

Antimicrobial Effect of Silver Nanoparticles Synthesized with *Bougainvillea Glabra* Extract on *Staphylococcus Aureus* and *Escherichia Coli*

Momeni, Mehdi; Asadi, Samer*⁺; Shanbedi, Mehdi

Department of Chemical Engineering, Kherad Institute of Higher Education,
Bushehr, I.R. IRAN

ABSTRACT: Considering the antimicrobial properties of silver and its enhanced level at nanoscale scale, it can be used to combat the various pathogens and microbial agents. The aim of this study was to investigate the antimicrobial effect of silver nanoparticles synthesized with *Bougainvillea Glabra* extract on standard strains of *Staphylococcus aureus* and *Escherichia coli*. In this study, silver nanoparticles were biosynthesized using the aquatic *Bougainvillea Glabra* extract under optimal conditions. The synthesis of silver nanoparticles was confirmed using UltraViolet-Visible (UV-Vis) spectroscopy and X-Ray Diffraction (XRD). Based on the X-ray diffraction pattern, the silver nanoparticles crystallite size was 21 nanometers. Transmission Electron Microscopes (TEMs) and Scanning Electron Microscopes (SEM) showed the synthesis of silver nanoparticles of about 23 nm in size and spherical morphology. Revitalizing and stabilizing agent groups were identified using Fourier-Transform InfraRed (FT-IR) spectroscopy. The mean diameter of the inhibition zone and the Minimum Inhibitory Concentration (MIC) were 27.6 mm and 3.12 µg/mL for *S. aureus* and 19.3 mm and 12.5 µg/mL for *E. coli*, respectively. Biological synthesis using *Bougainvillea Glabra* aquatic extract is a very inexpensive and cost-effective method. The ability of *Bougainvillea Glabra* to synthesize silver nanoparticles makes it possible to use this plant as a useful biological source for the synthesis of silver nanoparticles with suitable and practical sizes for medical and microbicide applications.

KEYWORDS: Silver Nanoparticles; Green Synthesis; *Bougainvillea Glabra*; Antibacterial Properties; Minimal Inhibitory Concentration.

INTRODUCTION

Nanoparticles are solid colloidal particles in the range of 1 to 100 nm. In the meantime, silver nanoparticles have been widely used because of their anti-bacterial properties. These particles are used in different sizes and shapes depending on the application, physical properties and the engaged living system. Of course, the use of them should

be in a range that would be ineffective on human cells, along with the destruction of microorganisms and foreign agents. Nowadays, the desire to produce and use nanoscale materials is increasing due to the interesting properties of these materials. Hence, there are methods for the preparation and manufacture of nanoscale materials,

* To whom correspondence should be addressed.

+ E-mail: samer.asadi@yahoo.com

1021-9986/2021/2/395-405

11/\$/6.01

including electric arc, chemical reduction [1], laser ablation [2] and microwave [3, 4]. However, nanoparticles derived from these methods have potential environmental hazards because of the use of hazardous chemicals, radiation, reactions in specific conditions (temperature and pressure) and costly materials and time-consuming materials [5]. Hence, there is an increasing need for a low-cost, non-toxic and non-harmful environment. One of the methods for producing nanoparticles is biological synthesis [6-8]. Green nanoparticle synthesis is a clean, non-toxic and environmentally friendly method that is related to different organisms, such as bacteria [9], fungi [10] yeast and plants [11]. Therefore, both single and multi-cell living organisms are used to produce intracellular and extracellular nanomaterials. Plants can be widely used due to environmental compatibility without causing environmental damage. Plants are also considered as the best choice for biological manufacture of nanoparticles due to the abundance and lack of specific conditions and nutrients for growth [12]. So far, biological production of silver nanoparticles has been carried out by many plants such as *Ocimum Sanctum* [13] and *Piper Longum* [14]. Silver nanoparticles, in addition to anti-bacterial properties, have anti-fungal and anti-inflammatory effects and environmentally friendly, non-stimulating and non-allergic, lack of resistance to microorganisms, heat resistance and high stability properties.

The main feature of silver nanoparticles is the anti-bacterial property of these particles. These particles shorten the process of wound healing by reducing the activity of metalloproteases and increasing the apoptosis of neutrophils and make the appearance of the scar natural. Moreover, they are effective in improving the collagen orientation and mechanical strength. Investigating wound dressings has shown that the use of silver for similar uses does not create toxicity and has no adverse effects on human cells. Recent studies have also shown that the use of silver nanoparticles in dermal ointments leads to the penetration of silver into the wound, absorption by the epidermal margin of the wound, accumulation in the wound's remnants, and ultimately the transfer to the blood circulation, which helps wound healing and prevents infection [15-18].

One of the important applications of silver nanoparticles is the role of these particles as drug and gene carriers because nanoparticles, in addition to increasing

the entry of these compounds into the body, produce synergistic effects against microorganisms and increase efficiency [19]. The use of silver nanoparticles in biosensors is another application. The dielectric properties of these particles in the biosensors allow the diagnosis of disorders and diseases (such as cancer). The plasmonic properties of silver nanoparticles have made them useful in medical imaging [20] and plasmonic sensors. The mechanism of operation of these sensors is that the light interacts with the electrons of the sensor surface and oscillates them, eventually the light is absorbed and diffused, and it allows detection of viruses and bacteria. In addition, the absorption of this light by the conjugated cells results in the production of thermal energy and destruction of the target cell (cancer cell) [21]. One of the most useful applications of these particles is the coating of vascular prosthesis and intravenous catheters with silver nanoparticles. The use of silver nanoparticles without bacterial resistance minimizes cloning and increases resistance to infection in prostheses. Therefore, the coating of medical tools with a layer of 2 to 20nm silver nanoparticle prevents the formation of biofilms and the accumulation of bacteria. Since there is a potential for volumetric contractions, creation of free space and bacterial accumulation after bone cement injections to the lesion, it is possible to use the bone cement and silver nanoparticle composite and use the anti-bacterial activity of these particles. In addition, studies on HMWPE cements containing silver nanoparticles showed a decrease in wear rate due to the addition of silver nanoparticles [22].

