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ORIGINAL ARTICLE

Synthesis and characterization of Silver nanoparticles from fruit extract of *Michelia Champaca* L.: Their antioxidant and antibacterial activity

Azharuddin Daphedar[1*], Madhu Ritti[2], Naganagouda Patil[2], Suprita Patil[2], Siddappa Kakkalameli[3], Tarikere Chandrashekharappa Taranath[2]

[1] *Anjuman E-Islam's Anjuman Degree College, Vijayapura, Karnataka-586 101, India.*
[2] *Environmental Biology Laboratory, P. G. Department of Studies in Botany, Karnatak University, Dharwad, Karnataka 580003, India.*
[3] *Davangere University, Shivagangotri, Davangere Karnataka-577 002, India.*

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Abstract

Plant mediated synthesis of silver nanoparticles (AgNPs) is considered as a representative approach in material synthesis for environmental benignity. In this paper, we report an eco-friendly (green) protocol for the preparation of AgNPs using fruit extract of Michelia champaca L. The color change in experimental solution from light brown to dark brown indicates the resonance of AgNPs and further confirmed by characteristic Surface Plasmon Resonance (SPR) absorption peak at 410 nm using UV-Vis spectroscopy. Fourier Transform Infrared Spectroscopy (FTIR) analysis confirms the presence of phytochemicals like phenols and flavonoids in the fruit extract which are responsible for reduction and stabilization of AgNPs. The biocapping molecules of nanoparticles were possibly stable and were negatively charged as revealed by zeta potential measurement. Further, the size and morphology of the nanoparticles were studied by using Atomic Force Microscopy (AFM) and High Resolution Transmission Electron Microscope (HRTEM) analysis. The AgNPs were evaluated for antioxidant and antibacterial activities. The DPPH radical scavenging assay showed good antioxidant activity of AgNPs (EC50= 532.16 µg/mL) when compared to both fruit extract (EC50= 261.08 µg/mL) and standard ascorbic acid (AS) (EC50= 426.04µg/mL). The AgNPs exhibited potent antibacterial activities against both gram positive and gram negative bacteria. The *Pseudomonas aeruginosa* (19.00±0.73a mm) showed the highest zone of inhibition at 1000µg/ml concentration of AgNPs solution followed by Staphylococcus aureus (9.00±0.14a mm), Bacillus cereus (10.00±0.19a mm) and Escherichia coli (10.66±0.18a mm). Finally, it can be concluded that AgNPs from *Michelia champaca* fruit extract showed distinctive free radical scavenging and potent antibacterial activity.

Keywords: *Antioxidant; Antibacterial Activity; Characterization; Michelia Champaca; Silver Nanoparticles.*

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INTRODUCTION

In the current scenario, metal based nanoparticles have been extensively investigated for a set of different biomedical applications [1]. Nanomaterials are ultimate fine particles with ranging from micrometers to several nanometers. In general, the very small particle enhances the reactivity of each particle, improved thermal conductivity, catalytic reactivity, nonlinear optical performance, and chemical stability due to their large surface area-to-volume ratio which increases their interaction with other molecules [2]. The advent of new nanomaterials is being perceived in many fields of science and technology, which include food industries, agriculture,

* Corresponding Author Email: azhar2887@gmail.com

material science, bioinformatics, cosmetics, textiles, medicine, environmental remediation, biotechnology, information technology and pharmaceuticals so on and so forth [3].

Over many years, silver nanoparticles (AgNPs) were verified to inhibit hundreds of microorganisms including pathogens such as bacteria, viruses and fungi [4]. The biogenic AgNPs also exerted proficient anti-cancer activity towards Triple-negative breast cancer (TNBC) cells in vitro and in vivo [5]. Silver ions and silver based chemicals are profoundly lethal to micro-organisms but not to human beings [6]. For synthesis of nanoparticles noble metals such as silver, gold, zinc, copper and iron are used because of their unique d-d transition characteristics [7]. Among all the metal nanoparticles AgNPs have been used as potent antioxidant, antifungal, anti-oxidant, antibacterial, anti-inflammatory and anticancer activities [8-12]. Currently, silver and its salts are widely employed as an antimicrobial agent for the topical treatment of burns, open wounds and chronic ulcers [13, 14].

