



Assessment of in vitro biopotency of bioderived silver nanoparticles from *Aegle marmelos* (L.) fruit extract

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Abstract

Aegle marmelos (L.) or bael commonly known as wood apple, is rich in bioactive compounds has diverse pharmacological importance and is widely employed for the synthesis of green nanoparticles. The current study aims to evaluate the phytochemical components of *A. marmelos* (L.) by biochemical assays and its antioxidant activity. Further, silver nanoparticles synthesized from *A. marmelos* (L.) fruit extract exhibited antibacterial effect against pathogens such as *Micrococcus luteus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*. The phytochemical screening revealed the phytochemicals such as saponins, flavonoids, alkaloids, phenols, and tannins. UV–Vis spectrophotometry confirms silver ions in the biosynthesized nanoparticles. Further, FT-IR revealed the chemical bonds of alkanes, amines, alcohols, and alkenes. Bioderived silver nanoparticles (AgNPs) showed the highest inhibitory activity against *Staphylococcus aureus* (29.33 ± 1.59 mm) followed by *Enterococcus faecalis* (20.14 ± 1.5 mm), *Micrococcus luteus* (19.33 ± 1.3 mm), *Pseudomonas aeruginosa* (16.23 ± 1.2 mm), and *Escherichia coli* (16.13 ± 1.5 mm) at the concentration of 100 μ l the highest. The antioxidant activity of different concentrations (25, 50, 100, and 200 μ l) of biosynthesized AgNPs were 40%, 46%, 57%, and 61%, respectively. The cytotoxic effect of bioderived nanoparticles on VERO cell lines by MTT assay showed increased cytotoxicity with an increase in concentration and the highest IC₅₀ value recorded at 1000 μ g/ml. These results indicate that the bioactive components of *A. marmelos* (L.) fruit extract exhibited potential antioxidant activity. Bioactive silver nanoparticles showed the highest antibacterial prospects that can be exploited in new drug and antiseptic lotion formulations of herbal origin with sustainable synthesis and application.

Keywords *Aegle marmelos* (L.) · Biochemical · Phytochemical · Antibacterial · Cytotoxic · Bioderived

Introduction

Nanotechnology, an interdisciplinary field of innovative science dealing with the study of materials in nano-range (1–100 nm) has revolutionized various fields in the last few decades. Green nanotechnology combines the principles of nanotechnology and green chemistry aiming to build environmental sustainability by adopting greener, safer and eco-friendly mechanisms. Green nanoparticles are synthesized by bottom-up approach where single-step bioreduction is carried out requiring only less amount of energy. Predominantly, plant extracts (sugars, proteins, polyphenols, terpenoids, phenolic acids, and alkaloids) are used for the bioreduction of metal ions. Polyphenols replace the capping/stabilizing/ reducing agents during bioreduction. Antioxidant protects the cells and tissues against damage by reactive oxygen species. Flavonoids in plants exhibit effective

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antioxidant potential. Almost all plant parts such as leaves, bark, seeds, fruits, and flowers can be utilized for metallic nanoparticle synthesis by green methods.

Plants are ubiquitous and widely employed in the synthesis of green nanoparticles due to less energy consumption, rapid and bulk synthesis, cost-effectiveness, and production of safe and non-toxic phytochemical derivatives. Both the magnetic and non-magnetic forms of bioderived silver nanoparticles benefit the biomedical field in diagnostics as well as in the treatment of cancer, diabetes mellitus, stroke, and malaria. The physical and chemical properties of bioderived silver nanoparticles such as reduced size, high affinity, larger surface area, unique optical and surface plasmon resonance property, high compatibility, fluorescence emission, and light-scattering ability aid in targeted drug delivery. It pierces the internal blood barrier and aids in more accurate diagnosis using magnetic resonance imaging (MRI) and computed tomography (CT) (Nguyen et al. 2022 and Tran et al. 2022). Silver nanoparticles (AgNPs) have received much recognition in various fields such as targeted drug delivery, dye degradation, water purification, biomedical device coating, and food packaging. Due to their antibacterial activity against human pathogens and safe eco-friendly nature, they are used in medical applications like antibacterial, antifungal, antiviral gels, creams, and wound healing bandages. AgNPs are synthesized from many plants extracts some of which include *Boswellia serrata* (Kora et al. 2012), *Alternaria alternate* (Agnihotri et al. 2014), *Citrus maxima* (Sarvamangala et al. 2013), *Desmodium gangeticum* (Thirunavoukkarasu et al. 2013), *Thevetia peruviana* (Rupiasih et al. 2013), *Piper pedicellatum* (Tamuly et al. 2013), *Centella asiatica* (L) (Rout et al. 2013), *Myrmecodia pendans* (Zuas et al. 2014), *Tectona grandis* (Nalvothula et al. 2014), and *Santalum album* (Ali et al. 2016), respectively.

Aegle marmelos (L.) (Family: Rutaceae), a tree native to India, traditionally known as wood apple or bael is used in Ayurveda, for the treatment of swollen joints, wound healing, pregnancy illness, snake bites, high blood pressure, eye problems, fever, burning sensation on skin, diarrhea, skin problem, and urinary troubles. The fruit contain bioactive compounds like marmelide, luvangetin, auraptene, psoralen, and tannin. The flavonoids act as antioxidants, saponins are responsible for foaming and antifungal property (Venkothdika et al. 2021). The fruit shell possesses a skin exfoliating agent, and the pulp has anti-inflammatory and antipyretic action. The leaf and stem bark extract from wood apple possesses anti-tumor and antimicrobial activity (Rajaram et al. 2018).

