Readers Insight
Publisher of open access books and journals

PJMLS

Print ISSN: 2707-4471. Online ISSN: 2707-448X

Research Article	Pak-Euro Journal of Medical and Life Sciences
DOI: 10.31580/pjmls.v4i4.2294	Copyright © All rights are reserved by Corresponding Author
Vol. 4 No. 4, 2021: pp. 291-302	
www.readersinsight.net/pjmls	
Submission: November 13, 2021	**Revised:** December 28, 2021 **Accepted:** December 31, 2021

RUELLIA NUDIFLORA-MEDIATED BIOLOGICAL SYNTHESIS OF SILVER NANOPARTICLES AND THEIR POTENTIAL ANTIOXIDANT, ANTIFUNGAL AND ANTIBACTERIAL APPLICATIONS AGAINST SELECTED MULTIDRUG RESISTANT BACTERIA

Safat Ullah[1ǂ], Nodia Shujaat[1], Rahmat Ali Khan[2], Aamir Sohail[3], Momin Khan[3], Hazrat Bilal[4], Imdad Ullah Khan[5], Manzoor Ahmad[6], Mirwaise Khan[5], Rahman Ullah[5], Baseer Ahmad[7], Rahat Ullah Khan[3*ǂ]

[1]**Department of Biochemistry,** Hazara University Mansehra, Pakistan

[2]**Department of Biotechnology,** University of Science and Technology, Bannu, Pakistan

[3]**Institute of Pathology and Diagnostic Medicine,** Khyber Medical University, Peshawar, Pakistan

[4]**School of Life Sciences**, Anhui University, Hefei, P.R. China

[5]**Department of Clinical Sciences,** Faculty of Veterinary and Animal Sciences, Gomal University, Dera Ismail Khan, Pakistan

[6]**Department of Animal Sciences,** Quaid -I-Azam University, Islamabad, Pakistan

[7]**Department of Animal Sciences,** Faculty of Veterinary and Animal Sciences, Mian Nawaz Sharif Agriculture University, Multan 25000, Pakistan

ǂThese authors contributed equally to this work

***Corresponding Author:** Rahat Ullah Khan. Email: rahatullahkhan@gu.edu.pk

Abstract

Among various Nano-technological fields, green chemistry is an eco-friendly, inexpensive, easy, and fast method for synthesizing nanoparticles. Therefore, it has influenced researchers and scientists from various fields to create an innovative way to produce nanoparticles. In the present study, silver nanoparticles (AgNPs) were synthesized using an aqueous leaves extract of Ruellia nudiflora. The synthesized nanoparticles were characterized through different techniques such as UV visible spectrophotometer, Fourier Transformed Infrared Spectroscopy (FTIR), X-ray diffraction (XRD), Scanning electron microscopy (SEM), and energy dispersive spectroscopy (EDX). Using the scanning electron microscope, the green synthesized (AgNPs) displayed a spherical shape with an average size of 16 nm. Synthesis of AgNPs was visually observed through a change in color from light brown to dark brown. The presence of AgNPs in the solution was confirmed by using UV-Vis Spectroscopy. After characterization, the AgNPs were tested against clinical isolated multiple drug-resistant Staphylococcus epidermidis, Escherichia coli, and Klebsiella pneumoniae by using the standard agar well diffusion method. The results revealed that AgNPs inhibited the growth of S. epidermidis, K. pneumoniae, and E. coli with inhibition zones of 9±2.1 mm, 15±2.4mm, and 13±2.3mm, respectively. AgNPs have also tested against Aspergillus flavus and Aspergillus niger. The antifungal activity against A. flavus and A. niger was 67% and 57% (in mm), respectively. Additionally, AgNPs also showed effective antioxidant activity against 2, 2-diphenyl-1-picrylhydrazyl (DPPH) as compared to plant extracts and positive control. It was concluded that green synthesized AgNPs showed promising antibacterial, antifungal, and antioxidant activity in vitro; however, this formulation may be tested in vivo to evaluate its efficacy further.

Keywords: *Silver nanoparticles, Multi-drug resistant isolates, Ruellia nudiflora, green synthesis, Antimicrobial activity, Antioxidant activity*

INTRODUCTION

In the past, a variety of pathogenic microbes have been eliminated with the help of medicinal plants. Plants contain active phenolic, alcoholic, alkaloids, and organic compounds, which play an influential role and have antioxidant potential against bacteria, fungi, and helminths. The plant extricates various organic compounds that are considered significant in the advanced field of nanotechnology (1). Nanotechnology is the new advanced research area where materials range from 1nm to 100nm is fabricated. The unique properties of nanotechnology give a great chance of its application in applied sciences (2). With nanotechnology, materials with improved and ameliorate physicochemical and optically active properties can also be synthesized. Nanotechnology also has an influential role in the field of pharmacology (3). The metal nanoparticles have an excellent potential to restrict the growth or kill the microbes (4).

Amid metal nanoparticles, silver (Ag) is one of the vital nanoparticles (5). Ag is the noble metal with a very high electrical conductivity with an atomic number 47 and is placed in transition metal d block elements. The silver nanoparticles have a prominent and influential anti-bacterial, anti-fungal, and antiviral role (6). Besides that, the coinage property of silver enables it to have great appliances in pottery and jewelry products (7). It serves as a resistor to rusting and catalyze redox reactions; that are why it has a catalytic property (8).

Moreover, Ag is also used to cure outer skin infections and urinary tract infections (9). Various researchers described the demerits of the synthesis of AgNPs through typical methods. These methods are slow, costly, and create environmental inconvenience using chemical reducing agents, i.e., NaBH4, Na3C6H5O7, and (CH3)2NCH. Also, AgNPs show high instability and need a supplementary capping agent that provides stability. On the other hand, the green synthesis of AgNPs attracted many scientists due to its natural and improved properties like the use of natural resources, fastness, and cost-effectivity. These instinctive characteristics are very crucial for medical purposes, precise and exact size of nanoparticles. Moreover, they create no contamination and are easy to handle (10).

Ruellia nudiflora is a Perennial flowering plant found in Mexico and Texas in partially shaded places at the height of 20 to 30cm. The plant belongs to the genus *Ruellia,* family Acanthaceae, and is considered very important in pharmacology (11). *Ruellia tuberosa* is a tropical, medicinal plant that is mainly found in South Asia. This Ruellia genus species is essential for treating oral bacterial infections, fungal infections, and insecticidal activity (12). Anti-inflammatory and anti-diabetic properties of *Ruellia* species are also reported (13).

