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Green synthesis of silver nanoparticles using methanolic fruit extract of *Aegle marmelos* and their antimicrobial potential against human bacterial pathogens

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ABSTRACT

Plant-based synthesis of nanoparticles has generated worldwide interest because of cost-effectiveness, eco-friendly nature and plethora of applications. In the present investigation, antimicrobial potential of silver nanoparticles (AgNPs) of methanolic extract of *Aegle marmelos* fruit has been investigated. Agar well diffusion method was used for determining antimicrobial activity of solvent extracts (*viz*., petroleum ether, chloroform, acetone, methanol and aqueous), and AgNPs. Among these, methanolic extract of *A. marmelos* showed highest inhibitory activity against *B. cereus* (16.17 ± 0.50 mm) followed by *P. aeruginosa* (13.33 ± 0.62 mm) and *E. coli*. Phytochemical analysis of methanolic extract of *A. marmelos* revealed the presence of tannins, saponins, steroids, alkaloids, flavonoids, and glycosides. AgNPs synthesized using *A. marmelos* methanolic extract, characterized by UV–Visible spectroscopy, atomic force microscopy, dynamic light scattering, and X-ray diffraction showed a peak at 436 nm and size ranged between 159 and 181 nm. Evaluation of the antimicrobial potential of green synthesized AgNPs recorded the highest inhibitory activity against *B. cereus* (19.25 ± 0.19 mm) followed by *P. aeruginosa* (16.50 ± 0.30 mm) and *S. dysentriae*. The minimum inhibitory concentration (MIC) of synthesized AgNPs was found to be in the range of 0.009875–0.0395 mg/100 μl which was quite lower than the MIC of crude extract i.e. 0.0781–0.3125 mg/100 μl. The results obtained indicated that the different crude extracts of *A. marmelos* plant as well as AgNPs have a strong and effective antimicrobial potential that provide a marvelous source for the development of new drug molecules of herbal origin which may be used for the welfare of humanity.

1. Introduction

Medicinal plants have served as a major source of drugs for centuries. India has a rich heritage of knowledge on plant-based drugs both for use in preventive and curative medicine from ancient times with mention of these in Ayurveda, Siddha, Homoeopathy and other reforms. Approximately 88% of the global population uses plant-based drugs or medicines as their first line of defense for maintaining health and combating many diseases.[1,2] The increasing failure of chemotherapeutics and development of bacterial resistance to currently available antibiotics has necessitated the search for novel and effective antimicrobial compounds from natural resources. Plant-derived medicines have gained immense importance in recent years because of their potential efficacy, and no side effects. Plants are a rich source of valuable secondary metabolites such as tannins, terpenes, terpenoids, alkaloids, flavonoids, coumarins, polysaccharides, glycosides, gum and phenols that are used by plants as defense mechanisms against invasion by many microorganisms, insects, and herbivores.[3,4] Plant-derived medicines are believed to be more acceptable to the human body in comparison to modern synthetic drugs.[5] Thus there is a need to derive the maximum benefit from the medicinal plants as well as the traditional system of medicine for providing adequate health services to the people in rural areas. The extracts of medicinal plants have

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wonderful antibacterial, antifungal, and antioxidant activities.[6] These activities can be improved many folds by a blend of modern day scientific inputs e.g. synthesizing nanoparticles.

Nanotechnology has emerged as a promising escalating field with plethora of applications in numerous areas including medicine, pharmacology, sensing devices, micro-electronics and drug delivery etc.[7] Among various nanoparticles, AgNPs are one of the most vital and fascinating nanomaterials that are involved in a range of biomedical applications and have been reported to possess antibacterial, antifungal, anti-viral, anti-angiogenic and anti-inflammatory properties.[8] Nanoparticles can be synthesized via chemical, physical and photochemical routes, carrying many threats to the environment as well as human beings.[9,10] Nanoparticles can be broadly grouped as organic nanoparticles which include carbon nanoparticles (fullerenes), inorganic nanoparticles including magnetic and noble metal nanoparticles (like gold and silver) and semiconductor nanoparticles (e.g. titanium oxide and zinc oxide). Inorganic metal nanoparticles (Gold and silver) are of increasing interest since they provide superior material properties with functional versatility. Due to their advantages over available chemical imaging drug agents besides size features, inorganic particles have been used as potential tools for medical imaging as well as for treating diseases.[11]

In recent years, green synthesis of nanoparticles using different plant parts/components is receiving considerable attention because of its simplicity, low cost, safety, and eco friendly nature. A number of plant extract mediated green synthesis of AgNPs have been reported in the literature.[12] Plant extract may act both as reducing agents and stabilizing agents in the synthesis of nanoparticles.[13] Previous workers reported the successful synthesis of AgNPs using various plant parts such as *Acacia concinna* fruit, *Syzygium cumini* fruit, *Dillenia indica* fruit, *Lagerstroemia speciosa* fruit, *Mimuosops elengi* fruit, *Embellica officinallis* fruit, *Arbutus unedo* leaf, *Stevia rebaudiana* leaf, *Azadirachta indica* leaf, *Nicotiana tobaccum* leaf, *Arbutus unedo* leaf, *Ocimum sanctum* leaf, *Eucalyptus globules* Leaf and *Ficus benghalensis* leaf etc.[8,12,14-22]