Studies have shown that 56 percent of the world's nanoparticle is allocated to silver nanoparticles [23]. Therefore, these particles are widely used in the development and improvement of the quality of many biological and medicinal products. Since the biocompatibility and toxicity of these particles to microorganisms and humans have not been fully determined, it is important to estimate the ability of nanoparticles based on the type of the engaged biological system and understand the mechanism of reaction. In addition, the nanoscale dimensions in these particles leads to the easy passage through the biological membrane and the effect (plant, animal, human, and microorganism) on cellular physiology. Accordingly, with increasing diameter, the contact surface is increased and

the penetration of these particles is increased [24]. In addition, the size of the nanoparticles is one of the most important factors in toxicity and ability to target cells [25]. The nanoparticle shape will also affect the contact surface and silver ion release. Since the tendency for proteins to join the sharp edges of is higher, the tendency to adhesion to cubic or triangular particles will be higher [26]. The above properties have transformed silver nanoparticles into a suitable material for medical applications. The synthesis of silver nanoparticles in optimal operating conditions has not been mentioned in similar literature. However, nanoparticle synthesis in optimal conditions makes nanoparticles ideal in shape and size and shows stronger antibacterial properties. Also in previous studies, images from TEM and SEM are not accurate, the particle size is not specified on the images, and there is no precise criterion for particle size measurement [27, 28].

Bougainvillea Glabra (Fig. 1) is an indigenous South American plant with four to eighteen convolvulus species. The purpose of this study was to investigate the antimicrobial activity of silver nanoparticles synthesized with aqueous extract of *Bougainvillea Glabra* on standard strains of *S. aureus* and *E. coli*.

EXPERIMENTAL SECTION

Materials

All of the chemicals used in this research had high purity. Silver nitrate salt (AgNO_3), hydrochloric acid (HCl), nitric acid (HNO_3), nutrient agar powder, Mueller Hinton Agar powder and Mueller Hinton Broth powder were purchased from MERCK-Germany. The applied microorganisms including *S. aureus* (ATCC: 6538) and *E. coli* (ATCC:35218) were provided by Microbiology Center of Shiraz Agricultural College (prepared from the Iranian Research Institute for Scientific Research) with specific IDs). Double – distilled water was used for distillation and washing.

Methods

Preparation of *Bougainvillea Glabra* extract

For this purpose, a certain amount of *Bougainvillea Glabra* was collected. Flower samples were washed twice for a specific period with double-distilled water twice a day to remove surface dust. To prepare *Bougainvillea Glabra* extract, 20 g of the sample was washed and dried

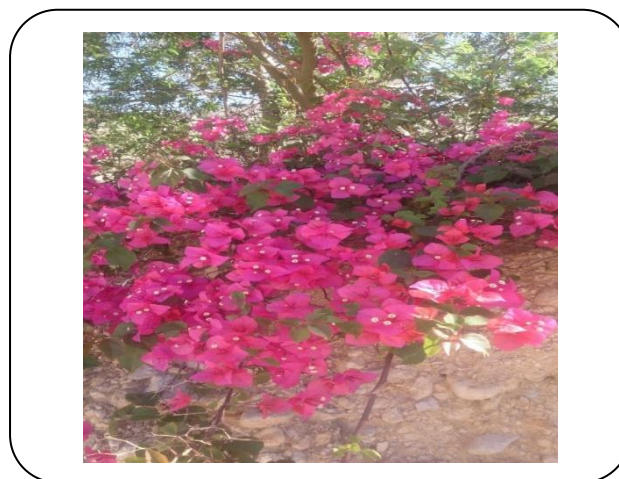


Fig. 1: *Bougainvillea Glabra*.

and boiled in 200 ml double-distilled water at 80 °C for 30 min. The resulting mixture was cooled and filtered with Whatman filter paper#1 and stored in a refrigerator at 6 °C for further tests.

Effect of pH on the synthesis of silver nanoparticles by the extract of *Bougainvillea Glabra*

Several previous studies have addressed to the effect of pH on the formation of nanoparticles [24-28]. Reports indicate that reaction pH does not have significant effect on the shape of nanoparticles, and only significantly affects their size. According to literature, the most optimum pH in bio-synthetic nanoparticles is between 7 and 8.5. This pH value is related to double distilled water. Since the external functional groups such as -OH are introduced into the medium by increasing pH, which affects the reduction and synthesis process, our experiments were performed at pH 7-8.5, for double distilled water.

Effect of extract volume on synthesis of silver nanoparticles by extract of *Bougainvillea Glabra*

Volumes of 2, 4, 6, and 8 ml of extract of *Bougainvillea Glabra* and 95 ml silver nitrate 0.003 M were first poured in Erlenmeyer flasks. The flasks were then placed on a Magnetic stirrer-Heater device at 70 °C and 150 rpm for 30 min. Their absorption was ultimately measured by a UV-Vis absorption spectrometer at a wavelength of 300 to 800 nm (Fig. 2). The desired volume of extract was selected according to the results.

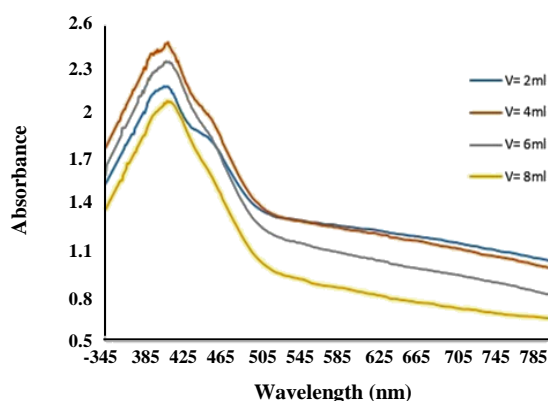


Fig. 2: Effect of extract volume on the synthesis of silver nanoparticles by Egyptian *Bougainvillea Glabra* extract (medium pH = 7.5, silver nitrate concentration = 0.003 mol, temperature = 70 °C, contact time = 30 min, speed = 150 rpm).