Many effective approaches have been devoted for the synthesis of silver nanoparticles including physical, chemical and biological methods. Nowadays, physico-chemical methods employ hazardous chemicals, high temperature, pressure; long reflux time of reaction and result in hazardous by-products which may poses a threat to the human health and environment [15-20]. Thus, there is an obvious need to discontinue this process and need to explore novel, cost effective, pollutant free, and green methods for production of nanoparticles. Biological methods involve the use of microorganisms/biomolecules for the nanoparticle synthesis. In this context, several reports are available. Wherein, wide array of biological system have been used such as algae [21], fungi [22], bacteria [23, 24] and plant extracts [25, 26] has been reported. Typically, plant extract mediated synthesis of nanoparticles is considered to be the best platform and providing natural biocapping agents for the stabilization of nanoparticles.

Michelia champaca Linn. is an (a) large evergreen trees and shrubs belonging to the family Magnoliaceae, native tropical and subtropical South and Southeast Asia [27]. Traditionally, the plant extract of *M. champaca* have been used for a wide variety of purposes or as ethno medicine such as antioxidant, antimicrobial [28], anti-inflammatory [29], leishmanicidal [30], anidiabetic [31] and antitumor activities [32]. The phytochemicals constituents of the medicinal plants contain several health beneficial properties which may protect the body from various diseases such as cancer, inflammatory, diabetic and cardiac disorders. In the present investigation, an attempt has been made to *M. champaca* fruit extract mediated synthesis of AgNPs. The prepared samples were characterized by AFM and HR-TEM analysis and the purity of the sample was tested by FTIR spectroscopy and UV–Visible spectroscopy. Antioxidant and antibacterial activity of AgNPs performed against both Gram positive and Gram negative bacteria.

MATERIALS AND METHODS

Collection of the plant material

The fruits of *Michelia champaca* L. (Synonym *Magnolia champaca* (L.) Baill. ex Pierre) belongs to the family Magnoliaceae were collected from the Karnatak University, campus Dharwad, Karnataka, India.

Preparation of fruit extract

The collected samples were washed 2-3 times with running tap water followed by Milli-Q water to remove dust and unwanted visible particles. The washed leaves were shade dried to remove the residual moisture and cut into small pieces. About 10g incised leaves were transferred to 250 mL of glass beaker containing 100 mL of double distilled water and boiled at 60˚C for 30 minutes. Filtration was done after the extract is cooled to room temperature with the help of Whatman filter paper No. 1 and stored in a refrigerator at 4˚ C for further analysis.

Phytosynthesis of silver nanoparticles

Fifteen milliliter *Michelia champaca* L. fruit extract was added to 250 mL Erlenmeyer conical flask containing 85 mL of silver nitrate ($AgNO_3$) solution and to the pH was adjusted to 8, 9, 10 using 0.01N HCl in and 0.01N NaOH. The change in color of the reaction mixture from light brown to dark brown was noted after the reaction period, which indicates the formation of silver nanoparticle which was confirmed by UV-Vis studies.

Characterization of silver nanoparticles (AgNPs)

The reduction of the silver ions and the formation of silver nanoparticles in aqueous solution were monitored by UV-Vis

spectrophotometry (Jasco Corporation, Tokyo, Japan) by scanning the absorbance in the range 300 to 600 nm at a resolution of 1 nm. The reaction mixtures of AgNPs were centrifuged at 3500 rpm for 30 min (Remi R-8C, Remi, Mumbai, India). The solid residues were centrifuged 2 to 3 times with 10 mL sterile distilled water to obtain the purified pellets of AgNPs and thereafter the suspension was oven-dried to obtain dry powder for FTIR measurement using KBr pellets and spectrum was recorded in the range 400- 4000 cm^{-1}. The colloidal solution of silver nanoparticles was subjected to zeta potential analysis using a Zetasizer (Nano ZS) Instrument (Malvern instrument). It is also used to measure small or dilute samples, and the samples at very low or high concentration by using dynamic light scattering (DLS) with 'NIBS' optics. The surface morphology and size of the silver nanoparticles was monitored using atomic force microscopy (AFM) and high resolution transmission electron microscope (HR-TEM).