In the present study, we reported the use of *Ruellia nudiflora* leaf extracts for the green synthesis of silver nanoparticles for the first time. The synthesized AgNPs were found to have good anti-bacterial, anti-fungal, and antioxidant activity. This data may help the future use of *Ruellia nudiflora* mediated green synthesized silver NPs against other medically significantly bacterial and fungal pathogens in the future.

MATERIALS AND METHODS

PLANT COLLECTION AND PROCESSING

The plant *Ruellia nudiflora* was collected from the hilly area of Bargahnatu, Frontier Region (FR) Bannu, Khyber Pakhtunkhwa, Pakistan. After collection, the plant was identified in the Department of Biotechnology, University of Science and Technology Bannu, Pakistan. The plant leaves were washed thoroughly with distilled water to remove dust particles. After washing, the leaves were shade-dried for ten days. The leaves were converted to a fine powder using a grinder. For the preparation of aqueous leaf extract, about 20 g powdered was mixed with 200 ml double deionized water and stirred magnetically at room temperature for 4 hours on an electronic shaker. The extract was filtered on Whatman filter paper and was thus centrifuged at 6000 × g for 5 min to remove cell debris. The resultant supernatant was subjected to filtration through a 0.2 μm filter and was used to synthesize AgNPs.

GREEN SYNTHESIS OF AgNPs

Silver nanoparticles were synthesized as previously described (14). About 10 mL of 1 mM aqueous silver nitrate (AgNO3) (Merck, Germany) solution was added to 5 mL aqueous leaf extract and was incubated in the dark for 30 min to 24 h to observe any change in the color of the solution, which might indicate silver nanoparticles (AgNP) formation. For further confirmation of silver nanoparticles in solution, the synthesized colloidal solution of plant extract and silver nitrate was subjected to UV-Vis Spectrophotometer at sequential time intervals of 30 min, one h, two h, three h, four h, five h, six h, and 24 h respectively. In the end, AgNPs were then lyophilized by using a lyophilizer machine (Merck, Germany).

CHARACTERIZATION OF AgNPs

The synthesized AgNPs were characterized by using the following techniques.

UV VISIBLE SPECTROSCOPY

After visual observation of the change mentioned earlier in color, the solution was analyzed through Spectrophotometer (UV-1800 Shimadzu) in the absorbance range of 200 nm to 800 nm. The appearance of peaks under a specific time between 350 nm to 480 nm indicates the presence of AgNPs in different time intervals.

X-RAY DIFFRACTION (XRD)

To study the crystalline structure and size of AgNPs, the X-Ray diffraction (JDX 3532 JEOL, Japan) technique was used with a running time of 0.55 seconds. The diffracted intensities were calculated from 10°-80° of 2ø angles (JCPDS, No.01-087-0719). About 1 mg of silver nanoparticle powder was precisely weighed and was used for XRD analysis. After analysis, the data was obtained through Cu Ka radiations with a wavelength of about 1.5406 A. Particles size was calculated using the Debye-Scherrer equation as described elsewhere (15).

FOURIER-TRANSFORM INFRARED SPECTROSCOPY (FTIR)

FTIR data was used to determine the functional groups of both leaf extracts and silver nanoparticles. The green synthesized AgNPs and plant extract were analyzed using FTIR spectroscopy (broker ECO.ATR, Japan) in the range of 500 to 4000 cm-1 to determine the functional groups responsible for AgNPs synthesis.

SCANNING ELECTRON MICROSCOPY

The size and surface morphology of silver nanoparticles was performed by using a scanning electron microscope (SEM) (JSM5910, JEOL, Japan). Before microscopy, copper grid carbon was coated with a thin film of AgNPs, mixed with distilled water, and allowed to dry in hot air for 5 min and was then examined at different magnifications through an electron microscope. The green synthesized AgNPs showed a spherical shape with an average size of 16 nm.

ENERGY DISPERSIVE SPECTROSCOPY (EDS)

The elemental composition was determined with the help of EDX by using an electron beam, as mentioned elsewhere (16).

BACTERIAL ISOLATES

A total of 120 clinical Isolates, (n=55) *E. coli*, (n= 43) *K. pneumoniae* and (n=22) *S. epidermidis* were collected from Khyber teaching hospital Peshawar, Pakistan. The antibiotic susceptibility testing of all isolates against seven antibiotics, amikacin, ceftriaxone, meropenem, tetracycline, chloramphenicol, clarithromycin, and levofloxacin, were performed by Disk Diffusion Assay. The multiple drug-resistant (MDR) isolates amongst the tested strains were selected for antibacterial testing of AgNPs via the standard agar well diffusion method (17).

ANTIBACTERIALACTIVITY OF AgNPs

The antibacterial activity of AgNPs was determined for ten MDR *E. coli*, 8 *K. pneumoniae*, and five *S. epidermidis* using the Muller Hilton agar (MHA). Briefly, the bacterial strains were sub-cultured in Luria-Bertani (LB) for 24 h and mixed with normal saline to equalize the turbidity among the sample accrediting to 0.5 McFarland standard solution. The freshly grown bacteria were then inoculated on MHA plates using a sterile cotton swab. The five wells in each Petri plate were loaded with AgNPs, AgNO3, plant extract, positive control (Clarithromycin), and negative control (DMSO), respectively. In antibacterial activity, AgNO3, AgNPs and control were used in equal concentration (20µg/mL). The plant extract was used in the concentration of 20 µg/mL, 300 µg/mL, 700 µg/mL, and 1000 µg/mL, respectively. The plates were incubated at 37 ℃ for 24 h. The zone of inhibition of each loaded well was measured in mm with scale. All experiments were performed in triplicate. The collective zone for each bacterium type was calculated as mean and standard deviation by GraphPad Prism.

ANTIFUNGAL ASSAY

The antifungal efficacy of silver NPs was determined using the agar tube dilution method with some modifications (18). Both fungal strains (*Aspergillus flavus* and *Aspergillus niger*) were cultured in

Sabraud Dextrose Agar (SDA). After sterilization, the media was poured into five different tubes, and the tubes were kept in a slant position for solidification. At the same time, each tube was inoculated with fungi and exposed to 60 µg/mL of each treatment i-e plant extract, AgNPs, AgNO3, positive control, and negative control, respectively. Five tubes were used for each fungal strain. Tube 1 was given silver nitrate, tube two plant extract; tube three silver nanoparticles, tube 4 Terbinafine, and tube 5 DMSO. Terbinafine was used as a positive control, while DMSO was used as a negative control. The tubes were incubated for one week at 28 ℃. The percentage inhibition was computed as;

% inhibition of fungal growth = [(100 – linear growth in experimental sample (mm)) / (linear growth in control in mm)] × 100

ANTIOXIDANT ASSAY

The scavenging assay was performed according to the literature as previously shown (19).