Effect of concentration of silver nitrate on the synthesis of silver nanoparticles by *Bougainvillea Glabra* extract

First, 95 ml of silver nitrate in concentrations of 0.01, 0.001, 0.003 and 0.027 M and 4 mL of extract were poured in flasks. The flasks were then placed on a Magnetic stirrer-Heater device at 70 °C and 150 rpm for 30 min. Finally, their absorption was measured by a UV-Vis absorption spectrometer at a wavelength of 300 to 800 nm (Fig. 3). The desired concentration of silver nitrate was selected according to the results.

Effect of temperature on the synthesis of silver nanoparticles by the extract of *Bougainvillea Glabra* extract

Initially, 95 ml of silver nitrate with the concentration of 0.01 M and 4 ml of Egyptian *Bougainvillea Glabra* extract were poured into several flasks. The flasks were then placed at different temperatures of 30, 45, 55, 65 and 85 °C at 150 rpm on a Magnetic stirrer-Heater device for 30 min. Their absorption was finally measured by a UV-Vis absorption spectrometer at a wavelength of 300 to 800 nm (Fig. 4). According to the results, optimum temperature was selected for synthesis of nanoparticles.

Effect of contact time on the synthesis of silver nanoparticles by *Bougainvillea Glabra* extract

First, 95 ml of silver nitrate with the concentration of 0.01 M and 4 ml of Egyptian *Bougainvillea Glabra* extract were poured into several flasks. The flasks were then placed at a temperature of 65 °C in 150 rpm on a Magnetic stirrer-Heater device at 10, 30, 60, 90 and 120 min. Finally,

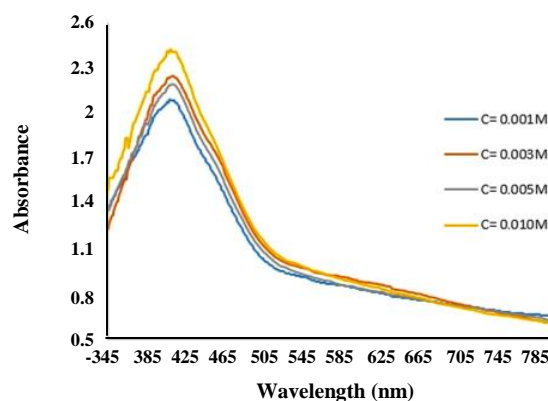


Fig. 3: Effect of concentration of silver nitrate on the synthesis of silver nanoparticles by Egyptian *Bougainvillea Glabra* extract (pH = 7.5, extract volume = 4 ml, temperature = 70 °C, contact time = 30 min, speed = 150 rpm).

their absorption was measured by a UV-Vis absorption spectrometer at a wavelength of 300 to 800 nm (Fig. 5). According to the results, the optimum contact time was selected for synthesis of nanoparticles.

Synthesis of silver nanoparticles

In this study, silver nanoparticles were synthesized in optimum conditions. Optimum conditions for synthesis of nanoparticles were determined by examining the extract's volume, silver nitrate solution's concentration, synthesis temperature and contact time. According to the results, 95 mL of silver nitrate solution at a concentration of 0.01M in double- distilled water was contacted with 4mL *Bougainvillea Glabra* extract on a shaker at 150 rpm at 65°C for 120 min. After completion of the solvent contact time, it was covered with paraffin with specific holes and placed at room temperature until complete evaporation, drying, and determining silver nanoparticles' precipitations.

Characterization of synthesized silver nanoparticles

The synthesis of silver nanoparticles was investigated using a UV-vis 1280 Ultraviolet Spectrophotometer made by Shimadzu Corporation in the range of 300 to 800 nm X-ray diffraction was performed using the XRD-D8-Advance Bruker device with an $\lambda = 0.15406$ nm radiation at an angle 2θ from 20 to 80 degrees. Using SEM-TESCAN-Vega3 scanning electron microscope and TEM-Philips-LEQ906E transmission electron microscopes, the morphology, size and image of the synthesized

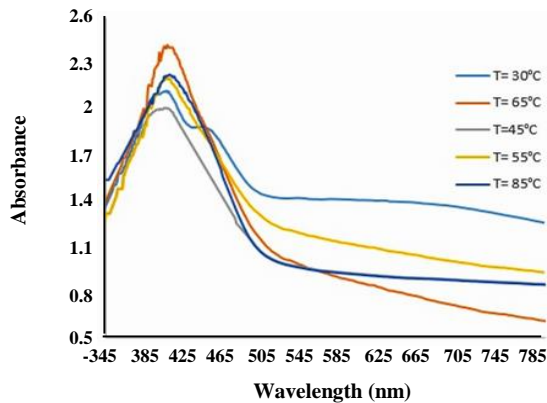


Fig. 4: Effect of temperature on the synthesis of silver nanoparticles by Egyptian *Bougainvillea Glabra* extract (pH = 7.5, extract volume = 4 ml, concentration of silver nitrate = 0.01 M, contact time = 30 min, speed = 150 rpm).

nanoparticles were studied. The Fourier Transform Spectrometry Test was performed using an FT-IR-PerkinElmer-Spectrum RXI to investigate the resilient and stabilizing foundations in an extract obtained from *Bougainvillea Glabra* in a range of 400 to 14000 cm [29].

MICROBIAL STRAINS AND ANTIBACTERIAL EFFECTS OF SYNTHESIED SILVER NANOPARTICLES

Microbial strains of *S. aureus* (ATCC: 6538) and *E. coli* (ATCC: 35218) were provided by Microbiology Center of Shiraz Agricultural College (prepared from the Iranian Research Institute for Scientific Research) with specific IDs) and prepared according to their respective instructions.

Antibacterial effect of bio-synthesized nanoparticles was investigated using agar well diffusion and minimum inhibitory concentration (MIC) was measured by micro dilution method.

