

## RESEARCH PAPER

## Green Synthesis of Silver Nanoparticles Using *Cuminum cyminum* Leaf Extract and Evaluation of Their Biological Activities

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### ABSTRACT

The aim of this study is to determine some phytochemical contents, to synthesize silver nanoparticles AgNPs using *Cuminum cyminum* (*C. cyminum*) leaf extract, to study the effects of different conditions on the rate of synthesis, and to evaluate their antibacterial and antifungal activities. AgNPs were synthesized using *C. cyminum* leaf extract as bioreducer agent and aqueous silver nitrate solution ( $\text{AgNO}_3$ ) as precursor at different conditions. The synthesized AgNPs were characterized using Ultraviolet-visible absorption spectroscopy (UV-vis), Scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD). In addition, the synthesized AgNPs were evaluated for their antibacterial and antifungal activities against 4 positive and negative bacteria using agar disc diffusion method and the fungus *Fusarium oxysporum* cultivated in Potatoes Dextrose Agar (PDA) medium, respectively. Results showed that the bioreduction process was quite fast and the synthesized AgNPs were nearly spherical in shape and the size range of 3-20 nm. The AgNPs formation was affected by different parameters including concentration of  $\text{AgNO}_3$  solution and the extract, temperature and the time of reaction. The best condition for AgNPs biosynthesis was achieved by 1-3 mM  $\text{AgNO}_3$  at 40 °C after 4 h. In addition, the leaf extract and AgNPs showed efficient antibacterial activities against Gram positive and negative bacteria, respectively.

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### INTRODUCTION

The silver nanoparticles (AgNPs) are extensively applied in different fields such as pharmaceuticals, food, agriculture and textile industries, and water treatment, and as antioxidant, antimicrobial, anti-cancer, cosmetics, ointments, and larvicide [1-7]. In recent years, the chemical methods for synthesis AgNPs have been extended. Recent advances have been made in the utilization of various water based synthesis routes towards the shape-controlled synthesis of diverse silver nano- and microstructures such as  $\text{Ag}_2\text{Se}$ ,  $\text{Ag}_2\text{CdI}_4$ /AgI,  $\text{ZnCr}_2\text{O}_4$ /Ag,  $\text{Ag}_2\text{S}$ ,  $\text{Ag}_2\text{CrO}_4$  and  $\text{Ag}_2\text{Cr}_2\text{O}_7$  in a diverse range of shapes and sizes from several nanometers to micrometers [8-13]. However, use of many of the chemical methods requires time

and money consuming and also these particles may have several hazards effects including cyto- and genotoxicity [14, 15]. Today, nontoxic and green methods using bacteria, fungi and yeasts have drawn the attention of researchers for synthesis of nanoparticles [16, 17]. However, the use of microbes as bioreducers is difficult and also very expensive in terms of industrial production costs [18]. Plants as renewable resources have been used to produce biodegraded nanomaterials, thereby this green synthesis provides an economic, eco-friendly, and clean synthesis route to AgNPs [19-21]. Many natural biomolecules in plants such as proteins, amino acids, polysaccharides, alkaloids, alcoholic compounds and vitamins could be involved in

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bioreduction, formation and stabilization of AgNPs [22]. In order to use the organisms for synthesis of metal nanoparticle in industrial scale, the yield and the production rate need to be considered and the bioreduction conditions in the reaction mixture to be optimized. The substrate concentration, the biocatalyst concentration, the electron donor and its concentration, pH, exposure time, temperature, buffer strength, mixing speed, and light need to be controlled and optimized [23-26].

*Cuminum cyminum* L. belongs to the family Apiaceae and is one of the old cultivated medicinal food herbs in Asia, Africa and Europe [27]. This species contains a large amount of metabolites like polyphenols, cuminaldehyde, sabinene and myrcene. Cumin is widely used in medicine for the treatment of dyspepsia, diarrhea, and jaundice, as it has stomachic, diuretic, carminative, and antispasmodic properties [28-33].

Recently, the growing microbial resistance against antibiotics and the development of resistant strains has shifted the interests of many scientists to focus on metallic nanoparticles application. Metallic nanoparticles are the most potential compounds having a significantly high specific surface area and a high fraction of surface atoms, unique optical, electronic, catalytic, antibacterial and magnetic properties. Antibacterial and antifungal activities of nanoparticles related to their features such as size and shape [34-38]. Among metals, silver exhibits a higher toxicity to microorganism. It has been found that AgNPs inhibited bacterial growth by a destructive effect on DNA, resulting in a loss of replication and

degradation of DNA. Therefore, silver nanoparticles that possess unique physicochemical properties have attracted abundance of interest in various fields, especially to produce antimicrobial agents. Synthesis and characterization of AgNPs using *C. cyminum* seed extract were reported and their antimicrobial activity shown only against *E. coli* [39-41].