The ban on certain drugs and chemical nanomaterials such as triclosan and triclocarban by FDA has opened up the avenues for bio-based nanomaterials in medical, environmental and agricultural fields. Solubility in the aqueous medium, oxidation under aerobic condition, flexibility for

surface modification in drug delivery, ease in synthesis and modifications, and capping ability with other plant-based molecules tailored for specific targets in any medium makes biogenic silver nanoparticles, a great choice of study. Additionally, employing *A. marmelos* (L.) with several properties such as antidiabetic, antimicrobial, larvicidal, catalytic, anti-carcinogenic, antipyretic, cytotoxicity for silver nanoparticle synthesis would be an added advantage for this study. *A. marmelos* (L.) leaf extract has been extensively employed for the synthesis of green nanoparticles in the form of metal oxides (Pathirana et al. 2020). The fruit and fruit peel extract of *A. marmelos* (L.) are scarcely studied for the combined antibacterial efficacy and cytotoxicity.

The present study aims to bring out the bioactive components of *Aegle marmelos* (L.) fruit extract and its antioxidant property by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Further, silver nanoparticles from the fruit extract of *Aegle marmelos* (L.) was investigated for the physiochemical nature, antibacterial, and cytotoxic activity. However, further studies on antifungal efficacy, enhanced activation by surface modifications and structural analysis, and application of synthesized green nanoparticles in drug delivery needs to be explored further.

Materials and methods

Materials

The chemicals such as ferric chloride, chloroform, concentrated sulphuric acid (H_2SO_4) lead acetate, dilute hydrochloric acid, picric acid, Bradford reagent, aqueous ammonia solution, silver nitrate, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Dimethyl sulfoxide (DMSO), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Merck laboratory, India. Nutrient agar and nutrient broth were purchased from Himedia, India. The fruits were collected from the local market in Chennai, India. The bacterial strains such as *Escherichia Coli* (MTCC 1089) (*E. coli*), *Micrococcus luteus* (MTCC 2452) (*M. luteus*), *Enterococcus faecalis* (MTCC 3159) (*E. faecalis*), *Staphylococcus aureus* (MTCC 1144) (*S. aureus*), and *Pseudomonas aeruginosa* (MTCC 1034) (*P. aeruginosa*) were procured from National Centre for Biological Sciences, Bangalore, India. VERO cell lines were procured from National Centre for Cell Science (NCCS), Pune, India. Sterile distilled water was used for the entire experiment.

Preparation of aqueous fruit extract

The rind of *Aegle marmelos* (L.) from the fresh fruit is separated, washed thoroughly with distilled water and air-dried. The dried rind was grounded to obtain a powder. About 10 g

of the powder was weighed and transferred to 100 ml of distilled water, mixed well and boiled for 1–2 h (Krupa and Raghavan 2014). The filtrate was collected using Whatmann No.1 filter paper and stored for further use.

Bioactive components

The presence of bioactive components like glycosides, alkaloids, tannins, flavonoids, saponins, and steroids was examined from the fruit peel extract of *Aegle marmelos* (L.) by qualitative phytochemical assays (Kushwah et al. 2019).

Bioderived nanoparticle synthesis

Fruit peel extract of *Aegle marmelos* (L.) (10 ml) was added to 90 ml of distilled water to prepare aqueous plant extract. 0.0153 g of silver nitrate (AgNO_3) was augmented to aqueous extract and boiled for 10 min at 80 °C. Colour change to brown was visualized which confirms the reduction of silver nitrate to silver ions (Krupa and Raghavan, 2014). The solution was centrifuged at 5000 rpm for 20 min. The settled particles were transferred into glass plates and dried in a hot air oven and stored for future purposes.

Analysis of biosynthesized silver nanoparticles

The characteristics of bioderived silver nanoparticles were determined by techniques such as UV–Vis spectrophotometer and Fourier Transform Infrared (FT-IR) spectroscopic analysis. Silver ion reduction from AgNO_3 to AgNPs was confirmed by scanning in UV–Vis spectrophotometer at the wavelength range from 300 to 700 nm for absorption peaks at the respective wavelength. The determination of functional groups from silver nanoparticles was determined with FT-IR Spectrophotometer.

Antioxidant activity

The free radical scavenging capacity of the bioderived silver nanoparticles (25, 50, 100, 200 μl) was determined using DPPH assay. Absorbance was recorded at 517 nm against blank and the scavenging property was estimated as the percentage of radical scavenging.

Antibacterial efficacy

The antibacterial efficacy of bioderived silver nanoparticles (0.5 $\mu\text{g}/\mu\text{l}$ concentration) at different volumes (25, 50, 100 μl) were examined for antibacterial activity against certain gram-positive and gram-negative bacterial strains by well diffusion method on Muller Hinton agar upon incubation at 37 °C for 24 h. The bacterial strains such as *M. luteus*, *E. faecalis*, *S. aureus*, *P. aeruginosa*, and *E. coli* were used for

examination. The extent of antibacterial efficacy of AgNPs was examined by zone of inhibition around the well.

