

## Green Biosynthesis of Silver Nanoparticles Using *Glycyrrhiza glabra* L. Extract and Evaluation of their Selective Antimicrobial Activity

R. Karamian\* and M. Asadbegy

*Department of Biology, Faculty of Science, Bu-Ali Sina University, P. O. Box: 65175/4161, Hamedan, Iran*

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The aim of the present study is to determine the content of some phytochemicals in stem and leaf extracts of the medicinal plant of *Glycyrrhiza glabra*, and also to design a green method for biosynthesis of silver nanoparticles by these extracts as stabilizers and bioreducers. The properties of the synthesized silver nanoparticles (AgNPs) were monitored using UV-Vis and fully characterized by scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD). The effect of different parameters including concentration of the extracts and silver nitrate salt, reaction time, temperature and their interaction on bio-reduction process were also discussed. In addition, antibacterial and antifungal activities of the extracts and the synthesized AgNPs were assessed against 4 positive and negative bacteria by disc diffusion method and also the fungus *Fusarium oxysporum*. Results indicated that the bio-reduction process was relatively fast and the resulting nanoparticles were nearly spherical in their shapes with the size range of 5-45 nm. The best conditions for biosynthesis were achieved in 1-3 mmol AgNO<sub>3</sub>, at 60 °C temperature and 4 h reaction time. All the extracts and their corresponding nanoparticles had good inhibition activities against bacteria tested. However, the extracts showed more inhibition activity against Gram positive bacteria, while the synthesized AgNPs were more active against Gram negative bacteria. This is the first report on the green synthesis of AgNPs using *G. glabra* extract followed by an estimation of their biological activities. So, the present work can open up a new and promising insight into the course of rational design, green synthesis and applications of task-specific nanoparticles for various purposes.

**Keywords:** *Glycyrrhiza glabra* L., Green synthesis, Silver nanoparticles, Antibacterial activity, Antifungal activity

### INTRODUCTION

Among the nanoparticles, the silver nanoparticles are of particular importance due to a wide range of their characteristics and applications in different fields such as pharmaceutical agents [1,2], food industries [3], agriculture [4], textile industries [5], water treatment [6], as an antioxidant [7], antimicrobial [8], anti-cancer [9] cosmetics [10], ointments [11] and larvicides [12,13]. Although in recent years, chemical methods for the synthesis of nanoparticles have been developed, the use of the reported methods to produce nanoparticles requires time and money

consuming. Also, the introduced particles have several disadvantages such as hazards effects, including cyto- and genotoxicity [14,15]. Nowadays, nontoxic mild and green methods are attractive for the synthesis of nanoparticles [8,14]. Recently, the use of bacteria, fungi and yeasts for biosynthesis of metal nanoparticles as a green method has drawn the attention of researchers [8,16,17]. However, the use of microbes as bioreductants is difficult and also very expensive in terms of industrial production costs [18]. The use of other biological sources including plant extracts for biosynthesis of silver nanoparticles is going to be extending, because plant metabolites have remarkable possibility of rapid and simple synthesis, thereby, this method is economical and cost-efficient [8,18-20]. As the primary and

\*Corresponding author. E-mail: [karamian.roya@gmail.com](mailto:karamian.roya@gmail.com)

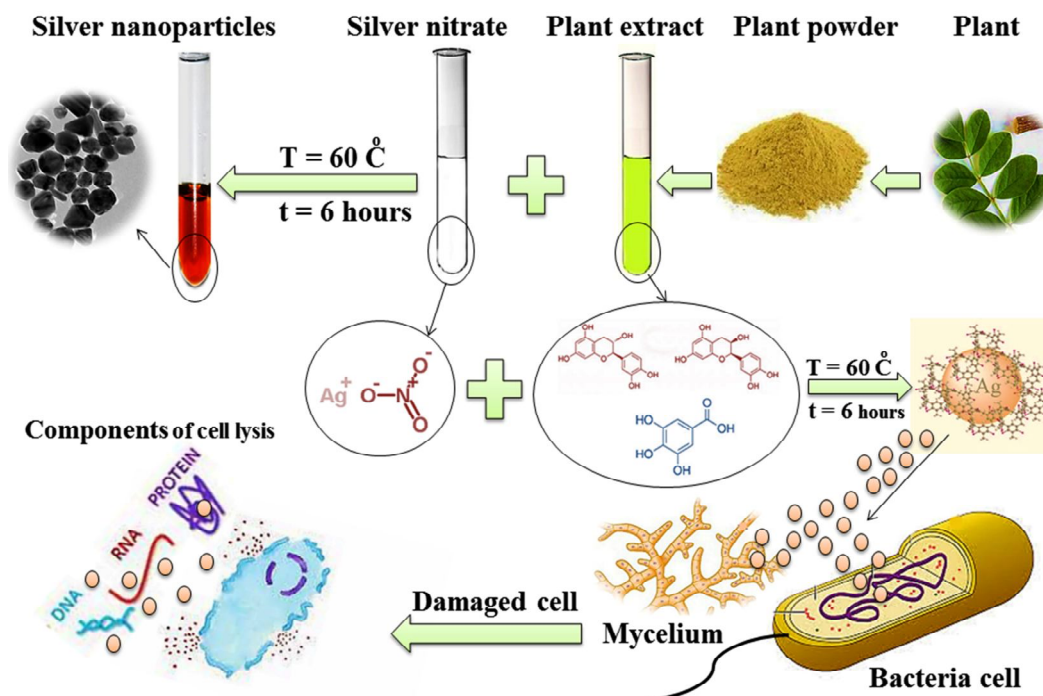


Fig. 1. Schematic illustration representing green synthesis of AgNPs by aqueous extract of *G. glabra*.

Table 1. Phytochemical Content of *G. Glabra* Extract

	Sample	
	Leaf	Stem
Total phenol content (mg ml <sup>-1</sup> )	6.59 ± 0.82 <sup>a</sup>	4.27 ± 0.63 <sup>b</sup>
Total flavonoid content (mg ml <sup>-1</sup> )	2.15 ± 0.35 <sup>a</sup>	2.57 ± 0.09 <sup>a</sup>
Starch (mg ml <sup>-1</sup> )	0.049 ± 0.04 <sup>a</sup>	0.038 ± 0.03 <sup>a</sup>
Total reducing sugar (mg ml <sup>-1</sup> )	0.122 ± 0.005 <sup>a</sup>	0.061 ± 0.002 <sup>b</sup>
Ascorbic acid (mg ml <sup>-1</sup> )	0.365 ± 0.010 <sup>a</sup>	0.058 ± 0.003 <sup>b</sup>

Experiment was performed in triplicate and expressed as mean ± SD. Values along column with different superscripts are significantly different ( $P < 0.05$ ).

secondary metabolites of plants such as carbohydrates, polyphenols and terpenoids are good hydrogen donors, they may act as bioreducing agents in the biosynthesis of nanoparticles [22]. However, these compounds exist in different amounts in various plant parts. *Glycyrrhiza glabra*

L. belongs to the family of Fabaceae, and is one of the most important crude drugs in the world. It contains a large amount of metabolites like polyphenols and glycyrrhizin in roots as an Oleanane-type triterpenoid saponin [23]. Glycyrrhizin exhibits a wide range of biological properties

such as anti-inflammatory, antiulcer, hepatoprotective, antiallergy and antiviral activity [24-29].