The aim of this study is to determine the content of some metabolites including phenols, flavonoids, reducing sugars, starch and ascorbic acid in *C. cyminum* leaf extract and to biosynthesis AgNPs using this extract for the first time. The effects of different parameters such as concentration of  $AgNO_3$  solution and the extract, temperature and time on the reaction rate were also examined. The morphology and structure of the AgNPs were characterized using Scanning electron microscopy (SEM), UV-Vis absorption spectroscopy, X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR). In addition, the AgNPs were evaluated for their antibacterial and antifungal activities against 4 bacterial strains and the fungus *F. oxysporum*, respectively (Fig. 1).

## MATERIALS AND METHODS

### Plant material

*Cuminum cyminum* L. seeds were provided from Pakan Bazer Company, Isfahan, Iran.

### Preparation of leaf extract

Air-dried and powdered leaves of *C. cyminum* (5 g) were mixed with 50 mL of sterile distilled water. The mixture was boiled for 5 min to denature the

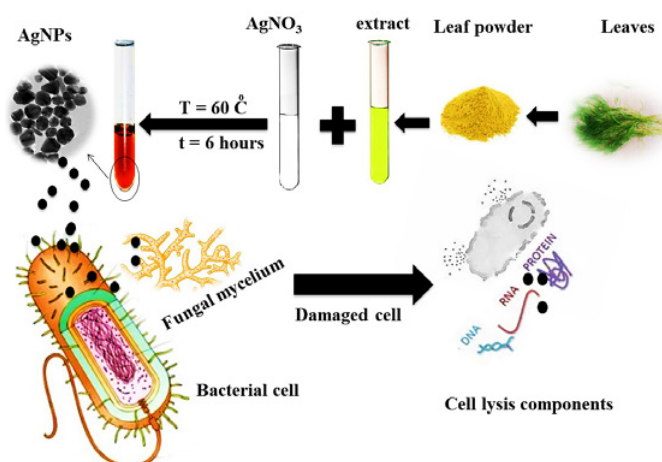


Fig. 1. Schematic illustration of green synthesis of silver nanoparticles by aqueous leaf extract of *Cuminum cyminum* L. Abbreviations: T, temperature; t, time.

enzymes in the extract and then filtered through a Whatman filter paper (No. 1) and stored at 4 °C for further use.

#### Phytochemical analysis

##### Determination of total phenol content

Briefly, 3 mL of a reaction mixture containing 0.125 mL of the extract or gallic acid (standard phenolic compound), 1.5 mL deionized water, 0.125 mL Folin-Ciocalteu reagent, and 1.25 mL 7% Na<sub>2</sub>CO<sub>3</sub> solution was allowed to stand for 90 min and then amount of total phenols was determined by colorimetry at 760 nm and calculated as mg/mL from calibration curve of gallic acid standard solution (0-0.25 mg/mL in methanol: water (50:50 v/v)) [42].

##### Determination of total flavonoid content

Briefly, 1 mL of a reaction mixture containing 0.5 mL of the leaf extract and 0.5 mL 2% AlCl<sub>3</sub> was incubated at 25 °C temperature for 10 min and its absorbance was measured at 368 nm by a double beam Perkin Elmer UV/visible spectrophotometer [43]. The amount of total flavonoids was calculated as mg/mL from calibration curve of quercetin standard solution (12.5-100 µg/mL in methanol).

##### Determination of starch content

The anthrone method was used to determine starch content [44]. Briefly, 5 g of leaf powder was washed using 70% ethanol several times. The residue was dried and boiled in 100 mL of distilled water to obtain the extract without any sugars. A 1 mL sample of the extract was evaporated to dryness and reconstituted in 60% perchloric acid. 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 from calibration curve of glucose (12.5-100 µg/mL in distilled water).

##### Determination of total reducing sugar content

Briefly, 1 mL of the 1% aqueous extract was mixed with 1 mL dinitrosalicylic reagent and kept at 100 °C for 5 min. Total reducing sugar content was determined by colorimetry at 630 nm and quantified from calibration curve of maltose (12.5-100 µg/mL in distilled water) [45].