DPPH Radical Scavenging Assay

The capability of Ag-NPs for scavenging DPPH (2, 2-diphenyl 1picrylhydrazyl) radical was evaluated by the method described by Blois [33] with slight modifications. Four mg of DPPH was dissolved in 100 ml of methanol to prepare 0.1 mM DPPH solution. A series of dilution of Ag-NPs was formulated using distilled water to make the different concentrations (10, 20, 40, 60, 80, 100 µg/ml). The 3 ml of methanol solution containing DPPH mixed with the different concentrations of AgNPs solution. To 1 ml of sample 2 ml of DPPH was added and incubated in the dark for 30 minutes. After 30 minutes, the reduction of DPPH radicals was monitored using UV-Vis spectrophotometer by measuring the absorption at 517 nm against a blank (methanol). Ascorbic acid (AS) was used as a standard.

Antibacterial activity

The antibacterial activity of biosynthesized AgNPs was investigated using the disc diffusion method against Gram positive (*Staphylococcus aureus, Bacillus cereus*) and Gram negative (*Pseudomonas aeruginosa, Escherichia coli*) obtained from CSIR (Council of Scientific and Industrial Research) lab were used as test organisms. The stock cultures of bacteria was inoculated to the broth media and grown at 37˚C for 18 hrs. Thereafter, Muller-Hinton Agar was poured to agar plates and then each plates were inoculated with 18 hr old cultures (100µl, 10^{-4} cfu), spread evenly on the plate. The Hi-media sterile disc 6 mm diameter was impregnated with silver nanoparticles in different concentrations (200, 400, 800 and 1000 µg/ml). These discs were gently pressed in Muller-Hinton agar plates and incubated for 24 hrs. After incubation, petri dishes were observed for the zone of inhibition (in mm including disc) using the Hi-media antimicrobial zone scale.

Statistical analysis

Statistical analyses were done by One way Analysis of Variance (ANOVA) using IBM, SPSS statistical version 20. The data was evaluated for mean by three replicates, expressed as mean ± standard error followed by Duncan's multiple range tests with significant $p<0.05$ level.

RESULTS AND DISCUSSION

UV-Visible Spectroscopy

UV- Visible spectroscopy is one of the most important tools to analyze the formation of silver nanoparticles in aqueous solution. The reduction of silver ions to silver nanoparticles exhibited color change from light brown to dark brown within 10 minutes indicating the formation of AgNPs. Similar changes in color have also been observed in previous studies (Fig. 1) [34]. Absorption spectra of AgNPs have shown a prominent peak at 410 nm at pH 9, which is due to surface plasmon resonance (SPR) of AgNPs [35]. This is similar to that of the surface plasmon resonance of the absorption peak of AgNPs prepared by Dakhil [36] and Anes Al-Sharqi *et al.* [37]. Different parameters like temperature, pH, and concentration of silver nitrate play a significant role in the formation of AgNPs. The temperature was considered as the first factor, as the temperature increases, the rate of formation of AgNPs is also increases [38]. The pH was considered as the second factor, at the acidic condition the formation of AgNPs is suppressed but at in the basic condition, it enhances the rate of formation of AgNPs. From the results, it is evident that the formation of AgNPs mainly depends on pH of the reaction medium.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis was carried out to identify possible involvement of biomolecules in the synthesis, capping and stabilization of AgNPs. The