The emerge of antibiotic-resistant bacteria has become a great health challenge in recent years, because most of the microbes, such as bacteria and fungi, have shown resistance to classical antimicrobial agents [30-32]. Silver exhibited strong activity to prevent growth of bacteria, and is well known as an effective material for treating diseases [33]. Antimicrobial potential of silver salts is due to their ability in rapid release of the silver ions [34]. Silver nanoparticles possessing unique physicochemical properties have attracted abundance of interest in various fields, especially product antimicrobial agents [35-37]. Literature survey shows that biological activity of all nanoparticles is related to their features such as size and shape. The aim of the present study is to determine the phytochemical content (total phenol, flavonoid, reducing sugars, starch and ascorbic acid) of the aqueous leaf and stem extracts of *G. glabra* and biosynthesis of AgNPs by using these extracts. In addition, the resulting nanoparticles were analyzed for their antibacterial activity against 4 Gram positive and Gram negative bacteria using disc diffusion method, and also their antifungal activity against the fungus *F. oxysporum* (Fig. 1). The morphology and structure of the synthesized AgNPs were characterized using scanning electron microscopy (SEM), UV-Vis absorption spectroscopy and X-ray diffraction (XRD). In addition, the effect of different parameters such as concentration of silver nitrate salt and the extracts, temperature and also reaction time on the rate of synthesis were also studied.

## EXPERIMENTS

The chemical compounds used in this study were purchased from Merck (Germany) and Sigma (USA) companies. *G. glabra* was collected randomly from Kermanshah and Qazvin provinces, NW and W Iran, respectively, and kept the vouchers at BASU herbarium of Bu-Ali Sina University. The dried aerial parts were powdered and then used for extraction.

### Preparation of the Extracts

The extracts were prepared by putting 5 g plant powder (stem or leaf) into 50 ml sterile distilled water, boiling the

mixture for 5 min and then decanting it. Then, the extracts were filtered off through a Whatman filter (No. 1) and stored at 4 °C for further use.

### Phytochemical Analysis

**Determination of total phenol content.** Briefly, a reaction mixture (3 ml) containing 0.125 ml of each extract or gallic acid (standard phenolic compound), 1.5 ml deionized water, 0.125 ml Folin-Ciocalteu reagent, and 1.25 ml Na<sub>2</sub>CO<sub>3</sub> (7%) was incubated for 5 min at 25 °C. The mixture was allowed to stay for 90 min, and then, total phenol content was determined by a double beam Perkin Elmer UV-Vis spectrophotometer at 760 nm [38]. The amount of total phenol content was calculated as mg ml<sup>-1</sup> from calibration curve of gallic acid standard solution (0-0.25 mg ml<sup>-1</sup> in methanol: water, 50:50 v/v).

**Determination of total flavonoid content.** Briefly, a reaction mixture (1 ml) containing 0.5 ml of each extract and 0.5 ml AlCl<sub>3</sub> (2%) was incubated at 25 °C for 10 min and then the absorbance was measured at 368 nm by a double beam Perkin Elmer UV-Vis spectrophotometer [39]. The amount of total flavonoid content was calculated as mg ml<sup>-1</sup> from calibration curve of standard quercetin solution (12.5-100 µg ml<sup>-1</sup> quercetin in methanol).

**Determination of starch content.** The anthrone method was used to determine the starch content [40]. Briefly, 5 g of the dried plant powder was washed using ethanol (70%) several times. To obtain the extract without any sugar, the residue was dried and boiled in 100 ml distilled water. A 1 ml sample of the extract was evaporated to dryness and reconstituted in perchloric acid (60%). There after, 4 ml anthrone reagent was added, followed by boiling in a water bath for 8 min. The absorbance of the samples was recorded at 630 nm. The content of starch was calculated as mg ml<sup>-1</sup> from calibration curve of standard glucose solution (12.5-100 µg ml<sup>-1</sup> glucose in distilled water).

**Determination of total reducing sugar.** Briefly, 1 ml dinitrosalicylic reagent was added to 1 ml of 1% aqueous extract. The mixture was kept at 100 °C for 5 min, and the reducing sugars were determined by colorimetry at 630 nm [41]. Total reducing sugar was quantified from calibration curve of standard maltose solution (12.5-100 µg ml<sup>-1</sup> glucose in distilled water).

**Determination of ascorbic acid content.** Briefly, 2 ml

of the aqueous extract was evaporated to dryness by rotary apparatus, and then, reconstituted in oxalic acid (4%). In the following, the sample was brominated and 1 ml 2,4-dinitrophenyl hydrazine reagent was added, followed by two drops of thiourea (10%) and mixing thoroughly. The samples were incubated at 37 °C for 3 h. The orange-red osazone crystals formed were dissolved in 7 ml sulfuric acid (80%), and then, the absorbance was measured at 540 nm. The content of ascorbic acid in the extract was quantified from calibration curve of the standard ascorbic acid [42].

### Synthesis and Characterization of Silver Nanoparticles

For AgNPs synthesis, 25 ml of aqueous silver nitrate ( $\text{AgNO}_3$ ) solution (0.5-3 mmol) was mixed with 5 ml of the extract (0.2-1  $\text{mg ml}^{-1}$ ) as a bioreductant agent. Biosynthesis was done in different times (0-4 h), and at various temperatures (4-60 °C). In the following, the samples were monitored for changes of their absorptions by UV-Vis spectrophotometer in the range of 300-800 nm. Then, the dried nanoparticle powders was obtained by centrifugation at 12000 rpm for 15 min at 25 °C. The features (size and morphology) of the synthesized AgNPs were characterized by scanning electron microscopy (SEM, Philips-CMC-300 KV) and X-ray diffractometer (Italstructure ADP2000 XRD diffractometer). The extracts and nanoparticles were also subjected to Fourier transform infrared (FTIR, Perkin Elmer GX FT-IR spectrometer) spectroscopy. In addition, the effect of temperature, concentration of  $\text{AgNO}_3$ , and the extract on the rate of synthesis and the size of nanoparticles were discussed.

**X-ray diffraction analysis.** The synthesized AgNPs (after 24 h reaction of 1 mmol  $\text{AgNO}_3$  solution with the stem and leaf extracts) were centrifuged at 12000 rpm for 15 min at 25 °C, after which the pellet was redispersed in sterile distilled water, and then dried. The samples were studied using X-ray diffraction. The diffraction data for thin films of the dried samples were analyzed by an X-ray diffractometer (Italstructure ADP2000 XRD diffractometer) and the size of the synthesized AgNPs was calculated using Scherrer's formula:

$$d = 0.9\lambda/\beta\cos\theta$$

where 0.9 is the shape factor,  $\lambda$  is the X-ray wavelength (typically 1.54 Å),  $\beta$  is the full width at half maximum intensity in radians, and  $\theta$  is the Bragg angle.

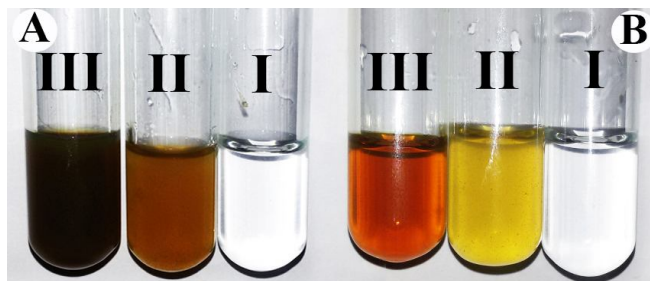
**Fourier transform infrared spectroscopy.** The stem and leaf extracts before and after bioreduction were subjected to Fourier transform infrared analysis (FTIR, Perkin Elmer GX FT-IR spectrometer in KBr pellets). The nanoparticle powder was mixed with KBr and exposed to an infrared source of 4000-400  $\text{cm}^{-1}$ .

**Scanning electron microscopy.** Scanning electron microscopy of the samples was performed using a Philips-CMC-300 KV SEM machine. This study was undertaken to know the size and shape of the synthesized AgNPs using the extracts. Thin films of the samples were prepared by just dropping of the samples on a foil, then, extra solution was removed in an oven at 80 °C and the images of nanoparticles were taken.