Agar well diffusion test

A- Preparation of nutrient agar culture medium

First, 20 g of nutrient agar powder (Merck Germany) is added to 1000 ml of distilled water, and heated until it reaches the boiling point and complete solution so that the solution becomes completely transparent. The solution was then placed in an autoclave at a temperature of 121 °C for 15 minutes so that it is completely sterilized. The culture medium was used for bacterial test after cooling down.

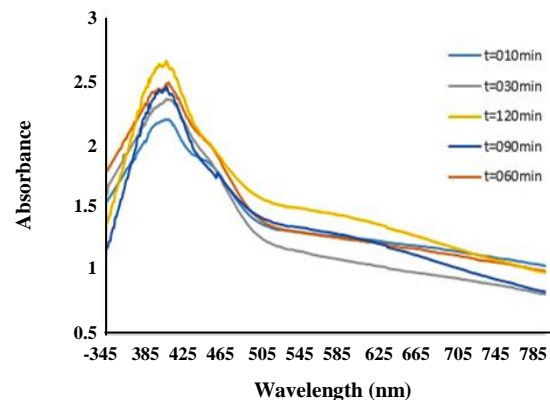


Fig. 5: Effect of time on the synthesis of silver nanoparticles by Egyptian *Bougainvillea Glabra* extract (pH = 7.5, extract volume = 4 ml, concentration of silver nitrate = 0.01 M, temperature = 65 °C, and speed = 150 Rpm).

B- Preparation of the Mueller Hinton Agar culture medium

First, 34 g of Mueller Hinton Agar (Merck Germany) powder is added to 1000 mL of distilled water, and heated until it reaches the boiling point and complete solution so that the solution becomes completely transparent. The solution was then placed in an autoclave at a temperature of 121 °C for 15 minutes so that it is completely sterilized. The culture medium was used for bacterial test after cooling down.

The bacteria were first cultured in nutrient agar medium and Mueller Hinton Agar medium was used to culture microorganisms in a plate. In each plate, a bacterial suspension with concentration of 0.5 McFarland prepared by nutrient agar medium (equivalent to a concentration of 1.5×10^8 bacteria), was cultured in a Mueller Hinton Agar culture medium using swab in three directions. Then, using the end of the sampler head, three wells were created at a depth of 4 mm at specified intervals. 0.03 mL of silver nanoparticles, silver nitrate solution and plant extract was poured into the wells and placed at 37 °C for 24 hours. In the end, the diameter of the inhibition zone of the bacteria was measured by the caliper and the mean diameter of the well was calculated.

Minimum Inhibitory Concentration test (MIC)

A- Preparation of Mueller Hinton Broth medium

First 21 g of Mueller Hinton Broth powder (Merck Germany) powder is added to 1000 mL of distilled water and dissolved thoroughly. The solution was then placed

an autoclave at a temperature of 121 °C for 15 minutes so that it is completely sterilized. The culture medium was used for bacterial test after cooling down.

The minimum inhibitory concentration is defined as the concentration of an antibiotic that can inhibit bacterial growth *in vitro*. For this purpose, the bacterial strains mentioned in the above test were used. In this test, 96-well microplates were used (micro dilution method). At first, different concentrations of silver nanoparticles were prepared (0.78, 1.6, 3.2, 6.25, 12.5, 25, 50 and 100 µg/mL). Then, from each of the prepared concentrations, 70 microliters were poured into eight wells of microplate. Then, amount of 70 µL of Mueller Hinton Broth medium of Merck Germany plus 70 µL of bacterial suspension at a concentration of 0.5 McFarland (equivalent to a concentration of 1.5×10^8 µg of bacteria) was added to eight microplates wells containing nanoparticles. Accordingly, the final volume in each microplate was 210 µL. In addition to eight wells, a well with culture medium and nanoparticles without inoculation of bacteria was considered as a negative control to consider the turbidity of silver nanoparticles and a well with culture medium and bacteria without inoculation of nanoparticles was considered as a positive control. Accordingly the effect of nanoparticles on the growth of the tested bacteria was compared with the turbidity of this well. Samples were kept at incubator for 24 hours at 37 °C. The first well (microtitre) without turbidity was reported as inhibitory concentration in µL /mL.

RESULTS AND DISCUSSION

Synthesis of silver nanoparticles

Silver nanoparticles were synthesized by *Bougainvillea Glabra* extract from a silver nitrate solution under optimum conditions. Fig. 6 shows the color change of the extract from pink to dark brown, which indicates the formation of nanoparticles.

4.2. UV-Visible spectroscopy

Absorption spectroscopy makes it possible to track the production of silver nanoparticles in the media due to surface plasmon resonance of particles in the medium. Fig. 7 shows the UV-Visible absorption for *Bougainvillea Glabra* extract, which indicates the existence of silver in the range of 420 nm. Fig. 8 also shows UV-Visible standard absorption in the range of 300 to 800 nanometers



Fig. 6: Color change of *Bougainvillea Glabra* extract after the formation of silver nanoparticles.

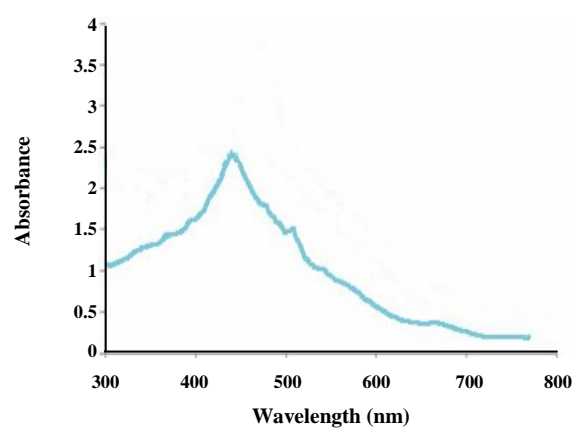


Fig. 7: UV-Visible absorption spectrum of silver nanoparticles by *Bougainvillea Glabra* extract.