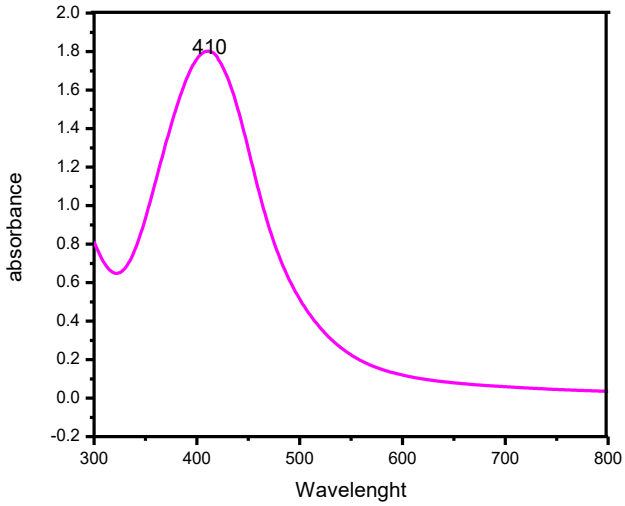

Fig.1. UV-Visible absorption spectra of AgNPs synthesized by aqueous fruit extract of *M. champaca.*

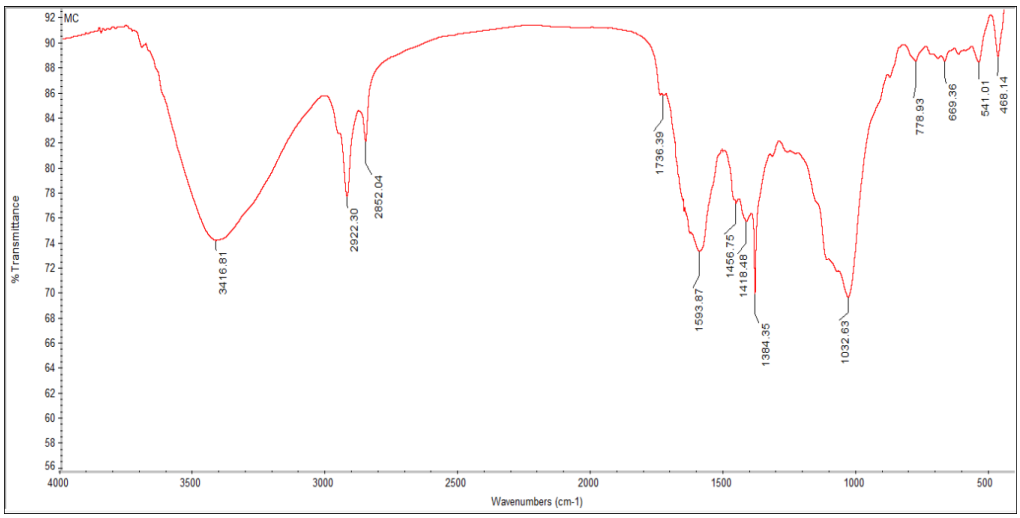

Fig.2. FTIR spectrum of AgNPs synthesized using fruit extract of *M. champaca.*

FTIR spectra of AgNPs synthesized by the fruit extract of *M. champaca* shows prominent bands at 3416.81 cm^{-1}, 2922.30 cm^{-1}, 1736.39 cm^{-1}, 1456.75 cm^{-1}, 1384.35 cm^{-1}, 1032.63 cm^{-1}, 778.93 cm^{-1}, 669.36 cm^{-1} and 541.01 cm^{-1} respectively (Fig. 2 and Table 1). The absorption peak at 3416.81 cm^{-1} can be assigned to O–H stretching due to carboxylic acids and also shows the presence of ascorbic acid [39]. The peak located at 2922.30 cm^{-1} could be assigned to O–H stretching of alcohols and phenolic compounds [40]. The intense bands at 1384.35 cm^{-1} and 1032.63 cm^{-1} may arose due to the -NO_3 stretching which comes from silver nitrate and C-S stretch (CH2-S) of thiol or thioether/ -C-O-C- bonds [41]. The bands at 778.93 cm^{-1}, 669.36 cm^{-1} and 541.01 cm^{-1} could be attributed to the bending vibration of S-H moiety bonded to the CH_2 group and CH out of plane bending vibrations of substituted ethylene system CH=CH and C-Cl, C-Br stretching vibrations of alkyl halides. Therefore, these biomolecules might have interfered in the reducing, capping and efficient stabilization of synthesized silver nanoparticles.

