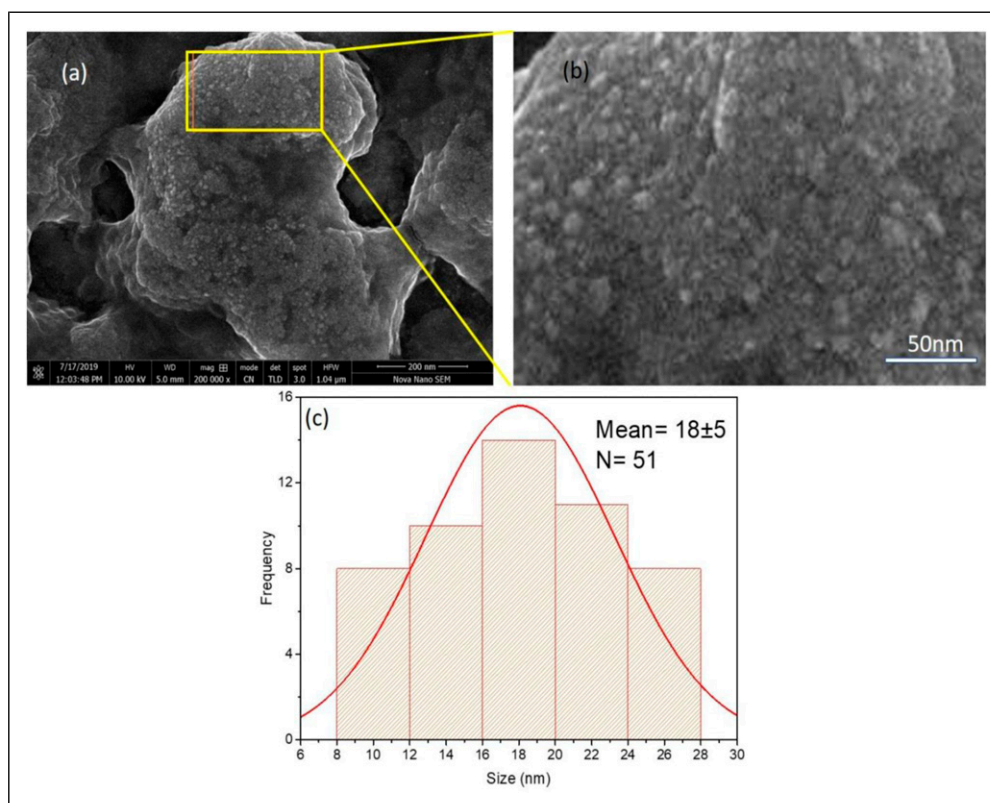
Figure 6. (a) SEM image of AgNPs, (b) Particular section magnified image (c) Particle size distribution histogram.

Table 3. Results obtained from UV-VIS and SEM analysis.

Sample	Color	Absorbance Peak	Shape	Average size (nm)
AgNPs	Red	439	Spherical	18
AgNPs	Black	419	Spherical	18

extracts like leaf extracts of *Acalypha indica*,[17] *Solidago altissima*,[16] *Xanthium strumerium L*,[22] *Murraya koenigii* (curry leaf),[23] *Ocimum sanctum* (Tulsi leaf)[8,15] seed extracts of *Acacia farnesiana*,[24] *Macrotyloma uniflorum*,[26] root extracts of *Trianthema decandra*,[27] stem extracts of *Ocimum sanctum*,[8] and even fruit extracts of *Musa paradisiaca* (banana) peels[28] and *Carica papaya*.[31] In these studies, phytocompounds in the plant extract serve as reducing and/or capping agent in the reaction with silver nitrate ($AgNO_3$), a commonly used precursor in silver nanoparticle synthesis.[32]. According to Šileikaitė et al.,[33] during the synthesis of silver nanoparticles by green synthesis method, the original mechanism happened can be explained by liquefying the metallic silver nitrate into deionized water. Initially, silver nitrate changes into silver ions. As the *M. oleifera* leaves extract added into $AgNO_3$ reduction process occurred and silver ions reduced into free metallic atoms (Ag). MLE works as a reducing agent as well as a capping agent and controls the agglomeration. Under the necessary conditions such as constant stirring and temperature, free silver atoms

agglomerated and provided the basis for the formation of colloidal silver nanoparticles.

The surface Plasmon resonance shifted toward red shift by increasing concentration and resulted stable particles because larger functional ligand as reported by Bar et al.[13] But our prepared extract was too much thick and on large concentration there was no peak observed in UV-VIS spectra. As we reduced the concentration, the SPR shifted towards blue correspondingly that resulted smaller size particles which are more stable because of large surface area. By diluting the extract, clear peak was obtained but peak position remained same. We used to optimize AgNPs for further characterization.