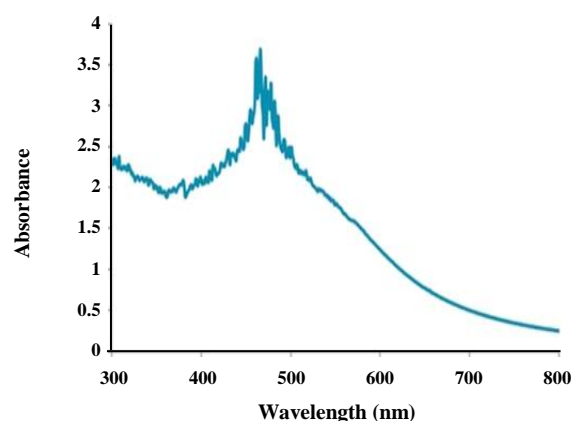


Fig. 8: Standard UV-Visible absorption spectrum of silver nanoparticles.

for silver nanoparticles. Since various plants with different reduction properties can be used in the synthesis of silver nanoparticles through biological approaches, the shape and size of nanoparticles will be different, so any plant species can show a different range of wavelengths. The silver nanoparticle absorption ranges between 420 and 580 nm, which will vary based on the shape and size of nanoparticles in the wavelength range. There are several peaks in the standard form of silver nanoparticles ranged from 450 to 500 nm due to the diversity of the synthesized particles in terms of shape and size, and each of these particles applies its own absorption and peak.

X-ray diffraction

X-ray diffraction analysis was used for further investigation and study of the crystalline structure of synthesized silver nanoparticles. According to Fig. 9, Miller indices at (111), (200), (220) and (311), respectively, which are related to 38.3043° , 44.2739° , 64.6300° and 77.4877° in silver nanoparticles synthesized with *Bougainvillea Glabra* extract are proof of the successful synthesis of silver nanoparticles and presence of spherical silver nanocrystals in the extract. The presence of sharp peaks in patterns shows a high degree of crystallinity for nanoparticles. Also, the difference in the width of the peaks in the XRD spectra shows the variety of crystalline grain sizes. The size of the synthesized crystalline grains was estimated as 21 nm for *Bougainvillea Glabra* extract by the Debye-Scherrer relation, which was in good agreement with the results of electron microscopy images.

TEM and SEM imaging

In order to compare the size, morphology and uniformity of the nanoparticle distribution, the TEM and SEM were used (Figs. 10 and 11). Using TEM the formation of spherical silver nanoparticles with a mean size of 23 nm was proven. The size of the silver nanoparticles in the current study was estimated to be between 21 and 53 nm, while in a similar study in 2016 [28], the size of the nanoparticles was up to 83 nm.

FT-IR Spectroscopy of synthesized silver nanoparticles

The FT-IR spectra of the biosynthesized silver nanoparticles are shown in Fig. 12. The FT-IR spectrum shown that the silver nanoparticles have layers of

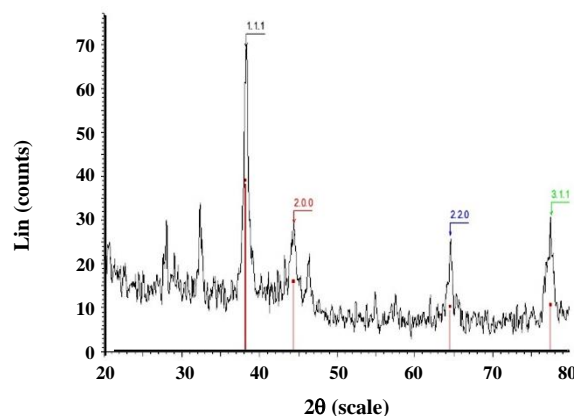


Fig. 9: XRD spectrum of silver nanoparticles synthesized with *Bougainvillea Glabra* extract.

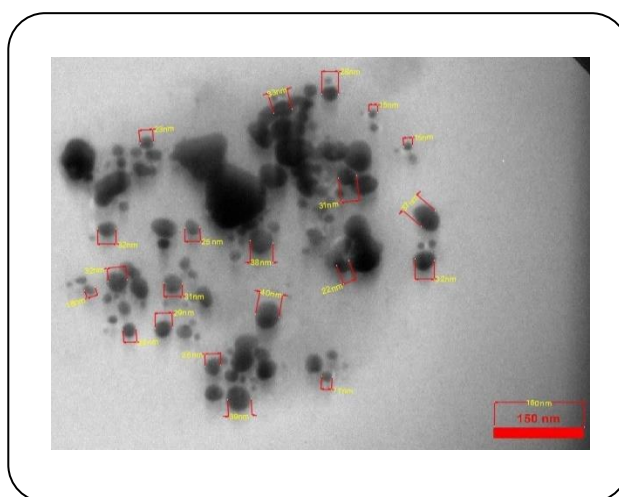


Fig. 10: Image of biosynthesized silver nanoparticles by TEM.

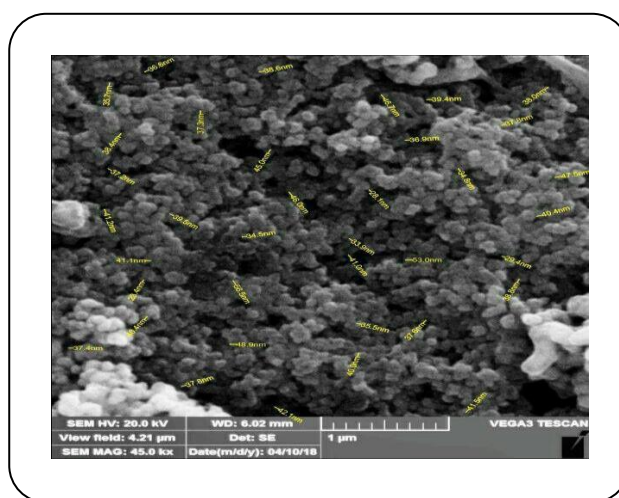


Fig. 11: Image of biosynthesized silver nanoparticles by SEM.