Cytotoxic assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide) assay was used to determine the cytotoxicity assay or cell viability of AgNPs on the VERO cell line. The cell line was kept alive in DMEM (Dulbecco's Modified Eagle Medium) supplemented with penicillin (100 U/ml), 10% Fetal Bovine Serum (FBS), and streptomycin (100 g/ml) at 37 °C in a humid environment with 50 ng/ml CO_2 . In 24-well plates with 1×10^5 cells per well, the cells were mounted and incubated at 37 °C with 5 percent CO_2 . After the cells had reached confluence, different quantities of AgNPs were applied, and the cells were then incubated for 24 h. Phosphate-buffered saline (pH 7.4) or DMEM without serum were used to rinse the cells. 100 l/well of 0.5 percent MTT was added for 4 h, and then 1 ml of DMSO was added. Using DMSO as a blank, the UV–Vis Spectrophotometer was used to analyze the absorbance at 570 nm. Additionally, the percentage of viable cells was calculated, and the IC50 the dose needed to cause 50% inhibition was established.

Results and discussion

The bioactive phytochemicals extracted from the fruit pulp of *Aegle marmelos* (L.) resulted in the synthesis of silver nanoparticles (AgNPs) by bioreduction of silver ions. Bioderived silver nanoparticles were characterized by UV–Vis spectrophotometer and FT-IR. Further, the antibacterial efficacy and cytotoxic activity of the bioderived AgNPs were determined with bacterial strains and VERO cell line. Table 1. represents the various bioactive compounds with antibacterial activity range, nanoparticle size of *Aegle marmelos* (L.) derived Ag nanoparticles. The different types of extracts from *Aegle marmelos* (L.) leaves and fruits possess different bioactive compounds with various range of size of silver nanoparticles depending upon the conditions such as pH, temperature, reducing agent and method of the green synthesis process. It was also evidenced that the antibacterial activities of *Aegle marmelos* (L.) derived AgNPs connected with the NPs size, shape, type of extract and nature of bacterial species. From Table 1, methanolic fruit extract and aqueous leaves extract of *Aegle marmelos* (L.) derived AgNPs plays the main role in bioactive molecules elevation and activities. Comparatively the antibacterial activity of *S. dysenteriae* (16.50 ± 0.30) in fruit extract and *P. aeruginosa* (20) in leaves extract showed evidence of excellent activity when compared with other bacterial species.

Table 1 Bioactive compounds and antibacterial activity from *Aegle marmelos* (L.) derived AgNPs

S.No	Type of <i>Aegle marmelos</i> (L.) extract	Reactive chemical compound	Name of the bacteria and activity range (mm)	Size of AgNPs (nm)	Authors
1	Methanolic fruit extract	Tannins, saponins, steroids, alkaloids, flavonoids, and glycosides	<i>B. cereus</i> (16.17±0.50) <i>P. aeruginosa</i> (13.33±0.62) <i>S. dysenteriae</i> (16.50±0.30)	70–192	(Devi et al. 2020a, b)
2	Aqueous leaves extract	Skimmianine, lupeol, citral, cineol, citronellal, aegelin, marmesin, eugenol, cumin aldehyde	<i>E. coli</i> (14) <i>S. aureus</i> (16) <i>S. typhi</i> (18) <i>P. aeruginosa</i> (20)	104–129	(Subasri et al. 2019)
3	Aqueous fruit extract	Alkaloids of which aegeline (N-[2 hydroxy-2(4 methoxyphenyl)ethyl]-3-phenyl-2 propenamide)	<i>P.aeruginosa</i> NV2 (5.0±0.12) <i>Microbacterium</i> sp. NV4 (5.2±0.76) <i>P. otitidis</i> NV1 (4.0±0.67) <i>S.hominis</i> NV5 (3.0±0.92) <i>E. cloacae</i> NV3 (2.1±0.91)	34.7	(Krupa and Raghavan, 2014)
4	Aqueous leaves extract	Flavonoids, alkaloids and terpenoids	No bacterial activity		(Banu et al. 2018)
5	Aqueous leaves extract	NA	<i>B. subtilis</i> (4) <i>P. aeruginosa</i> (8)	NA	(Christopher et al. 2015)
6	Aqueous leaves extract	Phenols	NA	60	(Korukonda and Paria, 2012)
7	Aqueous leaves extract	NA	<i>S. pyogenes</i> (8±0.3) <i>E.coli</i> (6±0.1) <i>P. aeruginosa</i> (6±0.05) <i>S. aureus</i> (7±0.05) <i>A.hydrophila</i> (7±0.4)	1.54	(Patil et al. 2015)
8	Aqueous leaves extract	Phenolic acid, flavonoids, quinone	<i>B. megaterium</i> (5±0.15) <i>B. aryabhatai</i> (5±0.25) <i>S. aureus</i> (6±0.15) <i>S. marcescens</i> (7±0.15) <i>P. putida</i> (5±0.01)	5–30	(Sampath et al. 2021)
9	Aqueous and solvent (chloroform and ethanol) leaves extract	Alkaloids carbohydrates glycosides saponins, phytosterols, phenols, tannins, flavonoids, protein, amino acids, diterpenes,	<i>E. carotovora</i> and <i>P. syringae</i>	NA	(Samrot et al. 2019)