##### Determination of ascorbic acid content

Briefly, 2 mL of the leaf extract was evaporated to dryness and then reconstitution in 4% oxalic

acid. Then the sample was brominated and 1 mL 2,4-dinitrophenyl hydrazine reagent was added, followed by two drops of 10% thiourea and mixing thoroughly. The sample was incubated at 37 °C for 3 h. The orange-red osazone crystals formed were dissolved in 7 mL 80% sulfuric acid and then absorbance was measured at 540 nm. The content of ascorbic acid in the extract was quantified by comparison with a standard curve for ascorbic acid [46].

##### Synthesis and characterization of silver nanoparticles

Briefly, 25 mL of aqueous AgNO<sub>3</sub> solution (0-3 mM) was mixed with 5 mL of the aqueous extract (0.2-1 mg/mL) as bioreduction agent. The biosynthesis was done in different times (0-4 h) and at different temperatures (4-60 °C). Then the effects of temperature, concentration of AgNO<sub>3</sub> and the extract and the time of reaction on the rate of synthesis and the size of AgNPs were discussed. Then, the samples were analysed for change of absorbance by UV-Vis spectrophotometer in the range of 300-600 nm. A dry nanoparticle powder was obtained by centrifugation at 12,000 rpm for 15 min at 25 °C. Then the features of AgNPs were characterized by scanning electron microscopy (SEM, Philips-CMC-300 KV). Also, the size distribution and crystal structure of AgNPs were determined and calculated from the SEM images and X-ray diffractometer (Italstructure ADP2000 XRD diffractometer). The AgNPs were also subjected to Fourier transform infrared (FTIR, Perkin Elmer GX FT-IR spectrometer) spectroscopy measurement.

##### X-ray diffraction analysis

After a 24-h reaction of 1 mM AgNO<sub>3</sub> solution with the leaf extract, the synthesized AgNPs were centrifuged at 12,000 rpm for 20 min at 25 °C followed by redispersion of the AgNPs pellet into 1 ml deionized water and then dried. Phase formation of the bioreduced nanoparticles was studied using x-ray diffraction. Diffraction data for thin thoroughly dried nanoparticle films on glass slides were recorded on an X-ray diffractometer (Italstructure ADP2000 XRD diffractometer) operated at a voltage of 40 kV and a current of 30 mA with Cu Kα (1.54 Å) source and the nanoparticles size was calculated using Scherrer's formula:

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

Where,  $d$  is the average crystallite domain size perpendicular to the reflecting planes, 0.9 is the shape factor,  $\lambda$  is the X-ray wavelength (typically 1.54 Å),  $\beta$  is the full width at half the maximum intensity in radians, and  $\theta$  is the Bragg angle.

#### Fourier transform infrared spectroscopy

The synthesized AgNPs were subjected to Fourier transform infrared (FTIR, Perkin Elmer GX FT-IR spectrometer in KBr pellets) based on the X-ray method. The nanoparticles powder was mixed with KBr and exposed to an infrared source of 400-4000  $\text{cm}^{-1}$ . A similar process was used to study *C. cyminum* leaf extract before and after bioreduction.

#### Scanning electron microscopy

Scanning electron microscopy of the obtained samples based on the X-ray and FTIR methods was performed using a Philips-CMC-300 KV SEM machine to know the size and shape of the AgNPs. Thin films of the samples were prepared by just dropping of the samples on a foil, and then removing of extra solution in an oven at 80 °C. In the following, the images of AgNPs were taken.

#### Antibacterial and antifungal activities

The leaf extract, AgNPs and  $\text{AgNO}_3$  were subjected to antibacterial and antifungal screening against four bacterial strains including *Bacillus cereus* (PTCC 1247), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 35218) and *Pseudomonas aeruginosa* (ATCC 27853) and also the fungus *Fusarium oxysporum*, respectively [47]. The antibacterial activity was assessed by disc diffusion method and inhibition zone was recorded. All samples were dissolved in DMSO to make different concentrations (1, 0.1, 0.01 mg/mL) and then sterilized by filtration (0.45  $\mu\text{m}$  Millipore). All tests were carried using 0.5 Mc Farland concentration (10 mL of suspension containing  $1.5 \times 10^8$  bacteria/mL). DMSO was used as negative reference standard and gentamicin, penicillin and streptomycin were used as positive reference standards. To evaluate the antifungal activity, *Fusarium oxysporum* was cultivated in Potatoes Dextrose Agar (PDA) medium and two

concentrations (500 and 1000 ppm) of the samples were added to the cultivation media. After a 7-days incubation, the radial growth of fungal mycelia was recorded using following formula:

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

Where,  $R$  is the radial growth of fungal mycelia on the control plate (double distilled water) 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 are expressed as means  $\pm$  standard deviation.