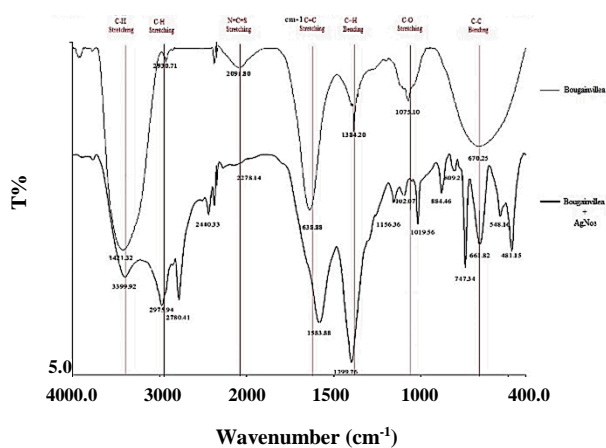


Fig. 12: Overlap of FT-IR spectra of *Bougainvillea Glabra* extract and silver nanoparticles biosynthesized by the *Bougainvillea Glabra* extract.

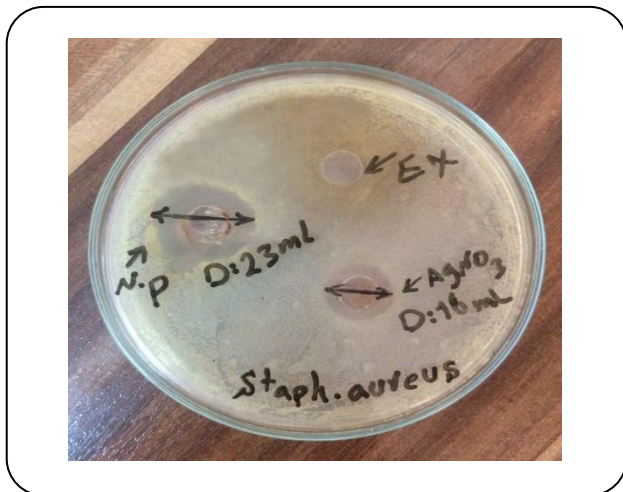


Fig. 13: Antibacterial test on *S. aureus*.

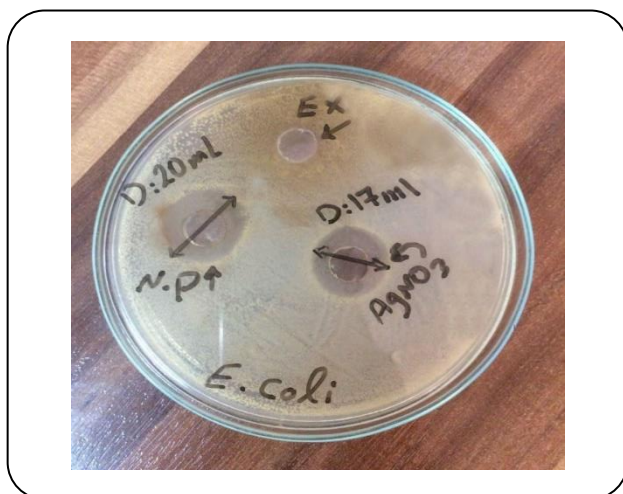


Fig. 14: Antibacterial test on *E. coli*.

the compounds in the extract that can both reduce the silver ions and contribute to stabilize the biosynthesized nanoparticles. Considering the similarity of the spectral pattern for the *Bougainvillea Glabra* extract and the silver nanoparticles, it can be concluded that the nanoparticles contain the compounds present in the extract, which usually surround nanoparticles in a layer around. Common bands in the FT-IR spectrum of the extract and nanoparticles are as follows: Band 3399 for stretching vibration of type I amine of functional group N-H and alcoholic stretching vibration of functional group O-H; band 2975 for aliphatic stretching vibration of functional group C-H; band 2278 for isocyanate stretching vibration of functional group N=C=S; band 1583 for stretching vibrations of functional group C=C; band 1384 for bending vibration of functional group C-H; bands 1019, 1102 and 1156 for stretching vibrations of functional group C-O; and band 661 for bending vibration of functional group C-C. The presence of these bands can be a reason for the presence of proteins in the structure of nanoparticles. The presence of these materials in layers around the nanoparticles will also increase their stability.

Antibacterial effects

Agar well diffusion test results

As shown in Figs. 13 and 14, the nanoparticles produced by the *Bougainvillea Glabra* extract produced an antimicrobial effect on both bacteria. The results were different for gram-positive and gram-negative bacteria. Silver nanoparticles penetrate into the bacteria by connecting to the membrane, destroying it and disrupting its activity. The inhibition zone in *S. aureus* bacteria is more than *E. coli* bacteria, which can be explained by the difference in the structure and composition of the membrane of gram positive and gram-negative bacteria. As shown in Table 1, the mean diameter of the inhibition zone was measured in nanoparticles produced from *Bougainvillea Glabra* extract with three replications (*S. aureus*, 27.6 mm, and *E. coli* 19.3 mm).

Minimum inhibitory concentration (MIC)

The results of MIC test on strains of *S. aureus* and *E. coli* are presented in Table 2. The size and shape of the silver nanoparticles are major contributors to their antibacterial properties, so that reducing the size of the particles results in proper engagement with bacteria

Table 1: Agar well diffusion test results of silver nanoparticles, *Bougainvillea Glabra* extract and silver nitrate.

Sample	Bacterial Strain	inhibition zones (mm)			Average zone of inhibition (mm)
		First iteration	Second iteration	Third iteration	
Paper Flower Extract	<i>S. aureus</i>	5	5	5	5
Silver Nanoparticles	<i>S. aureus</i>	23	30	30	27.6
Silver Nitrate	<i>S. aureus</i>	14	17	17	16
Paper Flower Extract	<i>E. coli</i>	5	5	5	5
Silver Nanoparticles	<i>E. coli</i>	21	20	17	19.3
Silver nitrate	<i>E. coli</i>	17	17	14	16

increases this property. Reducing the size of silver particles increases the release of silver from the surface and provides more antibacterial properties. In different sources, the antibacterial effect of silver nanoparticles is attributed to destabilizing membrane potential and the reducing cell adenosine triphosphate levels, binding to functional groups of proteins and the loss of the main properties of proteins and enzymes, intrusion into cells and producing hydrogen peroxide and finally, the death of bacteria. Particle shape is also effective on antibacterial effect, and spherical morphology has the ability to engage with bacteria and ultimately to destroy bacteria [30]. Researches show that the best silver nanoparticles' morphologies to prevent bacterial growth are angled amorphous nanoparticles, spherical nanoparticles and nano rods, because they have the ability to more engage with bacteria, respectively [31, 32]. Therefore, the appropriate antibacterial properties of silver nanoparticles synthesized with *Bougainvillea Glabra* can be attributed to their small size and sphericity.