NA not available

Phytochemical characterization of bioactive components

Substantial use of bael in Ayurveda and traditional medicine has been attributed to its nutritional phytochemicals. The phytochemical screening of *A. marmelos* (L.) fruit extract

showed phytochemicals such as alkaloids, flavonoids, terpenoids, phenols, protein, and carbohydrates (Fig. 1). The phytochemical analysis of the fruit extract for the presence of alkaloids, flavonoids, terpenoids, phenols, protein, and carbohydrates showed the presence of these compounds by colour formation. The presence of flavonoids in the extract

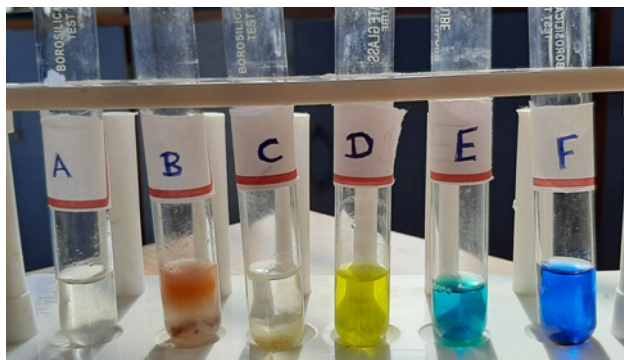


Fig. 1 Phytochemical analysis of *A. marmelos* (*L.*) fruit extract

was confirmed by the formation of brownish-green colour by the addition of ferric chloride solution. The appearance of reddish-brown colour by the addition of chloroform and concentrated sulphuric acid confirmed the presence of terpenoids. Similarly white precipitate confirmed the presence of phenols, blue colour denoted proteins and pink-red colour showed the presence of carbohydrates when the respective phytochemical analysis was performed. The phytochemicals such as alkaloids, coumarins, flavonoids, tannins, and phenolic acids have been reported in bael. Apart from these, amino acids, fatty acids, vitamins, carbohydrates, minerals, organic acids, and fibres also make bael fruit highly rich in nutrients with many health benefits.

The flavonoids and polyphenols include coumarins, alkaloids, carotenoids and polysaccharides (Gurjar et al. 2019). Certain phenols found in bael include arbutin, p-coumaric acid, caffeic acid, quinic acid, chlorogenic acid, protocatechuic acid and p-coumaroyl (Bharadwaj and Nandal, 2015). The phytochemicals generally act as reducing agents, stabilizing agents and solvent mediums for the synthesis of green nanoparticles (Ovais et al. 2018a, b). The phenolic compounds with metal-chelating ability having antioxidant potential serve as reducing agents and aid in the oxidation process that favours the synthesis of AgNPs (Ovais et al. 2018a, b). Flavonoids, are the secondary metabolites capable of donating hydrogen atoms or electrons act as reducing agents (Zhou et al. 2010). Free hydrogen produced during the formation of flavonoid (luteolin) aided in the reduction of Ag^+ ions in AgNPs synthesis. During the bioreduction of metal ions, the -OH group of the flavonoid quercetin was oxidised to carbonyl groups. (Ghoreishi et al. 2011). Due to the near proximity of the oxo and hydroxyl groups, as well as the catechol moiety, the hydroxyl functions of flavonoids play a crucial role in reducing Ag^+ ions to metallic silver and chelation of metals. Terpenoids, derivatives of essential oils contain hydrocarbons and functional groups like organic acids, ethers, esters, lactones, alcohols, aldehydes, ketones, phenols and phenolic ethers (Edris 2007) and aid

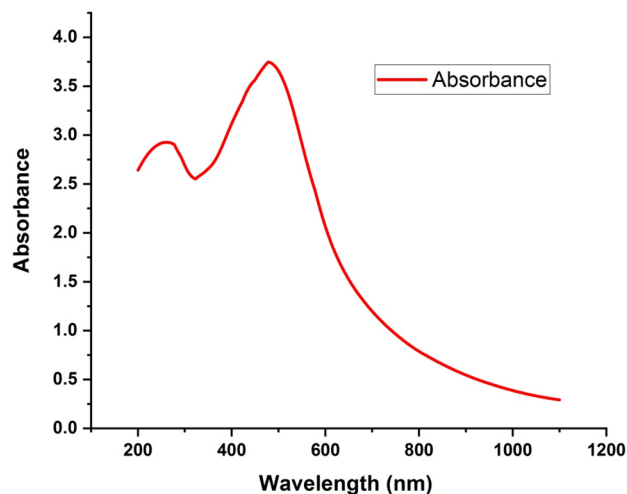


Fig. 2 UV-Vis absorption spectrum of biosynthesized AgNPs of *A. marmelos*

in the reduction as well as complexing agents (Peddi and Sadeh 2015). The carbohydrates such as glucose also have a higher reduction potential and the synthesis of AgNPs using plant extracts were strongly influenced by polyphenols and carbohydrate content (Kumar et al. 2019).