Zeta potential analysis

Zeta potential results of silver nanoparticles shows z-average value of 37.4 nm size of the nanoparticles with polydispersity index 0.327

Table 1. FTIR absorption peaks and their functional groups of fruit extract and AgNPs synthesized by *M. champaca*.

Sl. No	Absorbance peak of AgNPs (cm^{-1})	Functional Groups
1	3416.81	N-H/O-H stretching
2	2922.30	Methylene symmetric vibrational mode
3	1736.39	Carbonyl stretching
4	1456.75	Methylene scissoring vibrations
5	1384.35	-NO3 stretching which comes from silver nitrate
6	1032.63	C-S stretch (CH2-S) of thiol or thioether/ -C-O-C- bonds
7	778.93	Bending vibration in the S-H moiety bonded to the CH2 group
8	669.36	CH out of plane bending vibrations of substituted ethylene system –CH=CH
9	541.01	C-Cl, C-Br stretching vibrations of alkyl halides

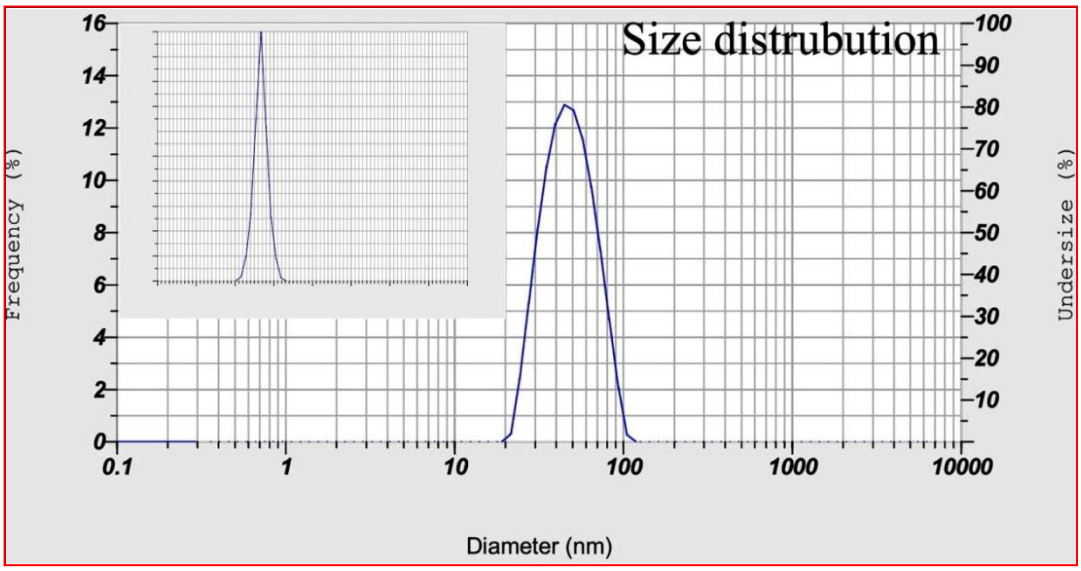

Fig.3. The graph showing particle size distribution and zeta potential of synthesized AgNPs.

(Fig. 3). The zeta potential results show negative values (-20.1mV) of the reaction mixture that play a key role in capping of the nanoparticles and which are negatively charged. The negative values of zeta potential analysis indicate that the capping molecules of AgNPs are negatively charged and the nanoparticles are fairly stable. It was notable that the synthesized silver nanoparticles can be stable in a wider range of pH [42]. As the concentration of reaction mixture increases, there was a decrease in the particle size due to the involvement of biomolecules acting as a reducing agent and capping of silver ions. A similar effect was observed in the leaf extract of *Magnolia kobus* [43].