## RESULTS AND DISCUSSION

#### Phytochemical analysis

Results from biochemical analysis of *C. cyminum* leaf extract revealed a high level of total phenol ( $8.46 \pm 0.92$  mg/mL), but a low level of total flavonoid ( $0.008 \pm 0.07$  mg/mL). Ascorbic acid content was also found almost high ( $0.627 \pm 0.023$  mg/mL) followed by starch ( $0.223 \pm 0.032$  mg/mL) and total reducing sugars ( $0.062 \pm 0.005$  mg/mL) in the extract (Table 1).

#### Characterization of the synthesized silver nanoparticles

AgNPs were successfully synthesized from aqueous  $\text{AgNO}_3$  solution using *C. cyminum* leaf extract in a continuously heated and stirred mixture. The colorless reaction mixture slowly changed to a dark-brownish suspension after several minutes of reaction (Fig. 2). Color changes of the reaction mixture 240 min after bioreduction process, which were recorded by UV/vis spectrophotometer at 300-600 nm showed a maximum peak around 400 nm (Fig. 3). In particular, absorbance in the range of 420-450 nm has been used as an indicator to confirm the reduction of  $\text{Ag}^+$  to metallic Ag [48, 49]. The development of intense yellowish brown color owing to the surface plasmon resonance confirmed the synthesis of the silver nanoparticles. *C. cyminum* is a medicinal

Table 1. Phytochemical content (mg/mL) of *C. cyminum* leaf extract.

Sample	Total phenol	Total flavonoid	Starch	Total reducing sugar	Ascorbic acid
Leaf extract	$8.46 \pm 0.92$	$0.008 \pm 0.07$	$0.223 \pm 0.032$	$0.062 \pm 0.005$	$0.627 \pm 0.023$

Experiment was performed in triplicate and expressed as mean  $\pm$  SD.

plant rich in important secondary metabolites like terpenoids, polyphenols and flavonoids. The possible functional groups like alcohols and alkenes present in the cumin extract are responsible for capping and reducing of silver nanoparticles, were identified by FTIR analysis. In the other hand, high flavonoid and phenolic contents in *C. cyminum*

leaf extract strongly supported the potential of this extract to bioreduce  $\text{Ag}^+$  to  $\text{Ag}^0$ . In addition our results showed that the AgNPs synthesis depended on the concentration of  $\text{AgNO}_3$  solution and the leaf extract (Figs. 4, 5). The maximum rate of synthesis was found at 1.5 mM  $\text{AgNO}_3$  and 1 mg/mL the extract, especially 180-240 min

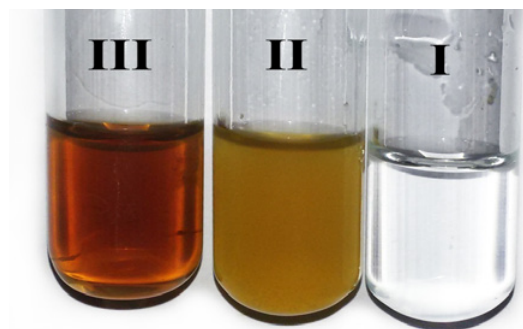


Fig. 2.  $\text{AgNO}_3$  solution (I), *C. cyminum* leaf extract (II) and reaction mixture ( $\text{AgNO}_3$  + leaf extract) at 60 °C (III).

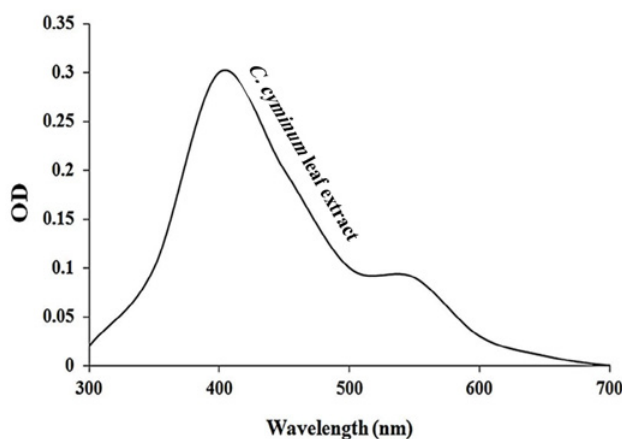


Fig. 3. UV-visible spectrum recorded as a function of reaction time of 1 mM  $\text{AgNO}_3$  solution with *C. cyminum* leaf extract at 60 °C.