CONCLUSIONS

In the present study, the effects of silver nanoparticles on standard strains of *E. coli* and *S. aureus* were investigated. It was found that silver nanoparticles synthesized with *Bougainvillea Glabra* extract have significant antibacterial properties. According to this study, the mean diameter of the inhibition zone of bacteria and the minimum inhibitory concentration were 27.6mm and 3.12µg/ml for *S. aureus* and 19.3mm and 12.5µg/mL for *E. coli* respectively. According to the results for two bacteria of *E. coli* and *S. aureus*, the nanoparticles

synthesized in the present study had an antimicrobial activity higher than those of the same studies. Therefore, it can be concluded that these nanoparticles can be used as an effective disinfectant for disinfection of hospital wastes, sterilization of the operating room environment and any other activity that needs to be controlled against pathogens.

Received : Sep. 9, 2019 ; Accepted : Dec. 2, 2019

REFERENCES

- [1] Faure C., Derre A., Neri W., *Spontaneous Formation of Silver Nanoparticles in Multilamellar Vesicles*, *J. Phys. Chem. B*, **107** (20): 4738–4746 (2003).
<https://doi.org/10.1021/jp027449u>.
- [2] Zhang Y., Chen F., Zhuang J., Tang Y., Wang D., Wang Y., A. Dong, N. Rena, *Synthesis of Silver Nanoparticles via Electrochemical Reduction on Compact Zeolite Film Modified Electrodes*, *Chem. Commun.*, **23**: 2814-2815 (2002).
<https://doi.org/10.1039/B208222E>
- [3] Fatimah I., *Green Synthesis of Silver Nanoparticles Using Extract of Parkia Speciosa Hassk Pods Assisted by Microwave Irradiation*, *Journal of Advanced Research*, **7**(6): 961–969 (2016).
<https://doi.org/10.1016/j.jare.2016.10.002>.
- [4] Susan W.P. Wijnhoven, Willie J.G.M. Peijnenburg, Carla A. Herberts, Werner I. Hagens, Agnes G. Oomen, Evelyn H.W. Heugens, Boris Roszek, Julia Bisschops, Ilse Gosens. et al, *Nanosilver-A Review of Available Data and Knowledge Gaps in Human and Environmental Risk Assessment*, *Nanotoxicology*, **3**: 109–138 (2009).
<https://doi.org/10.1080/17435390902725914>.

- [5] Senapati S., Syde A., Moez S., Kumar A., Ahmah A., "Intracellular Synthesis of Gold Nanoparticles Using Alga *Tetraselmis Kochinensis*", National Chemical Laboratory, Pune, India, *Materials Letters*, **2**: 275-281 (2012).
<https://doi.org/10.1016/j.matlet.2012.04.009>.
- [6] Rajeshkumar S.H., Malarkodi C.H., Gnanajobitha G., Paulkumar K., Vanaja M., Kannan C.H., Annadurai G., *Seaweed-Mediated Synthesis of Gold Nanoparticles Using Turbinaria Conoides and its Characterization*, *Journal of Nanostructure in Chemistry*, **3**: 44-50 (2013).
<https://doi.org/10.1186/2193-8865-3-44>.
- [7] Sadeghi B., Rostami A., Momeni S.S., *Facile Green Synthesis of Silver Nanoparticles Using Seed Aqueous Extract of Pistacia atlantica and Its Antibacterial Activity*, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **134**: 326-332 (2015).
<https://doi.org/10.1016/j.saa.2014.05.078>.
- [8] Salehi M., Reisia N., Mehrabian S., *Antibacterial Effect of External Shell of Pistacia Vera Extract*, *Journal. Islamic Azad University Microbial Biotech Research*, **3(1)**: 53-59 (2011).
- [9] Shiv Sh., Raia A., Ahmadb A., Sastrya M., *Rapid Synthesis of Au, Ag, and Bimetallic Au Core-Ag Shell Nanoparticles Using Neem (Azadirachta indica) Leaf broth*, *Journal of Colloid and Interface Science*, **275**: 496-502 (2004).
<https://doi.org/10.1016/j.jcis.2004.03.003>.
- [10] Gajbhiye M., Kesharwani A., Ingle A., Gade A., Rai M., *Fungus-Mediated Synthesis of Silver Nanoparticles and Their A- Ctiveity Against Pathogenic Fungi in Com- Bination with Fluconazole*, *Nano med NBM*, **5(4)**:382-386 (2009).
<https://doi.org/10.1016/j.nano.2009.06.005>.
- [11] Singh P.K., Bhardwaj K., Dubey P., Prabhune A., *UV-Assisted Size Sampling and Antibacterial Screening of Lantana camara Leaf Extract Synthesized Silver Nanoparticles*, *RSC Advances*, **5**: 24513-24520 (2015).
<https://doi.org/10.1039/C4RA17233G>.
- [12] Mittal A.K., Chisti Y., Banerjee U.Ch., *Synthesis of Metallic Nanoparticles Using Plant Extracts*, *Biotechnol Adv*, **31(2)**: 346-356 (2013).
<https://doi.org/10.1016/j.biotechadv.2013.01.003>
- [13] Daizy P.H., Unnib C., *Extracellular Biosynthesis of Gold and Silver Nanoparticles Using Krishna Tulsi (Ocimum Sanctum) Leaf*, *Physica E: Low-Dimensional Systems And Nanostructures*, **43(7)**: 1318-1322 (2011).
<https://doi.org/10.1016/j.physe.2010.10.006>.
- [14] Jacob S.J., Finub J.S., Narayanan A., *Synthesis of Silver Nanoparticles Using Piper Longum Leaf Extracts and Its Cytotoxic Activity Against Hep-2 Cell Line*, *Colloids and Surfaces B: Biointerfaces*, **91**: 212-214 (2012).
<https://doi.org/10.1016/j.colsurfb.2011.11.001>
- [15] Ahmadi F., Abolghasemi S., Parhizgar N., Moradpour F., *Effect of Silver Nanoparticles on Common Bacteria in Hospital Surfaces*, *Jundishapur Journal of Microbiology*, **6(3)**: 209-214 (2013).
<https://doi.org/10.5812/jjm.4585>.
- [16] Shahverdi A.R., Fakhimi A., Shahverdi H.R., Minain S., *Synthesis and effect of Silver Nanoparticles on the Antibacterial Activity of Different Antibiotics Against Staphylococcus Aures and Escherichia Coli*, *Nanomedician: Nanotechnology, Biology and Medicin*, **2(3)**: 168 (2007).
<https://doi.org/10.1016/j.nano.2007.02.001>.
- [17] Lee S.H., Sung K., Chung T.M., Lee S.G., Min K.D., Koo S., Kim C.G., *Preparation of Silver Nanoparticles and Antibiotic Test of [ts Polycarbonate Films Composite*, *Journal of Nanoscience and Nanotechnology*, **8(9)**: 4734-4737 (2008).
<https://doi.org/10.1166/jnn.2008.IC55>.
- [18] Wong K.Y., *Silver Nanoparticles in Medicine: Is the Panacea Here* *Nanomedician, Nanotechnology, Biology and Medicine*, **8(6)**: 935-940 (2012).
- [19] Ogston A., *On Abscesses*, *Classics in Infectious Diseases, Rev Infect Dis*, **6(1)**:122-28 (1984).
<https://doi.org/10.1093/clinids/6.1.122>. PMID 6369479.
- [20] Zhou W., Ma Y., Yang H., Ding Y., Luo X., *A Label-Free Biosensor Based on Silver Nanoparticles Array for Clinical Detection of Serum P53 In Head and Neck Squamous Cell Carcinoma*, *International Journal of Naomedicine*, **6**: 381-386 (2011).
<https://doi.org/10.2147/IJN.S13249>.
- [21] Keare M.D., Bukhari S., Swann A., Spier P., McLaren I., Myers J., *Reduction of Catheter-Related Colonisation by the Use of a Silver Zeolite-Impregnated Central Vascular Catheter in Adult Critical Care*, *Journal of Infection*, **54 (2)**: 146-150 (2007).
<https://doi.org/10.1016/j.jinf.2006.03.002>.