Atomic force microscopy (AFM) and High resolution transmission electron microscopy (HR-TEM) analysis

The surface morphology and size of the AgNPs was analyzed by atomic force microscopy (Fig. 4). The size of the nanoparticles was found to be 39.06 nm and is spherical in shape (Fig. 4a). The 3D image represents the intense peaks of nanoparticles (Fig. 4b). Topography image represents the particle size distribution of the silver nanoparticles Fig. (4c). Finally, zeta potential results compared with the AFM analysis were found to be very similar. Further, HR-TEM image confirms the formation of AgNPs with different size and shape. The shapes of the nanoparticles are spherical and size was found to be 10-65 nm (Fig. 5).

Antioxidant and antibacterial activity

In the present study, the antioxidant potential of silver nanoparticles synthesized by *M. champaca* fruit extract was assessed by using DPPH scavenging assay (Fig. 6). The DPPH

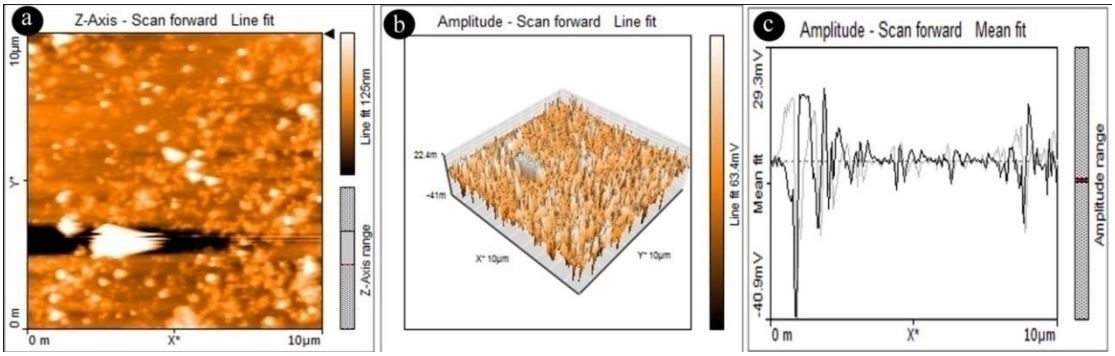

Fig.4. Atomic force microscopy (AFM) images of AgNPs synthesized by fruit extract of *M. champaca* a) 2D image b) 3D image c) Distribution of nanoparticles.

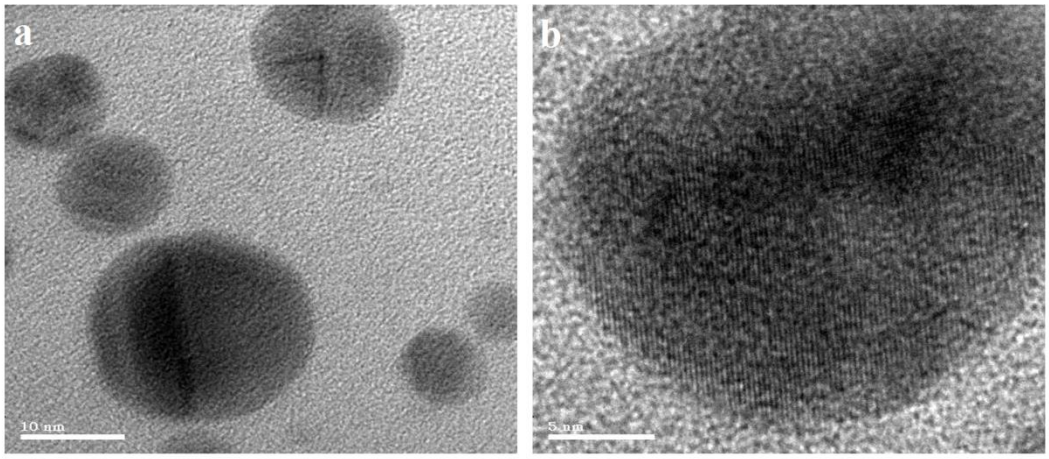

Fig.5. HR-TEM images of AgNPs synthesize by fruit extract of *M. champaca*.