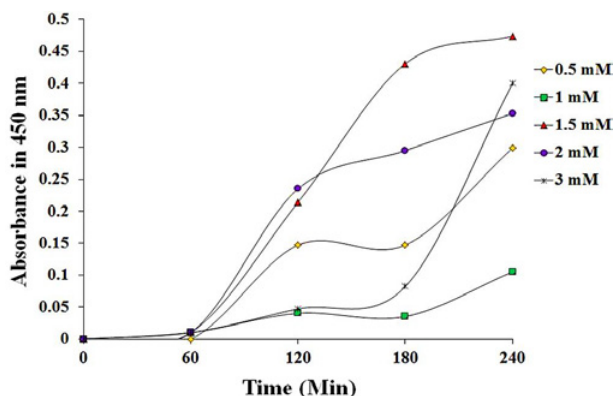


Fig. 4. UV/visible spectra of the reaction mixture at different concentrations of  $\text{AgNO}_3$  solution and different times.

after reaction (Fig. 6). As the time increased, the intensity of the absorbance increased, indicating increases in the amount of AgNPs produced from the mixture. In addition, temperature changes (4-60 °C) affect the AgNPs formation, so the highest rate was achieved at 40 °C. However, variation in the reaction kinetics at lower temperatures than 40 °C showed that the synthesis does not follow a regular pattern in this temperature range (Fig. 5). The AgNPs formed using by *C. cyminum* leaf extract were found to be very stable, possibly because some of metabolites such as starch present in the extract that prevented agglomeration, even after several days. In fact, the significant starch content in *C. cyminum* leaf extract reflects the capping properties of the extract, and starch is widely used in various synthetic processes for capping and stabilizing silver nanoparticles [50-52]. In addition, the complete reduction of  $Ag^+$  within 4

hours indicates that synthesis was much faster as compared with some other plants used for AgNPs synthesis [53]. Similar protocols have been used to synthesis of silver nanoparticles by a large number of plants as presented in Table 2.

*X-ray diffraction analysis*

In X-ray diffraction analysis phase formation was confirmed by characteristic peaks containing (111), (200), (220), and (311) and broadening of the peak indicated a smaller particle size (Fig. 7). The crystalline nature of the biosynthesized silver nanoparticles was confirmed by XRD data. The crystalline size of the synthesized particles which were calculated using Scherrer formula was ranged from 6 to 35 nm with an average diameter of 12 nm. This result is in agreement with Jeyashree and Revathi, who studied on AgNPs by using *C. cyminum* seed extract. In addition,

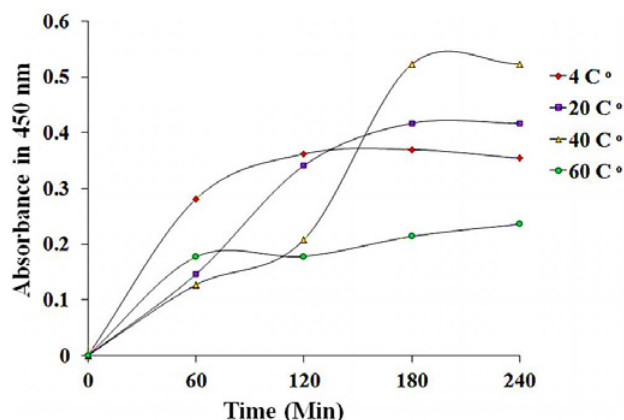


Fig. 5. Time course of AgNPs formation at different temperatures.

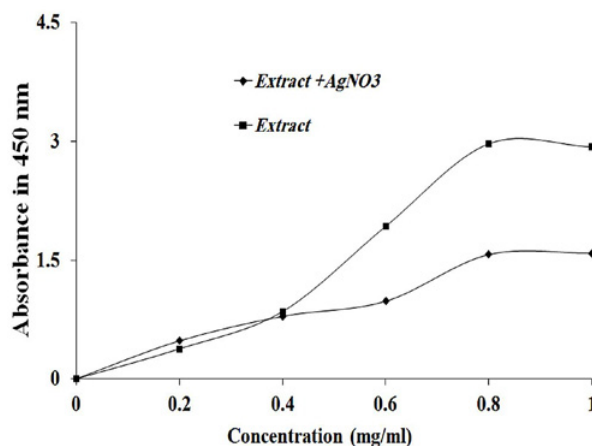


Fig. 6. UV/visible spectra of *C. cyminum* leaf extract at different concentrations alone and along with 1 mM  $AgNO_3$  solution at 60 °C.

there are some other peaks, which are not identified. It seems that they can be related to the unreduced  $\text{AgNO}_3$  molecules or some contaminants exist in the plant extract.