Bioderived nanoparticle synthesis and characterization

The silver ion was formed by adding the silver nitrate solution to the fruit peel extract. The brown colour formation confirms the presence of silver ions. The oxidised version of the functional groups in the plant extract, which operate as antioxidants, served to cap the Ag nanoparticles (Dimkpa et al. 2013). These bioactive compounds in bael play an essential role in the reduction of silver ions (Devi et al. 2020a, b) towards silver nanoparticle formation. The synthesized nanoparticle was analyzed by UV-Vis spectrophotometer from 300–700 nm. The highest absorption peak was observed at 453 nm which indicates the formation of silver ions (Fig. 2). Biosynthesized silver nanoparticles were analyzed by UV-Vis spectrophotometer which showed the spectral peaks at 400, 430, and 459 nm from *Lycopersicon esculentum*, *A. marmelos* (fruit pulp extract), and *Rhynchosyche ellipticum* (Sunita and Palaniswamy, 2017, Hazarika et al. 2014). The formation of silver nanoparticles was influenced by the phytochemical components. The components also had a significant impact on the structure of ruthenium and cerium oxides. Cerium materials with a CeFe_2O_4 composition exhibited inverse spinel, whereas ruthenium materials featured a combination of carbonous iron oxides and RuO_2 . Ruthenium materials

showed enhanced photocatalytic activity and degraded Congo red with the removal efficiency of 55% after a 6-h reaction following first-order kinetics (Hernández et al. 2022). Ruthenium and lanthanides in the form of iron oxides gain importance in the photo-oxidation of Congo red (Nguyen et al. 2022).

Further characterization of silver nanoparticles with FT-IR spectrophotometer showed vibration stretches at different wave numbers (Fig. 3). O–H stretching vibrations between 3200 and 3400 cm^{-1} denotes phenols and alcohols, C=C stretching between 1575 and 1675 cm^{-1} indicates alkenes and aromatics, peak at 1029 cm^{-1} indicates C–N stretching and denotes the presence of aliphatic amines. Similarly, peak at 666 cm^{-1} indicates C=C stretching shows the alkenes group. The peak between 600 and 562 cm^{-1} indicates C–I stretching and the presence of halo compounds such as chloroalkanes and bromoalkanes. The stretching vibrations of O–H for polyphenols, C–O for alkenes and alcohols,

ether stretching vibration of C–O group, and C–H for aromatic compounds were witnessed by FT-IR for *A. marmelos* extract (Kushwah et al. 2019). Infrared bands at 3271, 1637, and 386 cm^{-1} indicated the stretching of O–H/N–H, C=O in the amine group and Ag metal in the screening of silver nanoparticles from tea leaf extract (Loo et al. 2012).

Apart from nanomaterial detection, the structural characterization with UV–Vis and FT-IR can be extended to particles and doped materials such as polymers. UV–Vis spectral analysis of ZnO and Ni-doped ZnO showed absorption peaks at 342 nm and 400 nm. The absorption in the visible region (redshift) was due to sp–d exchange interactions, and oxygen deficiency. The interactions between the particle and polymer were evident by the peaks at 3026 cm^{-1} (O–H stretching), 2924 cm^{-1} and 1600 cm^{-1} represented C–H and C=C bonds. Peaks at 1155, 1068, and 697 cm^{-1} denote Ni–O due to π –d electron charge transfer and the characteristic peak at 416 cm^{-1} represents Zn–O (Minhas et al. 2019).

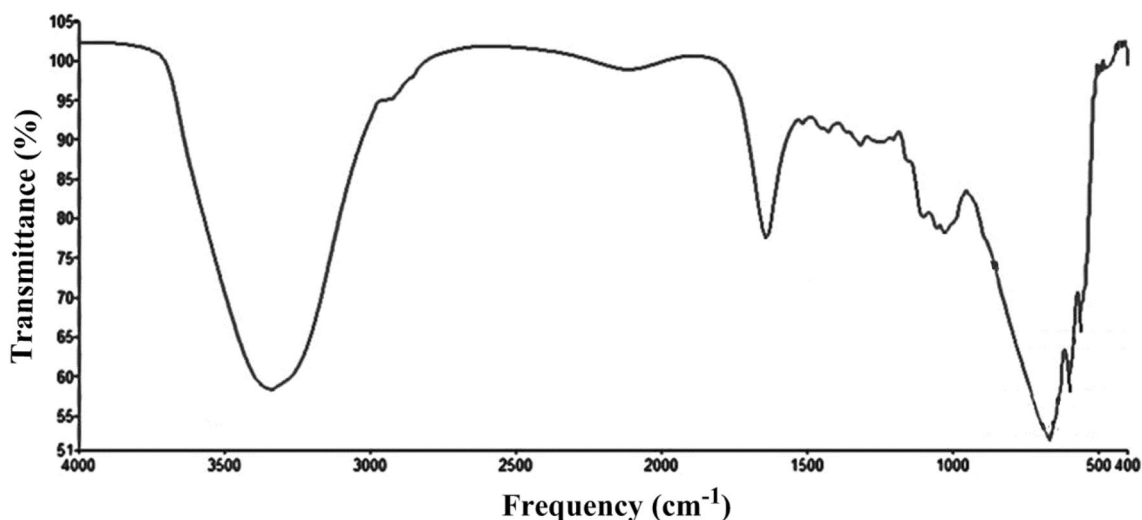
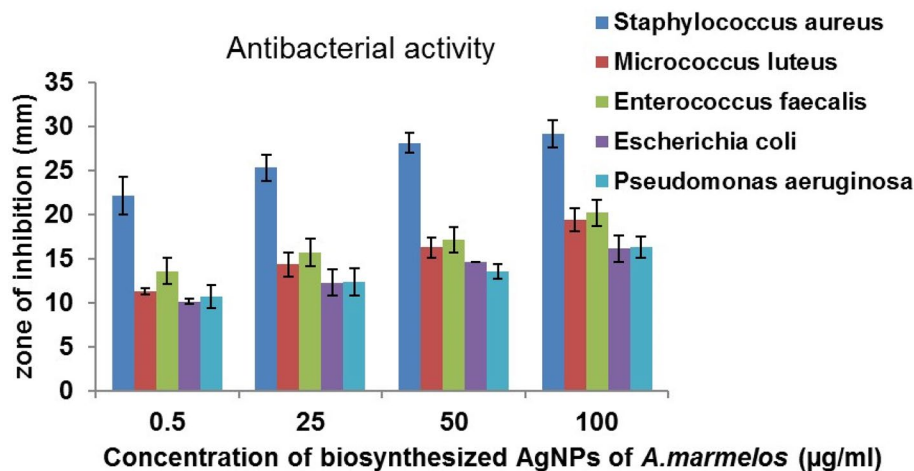