In the present study, AgNPs were synthesized using a methanolic extract of *A. marmelos* fruit. *A. marmelos* (commonly known as bael) is a hardy, deciduous tree found in India, Sri Lanka, Burma, Bangladesh, Pakistan, Thailand as well as southeastern Asian countries and used in Indian traditional system of medicine to treat myriad of illnesses and diseases.[25,28] *A. marmelos* is widely used in Ayurvedic and folk medicines to cure peptic ulcers, tuberculosis, headache, hypertension, dysentery, and diabetes, etc. This plant possesses the antimicrobial, anti-diarrheal, anti-diabetic, anti-ulcerative colitis, hepatoprotective, cardioprotective and radioprotective properties. *A. marmelos* contains different phytoconstituents such as alkaloids, coumarins, terpenoids, fatty acids, and amino acids etc.[23,24] Therefore, the present study was conducted to investigate the antimicrobial potential of AgNPs synthesized using *A. marmelos* fruit extract against various human pathogenic bacteria.

2. Materials and methods

2.1. Collection of plant material

Plant material *i.e.* leaves and fruits of *A. marmelos* were collected form Swarghat area in district Bilaspur, Himachal Pradesh. Plant materials were washed, air dried separately in shade, coarsely powdered and stored in the neatly labeled air tight plastic container until further use.

2.2. Test organisms

The bacterial pathogens *viz*., Gram positive (*Bacillus cereus*, *Staphylococcus aureus)* and Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella dysenteriae*, *Yersinia pestis)* were procured from Indira Gandhi Medical College and Hospital (IGMC), Shimla (H.P.) and maintained on nutrient agar.

Selected test organisms and antibiotics (Table S1).

2.3. Preparation of plant extracts

Cold percolation method was used for the preparation of plant extracts as per the method detailed by Rosenthaler.[25] The dried powdered sample was soaked in different solvents (1:10 ratio) and extracted for 24 h at room temperature with continuous agitation at 150 rpm. Filtrates of the extracts were evaporated to dryness at 40 °C. The dried extracts were further powdered and then resuspended in universal solvent DMSO (Dimethyl sulfoxide) to bring to 100 mg/ml concentration.

2.4. Determination of antimicrobial potential of plant extracts

The effect of different plant extracts on pathogenic strains was assayed by agar well diffusion assay and zones of inhibition (mm) were recorded after 24 h of incubation. The MIC of the plant extract required to inhibit the growth of microorganisms was analyzed by Resazurin dye method using plant extract having 2 fold serial dilutions (Thakur et al., 2017).

2.5. Phytochemical analysis

Phytochemical analysis of fruit and leaf methanolic extract for the identification of phytochemicals like carbohydrates, soluble starch, tannins, alkaloid, steroids, saponin, terpenoids, and flavonoids was carried out using methodology described by Thakur et al.[15]

2.6. Synthesis of AgNPs

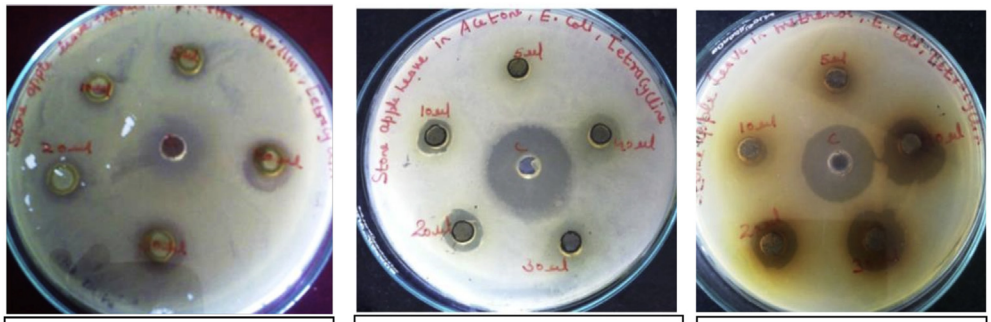
1 mM aqueous solution of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of AgNPs. Synthesis of AgNPs from silver nitrate solution takes place by co-precipitation using fruit extract of *A. marmelos* which acts as reducing and capping agent. Characterization of biologically synthesized AgNPs was carried out using UV–Vis spectrophotometer, XRD, AFM, and DLS studies.

3. Results

In this study, the antimicrobial efficiency of different extracts (*viz*., petroleum ether, chloroform, acetone, methanol and aqueous) of *A. marmelos* plant (i.e. leaf and fruit extracts) were tested against both Gram positive (*B. cereus, S. aureus)* as well as Gram negative (*E. coli, P. aeruginosa, S. typhi, S. dysentriae, Y. pestis)* bacterial pathogens by using the agar well diffusion method. The results of the antimicrobial effects of different parts (i.e. leaf and fruit) of the *A. marmelos* solvent extracts are presented in Table S2.

