J Nanostruct 9(1): 74-85, Winter 2019

RESEARCH PAPER

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

Roya Karamian*, Jamalaldin Kamalnejad

Department of Biology, Faculty of Science, Bu-Ali Sina University, Hamedan, Iran

ARTICLE INFO

Article History:
Received 04 October 2018
Accepted 28 November 2018
Published 01 January 2019

Keywords:
Antibacterial Activity
Antifungal Activity
Cuminum Cyminum
Green Synthesis
Silver Nanoparticles

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 antibaterial and antifungal activities. AgNPs were synthesized using *C. cyminum* leaf extract as bioreducer agent and aqueous silver nitrate solution ($AgNO_3$) as precursor at different conditions. The synthesized AgNPs were characterized using Ultraviolet-visible absorption spectroscopy (UV-vis), Scaning 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 $AgNO_3$ solution and the extract, temperature and the time of reaction. The best condition for AgNPs biosynthesis was achieved by 1-3 mM $AgNO_3$ at 40 °C after 4 h. In addition, the leaf extract and AgNPs showed efficient antibacterial activites against Gram positive and negative bacteria, respectively.

How to cite this article
Karamian R, Kamalnejad J. Green Synthesis of Silver Nanoparticles Using Cuminum cyminum Leaf Extract and Evaluation of Their Biological Activities. J Nanostruct, 2019; 9(1): 74-85. DOI: 10.22052/JNS.2019.01.008

INTRODUCTION

The silver nanoparticles (AgNPs) are extensively applied in different fields such as pharmaceutics, 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 Ag_2Se, Ag_2CdI_4/AgI, $ZnCr_2O_4$/Ag, Ag_2S, Ag_2CrO_4 and $Ag_2Cr_2O_7$ 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 bioreductors 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

* *Corresponding Author Email: r_karamian@basu.ac.ir*

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. Metalic nanoparticles are the most potential compounds having a significatly high specific surface area and a high fraction of surface atoms, unique optical, electronic, catalytic, anti-bacterial 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

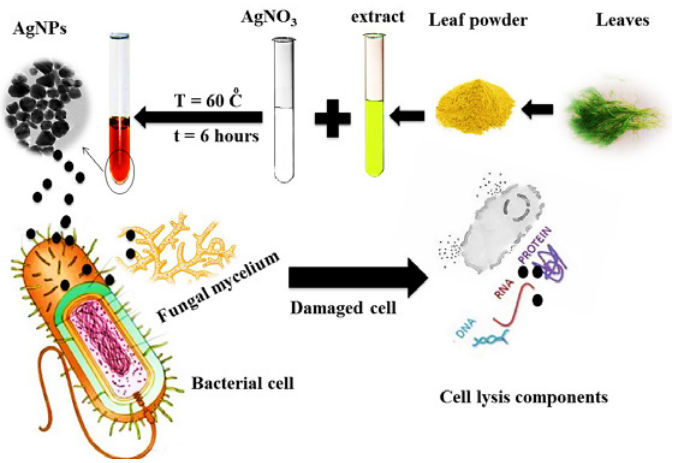

Fig. 1. Schematic illustration of green synthesis of silver nanoparticles by aqueous leaf extract of Cuminum cyminum L. Abbreviations: T, temperature; t, time.

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_2CO_3 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 contaning 0.5 mL of the leaf extract and 0.5 mL 2% $AlCl_3$ 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 obsorbance 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 $AgNO_3$ 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, cocentration of $AgNO_3$ and the extract and the time of raction on the rate of synthesis and the size of AgNPs were discussed. Then, the samples were analysed for change of absorbtion 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 scaning electron microscopy (SEM, Philips-CMC-300 KV). Also, the size distribution and crystal structure of AgNPs were determianed 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 $AgNO_3$ 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 A°) 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, λ is the X-ray wavelength (typically 1.54 A°), β is the full width at half the maximum intensity in radians, and θ 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 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 $AgNO_3$ were subjected to antibacterial and antifungal screening against four bacterial strians including *Bacillus cereus* (PTCC 1247), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 35218) and *Pseudomonas aeruginosa* (ATCC 27853) and also the fangus *Fusarium oxysporum*, respectivily [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 µm Millipore). All tests were carried using 0.5 Mc Farland concentration (10 mL of suspension containing 1.5×10^8 bacteria/mL). DMSO was used as negative reference standard and gentamicin, penicillin and streptomycin were used as positive reference standards. To evaluate the antifungul 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:

Antifungal activity (%) = (R-r/R) × 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 ± standard deviation.