Fig. 3 FT-IR spectrum of biosynthesized AgNPs of *A. marmelos*

Fig. 4 Antibacterial activity of biosynthesized AgNPs of *A. marmelos* on different bacterial strains



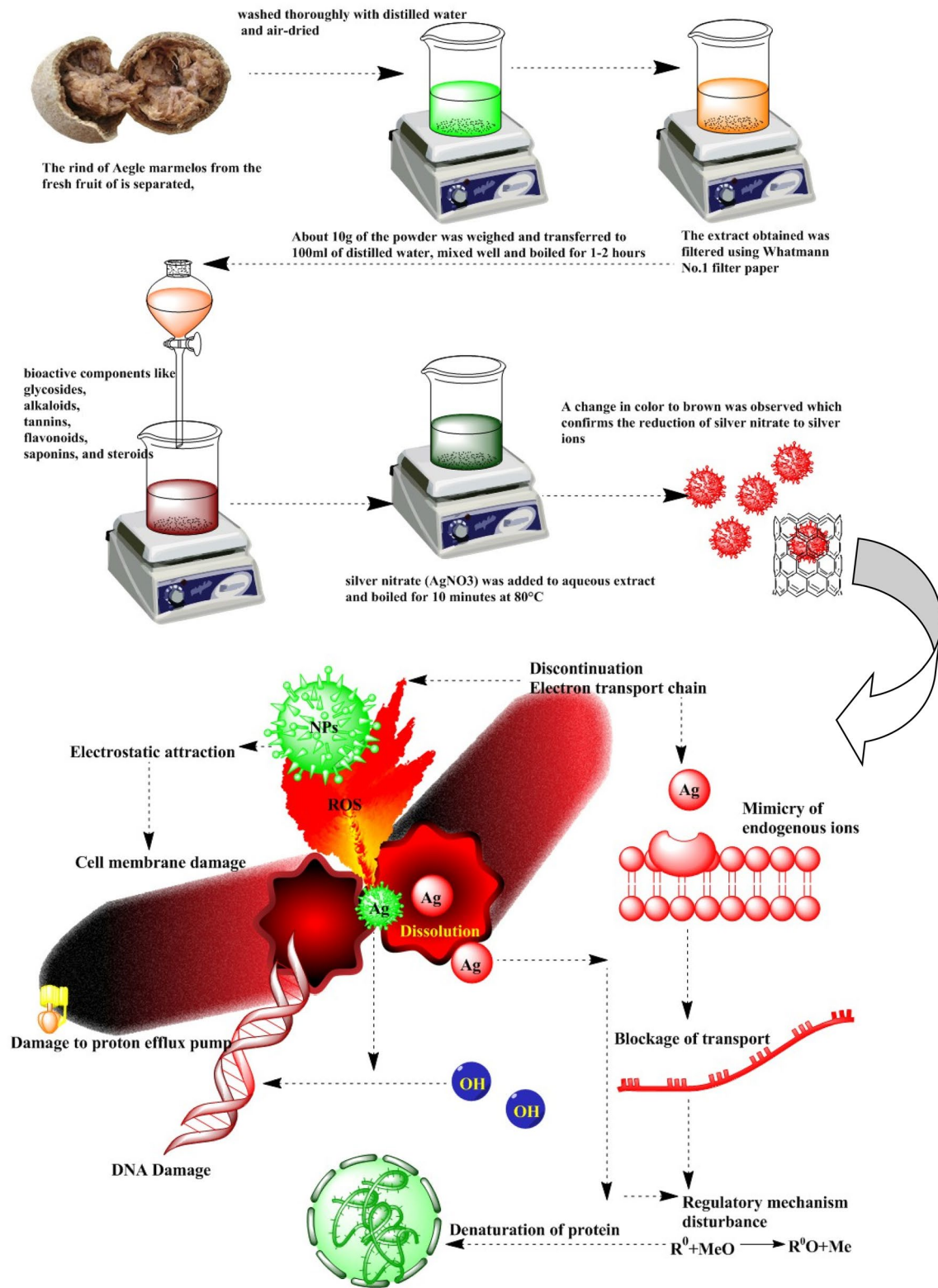


Fig. 5 Schematic diagram synthesis of NPs and antimicrobial mechanism of silver nanoparticles on bacterial strains