3.1. Antimicrobial activity assay of various solvent extracts of *A. marmelos* leaf

The petroleum ether extract was found effective against almost all the tested pathogens whereas no inhibitory activity was observed against *Y. pestis.* The maximum zone of inhibition 9.5 ± 0.07 mm at the concentration of 40 μl was recorded against *S. dysentriae* and minimum with *S. aureus* (7 ± 0.44 mm) (Table S3 and Fig. 1A). The acetone extract was shown maximum inhibition zone against *S. aureus* (10.33 ± 0.22 mm) followed by *S. typhi* (10 ± 0.49 mm) and minimum with *E. coli* (8.33 ± 0.57 mm) at the



(A) Inhibition zone of petroleum ether extract on *B. cereus*

(B) Inhibition zone of acetone extract on *E. coli*

(B) Inhibition zone of methanol extract on *E. coli*

Fig. 1. Effect of different extracts of *A. marmelos* leaf against various pathogens (A) Petroleum ether extract on *B. cereus*, (B) Acetone extract on *E. coli* and (C) Methanol extract on *E. coli*.

concentration of 40 μl (Table S3 and Fig. 1B). The methanolic extract showed maximum inhibitory effect against *S. typhi* (10.33 ± 0.60 mm) followed by *Y. pestis* (10.23 ± 0.18 mm), *B. cereus* (10.15 ± 0.62 mm) and *S. dysentriae* (10 ± 0.15 mm) at concentration of 40 μl (Table 1 and Fig. 1C). The methanol extract was almost equally effective against *E. coli*, *S. aureus*, and *P. aeruginosa*.

3.2. Antimicrobial activity assay of various solvent extracts of *A. marmelos* fruit

The petroleum ether extract showed maximum activity against *Y. pestis* (12.25 ± 0.18 mm) followed by *P. aeruginosa* (12.21 ± 0.18 mm) and *B. cereus* (11.33 ± 0.54 mm). The least activity was observed against *E. coli* (10.23 ± 0.17 mm) at the concentration of 40 μl (Table S3 and Fig. 2A). Chloroform extract showed activity against pathogenic strains i.e. *S. typhi, B. cereus, S. dysentriae, S. aureus* and *E. coli* (Table S3 and Fig. 2B). Maximum zone of clearance was observed in case of *B. cereus* (13.15 ± 0.62 mm) followed by *S. typhi* (13 ± 0.10 mm) at a concentration of 40 μl. The zone of inhibition in case of *B. cereus* (13.15 ± 0.62 mm) was quite comparable to the positive control (15.1 ± 0.61 mm). The acetone extract was found most effective against *P. aeruginosa* (13 ± 0.15 mm) which was followed by *E. coli* (12.22 ± 0.18 mm) and least effective against *B. cereus* (9.22 ± 0.16 mm) at the concentration of 40 μl (Table S3 and Fig. 2C). Methanolic extract showed good antimicrobial potential against almost all the pathogenic strains. The highest inhibitory effect was observed against *B. cereus* (16.17 ± 0.50 mm) which was better than positive control i.e. tetracycline (15 ± 0.15 mm), followed by *P. aeruginosa* (13.33 ± 0.62 mm) and *E. coli* (13.22 ± 0.18 mm at 40 μl conc.) and least against *S.aureus* (11.25 ± 0.85 mm) (Table 1 and Fig. 2D). Aqueous extract of *A. marmelos* fruit showed activity against pathogenic strains i.e. *S. typhi, B. cereus, P. aeruginosa, Y. pestis* and *E.coli*. The maximum zone of inhibition was recorded against *P. aeruginosa* (13.22 ± 0.40 mm) followed by *B. cereus* (12.32 ± 0.67 mm) and least with *Y. pestis* i.e. (10.33 ± 0.22 mm) at the concentration of 40 μl (Table S3).

3.3. MIC of methanolic extract of leaf of *A. marmelos*

MIC of methanolic extract of leaves of *A. marmelos* was found similar in case of *S. aureus*, *Y. pestis, E. coli,* and *B. cereus* i.e. 0.625 mg/100 μl (Table 2, Table S4 and Fig. 3A). However, 1.25 mg/100 μl concentration of the extract was required to inhibit the growth of *S. typhi* and *P. aeruginosa*. In case of *S. dysentriae,* lowest concentration required to inhibit the growth was 2.5 mg/100 μl.

3.4. The MIC of methanolic extract of fruit of *A. marmelos*

In case of methanolic extract of *A. marmelos* fruit, MIC value of 0.0781 mg/100 μl was recorded against *S. aureus*, *Y. pestis* and *B. cereus* (Table 2, Table S5 and Fig. 3B). Similar MIC was recorded in case of *E. coli, S. typhi*, and *P. aeruginosa* i.e 0.1562 mg/100 μl. However, 0.3125 mg/100 μl concentration of the extract was

Table 1
Antimicrobial activity of methanolic extract of *Aegle marmelos* leaf and fruit on various pathogenic strains.