RESULTS AND DISSCUSION

Phytochemical analysis

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

Characterization of the synthesized silver nano--particles

AgNPs were successfully synthesized from aqueous $AgNO_3$ 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 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 ± 0.92	0.008 ± 0.07	0.223 ± 0.032	0.062 ± 0.005	0.627 ± 0.023

Experiment was performed in triplicate and expressed as mean ± SD.

plant rich in important secondary metabolites like terpenoides, 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 Ag^{+} to Ag^{0}. In addition our results showed that the AgNPs synthesis depended on the concentration of $AgNO_3$ solution and the leaf extract (Figs. 4, 5). The maximum rate of synthesis was found at 1.5 mM $AgNO_3$ and 1 mg/mL the extract, especially 180-240 min

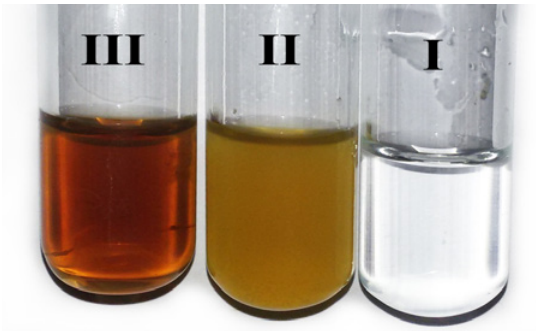

Fig. 2. $AgNO_3$ solution (I), C. cyminum leaf extract (II) and reaction mixture ($AgNO_3$ + leaf extract) at 60 °C (III).

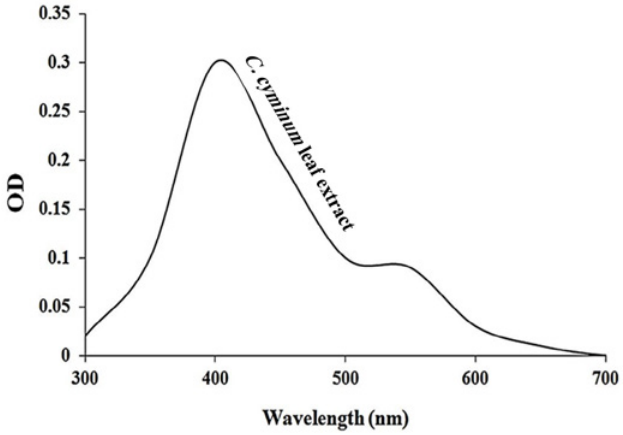

Fig. 3. UV-visible spectrum recorded as a function of reaction time of 1 mM $AgNO_3$ solution with C. cyminum leaf extract at 60 °C.

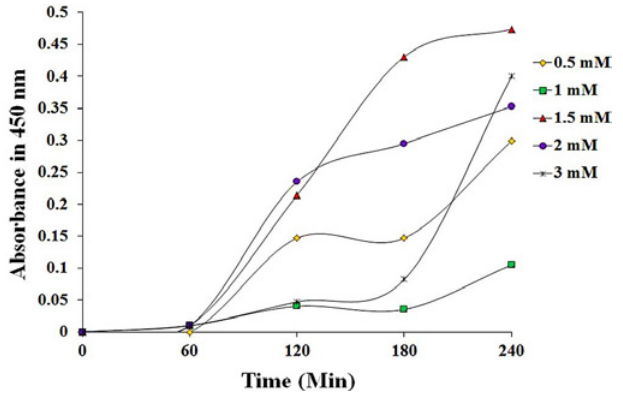

Fig. 4. UV/visible spectra of the reaction mixture at different concentrations of $AgNO_3$ solution and different times.

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,

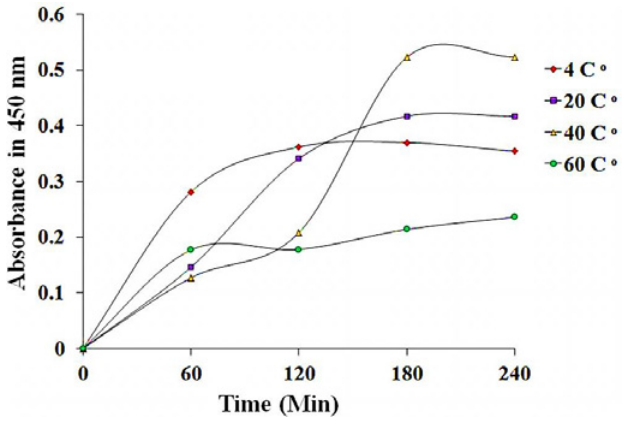

Fig. 5. Time course of AgNPs formation at different temperatures.