PERPARATION OF STOCK SOLUTION

For the preparation of stock, 3g powder DPPH was dissolved in 50 mL of methanol and was kept in the dark at room temperature for 30 minutes. Ascorbic acid was used as a standard, and a stock solution of silver nanoparticles and plant extract was prepared by adding 1mg: 1mL for each. The stock solutions were further fractionalized up to 100 µg/mL, 150 µg/mL, 300 µg/mL, 500 µg/mL, and 1000 µg/mL. Absorption spectra for each fraction were recorded after 30 minutes by using ultraviolet-visible spectroscopy at 517 nm. The scavenging potential was calculated with the formula percentage scavenging =a1- a2/a1 ×100

RESULTS

The plant extract, when incubated with AgNO3 solution, the color of the solution was changed from light brown to dark brown after 24 hrs. (Fig. 1). It indicated that the plant extract reduced the ionic silver to 0 oxidation state, which probably has a range of size in nanometers. The phytochemicals inside the plant leaves may be responsible for AgNPs synthesis. Further analysis with a spectrophotometer, the peaks were obtained between 350 to 480, indicating AgNPs during different time intervals.

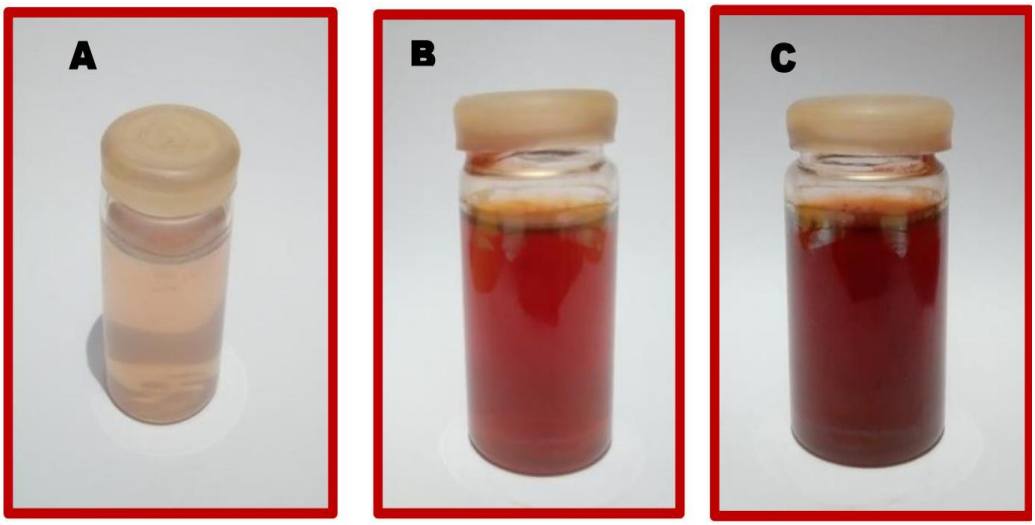

Fig. 1. Green synthesized AgNPs at: (A) 30 minutes, (B) 2 hours and (C) 24 hours incubations.

CONFIRMATION OF SILVER NPs

After spectrophotometry, different peaks were obtained that confirmed the formation of AgNPs in the solution. The highest peak intensity was observed at a 24 h interval, indicating a high concentration of nanoparticles (Fig. 2). Over time, nanoparticles' intensity and absorption increased, indicating their proportional stability with a time-lapse.

CHARACTERIZATION OF NPs

SCANNING ELECTRON MICROSCOPY

The AgNPs were round in shape, having a size of 16 nm **(Fig. 3).** Before SEM, the nanoparticles were centrifuged and powdered for microscopy. The average size (16 nm) of AgNPs obtained through SEM was the same as found with XRD.