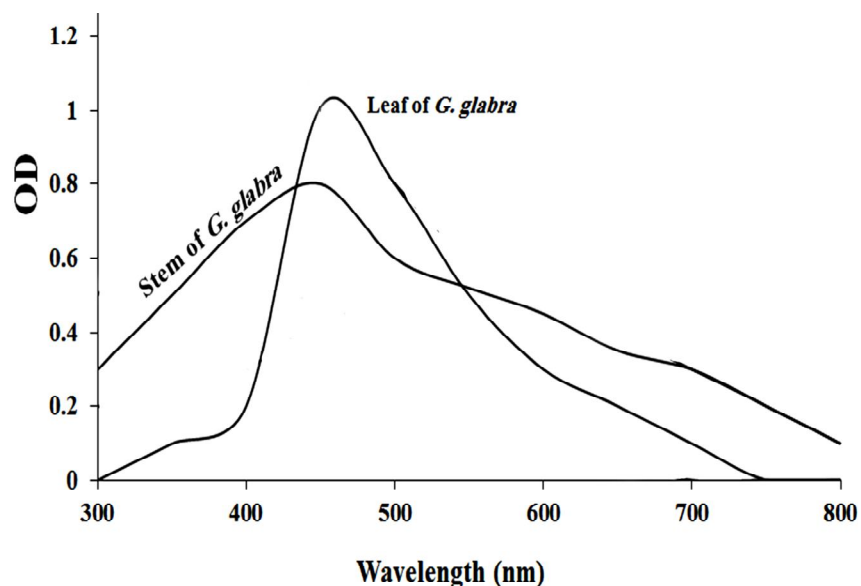
### Antibacterial Activity

The extracts and the synthesized AgNPs were evaluated for their antibacterial activities against 4 bacterial strains including *Bacillus cereus* (PTCC 1247), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 27853), and also antifungal activity against *Fusarium oxysporum* [43]. The antibacterial activity was assessed by disc diffusion method, and the zone of inhibition was recorded. To evaluate the antifungal activity, Potatoes Dextrose Agar (PDA) medium was used, and radial growth diameter of fungal mycelium was recorded after 7 days incubation. In antibacterial activity assessment, the samples were dissolved in DMSO to make different concentrations (1, 0.1, 0.01  $\text{mg ml}^{-1}$ ), and then sterilized by filtration (0.45  $\mu\text{m}$  Millipore). All tests were carried using 0.5 McFarland concentration (10 ml suspension containing  $1.5 \times 10^8$  bacteria/ml). Negative reference standard was prepared using DMSO, and the positive references by Gentamicin, Penicillin and Streptomycin. For antifungal assay, the samples in two concentrations (500 and 1000 ppm in DMSO) were added to culture medium and the following formula was used to determine antifungal activity:

$$\text{Antifungal activity (\%)} = (R - r/R) \times 100$$



**Fig. 2.** Color change in reaction mixture of *G. glabra* leaf and stem extracts. (A) 1 mmol AgNO<sub>3</sub> solution (I), 1 mg ml<sup>-1</sup> leaf extracts (II), reaction mixture (AgNO<sub>3</sub> + leaf extract) at 60 °C (III). (B) 1 mmol AgNO<sub>3</sub> solution (I), 1 mg ml<sup>-1</sup> stem extract (II), reaction mixture (AgNO<sub>3</sub> + stem extract) at 60 °C (III).



**Fig. 3.** Ultraviolet-visible spectra of the reaction mixture of 1 mmol AgNO<sub>3</sub> and *G. glabra* leaf and stem extracts at 60 °C.

where R is the radial growth of fungal mycelia on the control plate (double distilled water was used as a control group) and r is the radial growth of fungal mycelia on the plate treated with the samples.

#### Statistical Analysis

Statistical analysis of variance was performed using student's t-test by SPSS program. Data are the average of triplicate analyses and expressed as means  $\pm$  standard deviation.

## RESULTS AND DISCUSSION

### Phytochemical Analysis

*G. glabra* is an important medicinal plant, known to be rich in important secondary metabolites like phenolics, flavonoids, carbohydrates, vitamins and glycyrrhizin, that might play a remarkable role in bioreduction of Ag<sup>+</sup> into AgNPs [23]. Phytochemical analysis of *G. glabra* leaf and stem extracts revealed a high level of total phenol content (4-6 mg ml<sup>-1</sup>). Similarly, the extracts were found to be rich

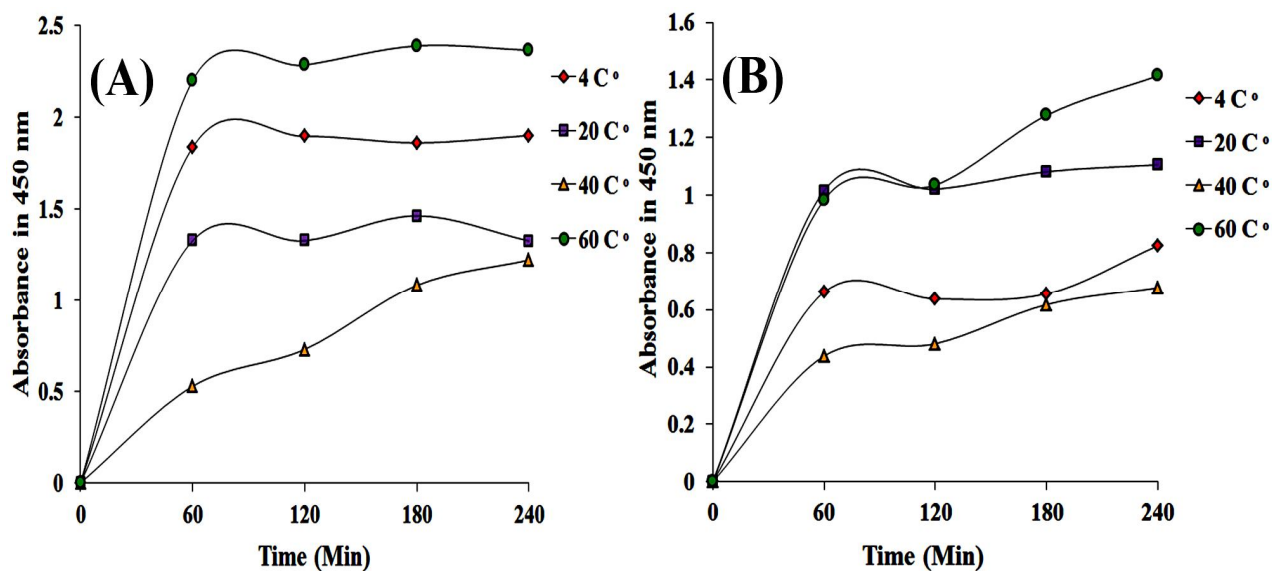


Fig. 4. Time course of AgNPs formation by (A) *G. glabra* leaf extract, and (B) *G. glabra* stem extract at different temperatures.