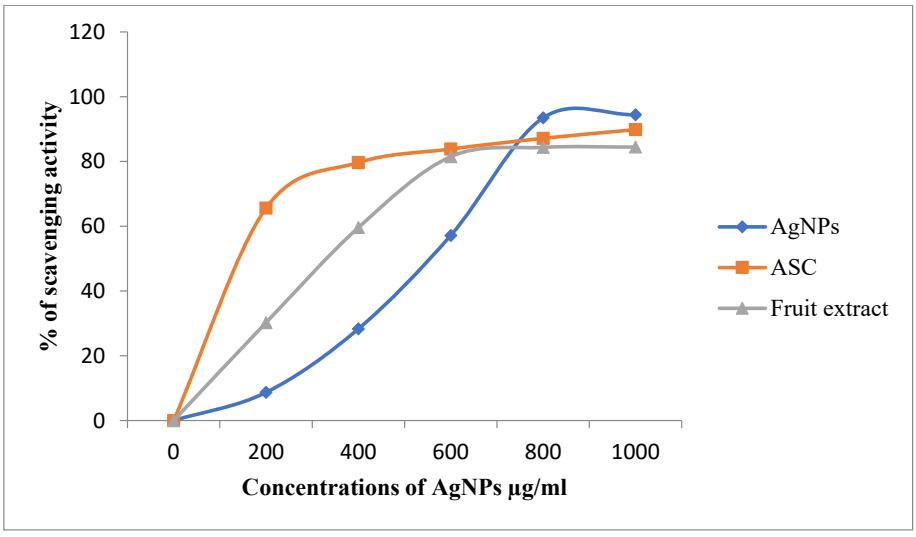

Fig.6. Antioxidant activities of AgNPs synthesized by fruit extract of *M. champaca*.

Table 2. Antimicrobial activity of *M. champaca* fruit extract synthesized by AgNPs showing zone of inhibition (in mm).

Concentrations of AgNPs (µg/ml)	Bacterial strains			
	Zone of inhibition (in mm)			
	Staphylococcus aureus	*Bacillus cerus*	*Escherichia coli*	*Pseudomonas aeruginosa*
5mM $AgNO_3$	2.33±0.13b	3.33±0.19b	3.33±0.11c	4.33±0.18b
200	0.00±0.00b	0.00±0.00c	0.00±0.00d	0.00±0.00c
400	0.00±0.00b	0.00±0.00c	4.00±0.32c	11.66±0.14b
600	7.00±0.15a	9.66±0.16a	7.33±0.45b	14.66±0.17a
1000	9.00±0.14a	10.00±0.19a	10.66±0.18a	19.00±0.73a
Fruit extract	-	-	-	-
Cefotoxime	14	17	19	24
DMSO	00	00	00	00

Results were expressed one way ANOVA as Mean±SE with significant at $p<0.05$.

assay was widely used to evaluate antioxidants properties of compounds for scavenging free radicals. From the results, it is evident that the synthesized silver nanoparticles exhibit good radical scavenging activity (EC50= 532.16 µg/mL) when compared to both standard Ascorbic acid (EC50= 426.04 µg/mL) and fruit extract (EC50= 261.08 µg/mL). Similar results were reported from the silver nanoparticles synthesized by leaf extracts of *Cymbopogon citrates* [44] and *Costus afer* [45]. Synthesis of silver nanoparticles by plant extracts exhibit the highest antioxidant activity as compared to other extracts. These plant extracts contain certain biochemical active compounds such as phenolic and flavonoid compounds that are main agents for the anti-oxidant activity, which plays a role in plant defense mechanism [46].