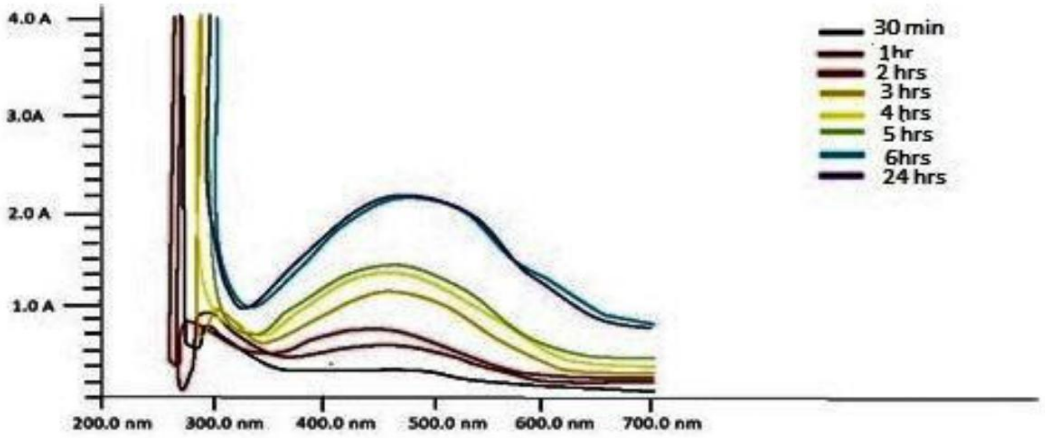

Fig. 2. UV spectra of AgNPs at different time intervals

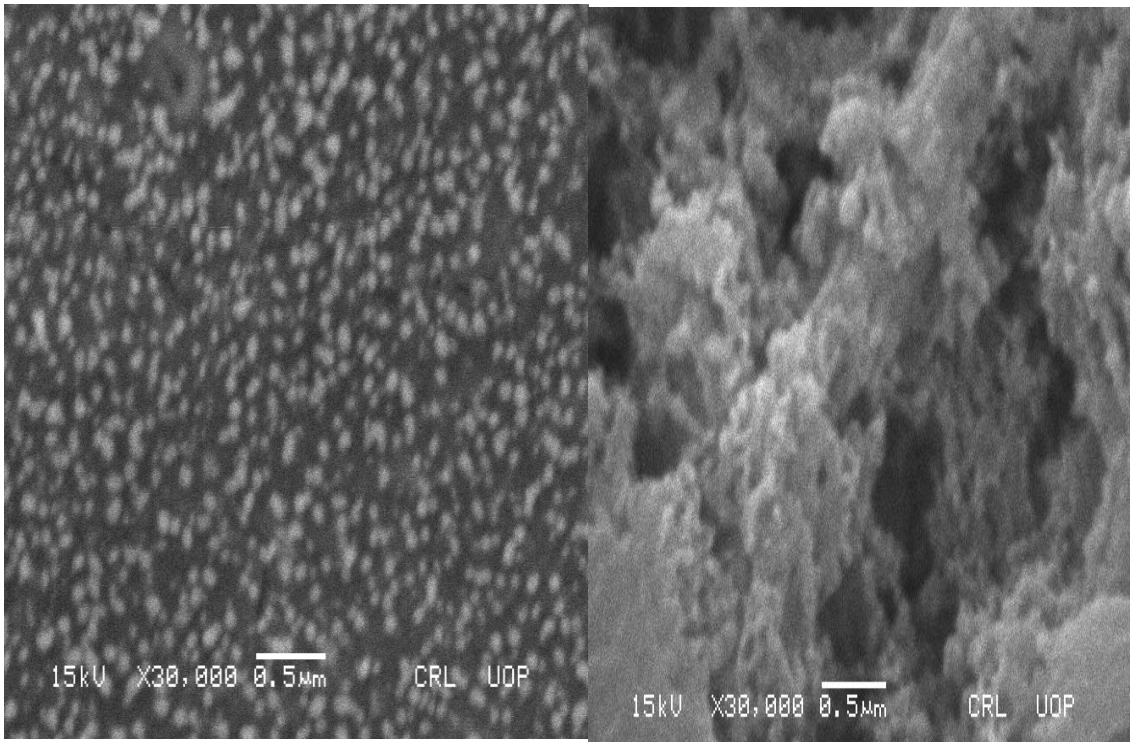

Fig. 3. Scanning electron micrograph of silver nanoparticles

ANALYSIS OF FUNCTIONAL GROUPS

Using Fourier transform infrared spectroscopy (FTIR), specific functional groups of plant extracts responsible for Ag ion reduction were determined. Different peaks were obtained for different functional groups, such as i-e 1701 cm-1 showed C=0 carbonyl, 2240 cm-1 showed nitryl, 3741 cm-1 showed N-H stretch, while 1550 cm-1 showed N-O functional groups (Fig. 4). These functional groups of plant extract were responsible for reducing Ag to silver nanoparticles and their capping and stability.

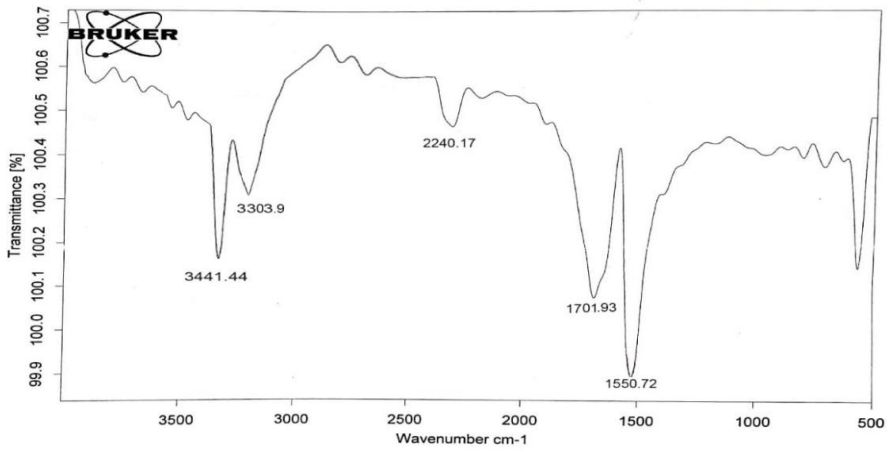

Fig. 4. FTIR spectra of green synthesized AgNPs

DETERMONATION OF ELEMENTAL COMPOSITION

The elemental composition was determined by using the EDX technique. The energy dispersive X-rays revealed the absorption peak at exactly 3KeV, representing silver 51% by weight in 100. The rest of them are carbon, oxygen, nitrogen, hydrogen in functional group form. The peaks of sodium, silicon, and chlorine that resulted from the glass materials used in this technique are shown in **Figure.5.**

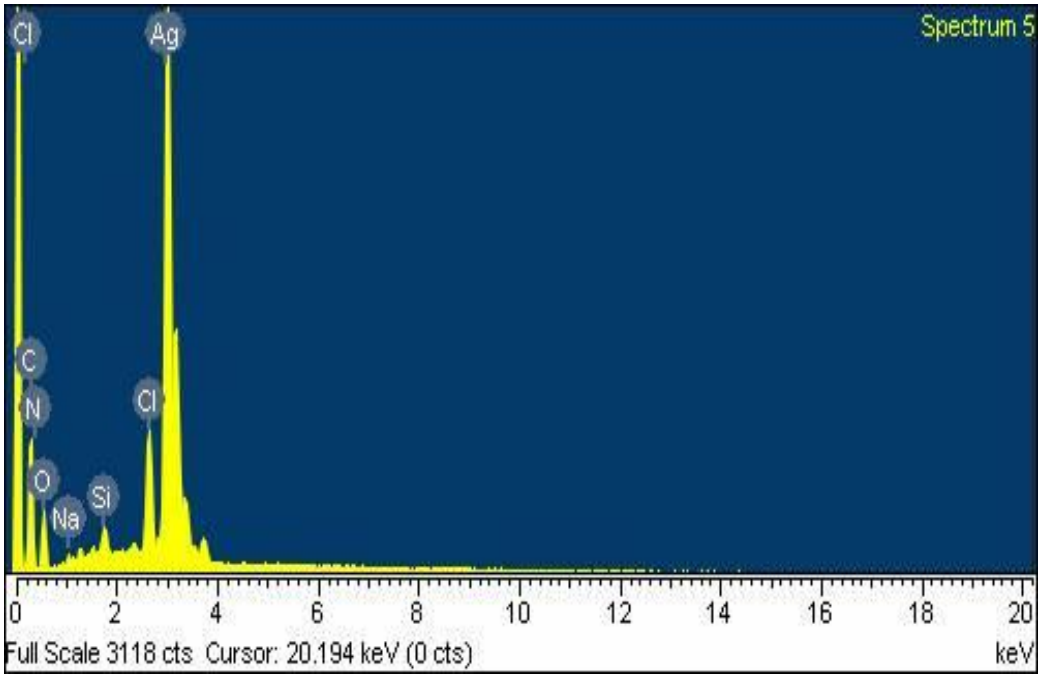

Fig. 5. EDX spectrum of silver NPs

DETERMONATION OF AVERAGE SIZE AND CRYSTALLINITY

For the determination of crystal lattices and average size, XRD was performed on powdered silver nanoparticles. The spectra obtained from XRD showed brags angle 111, 200, 220, 311, which reflect face-centered cubic lattice of silver nanoparticles with an average size of precisely 16nm, as represented in **Figure 6**. The lattice parameter was calculated using Bragg's equation. The present data are according to the reference number ICDD /ICSD and reference code of [JCPDS, No.01-087-0719].