The strength of MLE-AgNPs might be as a consequence of capping agents in *M. oleifera* leaves, like proteins, polysaccharides or phenolic composites as attributed by Khan et al.[34] The results are in accordance to Sur et al.[35] where the absorption peaks were arising at 401 nm due to the excitation of local plasmon and the fluctuations of the transmission electrons of gold and silver nanoparticles which resembled to the distinct transverse plasmon that was a spherical silver nanoparticle.

According to Öztürk et al.,[36] XRD showed the angle (2θ) peak at 37.8° which indicated the pure silver nanoparticles. Furthermore, X-ray diffraction investigations specified that these MOS-AgNPs (Nanoparticles) were crystalline in nature. There were also (2θ) angle peaks at 42°, 65°, and 78° companionable to 200, 220, and 311 silver planes respectively. Besides, the
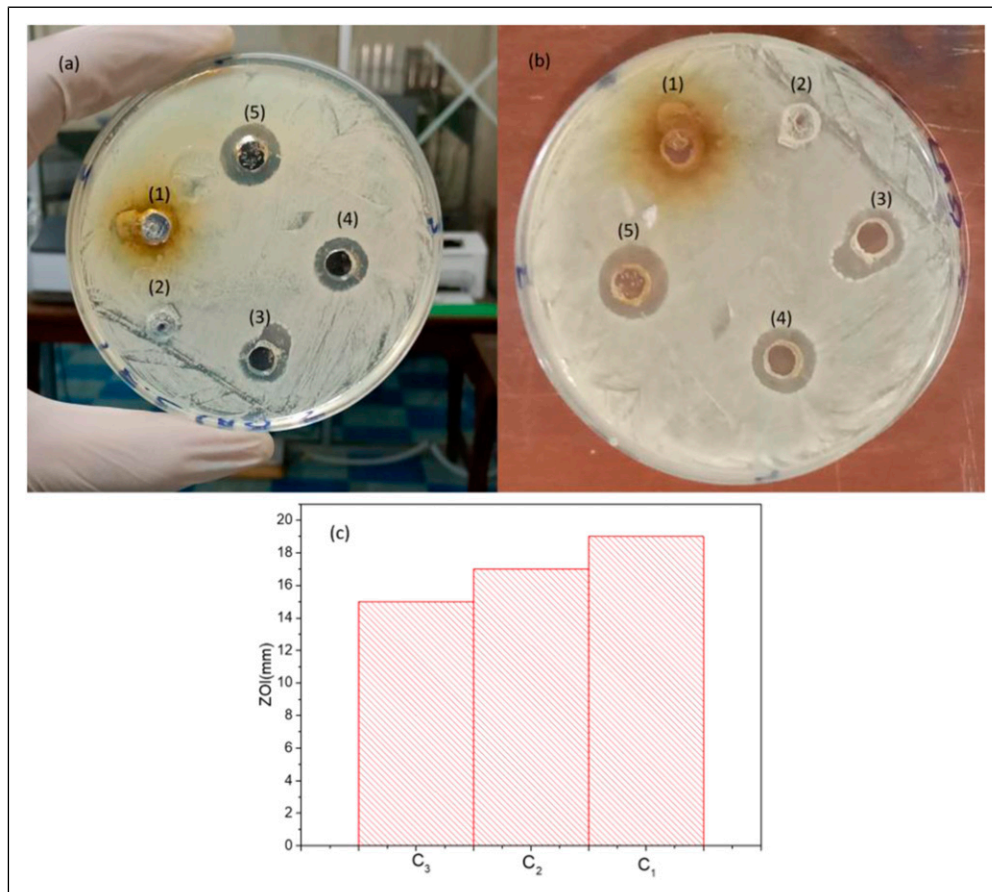
Figure 7. (a and b) Antibacterial activities of AgNPs against *E. coli*. (c) ZOI histogram.
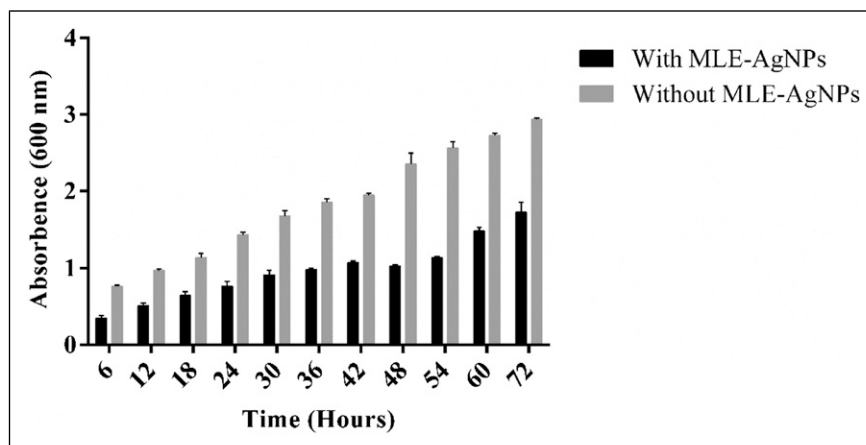

Figure 8. Effect of different concentrations of (MLE-AgNPs) on the growth of *E. coli*.

dispersed peaks in the XRD configuration might be due to the presence of biological levels that happened on the exterior of MOS-AgNPs.[37] investigated that the diffraction crests obtained were wide might be due to small crystalline sizes.