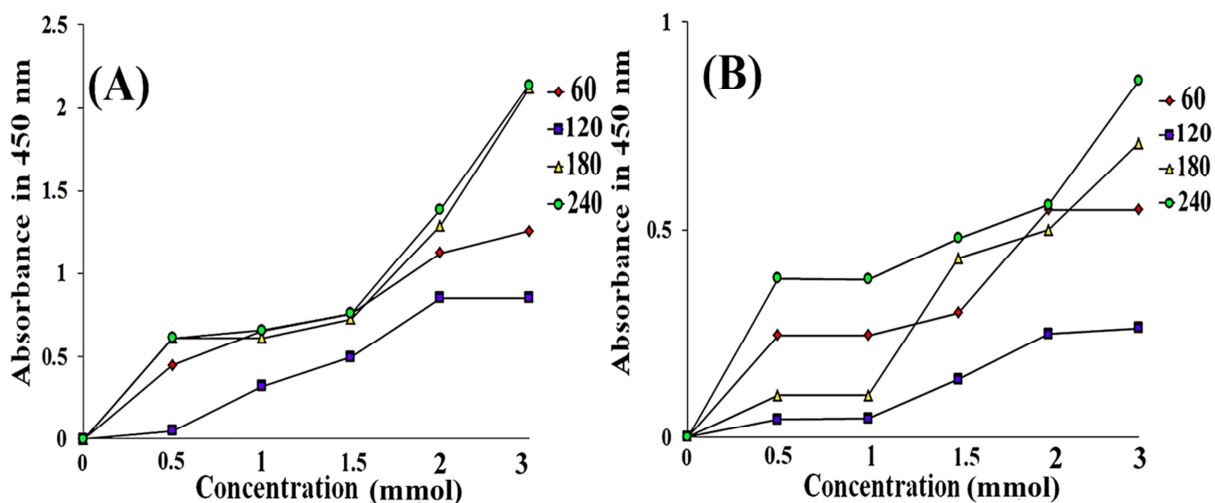
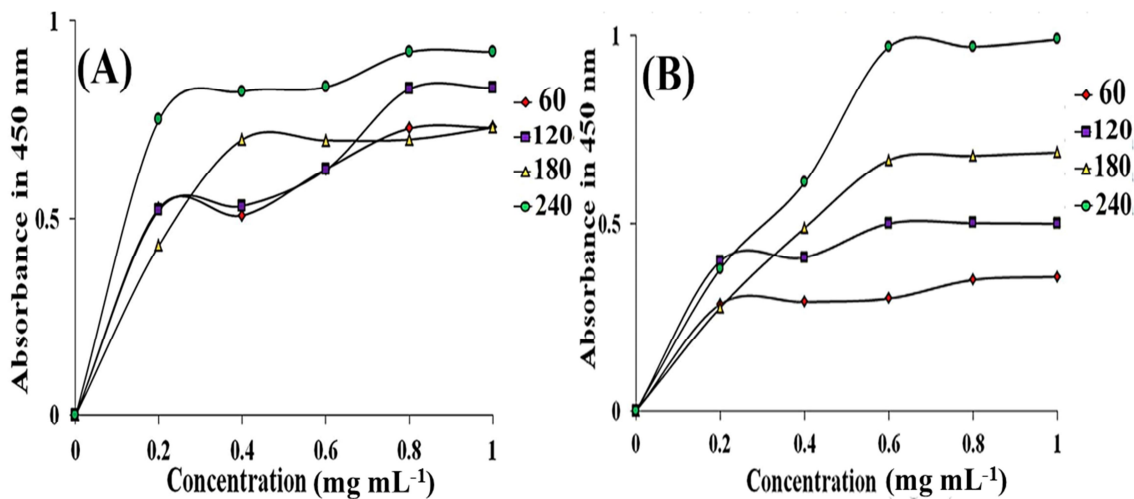


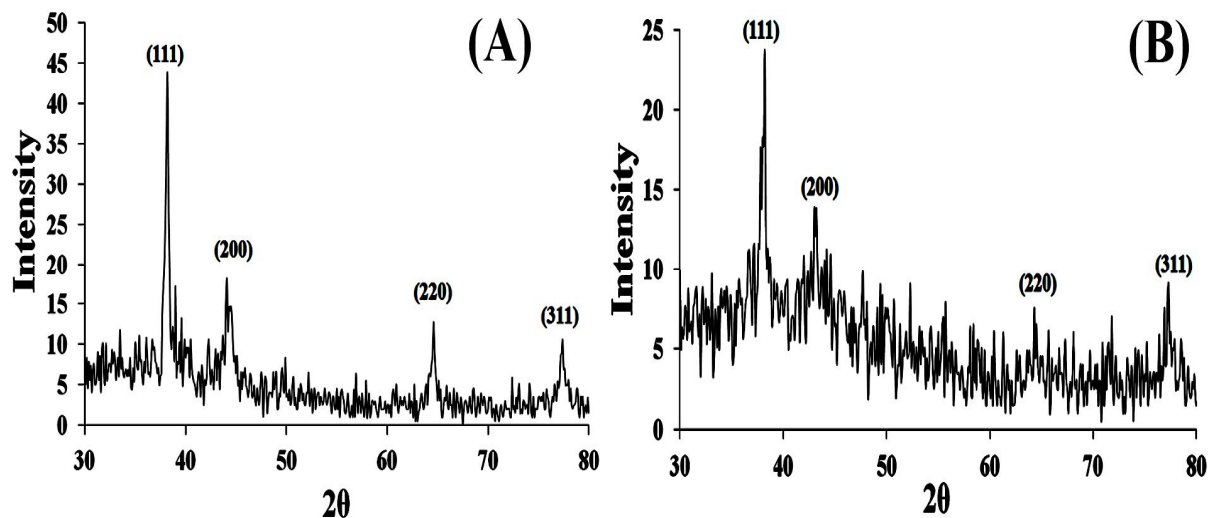
Fig. 5. The effects of reaction time (60-240 min) and different concentrations of  $\text{AgNO}_3$  (0.5-3 mmol) on formation of silver nanoparticles (A) *G. glabra* leaf extract ( $1 \text{ mg ml}^{-1}$ ) (B) *G. glabra* stem extract ( $1 \text{ mg ml}^{-1}$ ).

in flavonoids with no remarkable differences in two parts ( $P < 0.05$ ). It is shown that flavonoid complexes are one of the major components of *G. glabra* with high biological activity against inflammatory diseases and pyrexia [23]. Total reducing sugar was also found almost high (0.061-0.122

$\text{mg ml}^{-1}$ ), followed by starch (0.049-0.039  $\text{mg ml}^{-1}$ ). In addition, ascorbic acid was detected in the range of 0.365 to 0.058  $\text{mg ml}^{-1}$ . In general, our results indicated that the leaf extract has a higher phytochemical content compared to the stem extract (Table 1).



**Fig. 6.** The effects of reaction time (60-240 min) and concentration of the extracts (0.2-1 mg mL<sup>-1</sup>) on the formation of silver nanoparticles (1 mmol AgNO<sub>3</sub>) (A) *G. glabra* leaf extract as a bioreductant (B) *G. glabra* stem extract as a bioreductant.