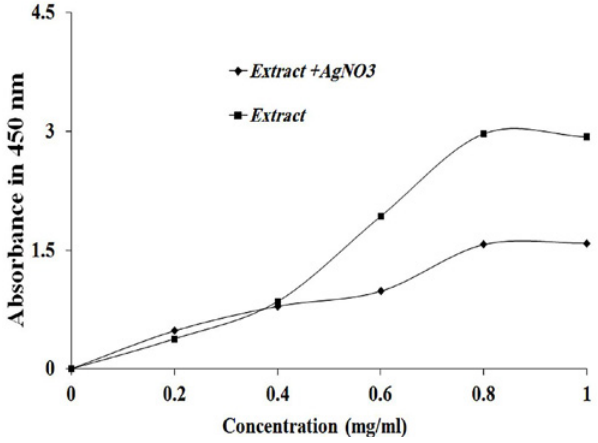

Fig. 6. UV/visible spectra of C. cyminum leaf extract at different concentrations alone and along with 1 mM $AgNO_3$ solution at 60 °C.

there are some other peaks, which are not identified. It seems that they can be related to the unreduced $AgNO_3$ 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 cm^{-1}. The absorbance bands at 1648 cm^{-1} and 1603 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 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 Ag^+ to 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 $AgNO_3$ 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
Vitex negundo	5 & 10-30	Leaves	Spherical & FCC	[54]
Thevetia peruviana	10-30	Latex	Spherical	[55]
Acorus calamus	31.83	Rhizome	Spherical	[56]
Boerhaavia diffusa	25	Whole plant	Spherical	[57]
Tea sinensis	20-90	Leaves	Spherical	[58]
Tribulus terrestris	16-28	Fruit	Spherical	[59]
Cocous nucifera	22	Inflorescence	Spherical	[60]
Abutilon indicum	7-17	Leaves	Spherical	[61]
Pistacia atlantica	10-50	Seeds	Spherical	[62]
Ziziphora tenuior	8-40	Leaves	Spherical	[63]
Premna herbacea	10-30	Leaves	Spherical	[64]
Calotropis procera	19-45	Whole plant	Spherical	[65]
Centella asiatica	30-50	Leaves	Spherical	[66]
Memecylon edule	20-50	Leaves	Triangular, Circular,	[67]
Nelumbo nucifera	25-80	Leaves	Spherical, Triangular	[68]
Datura metel	16-40	Leaves	Quasilinear	[69]

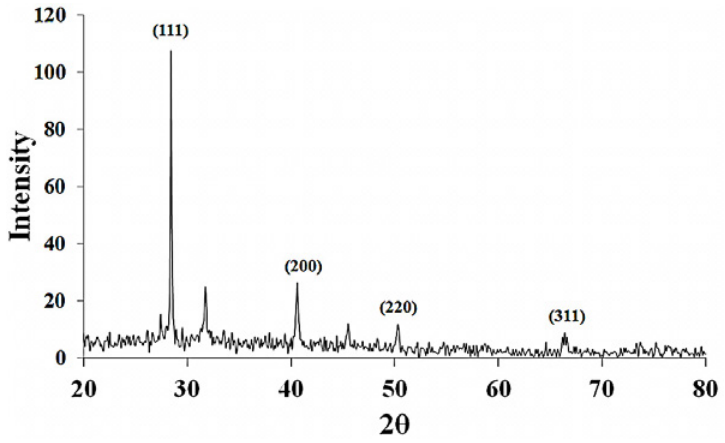

Fig. 7. Representative X-ray diffraction profile of the silver nanoparticles.