A similar study was conducted by Mehwish et al.[38] in which SEM image of the MOS-AgNPs (Silver nanoparticles with *M. oleifera* seeds) shown a uniform shape and globular

exterior structure. Chemical investigations of MOS-AgNPs showed the existence of silver and other components with silver masses. Ag was there as a result of the development of MOS-AgNPs, although the further elements were from *M. oleifera* extract. In the current learning, the average size of MOL-AgNPs was 10 to 25 nm which is in agreement with the results of[39] who found MOS-AgNPs size 10.2 nm and
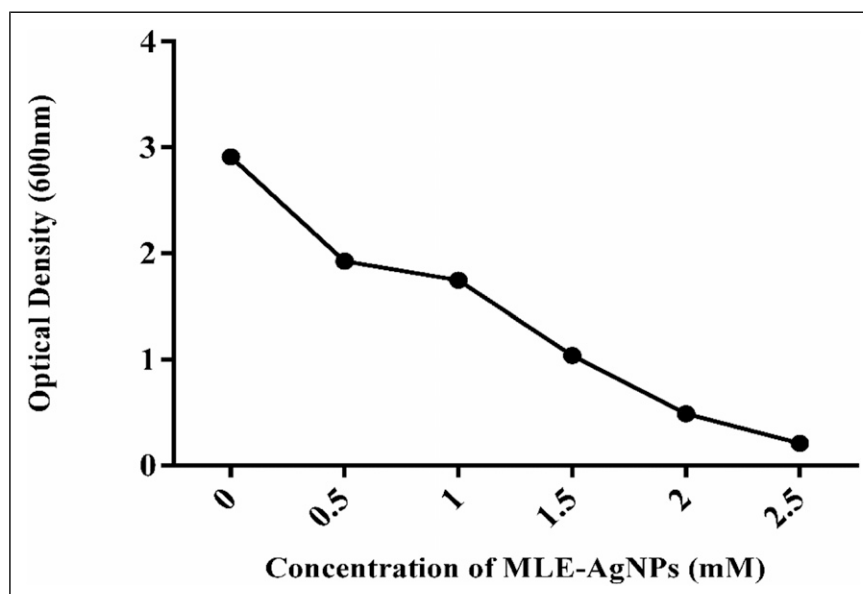
Figure 9. Effect of (MLE-AgNPs) on the growth of *E. coli* at different time interval.

Enicostemma axillare (Lam.) leaf extract with an average size of 18.12 nm respectively. According to Sur et al.,[35] the size of nanoparticles varies between 20 and 40 nm, while the average size of silver nanoparticles was typically about 30 nm with virtually globular in shape and nearly monodispersed in nature.

These results are in comparison with Masurkar et al.[40] where the antimicrobial activity of silver nanoparticles contrary to *E coli* was extensively described. According to the results, the rumors on the antibacterial action in contrast to *S. aureus* were inconstant where some claiming exceptional actions of AgNPs against *S aureus*, however, others reported nothing or insignificant activity against the same *S aureus* strain. These different results might be due to the dissimilar shapes of AgNPs acquiescent from variable synthetic procedures.[41] The results as suggested by Premanathan et al.[42] antibacterial activity of silver nanoparticles contrary to various bacteria was shape dependent. According to Khan et al.[43] who experimented silver nanoparticles on different Gram negative and Gram positive bacterial strains, the following order was observed *E. coli* > *P. aeruginosa* > *M. luteus* > *S. aureus* with the toxicity of silver nanoparticles. Therefore, it can be quantified from several results that the synthesized AgNPs are more operational against Gram negative bacteria than Gram positive bacteria. Similar to our results where *E coli* is more prone to even less concentration of MLE-AgNPs. Similar results were also found from green synthesis of silver nanoparticles using *Lysiloma acapulcensis* exhibit high-antimicrobial activity against *E. coli*, *P. aeruginosa*, and *S. aureus*.[44] In short, results represented that it is possible to synthesize silver nanoparticles from leaves extract of *M oleifera* which is efficient, simple and quick techniques with good antibacterial properties.

Conclusion

Conclusively, *M. leifera* leaves extract produce extracellular AgNPs with good stability power in the solution. The synthesized silver nanoparticles were operated as an active substrate against *E coli* which is recognized resistant against maximum commercially available drugs. This green synthesis of silver nanoparticles may prove a quick, cost free and suitable alternative of synthetic antibiotics against multi drugs resistance bacteria. Finally, biosynthesis of silver nanoparticles proved to be an ideal potential candidate for the medical application where antimicrobial activity is essential.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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