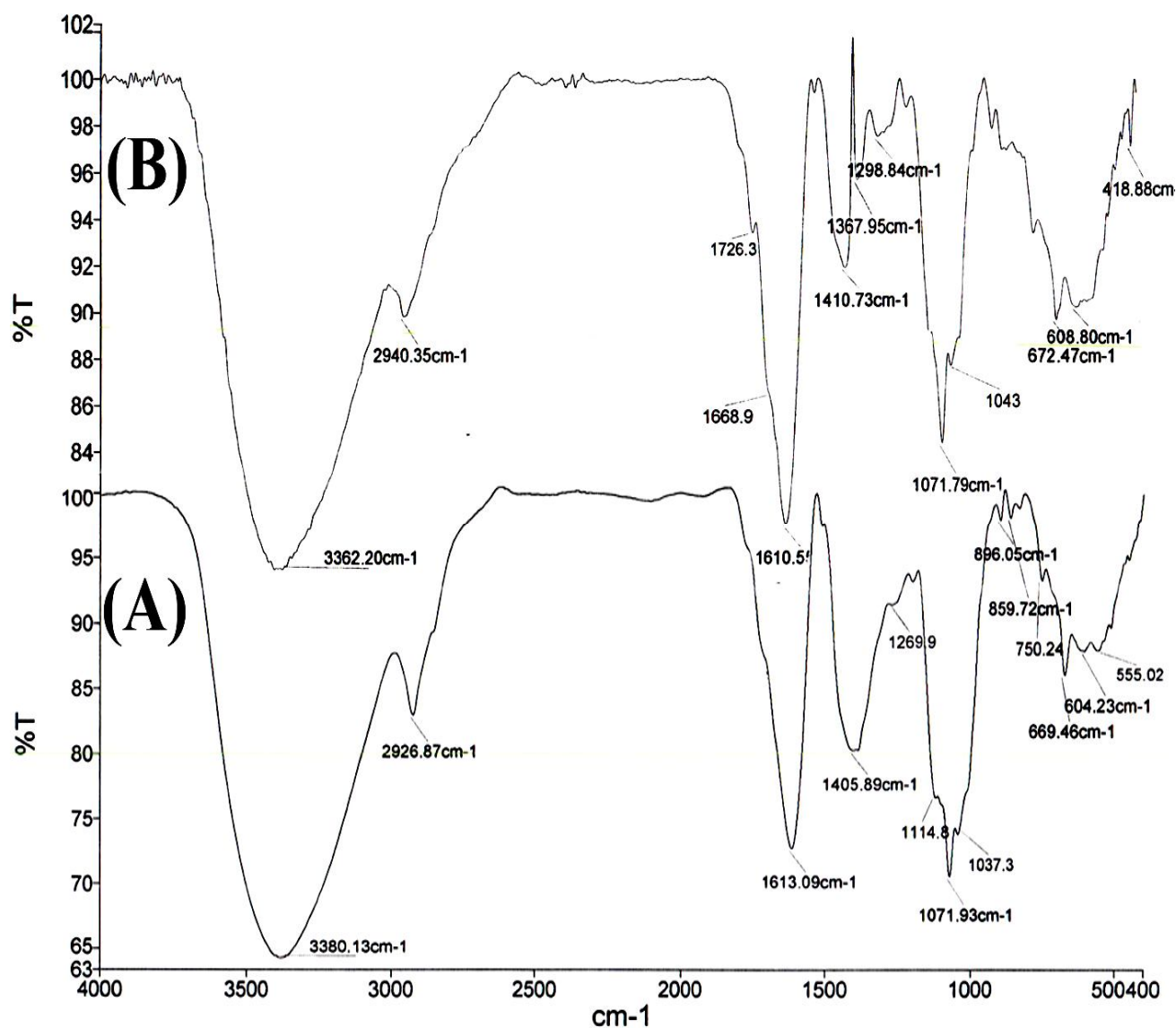
**Fig. 7.** Representative X-ray diffraction profile of the synthesized AgNPs. (A) The synthesized AgNPs by *G. glabra* leaf extract, and (B) *G. glabra* stem extract.

### Synthesis and Characterization of Silver Nanoparticles

The synthesized AgNPs formed by using *G. glabra* extracts, as a bio-reductant, were found to be very stable, possibly because of some metabolites such as starch present in the extracts that prevent agglomeration, even after several days. An intense brown coloration of the reaction mixture

indicates the synthesis of AgNPs (Fig. 2). The color changes of the reaction mixture containing the extracts (1 mg mL<sup>-1</sup>) and AgNO<sub>3</sub> (1 mmol) showed maximum peak in the range of 400-500 nm (Fig. 3). Study the effects of temperature, reaction time, different concentrations of AgNO<sub>3</sub> and the extracts, and their interaction by plotting absorbance at the peak wavelengths of silver nanoparticles





**Fig. 8.** Fourier transforms infrared absorption spectra of *G. glabra* leaf extract (A) after bioreduction, and (B) before bioreduction of Ag<sup>+</sup> ions at 60 °C.

indicates that temperature changes (4-60 °C) affect AgNPs formation, and the most rate of synthesis was obtained at 60 °C. However, the synthesis of AgNPs does not follow a regular pattern in the range of 4-40 °C (Figs. 4A and 4B). In addition, concentration of AgNO<sub>3</sub> and the extract had a remarkable effect on the synthesis of AgNPs in a dose-dependent manner. The maximum rate was found in 3 mmol AgNO<sub>3</sub>, especially 180-240 min after reaction with 1 mg ml<sup>-1</sup> extract (Figs. 5A and 5B) and in 1 mg ml<sup>-1</sup>

extract, especially 120-240 min after reaction with 3 mmol AgNO<sub>3</sub> (Figs. 6A and 6B).

**X-ray diffraction analysis.** The X-ray diffraction data for the dried powder was recorded, and phase formation was confirmed from characteristic peaks containing (111), (200), (220) and (311) (Fig. 7). In X-ray diffraction analysis, broadening of the peak indicates a smaller particle size. The crystallite size calculated using Scherrer's formula indicated that the size of particles is ranged from 5-45 nm with an