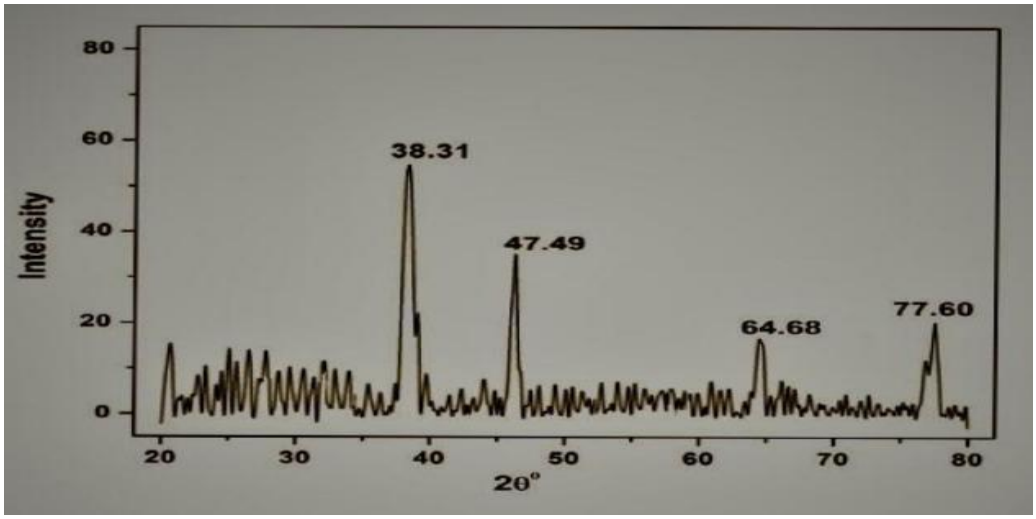

Fig. 6. XRD peaks of AgNPs

ANTIBACTERIAL TESTING

The antibiotic-resistant profiles of *S. epidermidis*, *E. coli*, and *K. pneumoniae* are presented in **figure 7**. The efficacy of synthesized silver nanoparticles was checked against MDR *S. epidermidis*, *E. coli*, and *K. pneumoniae*. The mean inhibition zones of AgNPs were 9±2.1 mm, 15±2.4mm, and 13±2.3mm against *S. epidermidis*, *E. coli*, and *K.pneumonae*, respectively **(Fig. 8).** DMSO was used as a negative control (no inhibition) while Clarithromycin was positive**.** Even using different concentrations, the polar plant extract showed no inhibition on tested bacteria.

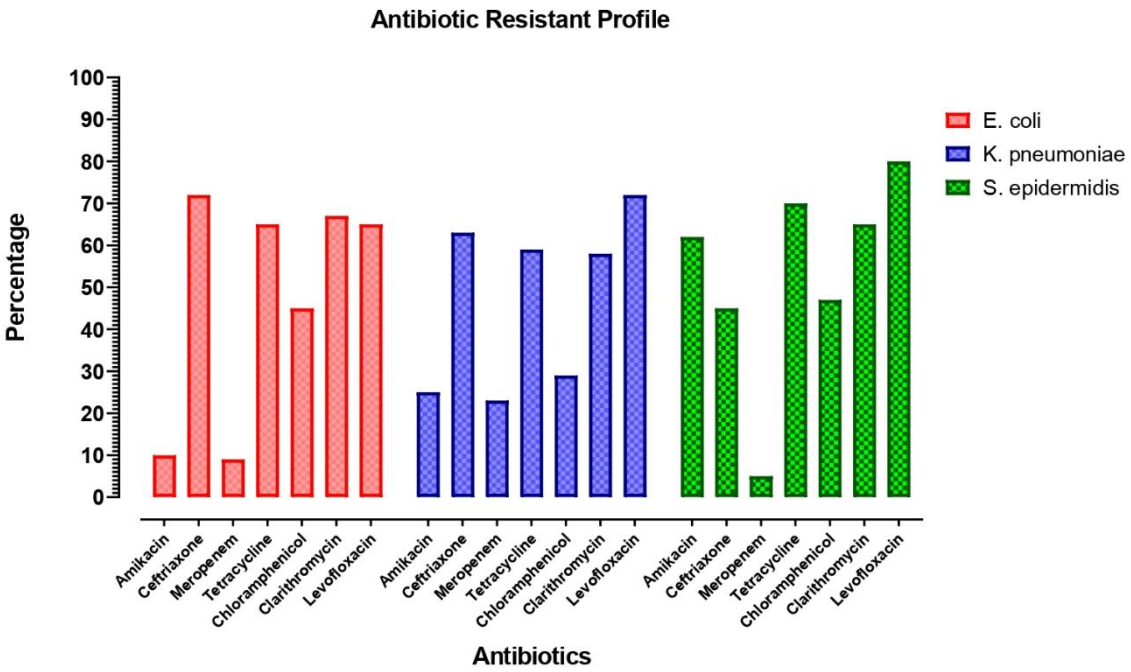

Fig. 7. Antibiotic resistant profile of *E. coli, K. pneumoniae* and *S. epidermidis*

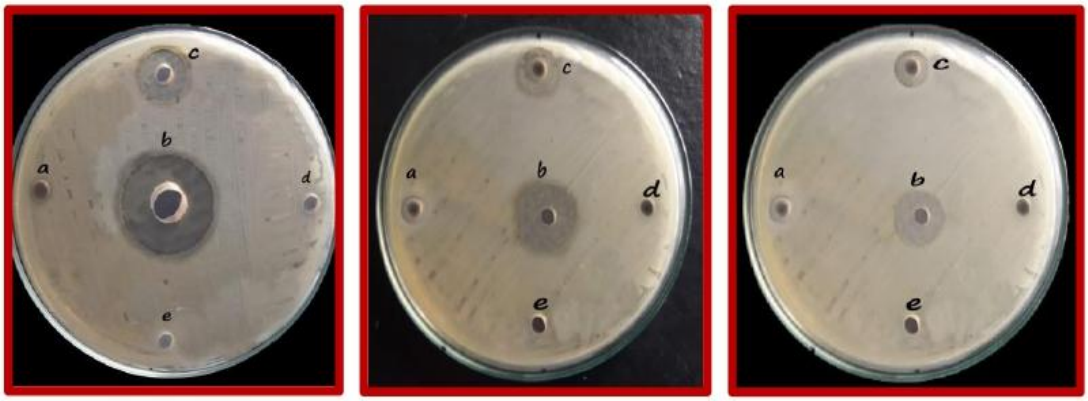

Fig. 8. Zones of inhibition of different compounds on tested bacteria
(a) $AgNO_3$ (b) AgNPs (c) +ve control (d) Plant extracts (e) –ve control

ANTIFUNGAL EFFICACY

The synthesized silver nanoparticles were tested against *A. flavus* and *A. niger* using the tube agar dilution method. Interestingly, the plant extract also causes inhibition of both fungal strains. The results are shown **(**Fig. 9**)** in the form of percent inhibition.