*Fourier transform infrared spectroscopy*

Results from FTIR absorption spectra of the samples indicated similarities between the spectra before and after bioreduction with some marginal shifts in the peak position that clearly proved the presence of the extract residual as reduction agent in the reaction mixture (Fig. 8). In addition, analysis of *C. cyminum* leaf extract strongly suggested the presence of alcoholic and phenolic compounds as the main parts of phytochemicals, which is supported by a strong peak at approximately  $3300\text{ cm}^{-1}$ . The absorbance bands at  $1648\text{ cm}^{-1}$  and  $1603\text{ cm}^{-1}$  after bioreduction are associated with bending of C–O–H and C–O stretches probably

because of primary and secondary reduction of the polyols (alcoholic and phenolic compounds) that can be responsible for reduction of  $\text{Ag}^+$  into AgNPs. These compounds along with the other water-soluble metabolites such as reducing sugars were believed to be the agents responsible for reducing the  $\text{Ag}^+$  to  $\text{Ag}^0$ , but their shape is believed to be controlled by chemical factors like ascorbic acid (Fig. 8).

*Scanning electron microscopy*

The SEM images confirmed the formation of AgNPs by the leaf extract and clearly showed that they were almost spherical in shape and 20 nm in size (Fig. 9).

*Antibacterial and antifungal activities*

In this study, *C. cyminum* leaf extract, AgNPs and  $\text{AgNO}_3$  dissolved in DMSO (0.0005 and 0.001

Table 2. Green synthesis of silver nanoparticles using different plant extracts.

Plant	Size (nm)	Plant's part	Shape	Ref
<i>Vitex negundo</i>	5 & 10-30	Leaves	Spherical & FCC	[54]
<i>Thevetia peruviana</i>	10-30	Latex	Spherical	[55]
<i>Acorus calamus</i>	31.83	Rhizome	Spherical	[56]
<i>Boerhaavia diffusa</i>	25	Whole plant	Spherical	[57]
<i>Tea sinensis</i>	20-90	Leaves	Spherical	[58]
<i>Tribulus terrestris</i>	16-28	Fruit	Spherical	[59]
<i>Cocous nucifera</i>	22	Inflorescence	Spherical	[60]
<i>Abutilon indicum</i>	7-17	Leaves	Spherical	[61]
<i>Pistacia atlantica</i>	10-50	Seeds	Spherical	[62]
<i>Ziziphora tenuior</i>	8-40	Leaves	Spherical	[63]
<i>Premna herbacea</i>	10-30	Leaves	Spherical	[64]
<i>Calotropis procera</i>	19-45	Whole plant	Spherical	[65]
<i>Centella asiatica</i>	30-50	Leaves	Spherical	[66]
<i>Memecylon edule</i>	20-50	Leaves	Triangular, Circular,	[67]
<i>Nelumbo nucifera</i>	25-80	Leaves	Spherical, Triangular	[68]
<i>Datura metel</i>	16-40	Leaves	Quasilinear	[69]

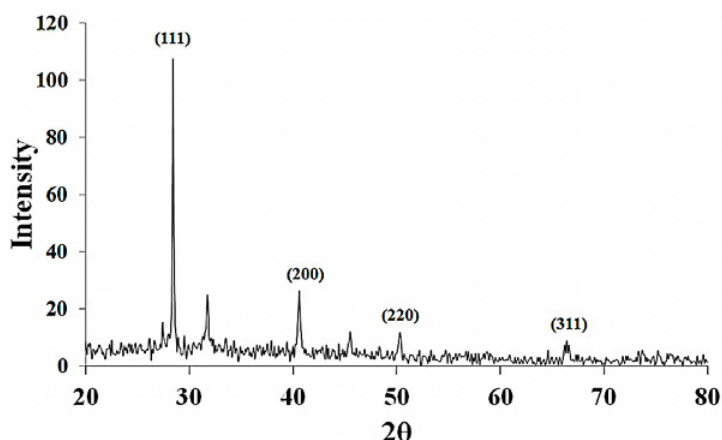


Fig. 7. Representative X-ray diffraction profile of the silver nanoparticles.