Plant extracts	Microorganisms	Stock solution of plant extract (100 mg/ml)					
			10 μl	20 μl	30 μl	40 μl	Positive control
Methanol	*E. coli*	Leaf	0	0	7.35 ± 0.23	9.0 ± 0.15	16.0 ± 0.15
		Fruit	7.25 ± 0.19	9.11 ± 0.53	11.13 ± 0.13	13.22 ± 0.18	15.0 ± 0.55
	S. aureus	Leaf	0	5.25 ± 0.65	7.33 ± 0.60	9.16 ± 0.54	17.5 ± 0.74
		Fruit	6.22 ± 0.52	7.11 ± 0.55	9.17 ± 0.50	13.0 ± 0.85	17.0 ± 0.49
	S. typhi	Leaf	0	0	9.23 ± 0.09	10.33 ± 0.60	20.0 ± 0.15
		Fruit	7.11 ± 0.15	8.0 ± 0.15	10.0 ± 0.44	12.23 ± 0.50	20.5 ± 0.30
	S. dysenteriae	Leaf	0	6.12 ± 0.48	8.0 ± 0.53	10.0 ± 0.15	25.5 ± 0.67
		Fruit	8.22 ± 0.16	9 ± 0.53	11.33 ± 0.87	13.15 ± 0.53	19.0 ± 0.59
	Y. pestis	Leaf	0	7.16 ± 0.62	9.0 ± 0.59	10.23 ± 0.18	19.5 ± 0.31
		Fruit	6.0 ± 0.15	8.22 ± 0.56	10.36 ± 0.58	12.15 ± 0.54	19.0 ± 0.15
	P.aeruginosa	Leaf	0	0	5.33 ± 0.22	8.32 ± 0.22	18.5 ± 0.51
		Fruit	8.28 ± 0.55	10.0 ± 0.15	12.0 ± 0.44	13.33 ± 0.62	18.5 ± 0.91
	B. cereus	Leaf	6.0 ± 0.15	7.11 ± 0.85	8.23 ± 0.18	10.15 ± 0.62	15.0 ± 0.49
		Fruit	7.22 ± 0.50	19.12 ± 0.16	14.25 ± 0.53	16.17 ± 0.50	15.0 ± 0.15

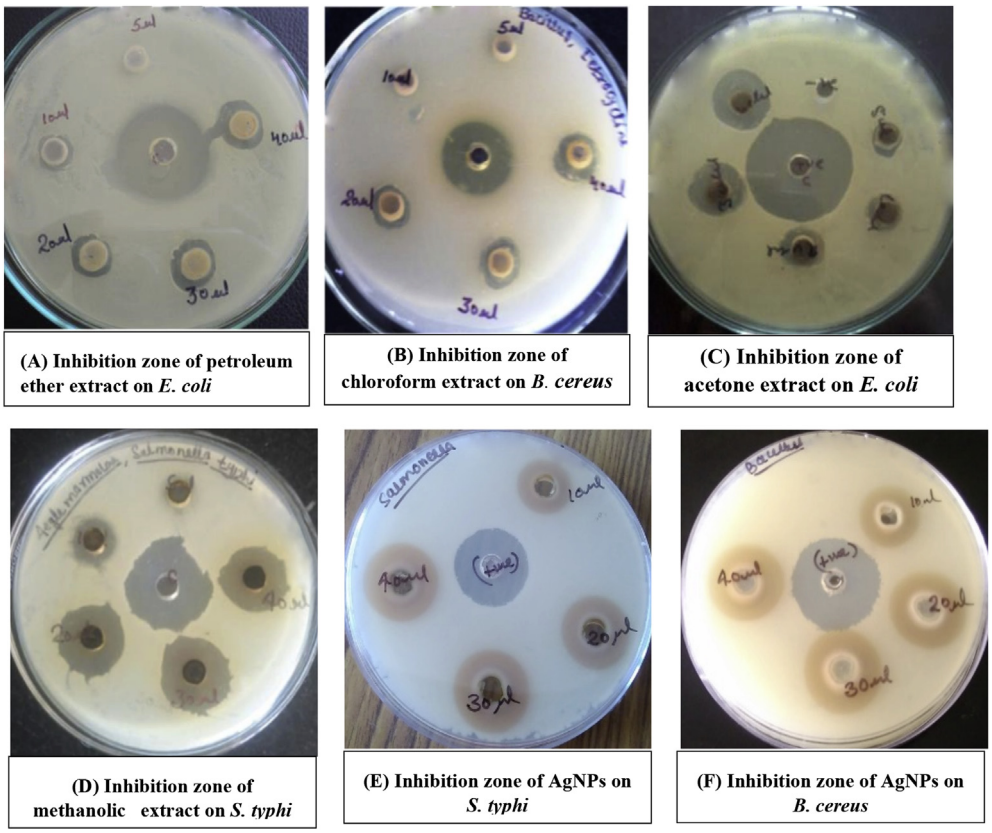


Fig. 2. Effect of different extracts of *A. marmelos* fruit and AgNPs against various pathogens (A) petroleum ether extract on *E. coli* (B) chloroform extract on *B. cereus* (C) Acetone extract on *E. coli* (D) methanolic extract on *S. typhi* (E) AgNPs on *S. typhi* (F) AgNPs on *B. cereus*.