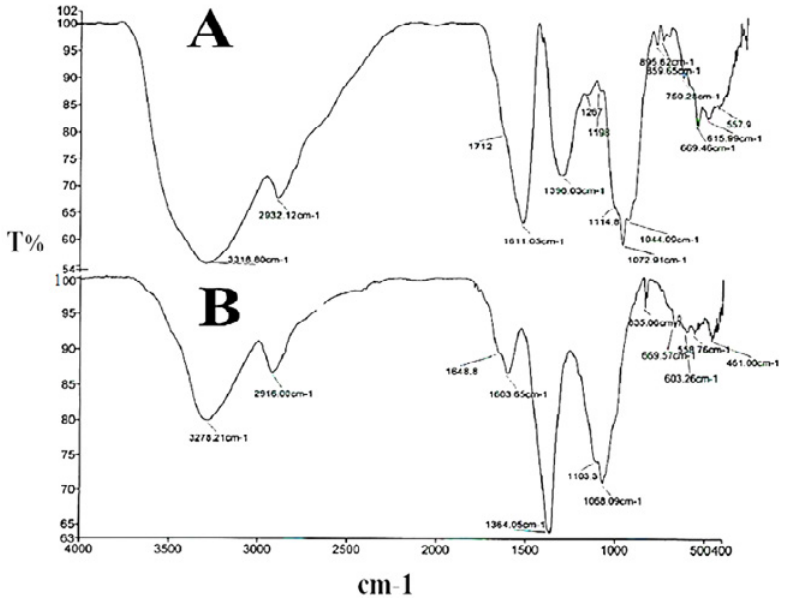

Fig. 8. Fourier transforms infrared absorption spectra of C. cyminum leaf extract (A) before bioreduction and (B) after bioreduction of Ag^{+} ions at 60 °C.

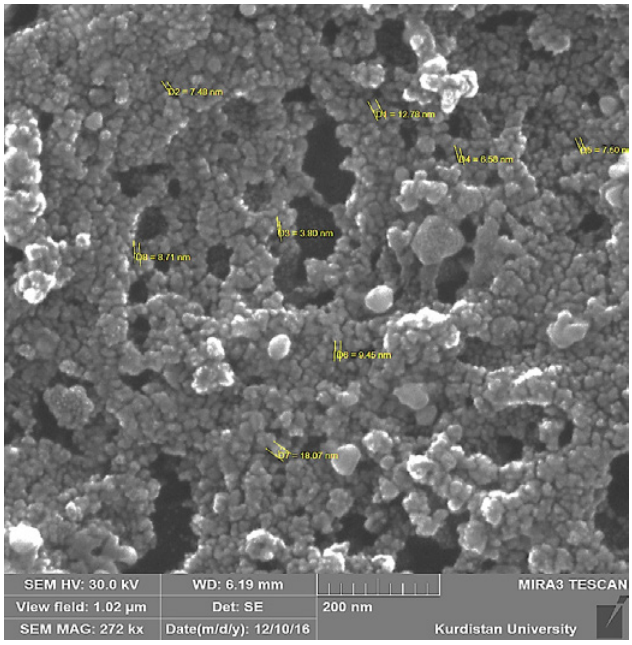

Fig. 9. Scanning electron micrograph of the synthesized silver nanoparticles.

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, $AgNO_3$ and standards (positive and negative controls).

Sample	Inhibition zone (mm)				
	Conc. (mg/mL)	*B. s (+)*	*S .a (+)*	*P. a (-)*	*E. c (-)*
Leaf extract	1	13 ± 0.41^a	13 ± 0.11^a	8 ± 0.30^a	9 ± 0.00
	0.1	9 ± 0.22^b	13 ± 0.52^a	7 ± 0.64^a	Na
	0.01	7 ± 0.00^c	10 ± 0.34^b	Na	Na
AgNPs	1	10 ± 0.12^a	17 ± 0.12^a	9 ± 0.00	8 ± 0.81
	0.1	7 ± 0.00^b	14 ± 0.16^b	Na	Na
	0.01	Na	9 ± 0.24^c	Na	Na
$AgNO_3$	1	8 ± 0.36	9 ± 0.11^a	8 ± 0.27^a	11 ± 0.15^a
	0.1	Na	7 ± 0.23^b	7 ± 0.55^a	9 ± 0.41^b
	0.01	Na	Na	Na	7 ± 0.00^c
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, $AgNO_3$ and negative control.