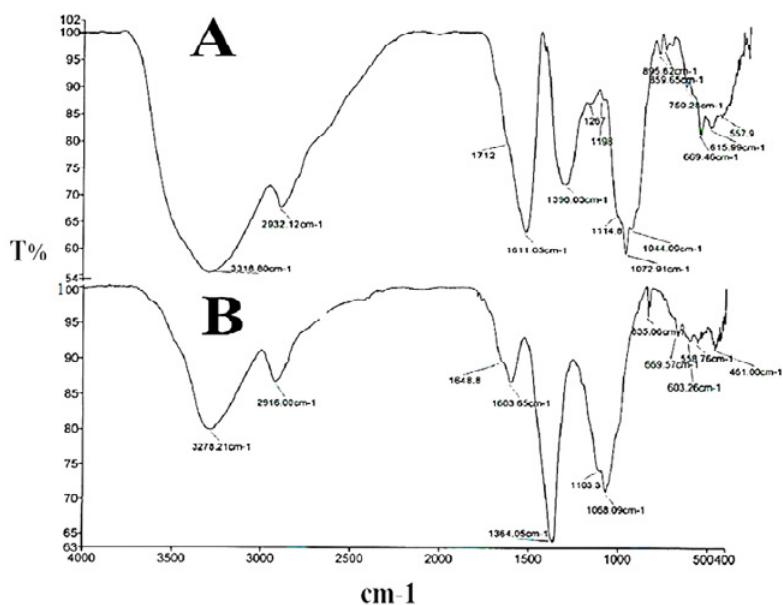


Fig. 8. Fourier transforms infrared absorption spectra of *C. cyminum* leaf extract (A) before bioreduction and (B) after bioreduction of  $Ag^+$  ions at 60 °C.

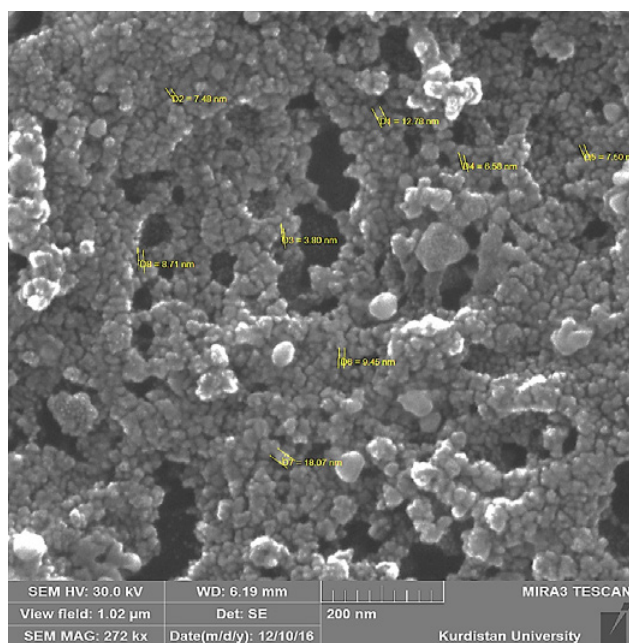


Fig. 9. Scanning electron micrograph of the synthesized silver nanoparticles.

mg/mL) were assessed for their antifungal activity and (0.01, 0.1 and 1 mg/mL) their antibacterial activity for the first time. The solvent DMSO used as negative control had no activity against all microbial strains used in this research (Table 3). The AgNPs showed more inhibition activity than those the other samples and also penicillin against some

bacteria tested such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Among antibiotics used here, streptomycin was the most effective, but penicillin had no effect on the bacteria tested. *Escherichia coli* was resistant to gentamicin and penicillin, but inhibited well by AgNPs. The progressive bacterial resistance against antibiotics



Table 3. Antibacterial activity of C. cyminum leaf extract, AgNPs, AgNO<sub>3</sub> and standards (positive and negative controls).