Table 2
Comparison of antimicrobial activity and MIC of synthesized AgNPs with crude methanolic extracts of *A. marmelos* fruit and leaf.

Bacterial isolates	*A. marmelos*					
	Zone of inhibition (mm)			MIC (mg/100 μl)		
	AgNPs	Fruit methanolic extract	Leaf methanolic extract	AgNPs	Fruit methanolic extract	Leaf methanolic extract
E. coli	15.15 ± 0.62	13.22 ± 0.18	9.0 ± 0.15	0.009875	0.1562	0.625
S. aureus	15.22 ± 0.52	13.00 ± 0.85	9.16 ± 0.54	0.01975	0.1562	0.625
S. typhi	14.50 ± 0.70	12.23 ± 0.50	10.33 ± 0.60	0.01975	0.0781	1.25
P. aeruginosa	16.50 ± 0.30	13.33 ± 0.62	10.0 ± 0.15	0.01975	0.1562	2.5
S. dysenteriae	15.90 ± 0.85	13.15 ± 0.53	10.23 ± 0.18	0.0781	0.3125	0.625
Y. pestis	14.65 ± 0.38	12.15 ± 0.54	8.32 ± 0.22	0.0395	0.0781	1.25
B. cereus	19.25 ± 0.19	16.17 ± 0.50	10.15 ± 0.62	0.0395	0.0781	0.625

required to inhibit the growth of *S. dysentriae*.

3.5. Phytochemical analysis of A. marmelos (methanolic extract of leaf and fruit)

The results of the preliminary qualitative phytochemical study of the methanolic extract of *A. marmelos* (i.e. leaf and fruit) showed the presence of tannins, saponins, alkaloids and flavonoids, steroids and glycosides (Table S6).

3.6. Characterization of silver nanoparticles (AgNPs)

3.6.1. Synthesis of AgNPs

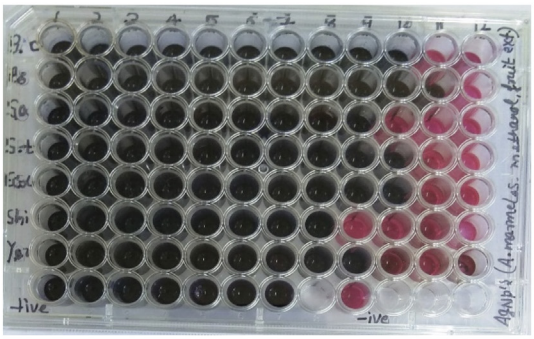
AgNPs exhibit new or improved properties depending upon their size, morphology, and distribution. In the present study, AgNPs were synthesized using *A. marmelos* fruit extract as a reducing agent and aqueous silver nitrate as the precursor. The synthesized silver nanoparticles were separated by centrifugation at 15,000 rpm for 20 min. The process was repeated by dispersion of pellets in water, to obtain colorless supernatant and AgNPs formation was confirmed by observing color change from colorless to dark-brownish. Green synthesized AgNPs were characterized by using UV–Vis spectrophotometer, XRD, atomic force microscopy, and dynamic light scattering studies.

3.6.2. UV–visible spectrophotometry

The reduction of Ag^+ ions to Ag^0 by *A. marmelos* fruit extract was monitored with the use of a UV–Vis spectrophotometer by recording the absorption spectra at the wavelength of 300–700 nm. UV–Vis spectral analysis showed that the maximum absorption was at 436 nm which confirmed the formation of AgNPs in the solution (Fig. 4a (B).

(A) MIC of methanolic leaf extract of *A. marmelos*

(B) MIC OF methanolic fruit extract of *A. marmelos*



(C) MIC of AgNPs of methanolic fruit extract

Fig. 3. MIC of methanolic extract of *A. marmelos* (A) leaf (B) fruit and (C) AgNPs.

3.6.3. XRD

XRD pattern for the synthesized nanoparticle has been presented in Fig. 4a (C). The X-ray diffraction (XRD) pattern of silver nanoparticles synthesized from methanolic fruit extract of *A. marmelos* was characterized using powdered AgNPs to confirm the silver particles and to know other structural details. The XRD analysis clearly revealed the crystalline nature of AgNPs and the XRD spectrum analysis showed different diffraction peaks at 38.130°, 44.353°, 64.429° and 77.55°. These results correspond to (111), (200), (220), (311) Bragg's reflection based silver nanoparticle. The size of the largest AgNPs as estimated from the FWHM of the peak using the Scherer formula was recoded as 181.36 nm and the smallest AgNPs as estimated from the FWHM peak was 159.76 nm. In addition to the Bragg peaks, representative of the face-centered-cubic structure of AgNPs, additional but unassigned peaks were also observed suggesting that the crystallization of bioorganic phase occurs on the surface of the AgNPs.