- [22] Prokopovich P., Kobrick M., Brousseau E., Perni S., Potent Antimicrobial Activity of Bone Cement Encapsulating Silver Nanoparticles Capped with Oleic Acid, *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, **103(2)**: 273–281 (2015).
<https://doi.org/10.1002/jbm.b.33196>.
- [23] Salari H.R., Kalbassi M.R., Johari A., Effect of Water Salinity on Acute Toxicity of Colloidal Silver Nanoparticles in Rainbow Trout (*Oncorhynchus Mykiss*) Larvae, *Iranian Journal of Health and Environment*, **5(1)**: 121-132 (2012).
- [24] Martirosyan A., Bazes A., Schneider YJ., *In Vitro* Toxicity Assessment of Silver Nanoparticles in the Presence of Phenolic Compounds--Preventive Agents Against the Harmful Effect, *Nanotoxicology*, **85**: 573-582 (2014).
<https://doi.org/10.3109/17435390.2013.812258>.
- [25] Nagal A., Singla R.K., Nanostructures Nano Materials, *Indo Global Journal of Pharmaceutical Sciences*, **3(2)**: 96-106 (2013).
- [26] Rai M.K., Yadav A.P., Silver nanoparticles as a New Generation of Antimicrobials, *Biotechnology Advances*, **27**: 76 -83 (2009).
<https://doi.org/10.1016/j.biotechadv.2008-09.002>.
- [27] Pareek N., Dhaliwal A.S., Malik C.P., Biogenic Synthesis of Silver Nanoparticles, Using *Bougainvillea spectabilis* Willd. Bract Extract, *National Academy Science Letters*, **35(5)**: 383-388 (2012).
<https://doi.org/10.1007/s40009-012-0067-1>.
- [28] Bharathi D., Kalaichelvan P.T., Atmaram V., Anbu S., Biogenic Synthesis of Silver Nanoparticles from Aqueous Flower Extract of *Bougainvillea Spectabilis* and Their Antibacterial Activity, *Journal of Medicinal Plants Studies*, **4(5)**: 248-252 (2016).
- [29] Bankara A., Joshi B., Kumara A.R., Zinjardea S., Banana Peel Extract Mediated Novel Route for the Synthesis of Silver Nanoparticles, *Colloids and surfaces A: Physicochem. Eng Aspects*, **368**: 58-63 (2010).
<https://doi.org/10.1016/j.colsurfa.2010.07.024>.
- [30] Suwanchawalit C., Chanhoml P., Sriprang P., Wongnawa S., Ag-doped TiO₂ Photocatalyst for Dye Decolorization under UV and Visible Irradiation, *PACCON*, **6**: 263-270 (2011).
- [31] Guzman M., Dille J., Godet S., Synthesis and Antibacterial Activity of Silver Nanoparticles against Gram-Positive and Gram-Negative Bacteria. *Nanomedicine: NBM*, **8**: 37–45 (2012).
<https://doi.org/10.1016/j.nano.2011.05.007>.
- [32] Ashkarran A.A., Antibacterial Properties of Silver-Doped TiO₂ Nanoparticles under Solar Simulated light, *Journal of Theoretical and Applied Physics*, **4-4**: 1-8 (2011).