The antibacterial activity of silver nanoparticles synthesized from the fruit extract of *M. champaca* was investigated using different bacterial strains such as *Staphylococcus aureus, Bacillus cereus, Escherichia coli,* and *Pseudomonas aeruginosa* (Table 2). Different concentrations (200-1000 µg/mL) of AgNPs were quantitatively evaluated on the basis of zone of inhibition and compared with the silver nitrate ($AgNO_3$) and aqueous plant extract (Table 2). Synthesized silver nanoparticles showed good antibacterial activity against the studied pathogens. Results showed that the *Pseudomonas aeruginosa* (19.00±0.73a mm) had the highest inhibition zone at the concentration of 1000 µg/mL and least activity was seen against *Staphylococcus aureus, Bacillus cereus* and *Escherichia coli*, which was stastically significant at ($P<0.05$). However, $AgNO_3$ and plant extract exhibit the least activity when compared to AgNPs (Table 2). Several researchers have reported that the green mediated silver nanoparticles exhibited good antibacterial activity when compared to silver nitrate [47, 48, 44]. The inhibitory action of the mechanism of silver nanoparticles remains unsure but partially known. It is believed that the silver nanoparticles attach with the bacterial cell membrane resulting in the inhibition of cell membrane synthesis such as permeability and respiration [49]. The study of Guzman et al. [50] reported that plasmolysis (cytoplasm separated from bacterial cell wall) in *P. aeruginosa* and the inhibition of bacterial cell wall synthesis in *S. aureus* bacteria. Bacterial interactions with surfaces are ubiquitous in nature, smaller particles having larger surface area is available for interaction and have a stronger bactericidal effect compared to larger particles [51]. The present results strongly suggest that the mechanism of AgNPs is predominantly based on reactive oxygen species (ROS), which affects cellular membrane damage, triggering initiation of glutathione level, lipid peroxidation reaction and consequently decreasing membrane fluidity leading to DNA damage, apoptosis or necrosis [52]. Another important factor for the dysfunction of the bacterial membrane integrity is the charge of nanoparticles. Positively charged nanoparticles alter the function of the electron transport chain (ETS) in bacteria by amino-functionalized polystyrene particles [53]. The antimicrobial activity of AgNPs shows difference in both gram positive and gram negative bacteria. *S. aureus* may have a stronger defense system due to the presence of thicker cell wall that prevents the action of AgNPs [54]. In addition the cell wall of gram negative bacteria possess stronger negative charge than the Gram positive bacteria due to the presence of lipopolysaccharides (LPS), which

promotes adhesion of Ag-NPs, causing the bacteria to be more susceptible to Ag-NPs' antimicrobial action [55, 56]. Hanady et al. [57] reported in their studies that synthesis of AgNPs from the leaf extract of *Catharanthus roseus* showed a strong in vitro antioxidant and antimicrobial activities against various pathogens. The AgNPs also enhanced the wound healing activity in mice by inhibiting the pathogenic bacterial growth in the wound area. Hence, the AgNPs could be used as an orthodox antibiotic in the treatment of infectious diseases caused by microorganisms.

CONCLUSION

The present study demonstrated the synthesis of silver nanoparticles which is an eco-friendly, rapid, cost-effective and green approach using *M. champaca* fruit extract. The fruit extract of AgNPs shows maximum stability at pH 9. The presence of water-soluble secondary metabolites (phenolic compounds) in the fruit extract was mainly responsible for reduction and stabilization (capping) of AgNPs. AFM and HR-TEM analysis revealed the size of the nanoparticles was found to be 39.06 nm and is spherical in shape. The silver nanoparticles showed good antioxidant and antibacterial activity against various pathogens. The zone of inhibition proves that the AgNPs have good antibacterial activity against *Pseudomonas aeruginosa* followed by *Staphylococcus aureus, Bacillus cereus,* and *Escherichia coli.* The ROS include $^{\cdot}OH$, $O_2^{\cdot-}$, and H_2O_2 generated when Ag-NP surfaces are irradiated, attack cell components leading to a loss of membrane permeability and ultimately reduced viability. The green approach is a valuable application in various fields of nanoscience such as biomedical, electrochemical and environmental because as they do not involve any toxic reagent.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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