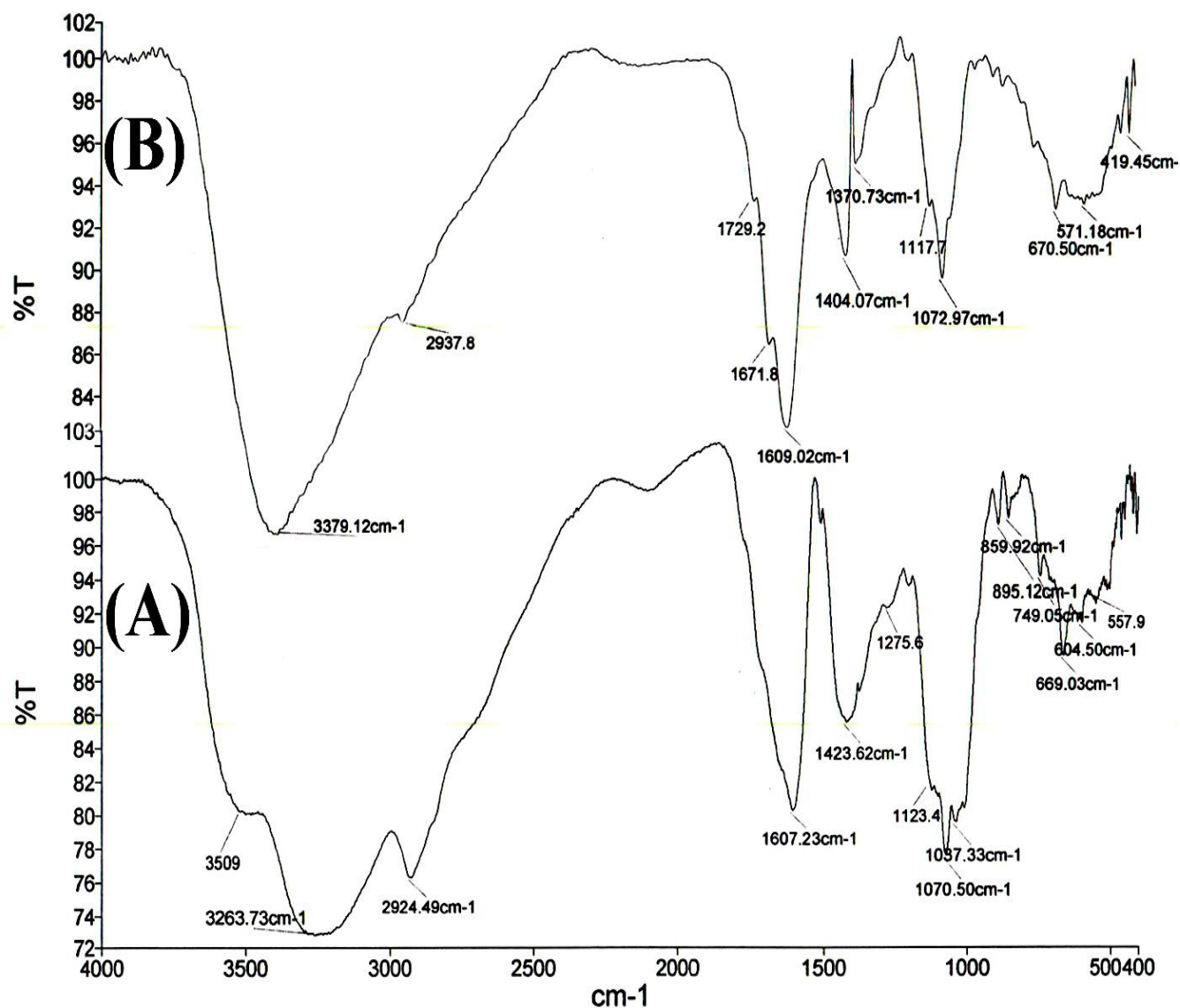


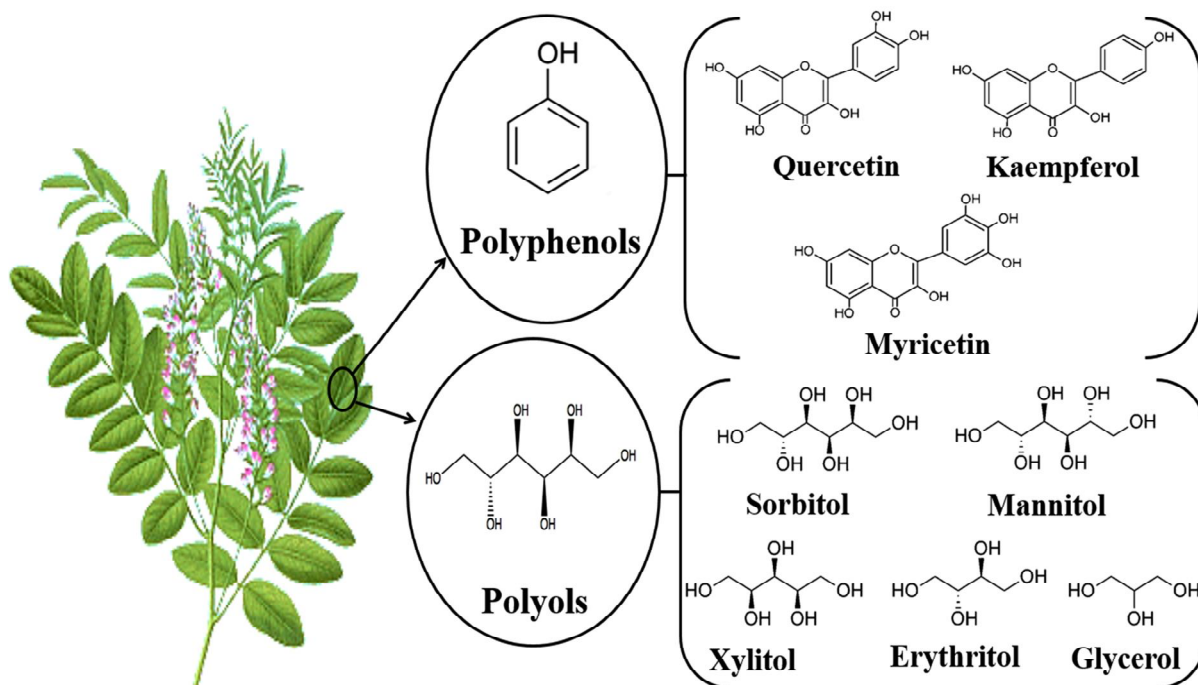
Fig. 9. Fourier transform infrared absorption spectra of *G. glabra* stem extract (A) after and (B) before bioreduction of  $\text{Ag}^+$  ions at 60 °C.

average diameter of 16 nm.

**FTIR analysis.** FTIR absorption spectra of the samples indicated similarity before and after bio-reduction, with some marginal shifts in peak position clearly indicating the presence of the extract residuals in the reaction mixture (Figs. 8 and 9). Phytochemical analysis of the stem and leaf extracts strongly suggested the presence of alcohols and phenolic compounds (polyphenols) as the main constituents that is supported by a strong peak at approximately 3350

$\text{cm}^{-1}$  (O-H stretch, H-bonded; alcohols, phenols). Polyphenols and polyols compounds have hydroxyl groups in their chemical structures that are strong hydrogen donors (Fig. 10). The absorbance bands at 1726.3  $\text{cm}^{-1}$  and 1668.9  $\text{cm}^{-1}$  after bio-reduction are associated with bending of C-O-H, and stretching of C-O, probably due to primary and secondary reduction of polyols that can be responsible for reduction of  $\text{Ag}^+$  into AgNPs [44].

**Scanning electron microscopy (SEM).** The SEM



**Fig. 10.** Structure of some polyols and polyphenols present in plant extracts.

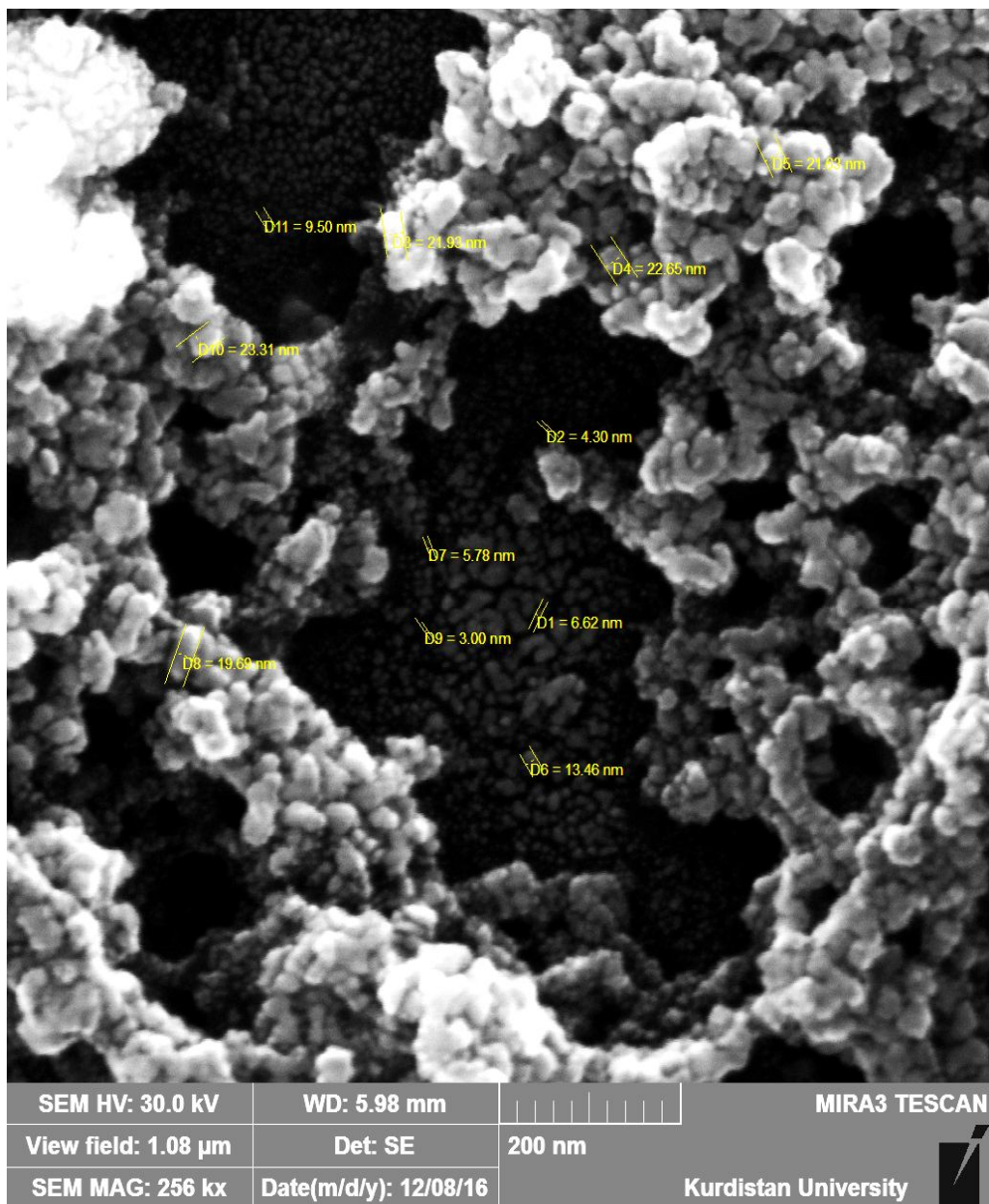
images confirmed formation of AgNPs by bio-reduction. The nanoparticles produced were nearly spherical in shape with a size range of 3-24 nm for the leaf extract and 5-30 nm for the stem extract (Figs. 11 and 12).