Antimicrobial efficacy of bioderived AgNPs

The silver ion particles exhibit antimicrobial property and were checked with a range of bacterial strains. The bacterial inhibitory efficacy improved with an increase in the concentration of biosynthesized silver nanoparticles. Bioderived AgNPs exerted high antibacterial activity over *Staphylococcus aureus* followed by *Enterococcus faecalis*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, and *Escherichia coli* (Fig. 4). Even the lowest concentration of 0.5 $\mu\text{g/ml}$ exerted inhibitory effects on the bacterial strains. The mechanism of antibacterial action of silver nanoparticles includes disintegration of cell membrane, protein inactivation, and inhibition in the replication of DNA (Fig. 5). The reaction of silver nanoparticles with DNA or other biomolecule leads to the formation of reactive oxygen species (ROS) causing cellular stress and damage. The bacterial peptides are changed by silver nanoparticles, which inhibits both growth and signal transmission. Bacterial cell lysis is caused by the disruption of the molecular conformation and the breaking of the H-bonds between the antiparallel base-pair strands by silver ions (Bapat et al. 2022). In the current study, the aqueous fruit extract of *Aegle marmelos* (L.) derived AgNP displayed significant antibacterial activity against *S. aureus* (28.12 ± 1.1), *M. luteus* (16.22 ± 1.1) and *E. faecalis* (17.12 ± 1.4) in 50 $\mu\text{g/ml}$. The five different pathogenic bacterial strains used in this study have comparable tendency in terms of zone of inhibition with different concentration of biosynthesized AgNPs from *Aegle marmelos* (L.). The bioactive molecules in the extract designate a good antibacterial effect on *S. aureus* compared with other pathogenic strain used (Behera et al. 2014). Our results are in line with the previous study by Kumar et al. (2019) where the stem and leaf extracts of *Aegle marmelos* (L.) reported significant antibacterial effect against *Escherichia coli* and *Pseudomonas aeruginosa*. Similarly the silver nanoparticles from apple extract had antibacterial efficacy against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, and MRSA strains (Ali et al. 2016). The leaf extract from *Santalum album* showed antimicrobial efficacy against several pathogenic and soil borne bacteria, highly effective against *Pseudomonas aeruginosa* (Swamy and Prasad, 2012). Silver nanoparticles from *Pelargonium graveolens*, *Actaea racemosa*, *Aloe sp.*, *Magnolia grandiflora*, *Sansevieria trifasciata*, *Impatiens balsamina*, and *Eucalyptus angophoroides*, exerted antibacterial efficacy against several G-positive and G-negative bacterial human pathogens (Okafor et al. 2013).

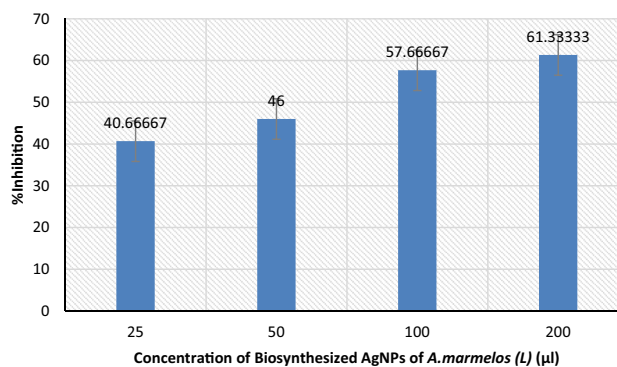


Fig. 6 Percent inhibition of biosynthesized AgNPs at different concentration by DPPH assay

Antioxidant activity and cytotoxicity of bioderived AgNPs

The synthesized silver nanoparticle was also tested for its antioxidant activity by DPPH scavenging assay. The maximum concentration (200 $\mu\text{g/ml}$) of percentage inhibition was recorded as 61.33 ± 0.59 . The percentage inhibition of bioderived silver nanoparticles increased with the increase in the concentration (Fig. 6). The in vitro cytotoxicity of AgNPs on VERO cell line for cell proliferation was analyzed by MTT assay. The dose-dependent inhibition was observed in AgNPs treated VERO cells and the increase in concentration of AgNPs (7.8, 15.6, 31.2, 62.5, 125, 250, 500, 1000 $\mu\text{g/ml}$) showed increased cytotoxicity in VERO cells (Fig. 7). The IC₅₀ value for VERO cells was recorded

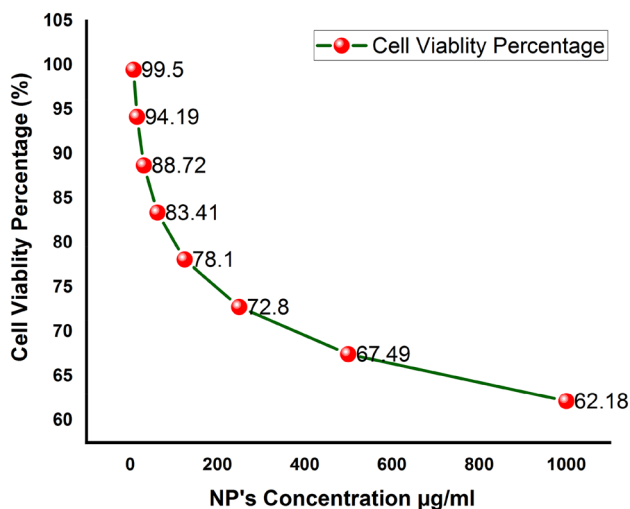


Fig. 7 Cytotoxicity effect of biosynthesized AgNPs on VERO ovarian cell lines (% Cell viability)