Sample	Conc. (mg/mL)	Inhibition zone (mm)			
		<i>B. s</i> (+)	<i>S. a</i> (+)	<i>P. a</i> (-)	<i>E. c</i> (-)
Leaf extract	1	13 ± 0.41 <sup>a</sup>	13 ± 0.11 <sup>a</sup>	8 ± 0.30 <sup>a</sup>	9 ± 0.00
	0.1	9 ± 0.22 <sup>b</sup>	13 ± 0.52 <sup>a</sup>	7 ± 0.64 <sup>a</sup>	Na
	0.01	7 ± 0.00 <sup>c</sup>	10 ± 0.34 <sup>b</sup>	Na	Na
AgNPs	1	10 ± 0.12 <sup>a</sup>	17 ± 0.12 <sup>a</sup>	9 ± 0.00	8 ± 0.81
	0.1	7 ± 0.00 <sup>b</sup>	14 ± 0.16 <sup>b</sup>	Na	Na
	0.01	Na	9 ± 0.24 <sup>c</sup>	Na	Na
AgNO <sub>3</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>
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
DMSO		Na	Na	Na	Na

Experiment was performed in triplicate and expressed as mean ± SD. Values in each row with different superscripts are significantly different ( $P < 0.05$ ). *P. a*: *Pseudomonas aeruginosa*, *S. a*: *Staphylococcus aureus*, *E. c*: *Escherichia coli*, *B. c*: *Bacillus cereus*, Na: no active.

Table 4. Antifungal activity of C. cyminum leaf extract, AgNPs, AgNO<sub>3</sub> and negative control.

Sample	Inhibition radial growth of fungal mycelium (%)	
	Concentration (ppm)	Percent (%)
Leaf extract	500	40.33 <sup>b</sup>
	1000	68.25 <sup>a</sup>
AgNPs	500	49.23 <sup>b</sup>
	1000	79.69 <sup>a</sup>
AgNO <sub>3</sub>	500	31.24 <sup>a</sup>
	1000	47.18 <sup>a</sup>
DMSO	-	Na

Experiment was performed in triplicate and expressed as mean ± SD. Values in each with different superscripts are significantly different ( $P < 0.05$ ). Na: no active.

and the development of resistant strains is serious problem and metallic nanoparticles are the most potential advantageous compounds with a high specific surface area for bacterial growth inhibition. Silver ion and silver-based compounds are highly toxic to micro-organisms, showing a strong biocidal effect against microbial species. Silver nanoparticles produced using microbes and plant extracts are known to exhibit potent antimicrobial activity [70]. This can be attributed to the fact that silver at low concentrations does not enter cells, but is adsorbed onto the bacterial surface [71]. Thus, silver ions by resisting dehydrogenation can disrupt respiration occurs across the cell membrane in bacteria [72]. Again, some hypotheses indicate that catalytic oxidation of silver ions, with nascent oxygen, reacts with bacterial cell membranes, leading to cell death. It

is well known that *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and also *Bacillus* species are agents of food poisoning [73, 74]. Our results indicated that *C. cyminum* leaf extract contains a remarkable amounts of phytochemicals such as polyphenoles, starch, reducing sugars and ascorbic acid may be used to treat bacterial infections. The synthesized AgNPs showed more inhibition activity than those the other samples against the bacteria tested. It was shown that biological activities of a compound is related to its major components. However, the synergistic or antagonistic effect of any components in minor percentages should be discussed [74, 75]. The inhibitory effect of the samples was higher than that of the antibiotics, however *Bacillus cereus* (+) was the most resistant bacterium against the samples (Table 2). In addition, *E. coli* (-) was

resistant to gentamicin and all the studied bacteria were resistant to penicillin. On the other hand, the leaf extract and AgNPs showed more antibacterial activity against Gram positive and Gram negative bacteria, respectively in comparison to AgNO<sub>3</sub>. In addition, our results indicated that the AgNPs inhibited mycelial growth of *Fusarium oxysporum* more than other examined samples (Table 4). It was reported that *Fusarium oxysporum* causes severe losses of the plant crop and even leads to its death. It 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 at infected plants [76, 77]. Most of synthetic fungicides usually are toxic to humans. Hence, interest to use of plant extracts and nanoparticles as antifungal agents has been increased [78-80]. Our finding about antifungal activity of the green synthesized AgNPs against various phytopathogenic fungi like *F. oxysporum* and other fungi were supported well by many reports [81-83].

#### CONCLUSIONS

In this work the biosynthesis of silver nanoparticles has been demonstrated using aqueous leaf extract of *C. cyminum*. This phytoassisted procedure is an environmentally safe, facile and cost-effective method. Qualitative analysis of the leaf extract revealed the presence of various components of importance including phenolics, reducing sugars and starch may be responsible for bioreduction of Ag<sup>+</sup> to Ag<sup>0</sup>. The results from this study clearly showed that the pathogenic strains tested are susceptible to the synthesized AgNPs, which confirms their potential upshot against some bacterial strains and the fungus *F. oxysporum*. This result can be utilized to expand the use of these nanoparticles in biomedicine in future.

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#### CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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