### Antibacterial Activity

Results from antibacterial activity assessment of the extracts and AgNPs as diameter of inhibition zones (in mm) are presented in Tables 2 and 3. Previous studies indicated that the synthesized nanoparticles using plant extracts have antimicrobial activity against a wide board of microorganisms [45-48]. The exact antibacterial performance of AgNPs is not completely understood. It is believed that AgNPs may interfere with cell wall synthesis, inhibit protein synthesis, interfere with nucleic acid synthesis, and inhibit metabolic pathways. It seems that electrostatic attraction between negatively charged bacterial cells and positively charged nanoparticles are probably crucial for the activity of nanoparticles as bactericidal materials. AgNPs attach to the surface of the cell membrane and disturb their functions, such as permeability and respiration. It is reasonable to state that the binding of the

particles to the bacteria depends on the interaction of the surface area available (shape and size of nanoparticles). Hence, smaller particles such as AgNPs having a larger surface area available for interaction will have a stronger bactericidal effect than larger particles [49]. It is well known that *Escherichia coli* (-), *Pseudomonas aeruginosa* (-), *Staphylococcus aureus* (+) and also *Bacillus* species are agents of food poisoning [50,51]. So a smaller dose of silver suspension nanoparticles is needed to inhibit the growth of these bacteria.

*F. oxysporum* is a fungus that lives in soil and enters through the roots of plant into the xylem tissue blocking the vascular system that finally prevents transport of water and nutrients in infected plants. It can cause severe losses of the plant crop even leading to its death [52,53]. In this research, the extracts and AgNPs were dissolved in DMSO as a negative control for assessment of antibacterial and antifungal activities. This solvent had no activity against all microbial strains tested (Table 2). All the samples (C<sub>1</sub>-C<sub>5</sub>) showed antibacterial activity, however C<sub>4</sub> sample (nanoparticles synthesized by stem extract) showed more antibacterial activity than others. *B. cereus* (+) was the most

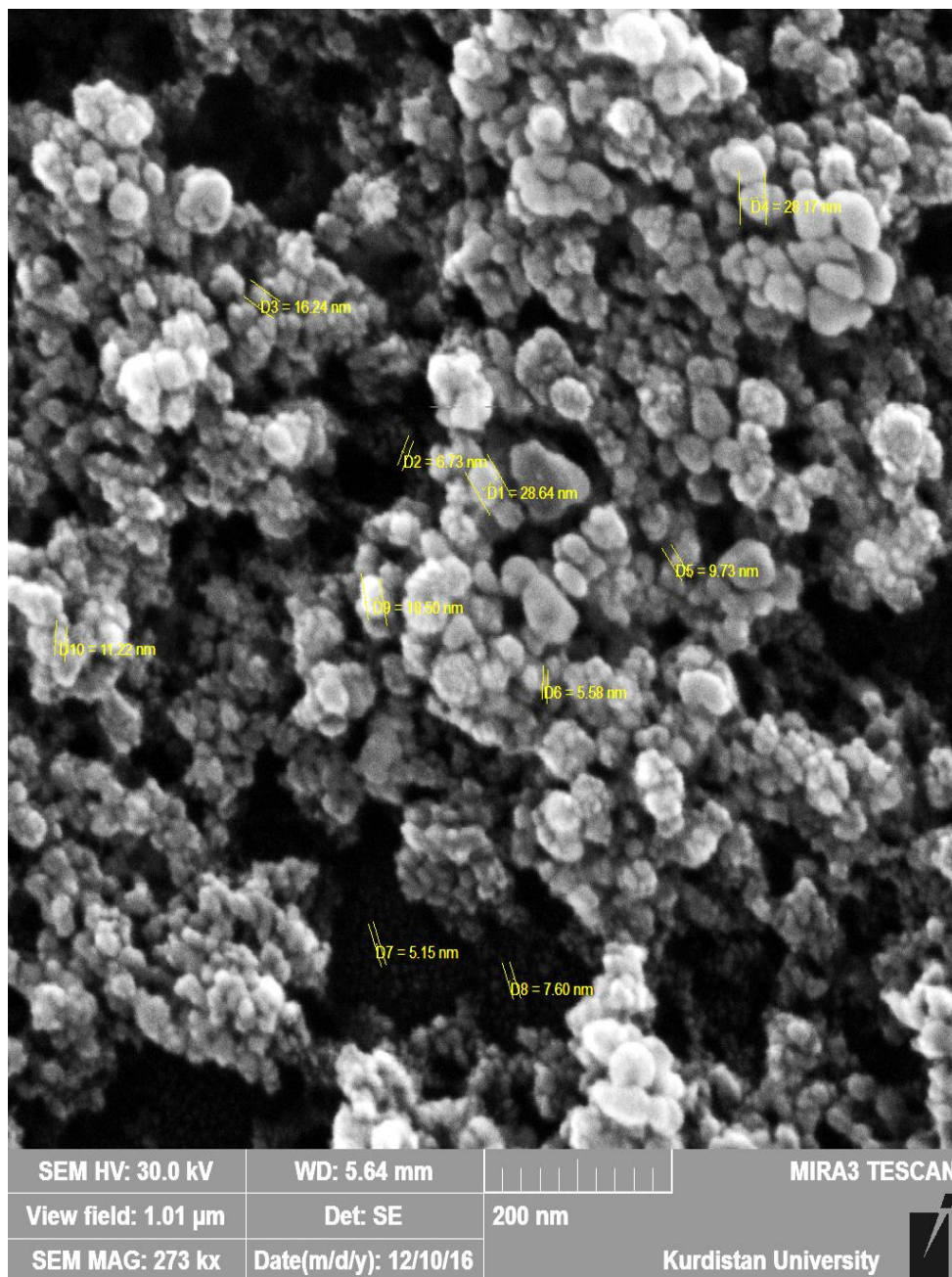


**Fig. 11.** Scanning electron micrograph of the synthesized AgNPs by *G. glabra* leaf extract.

resistant bacterium against all the samples and *E. coli* (-) was resistant to Gentamicin. All bacteria were resistant to Penicillin, and activity of the samples were higher than those of antibiotics (Table 2). In general, it was found that the extracts and AgNPs have more antibacterial activity against Gram positive and negative, respectively. Also, both

of extracts and AgNPs showed more antibacterial activity than silver salt ( $\text{AgNO}_3$ ). Previous studies showed that biological properties of a compound are related to its major components. However, the synergistic or antagonistic effect of any component in minor percentages was discussed [54,55]. Most of synthetic fungicides are usually toxic to