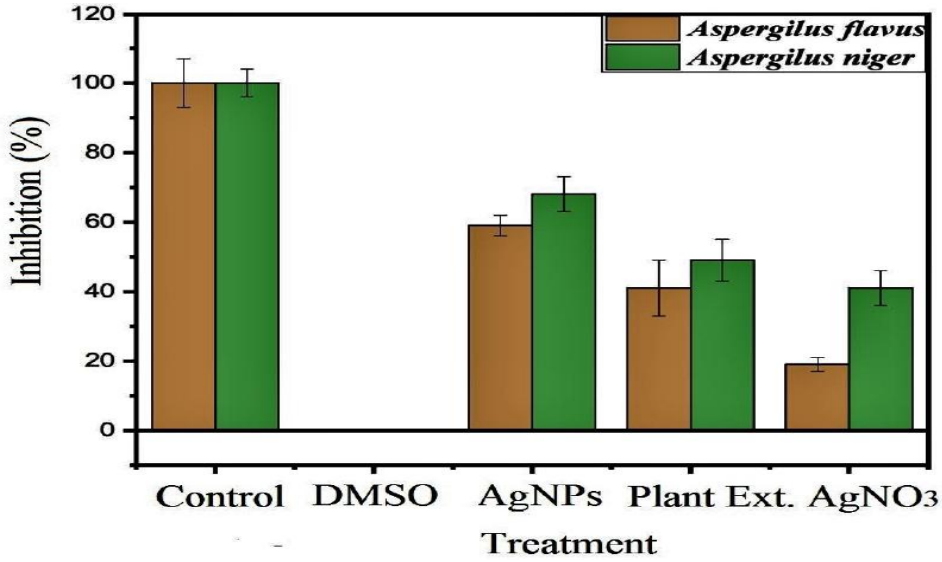

Fig. 9. Antifungal activity of *Ruellia mudiflora* mediated green synthesized AgNPs

ANTIOXIDANT ACTIVITY

Silver nanoparticles have shown a valuable free-radical inhibiting property and were compared with standard ascorbic acid. The highest scavenging ability of silver nanoparticles was recorded on 1000µg/mL of silver nanoparticles (Fig. 10)**.** The novel plant extract has also shown a promising scavenging ability for free radicals. DDPH was used as control.

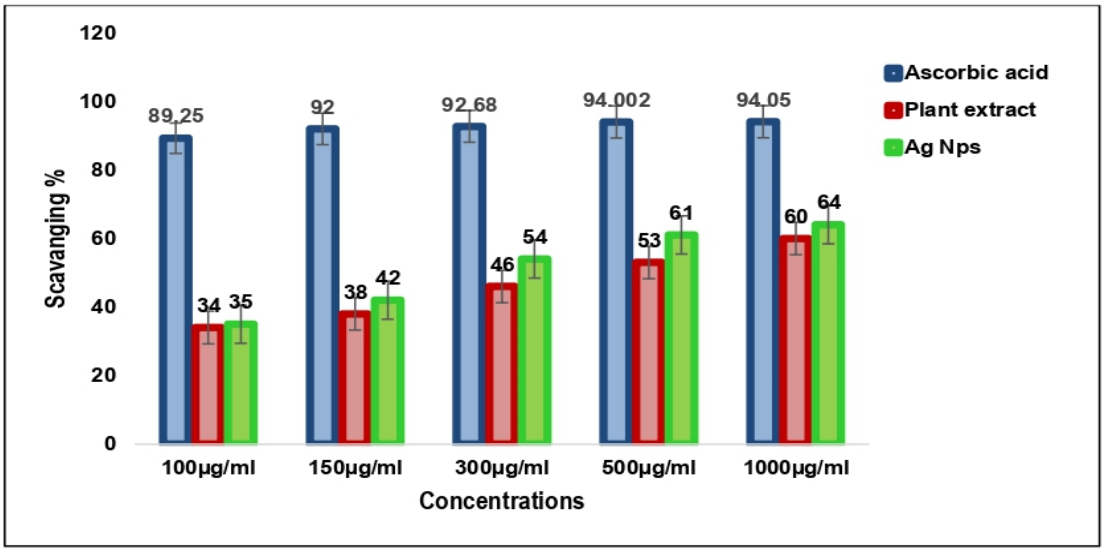

Fig. 10. The antioxidant potential of plant extract, silver nanoparticles and ascorbic acids