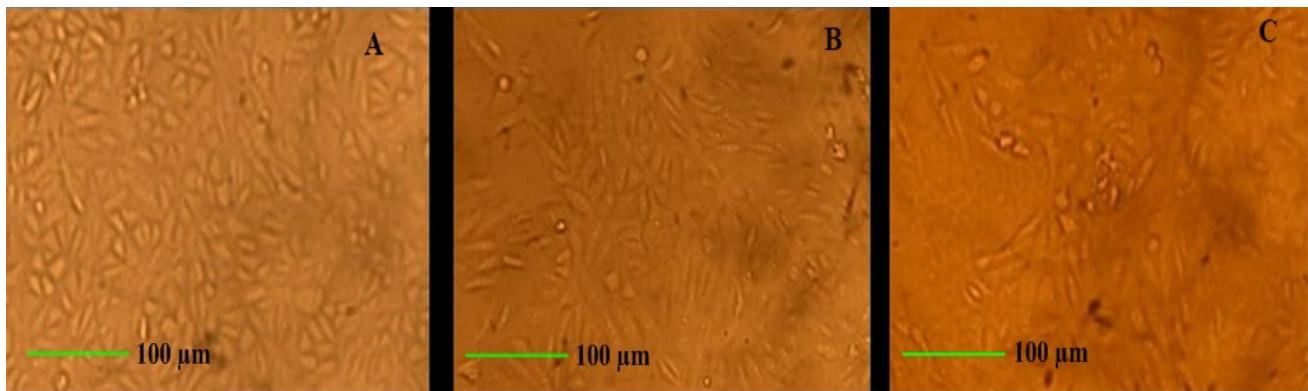


Fig. 8 Cytotoxic effect of biosynthesized AgNPs of *A. marmelos* on VERO cell lines **A.** control **B.** 7.88 µg/ml AgNP treated cell line **C.** 1000 µg/ml AgNP treated cell line. Scale ruler (10×) Inverted microscopy

at 1000 µg/ml. A prominent decrease in the cell viability was observed in AgNPs treated cells compared to the control (Fig. 8. A, B, C). Silver metal possess antibacterial, antifungal, and antioxidant properties (Kim et al. 2014; Jeeva et al. 2014). An earlier report on the inhibition of silver nanoparticles from *A. marmelos* fruit peel extract upon *E.coli* showed the inhibition zone of 16 ± 0.5 cm at 12.5 µg/ml (Kushwah et al. 2019). Wu et al. 2020 reported that phenolic compound enhances the antioxidant efficacy of silver nanoparticles. The increase in bioderived silver nanoparticles led to a high rate of toxicity and cell death in VERO cell line (Madhumithra et al. 2018). Ag nanoparticles from *Morinda citrifolia* root extract exhibit a cytotoxic effect on Hela cell lines (Suman et al. 2013). Apart from antimicrobial, antifungal, antioxidant and anticarcinogenic properties, biosynthesized silver nanoparticles from plant extracts find wider applications as plant growth promoters inhibiting rice pathogens (Ibrahim et al. 2019), effective controllers of vectors such as mosquitoes (Hatem et al. 2016), drug delivery vehicles (Kumar and Poornachandra 2015) protective food packaging (Kanmani and Lim, 2013), biomedical applications (Priyadarshini et al. 2013), dye degradation (Saravanan et al. 2017). The high surface area and improved optical properties of TiO₂ nanoparticles produced by sol-hydrothermal method followed by sonochemical activation showed enhanced photodegradation of methyl orange with 99.9% efficiency (Athawale et al. 2020). Carcia papaya leaves have significant therapeutic properties similar to *A. marmelos* (*L.*) was employed for the synthesis of fluorescent carbon dots and found to exhibit biological activities at a lower concentrations with maximal effect of free radical scavenging activity (27.6 µg/ml), antioxidant activity (23.00 µg/ml), in vitro anti-inflammatory activity (15.52 µg/ml) (Gudimella et al. 2022).

Conclusion

This study reports the antibacterial, cytotoxicity and antioxidant efficacy of green silver nanoparticles from the fruit peel extract of *A. marmelos* (*L.*). The fruit peel extract was extracted in a green way without any use of solvents. The aqueous fruit extract was found to possess bioactive compounds such as flavonoids, phenolic compounds, carbohydrates, and proteins. These bioactive compounds aided in the bioreduction and formation of silver ions. Biosynthesized silver nanoparticles exhibited antibacterial activity against several bacterial strains. AgNPs possess antioxidant activity and had cytotoxic effects on VERO cell lines. The extracted silver nanoparticle can be a potent antibacterial agent against several pathogenic bacteria and can be employed in biomedical applications. The limitation of the current study is predicting the bioactivities without knowing the exact active compounds and mechanism that is responsible for the evidenced activity with no appropriate scientific validation. Hence, further studies could be carried out from the bioactive compounds of biosynthesized AgNPs of *A. marmelos* (*L.*) for exploring antifungal, antidiabetic and anticarcinogenic activities for the formulation of novel antibiotics.

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Declarations

Conflict of interest The authors express no conflict of interests.

Ethical statement The manuscript is the author's original work which has not been published anywhere or being considered for publication elsewhere.

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