**Fig. 12.** Scanning electron micrograph of the synthesized AgNPs by *G. glabra* stem extract.

human. Hence, interest to use of plant extracts and nanoparticles as antifungal agents is being increased [45-48]. Our results indicated that all samples in both concentrations (C<sub>1</sub>-C<sub>5</sub>) represent different inhibitory effects

on the mycelial growth of *F. oxysporum* (Table 3). However, C<sub>3</sub> sample (nanoparticles synthesized by leaf extract) showed more inhibition activity. Our findings are supported by Krishnaraj *et al.* [56], Gopinath and Velusamy [57], and

**Table 2.** Antibacterial Activity of the Extracts, Synthesized Nanoparticles and Standards

Sample	Inhibition zone				
	Concentration (mg ml <sup>-1</sup> )	<i>B. s</i> (+)	<i>S. a</i> (+)	<i>P. a</i> (-)	<i>E. c</i> (-)
C <sub>1</sub>	1	10 ± 0.16 <sup>a</sup>	14 ± 0.11 <sup>a</sup>	7 ± 0.00	Na
	0.1	8 ± 0.27 <sup>b</sup>	13 ± 0.19 <sup>b</sup>	Na	Na
	0.01	Na	11 ± 0.34 <sup>c</sup>	Na	Na
C <sub>2</sub>	1	9 ± 0.22 <sup>a</sup>	15 ± 0.32 <sup>a</sup>	7 ± 0.53	7 ± 0.10
	0.1	7 ± 0.13 <sup>b</sup>	12 ± 0.12 <sup>b</sup>	Na	Na
	0.01	Na	8 ± 0.14 <sup>c</sup>	Na	Na
C <sub>3</sub>	1	Na	7 ± 0.22	17 ± 0.31 <sup>a</sup>	8 ± 0.31 <sup>a</sup>
	0.1	Na	Na	16 ± 0.28 <sup>a</sup>	8 ± 0.16 <sup>a</sup>
	0.01	Na	Na	16 ± 0.12 <sup>a</sup>	7 ± 0.21 <sup>b</sup>
C <sub>4</sub>	1	7 ± 0.26	12 ± 0.41 <sup>a</sup>	14 ± 0.17 <sup>a</sup>	12 ± 0.15 <sup>a</sup>
	0.1	Na	11 ± 0.32 <sup>b</sup>	11 ± 0.15 <sup>b</sup>	9 ± 0.36 <sup>b</sup>
	0.01	Na	8 ± 0.25 <sup>c</sup>	10 ± 0.26 <sup>c</sup>	9 ± 0.13 <sup>b</sup>
C <sub>5</sub>	1	8 ± 0.36	9 ± 0.11 <sup>a</sup>	8 ± 0.27 <sup>a</sup>	11 ± 0.15 <sup>a</sup>
	0.1	Na	7 ± 0.23 <sup>b</sup>	7 ± 0.55 <sup>a</sup>	9 ± 0.41 <sup>b</sup>
	0.01	Na	Na	Na	7 ± 0.00 <sup>c</sup>
Control (+)	Gentamicin	25 ± 0.12	33 ± 0.13	11 ± 0.17	Na
	Streptomycin	30 ± 0.18	18 ± 0.21	15 ± 0.16	15 ± 0.21
	Penicillin	Na	Na	Na	Na
Control (-)	DMSO	Na	Na	Na	Na

Experiment was performed in triplicate and expressed as mean ± SD. Values in each column with different superscripts are significantly different ( $P < 0.05$ ). (C<sub>1</sub>) Leaf extract, (C<sub>2</sub>) Stem extract, (C<sub>3</sub>) The synthesized AgNPs by leaf extract, (C<sub>4</sub>) The synthesized AgNPs by stem extract, and (C<sub>5</sub>) AgNO<sub>3</sub>. *P. a*: *Pseudomonas aeruginosa*, *S. a*: *Staphylococcus aureus*, *E. c*: *Escherichia coli*, *B. c*: *Bacillus cereus*, Na: No active.

**Table 3.** Antifungal Activity of the Extracts and Synthesized Nanoparticles

Sample	Inhibition radial growth of fungal mycelium	
	Concentration (ppm)	(%)
C <sub>1</sub>	500	36.24 ± 1.11 <sup>a</sup>
	1000	52.47 ± 1.42 <sup>b</sup>
C <sub>2</sub>	500	42.63 ± 0.10 <sup>a</sup>
	1000	57.13 ± 2.00 <sup>b</sup>
C <sub>3</sub>	500	51.26 ± 1.26 <sup>a</sup>
	1000	75.83 ± 1.65 <sup>b</sup>
C <sub>4</sub>	500	49.21 ± 1.73 <sup>a</sup>
	1000	66.68 ± 3.12 <sup>b</sup>
C <sub>5</sub>	500	31.24 ± 0.65 <sup>a</sup>
	1000	47.18 ± 1.23 <sup>b</sup>
DMSO	-	16.00 ± 0.31 <sup>b</sup>

Experiment was performed in triplicate and expressed as mean ± SD. Values in each column with different superscripts are significantly different ( $P < 0.05$ ). (C<sub>1</sub>) Leaf extract, (C<sub>2</sub>) Stem extract, (C<sub>3</sub>) The synthesized AgNPs by leaf extract (C<sub>4</sub>), The synthesized AgNPs by stem extract, and (C<sub>5</sub>) AgNO<sub>3</sub>.

Yehia and Ahmed [58], who reported that the green synthesized AgNPs possess strong antifungal activity against the various phytopathogenic fungi like *F. oxysporum*.

Experiment was performed in triplicate and expressed as mean ± SD. Values in each column with different superscripts are significantly different ( $P < 0.05$ ). (C<sub>1</sub>) Leaf extract, (C<sub>2</sub>) Stem extract, (C<sub>3</sub>). The synthesized AgNPs by leaf extract, (C<sub>4</sub>) The synthesized AgNPs by stem extract, and (C<sub>5</sub>) AgNO<sub>3</sub>. *P. a*: *Pseudomonas aeruginosa*, *S. a*: *Staphylococcus aureus*, *E. c*: *Escherichia coli*, *B. c*: *Bacillus cereus*, Na: No active.

Experiment was performed in triplicate and expressed as mean ± SD. Values in each column with different superscripts are significantly different ( $P < 0.05$ ). (C<sub>1</sub>) Leaf extract, (C<sub>2</sub>) Stem extract, (C<sub>3</sub>) The synthesized AgNPs by leaf extract (C<sub>4</sub>), The synthesized AgNPs by stem extract, and (C<sub>5</sub>) AgNO<sub>3</sub>.

## CONCLUSIONS

The results obtained from the present study clearly demonstrated the efficiency of *G. glabra* extract as easily

available agent in the rapid synthesis of stable silver nanoparticles for the first time. Also, this study showed that *G. glabra* extract has a high content of phytochemicals such as polyphenoles, starch, reducing sugars and ascorbic acid. Based on the kinetic studies and evidence obtained from FTIR analysis, our results suggested that phenolic compounds such as flavonoids are probably the main components responsible for nanoparticle biosynthesis. In addition, the extracts and the synthesized AgNPs have good antimicrobial potential that may help to develop new drug candidates for new antibiotics, which might be helpful in the treatment of diseases caused by a wide board of microbial species.

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