3.6.4. AFM

The atomic force microscopy (AFM) technique was used to study the surface morphology of biologically synthesized AgNPs and the 2D and 3D topographical views have been shown in Fig. 4a(D). Visualization of the surface properties of synthesized AgNPs was investigated by (NTEGRA NT- MDT) scanning probe microscope, in tapping mode, using high resonant-frequency (241 kHz) pyramidal cantilevers with silicon probes having force constants of 41 N/m. Scanning speed was set at 2 Hz. Silver nanoparticles synthesized from methanolic extract of *Aegle marmelos* were characterized for shape and surface morphology. The detailed analysis of the micrograph indicated that the particles were spherical in shape with a smooth surface, without any pinholes. AFM works by scanning a very sharp (end radius ca. 10 nm) probe along the sample surface, carefully maintaining the force between the probe and the surface.

3.6.5. DLS

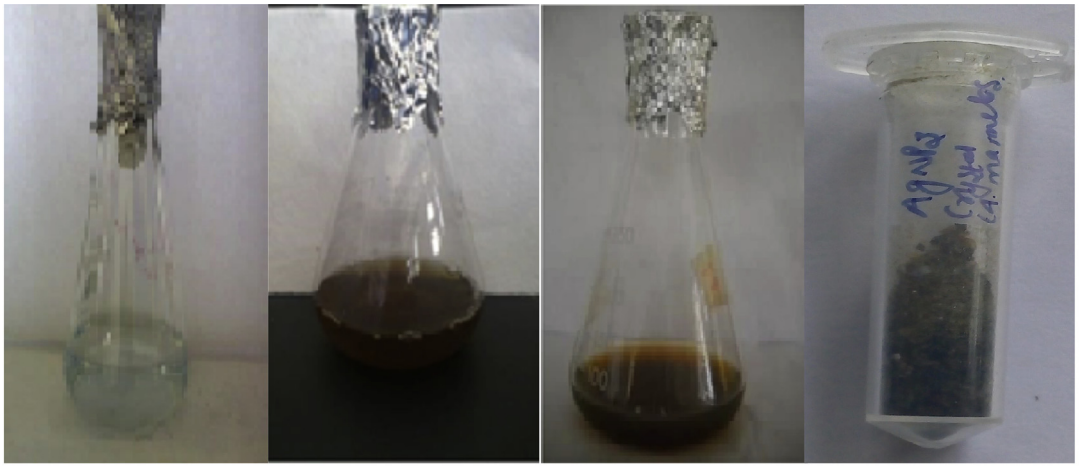
The particle size of the synthesized nanoparticles was determined by using dynamic light scattering (DLS) measurement technique which is used to determine the particle size by the random changes in the intensity of light scattered from a solution. This technique is also called as photon correlation spectroscopy (PCS) which is based on the laser diffraction method with multiple scattering technique employed to study the average particle size of silver nanoparticles. The DLS graph of methanolic extract of *Aegle marmelos* revealed that the particle size of AgNPs was in the range of 10–200 nm as shown in Fig. 4a (E).

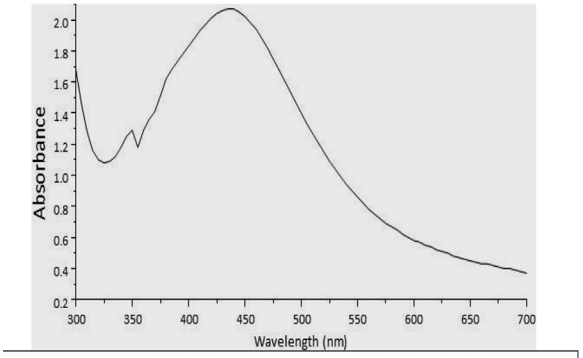
3.7. Antibacterial potential of nanoparticles

Antibacterial potential of green synthesized AgNPs were assessed against all the pathogenic strains and enhanced or highest inhibitory activity was recorded against *B. cereus* (19.25 ± 0.19 mm) followed by *P. aeruginosa* (16.50 ± 0.30 mm) *S. dysentriae* (15.90 ± 0.85 mm), *S. aureus* (15.22 ± 0.52 mm), *E. coli* (15.15 ± 0.62 mm), *Y. pestis* (14.65 ± 0.38 mm at 40 μl). The least inhibitory effect was observed against *S. typhi* (14.50 ± 0.70 mm) and. The data revealed that the AgNPs was more effective against the pathogenic microbes than the crude plant extract (Table 2, Fig. 2E and F).