DISCUSSION

In the present study, the silver nanoparticles were synthesized using an aqueous extract of plant *Ruellia nudiflora,* and the synthesized NPs were characterized by using different techniques such as UV-Visible spectroscopy, XRD, SEM, EDX, and FTIR. AgNPs were then assessed for their antifungal, antibacterial, and antioxidant activity. UV visible spectroscopy is the most crucial technique for the conformation, stability, and size determination of nanomaterials in an aqueous solution. The formation of AgNPs was confirmed due to a change in color of the solution mixture from light yellow to dark brown color which indicates thriving green synthesized AgNPs. The UV/Visible spectra of synthesized AgNPs were recorded between 350-480 nm absorbance range, which agrees with previous studies (20, 21). Maximum NPs synthesis was obtained by increasing reaction time from 30 min to 24 hrs. After 30 min, the spectra show no peak while increasing the time interval to 2 hrs and then 24 hours, respectively. We obtained sharp narrow UV-Visible spectra peaks that indicate the maximum nanoparticle synthesis. FTIR is the most common technique to determine the possible biomolecules responsible for the reduction, capping, and stabilization of AgNPs. [22] *Ruellia nudifluea* contains many functional groups that may be responsible for the reduction of Ag+ ions in solution. After analysis with FTIR spectroscopy, different peaks were obtained that represented a specific functional group. All peaks represent individual functional groups, i.e., 1701 cm-1 shows C=0 carbonyl, 2240 cm-1 shows nitryl, 3741 cm-1 shows N-H stretch, and 1550 cm-1 N-O functional groups, respectively. Our results are in substantial agreement with previous findings (23). Using a scanning electron microscope, the average size of AgNPs was 16 nm having a spherical shape with an irregular surface at room temperature. Our SEM results showed significant similarities to the previously described study (24). X-ray diffraction is the most crucial technique to determine the crystalized structure and size of crystalline nanoparticles. The diffracted intensities were calculated from 10°-80° of 2ø angles, which revealed expressive resemblance regarding the unit cell of face-centered-cubic (FCC) structure of metallic silver (joint committee for powder diffraction standards (JCPDS, No.01-087-0719). While comparing with previous studies, these results were in the permissible range (25). Due to its substantial antibacterial properties, silver NPs have been used widely in various fields such as health, water purification, the paint industry, pharmaceuticals, and food storage (26). In the present study, we tested the biosynthesized silver NPs against *S. epidermidis, K. pneumonia,* and *E. coli*. After treatment with AgNPs, different zones of inhibition were observed for *S.epidermidis* (9±2.1mm), *E.coli* (15±2.4mm), and *K. pneumonia* (13±2.3mm). The novel polar extract of *Ruellia nudiflora* showed no inhibition on any bacteria, even at a concentration of 1000 µg/ml. The highest inhibition zone was observed against *E. coli* and *K. pneumoniae* compared to *S. epidermidis*. This may be due to enhanced penetration of nanoparticles inside gram-negative bacterial cells due to its thin peptidoglycan cell wall layer and NPs small size and increased surface area for attachment to the bacterial surface. The inhibitory effect of silver nanoparticles is dependent upon the concentration of

silver nanoparticles. If we increase the concentration of silver nanoparticles, its antibacterial effect will also be increased. One of the possible inhibitory actions of silver NPs is the disruption of the cell membrane and release of silver ions that interfere with cell constituents and cause intracellular ATP leakage and cell death (27). Our results are strongly related to previously reported literature (28). The slight difference in both observations may be due to the use of different plants to synthesize silver NPs in both studies. Previous research on green-synthesized AgNPs also showed increased antibacterial activity against *E. coli* and *K. pneumoniae (29).* Minimum inhibitory concentration (MIC) assay of the *Ruellia nodiflora* mediated silver NPs could also be evaluated in future studies.

Researchers tried to find out new alternative sources to cope with fungal infections due to the emergence of drug-resistant fungal strains. The biosynthesized AgNPs were assessed against two pathogenic fungal strains. The synthesized AgNPs possessed excellent antifungal activity. We demonstrated high and low antifungal activity against *A. flavus* (67% in mm) and *A. niger* (57% in mm), respectively. Moreover, the aqueous plant extract also showed good antifungal activity against the tested fungal strains. It is already reported in early studies that green synthesized AgNPs have increased antifungal potential (30). A study conducted by Medda *et al.* reported that silver NPs have good antifungal activity against *Aspergillus* spp (31). Our results are very compatible with the previous literature. However, the cytotoxicity of synthesized silver NPs is missing in current data, which should be addressed in upcoming research studies. To determine the antioxidant activity of plant (*Ruellianodiflora*) extract and silver NPs, a DDPH scavenger assay was used with ascorbic acid as a positive control. We observed a direct relation between free radical scavenging activity and the concentration of both plant extract and green synthesized silver NPs. DDPH is a more stable compound that readily accepts hydrogen (electron) from silver nanoparticles (32). Due to this property, DDPH assay is widely used to determine the free radical scavenging ability of many compounds present in medicinal plants (33). The scavenging activity of *Ruellia nudiflora* mediated silver NPs was many folds greater than that of its aqueous extract. The highest scavenging ability of silver nanoparticles was recorded on 1000µg/mL of silver nanoparticles which was inadmissible range as studied previously (34).

CONCLUSION

We demonstrated the use of *Ruellia nudiflora* aqueous leaves extracts in the synthesis of silver nanoparticles. Later, the synthesized silver NPs were characterized by different techniques like SEM, XRD, UV, FTIR, and EDX. SEM images show that the AgNPs are spherical in shape and polydispersed. XRD peaks show Bragg's angle 111, 200, 220, and 311, reflecting face-centered cubic lattice of AgNPs with an average of 16nm. FTIR peaks revealed specific functional groups of plant extract i-e 1701 cm-1 shows C=0 carbonyl, 2240 cm-1 shows nitryl, 3741 cm-1 shows N-H stretch, and 1550 cm-1 shows N-O functional groups, respectively. The EDX absorption peaks of AgNPs were recorded as 3KeV, which represents 51% Ag by weight. The green synthesized AgNPs possess increased antibacterial activity with larger zone formation against *K. pneumoniae* (15±2.4mm) and *E. coli* (13±2.3mm) compared with that of *S. epidermidis* (9±2.1 mm). The antifungal activity against *A. flavus* and *A. niger* was 67% and 57% (in mm), respectively.

Conflict of Interest:

Authors have no conflicts of interest associated with this publication. As a corresponding author, I confirm that the manuscript has been read and approved for submission by all the named authors.

Ethical Approval:

We get clinical isolates from the laboratory as a secondary source, no patient data or figure is involved. Therefore, informed consent was not required from the patients or health care professional themselves.

Financial support and sponsorship:

No funding was received from any organization for the submitted work.

References:

1. Othman L, Sleiman A, Abdel-Massih RM. Antimicrobial activity of polyphenols and alkaloids in Middle Eastern plants. Front Microbiol. 2019;10:911.
2. Belkin A, Hubler A, Bezryadin A. Self-assembled wiggling nano-structures and the principle of maximum entropy production. Sci Rep. 2015;5:8323.