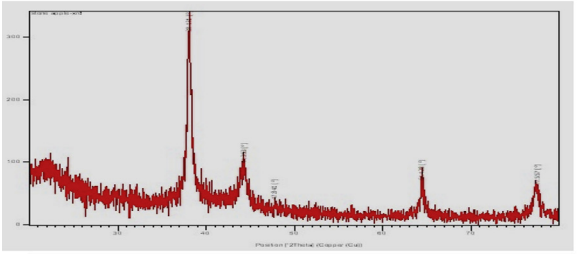
3.8. MIC of synthesized silver nanoparticles

From Table 2, it is clear that the silver nanoparticles were more

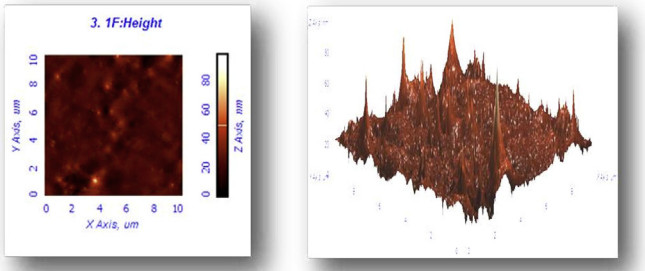




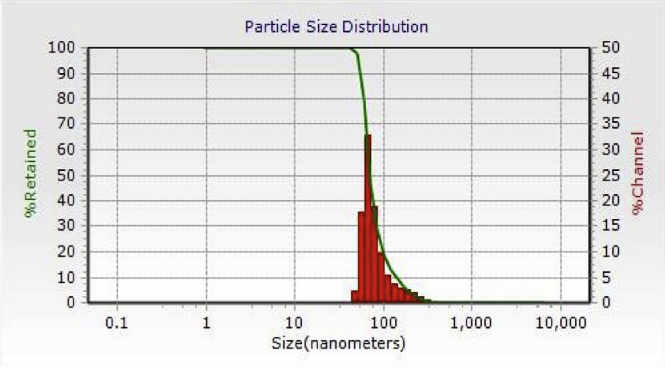
(B) UV- Visible spectrum of AgNPs of *A. marmelos* methanolic extract



(C) XRD pattern of AgNPs of *A. marmelos* methanolic extract



(D) AFM 2D and 3D images of AgNPs of *A. marmelos* methanol extract



(E) DLS spectrum of AgNPs of *A. marmelos* methanolic extract

Fig. 4. Green synthesis of silver nanoparticles: $AgNO_3$ Solution, $AgNO_3$+Plant extract, $AgNO_3$+Plant extract after 24 h, synthesized silver nanoparticles. A Characterization of AgNPs (B) UV-VIS Spectroscopy (C) X-Ray Diffraction (D) Atomic Force Microscopy (E) Dynamic Light Scattering.

effective potent antimicrobials than the crude plant extract. In the present study, the minimum concentration of *A. marmelos* AgNPs required to inhibit the growth of *P. aeruginosa* was 0.009875 mg/100 μl which was very lower in comparison to crude extract (Table 2 and Fig. 3C) followed by *S. typhi*, *B. cereus*, *E. coli*, and *S. dysentriae*. *Y. pestis* and *S. aureus* were found to be least affected in comparison to other pathogens. MIC of biosynthesized AgNPs was found to be in the range of 0.009875–0.0395 mg/100 μl which was quite lower than the MIC of crude extract (0.0781–0.3125 mg/100 μl) (Fig. 3C and Table S7).

4. Discussion

In the present study, different extracts of *A. marmelos* plant (i.e. leaf and fruit extracts) were evaluated for exploration of their antimicrobial activity against various Gram positive (*B. cereus, S. aureus*) as well as Gram negative (*E. coli, P. aeruginosa, S. typhi, S. dysentriae* and *Y. pestis*) bacterial strains. It was observed that all the extracts of plant showed varied extents of antimicrobial activity against pathogenic strains, albeit methanolic extract of *A. marmelos* exhibited the highest inhibitory activity against most of the tested

pathogens. Significant or higher activity of the methanolic extract against most of the bacterial strains investigated in this study is in agreement with earlier works which demonstrate that methanolic extract of the plant usually showed greater antimicrobial activities than other extracts.[15,26,27] This may be because of the organic nature of methanol and its high competence to dissolve or diffuse active antimicrobial constituents found in plants.

In the present study, methanolic extract of *A. marmelos* leaf showed maximum inhibitory effect against *S. typhi* (10.33 ± 0.60 mm at 40 μl) followed by *Y. pestis* (10.23 ± 0.18 mm) and *B. cereus* (10.15 ± 0.62 mm). Additionally, methanolic extract of *A. marmelos* fruit was found most effective against *B. cereus* (16.17 ± 0.50 mm at 40 μl) followed by *P. aeruginosa* (13.33 ± 0.62 mm) and *E. coli* (13.22 ± 0.18 mm). Pandey and Mishra[28] reported antimicrobial activity of different extracts of *A. marmelos* against *Bacillus subtilis, S. aureus, Klebsiella pneumonia*, and *E. coli*. Jyothi et al.[29] also confirmed that the methanolic extract of *A. marmelos* showed significant zone of inhibition against *E. coli, S. aureus*, *Pseudomonas vulgaris, B. subtilis, Enterococcus faecalis* and *Streptococcus faecalis*.

Plant extracts (crude) contain a plethora of phytochemicals (i.e. polyphenols, flavonoids, and alkaloids) which could be antimicrobial representatives. The phytochemical analysis revealed the presence of tannins, saponins, alkaloids and flavonoids, steroids and glycosides in the methanolic extract of *A. marmelos* which is in agreement with previous reports.[28,30,31] Several investigations showed the connection between antimicrobial potential of plant and the phytochemicals present in them.[29-34] Phenolic compounds are well known for their antibacterial, antifungal, antiviral and spasmolytic activities. Similarly, alkaloids of plant origin have also shown anti-inflammatory, antimicrobial and anticancer properties.

Various plants materials and their extracts provide an attractive platform for synthesis of nanoparticles as they are easily available, safe and free from toxic chemicals as well as provide natural capping agents for the stabilization of nanoparticles.[35] In the present study, methanolic crude extract obtained from the *A. marmelos* fruit was used as reducing agents to produce AgNPs via green route and the synthesized nanoparticles were characterized by UV–Vis spectroscopy, XRD, atomic force microscopy and dynamic light scattering studies. UV–Vis spectral analysis showed a surface plasmon resonance (SPR) band at the wavelength of 300–700 nm. This revealed a peak at 436 nm in *A. marmelos* fruit extract which confirmed the production of AgNPs. Krishnaraj et al.[36] monitored the AgNPs using UV–Vis absorption spectrum in the range of 200–800 nm. XRD pattern revealed the highest peak at $2\theta = 38.1^\circ$ and lowest peak at 77.5° which proved that the nanoparticle has the face centered cubic structure and size of synthesized AgNPs ranged from 159.76 nm to 181.36 nm. The size of nanoparticles obtained in this study is supported by results of Tippayawat et al.[37] who described the green synthesis of AgNPs from aloe vera plant extract and revealed the particle size of AgNPs in the range of 70–192 nm by XRD. Bar et al.[24] also reported the face-centered cubic structure of the AgNPs prepared from the fruit extract of *A. marmelos* using XRD studies.

Antimicrobial potential of green synthesized AgNPs were assessed against bacterial pathogens and it was observed that efficacy of AgNPs was much higher than the raw plant extract and also higher than the positive control. AgNPs obtained showed highest inhibitory activity against *B. cereus* (19.25 ± 0.19 mm at 40 μl) followed by *P. aeruginosa* (16.50 ± 0.30 mm), *S. dysentriae* (15.90 ± 0.85 mm), *E. coli* (15.15 ± 0.62), and *S. aureus* (15.22 ± 0.52 mm). Patil et al.[38] have also reported the effectiveness of AgNPs synthesized from *A. marmelos* leaf extract for *E. coli* (7–10 mm at 25–100 μl), *P. aeruginosa*, (7–10 mm) and *S. aureus* (6–9 mm) which are comparable to the results obtained in this study. MIC of biosynthesized AgNPs was found to be in the range of 0.009875–0.0395 mg/100 μl which was quite lower than the MIC of crude extract i.e. 0.0781–0.3125 mg/100 μl. It is noticeable that AgNPs were more potent antimicrobials than the crude plant extracts and can be effectively utilized in pharmaceutical, biotechnological as well as biomedical applications.

5. Conclusion

The findings of this study suggest that the crude methanolic extract of *A. marmelos* fruit exhibited good antimicrobial activity against bacterial pathogens which indicates the immense potential of *A. marmelos* fruit as effective antimicrobial agents that can be used in various modern medicines. Additionally, the current work presents the eco-friendly and cost-effective procedure for the synthesis of AgNPs by utilizing a renewable natural resource i.e. *A. marmelos* fruit extract, which exhibited excellent and much more antimicrobial activity then that of raw plant extracts. Therefore, this study will be helpful in the development of value-added products from *A. marmelos* plants for biomedical, pharmaceutical, biotechnological and nanotechnology-based industries and also could assist in the discovery of new and effective herbal medicines to fight against drug-resistant bacterial infections using green routes. However, further studies are needed for the isolation and identification of active components or antimicrobial compounds present in medicinal plant and also *in vitro* as well as *in vivo* studies are required for better understanding of toxicological aspects that will be related to AgNPs.

Conflicts of interest

We declare no conflict of interest involved in this study.

Taxonomy (classification by EVISE)

Identify the disease/health condition, the experimental approach, the methodology.

Solvent extraction, anti-microbial activity and MIC of extracts and silver nanoparticles by standard methods.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtcme.2019.04.007.

List of abbreviations

XRD	X-ray diffraction
DMSO	Dimethyl sulfoxide
MIC	Minimum inhibitory concentration
AgNPs	Silver nanoparticles
UV- Vis	UV–Visible spectroscopy
AFM	Atomic force microscopy

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