3. Jeevanandam J, Barhoum A, Chan YS, Dufresne A, Danquah MK. Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. Beilstein J Nanotechnol. 2018;9:1050-74.
4. Bankier C, Matharu RK, Cheong YK, Ren GG, Cloutman-Green E, Ciric L. Synergistic antibacterial effects of metallic nanoparticle combinations. Sci Rep. 2019;9:1-8.
5. Raj S, Mali SC, Trivedi R. Green synthesis and characterization of silver nanoparticles using Enicostemma axillare (Lam.) leaf extract. Biochem Biophys Res Commun. 2018;503:2814-9.
6. Hamouda RA, Hussein MH, Abo-elmagd RA, Bawazir SS. Synthesis and biological characterization of silver nanoparticles derived from the cyanobacterium Oscillatoria limnetica. Sci Rep. 2019;9:1-7.
7. Mikhailov OV, Mikhailova EO. Elemental Silver Nanoparticles: Biosynthesis and Bio Applications Materials. 2019;12:3177.
8. Smith DR, Fickett FR Low-temperature properties of silver. J Res Natl Inst Stand Technol. 995;100:119.
9. Politano AD, Campbell KT, Rosenberger LH, Sawyer RG.Use of silver in the prevention and treatment of infections: silver review. Surg Infect. 2013;14:8-20.
10. Iravani S, Korbekandi H, Mirmohammadi SV, Zolfaghari B. Synthesis of silver nanoparticles: chemical, physical and biological methods. Res Pharm Sci. 2014;9:385.
11. Zhuang Y, Tripp EA. The draft genome of Ruellia speciosa (beautiful wild petunia: acanthaceae). DNA Res. 2017;24:179-92.
12. Vasantharaj S, Sathiyavimal S, Senthilkumar P, LewisOscar F, Pugazhendhi A. Biosynthesis of iron oxide nanoparticles using leaf extract of Ruellia tuberosa: antimicrobial properties and their applications in photocatalytic degradation. J Potochem Photobiol B. 2019;192:74-82.
13. Ananthakrishnan M, Doss VA. Effect of 50% hydro-ethanolic leaf extracts of Ruellia tuberosa L. And dipteracanthus patulus (jacq.) on lipid profile in alloxan induced diabetic rats. Int J Prev Med. 2013;4:744.
14. Geethalakshmi R, Sarada DV. Gold and silver nanoparticles from Trianthema decandra: synthesis, characterization, and antimicrobial properties. Int J Nanomed icin. 2012;7:5375.
15. Varadavenkatesan T, Selvaraj R, Vinayagam R. Dye degradation and antibacterial activity of green synthesized silver nanoparticles using Ipomoea digitata Linn. flower extract. Int J Environ Sci Technol. 2019;16:2395-404.
16. Varadavenkatesan T, Vinayagam R, Selvaraj R. Structural characterization of silver nanoparticles phyto-mediated by a plant waste, seed hull of Vigna mungo and their biological applications. J Mol Struct. 2017;1147:629-35.
17. Charannya S, Duraivel D, Padminee K, Poorni S, Nishanthine C, Srinivasan MR. Comparative evaluation of antimicrobial efficacy of silver nanoparticles and 2% chlorhexidine gluconate when used alone and in combination assessed using agar diffusion method: An In vitro study. Contemporary clinical dentistry. 2018;9:204.
18. Haq MN, Wazir SM, Ullah F, Khan RA, Shah MS, Khatak A. Phytochemical and biological evaluation of defatted seeds of Jatropha curcas. Sains Malays. 2016;45:1435-42.
19. Nagaich U, Gulati N, Chauhan S. Antioxidant and antibacterial potential of silver nanoparticles: biogenic synthesis utilizing apple extract. J Pharma. 2016;6:1-8.
20. Okafor F, Janen A, Kukhtareva T, Edwards V, Curley M. Green synthesis of silver nanoparticles, their characterization, application and antibacterial activity Int J Environ Res Public Health. 2013;10:5221-38
21. Ajitha B, Reddy YA, Reddy PS. Green synthesis and characterization of silver nanoparticles using Lantana camara leaf extract. Mater Sci Eng. 2015;49:373-81.
22. Chinnasamy G, Chandrasekharan S, Bhatnagar S. Biosynthesis of silver nanoparticles from melia azedarach: Enhancement of antibacterial, wound healing, antidiabetic and antioxidant activities. Int J Nanomedicine. 2019;14:9823.
23. Balashanmugam P, Kalaichelvan , Cassia roxburghii DC. Biosynthesis characterization of silver nanoparticles using. aqueous extract, and coated on cotton cloth for effective antibacterial activity. Int J Nanomedicine PT. 2015;10:87.
24. Othman, A.M., et al,. *Biosynthesis and characterization of silver nanoparticles induced by fungal proteins and its application in different biological activities.* J Genet Eng Biotechnol 2019;17:8.
25. Dobrucka R. Biofabrication of platinum nanoparticles using Fumariae herba extract and their catalytic properties. Saudi J Biol Sci. 2019;26:31-7.
26. Murugan K, Senthilkumar B, Senbagam D, Al-Sohaibani S. Biosynthesis of silver nanoparticles using Acacia leucophloea extract and their antibacterial activity. Int J Nanomedicine. 2014;9:2431.

27. Nasar MQ, Khalil AT, Ali M, Shah M, Ayaz M, Shinwari ZK. Phytochemical analysis, Ephedra Procera CA Mey. Mediated green synthesis of silver nanoparticles, their cytotoxic and antimicrobial potentials. Medicina. 2019;55:369.
28. Loo YY, Rukayadi Y, Nor-Khaizura MA, Kuan CH, Chieng BW, Nishibuchi M, Radu S. In vitro antimicrobial activity of green synthesized silver nanoparticles against selected gram-negative foodborne pathogens. Front Microbiol. 2018;9:1555.
29. Mickymaray S. One-step synthesis of silver nanoparticles using saudi arabian desert seasonal plant Sisymbrium irio and antibacterial activity against multidrug-resistant bacterial strains. Biomolecules. 2019;9:662.
30. Ruiz-Romero P, Valdez-Salas B, González-Mendoza D, Mendez-Trujillo V. Antifungal effects of silver phytonanoparticles from Yucca shilerifera against strawberry soil-borne pathogens: Fusarium solani and Macrophomina phaseolina. Mycobiology. 2018;46:47-51.
31. Mallmann EJ, Cunha FA, Castro BN, Maciel AM, Menezes EA, Fechine PB. Antifungal activity of silver nanoparticles obtained by green synthesis. Revista do Instituto de Medicina Tropical de São Paulo. 2015;57:165-7.
32. Mohanta YK, Panda SK, Jayabalan R, Sharma N, Bastia AK, Mohanta TK. Antimicrobial, antioxidant and cytotoxic activity of silver nanoparticles synthesized by leaf extract of Erythrina suberosa (Roxb.). Front Mol Biosci. 2017;4:14.
33. Otunola GA, Afolayan AJ, Ajayi EO, Odeyemi SW. Characterization, antibacterial and antioxidant properties of silver nanoparticles synthesized from aqueous extracts of Allium sativum, Zingiber officinale, and Capsicum frutescens. Pharmacogn Mag. 2017;13:201-208..
34. Salari S, Bahabadi SE, Samzadeh-Kermani A, Yosefzaei F. In-vitro Evaluation of Antioxidant and Antibacterial Potential of GreenSynthesized Silver Nanoparticles Using Prosopis farcta Fruit Extract. Iran J Pharm Res. 2019;18:430-455.