Sample	Inhibition radial growth of fungal mycelium (%)	
	Concentration (ppm)	Percent (%)
Leaf extract	500	40.33^b
	1000	68.25^a
AgNPs	500	49.23^b
	1000	79.69^a
$AgNO_3$	500	31.24^a
	1000	47.18^a
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 activ

and the development of resistant strains is seious problem and metallic nanoparticles are the most potential advantagous 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 agansit 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 comparsion to $AgNO_3$. 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 xelym 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, interset to use of plant extracts and nanoparticles as antifungul 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 wel 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^+ to Ag^0. 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 fangus *F. oxysporum*. This result can be utilized to expand the use of these nanoparticles in biomedicine in future.

ACKNOWLEDGMENTS

The authors would like to thank Bu-Ali Sina University for financial support of this work.

CONFLICT OF INTERESTS

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

REFERENCES

1. Chaloupka K, Malam Y, Seifalian AM. Nanosilver as a new generation of nanoproduct in biomedical applications. Trends in Biotechnology. 2010;28(11):580-8.
2. Prow TW, Grice JE, Lin LL, Faye R, Butler M, Becker W, et al. Nanoparticles and microparticles for skin drug delivery. Advanced Drug Delivery Reviews. 2011;63(6):470-91.
3. Kelly FM, Johnston JH. Colored and Functional Silver Nanoparticle–Wool Fiber Composites. ACS Applied Materials & Interfaces. 2011;3(4):1083-92.
4. Dankovich TA, Gray DG. Bactericidal Paper Impregnated with Silver Nanoparticles for Point-of-Use Water Treatment. Environmental Science & Technology. 2011;45(5):1992-8.
5. Niraimathi KL, Sudha V, Lavanya R, Brindha P. Biosynthesis of silver nanoparticles using Alternanthera sessilis (Linn.) extract and their antimicrobial, antioxidant activities. Colloids and Surfaces B: Biointerfaces. 2013;102:288-91.
6. Sankar R, Karthik A, Prabu A, Karthik S, Shivashangari KS, Ravikumar V. Origanum vulgare mediated biosynthesis of silver nanoparticles for its antibacterial and anticancer activity. Colloids and Surfaces B: Biointerfaces. 2013;108:80-4.
7. Jagtap UB, Bapat VA. Green synthesis of silver nanoparticles using Artocarpus heterophyllus Lam. seed extract and its antibacterial activity. Industrial Crops and Products. 2013;46:132-7.
8. Ghanbari M, Soofivand F, Salavati-Niasari M. Simple synthesis and characterization of Ag 2 CdI 4 /AgI nanocomposite as an effective photocatalyst by co-precipitation method. Journal of Molecular Liquids. 2016;223:21-8.
9. Abbasi A, Hamadanian M, Salavati-Niasari M, Mortazavi-Derazkola S. Facile size-controlled preparation of highly photocatalytically active ZnCr2O4 and ZnCr2O4/Ag nanostructures for removal of organic contaminants. Journal of Colloid and Interface Science. 2017;500:276-84.
10. Jafari M, Sobhani A, Salavati-Niasari M. Effect of preparation conditions on synthesis of Ag2Se nanoparticles by simple sonochemical method assisted by thiourea. Journal of Industrial and Engineering Chemistry. 2014;20(5):3775-9.
11. Amiri O, Salavati-Niasari M, Farangi M. Enhancement of Dye-Sensitized solar cells performance by core shell Ag@organic (organic=2-nitroaniline, PVA, 4-choloroaniline and PVP): Effects of shell type on photocurrent. Electrochimica Acta. 2015;153:90-6.
12. Shakouri-Arani M, Salavati-Niasari M. Structural and spectroscopic characterization of prepared Ag2S nanoparticles with a novel sulfuring agent. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2014;133:463-71.
13. Soofivand F, Mohandes F, Salavati-Niasari M. Silver chromate and silver dichromate nanostructures: Sonochemical synthesis, characterization, and photocatalytic properties. Materials Research Bulletin. 2013;48(6):2084-94.
14. Mukherjee P, Roy M, Mandal BP, Dey GK, Mukherjee PK, Ghatak J, et al. Green synthesis of highly stabilized nanocrystalline silver particles by a non-pathogenic and agriculturally important fungusT. asperellum. Nanotechnology. 2008;19(7):075103.
15. Biazar E, Majdi, Zafari M, Avar M, Aminifard S, Zaeifi D, et al. Nanotoxicology and nanoparticle safety in biomedical designs. International Journal of Nanomedicine. 2011:1117.
16. Sastry M, Ahmad A, Khan MI, Kumar R. Biosynthesis of metal nanoparticles using fungi and actinomycete. Curr Sci, 2003; 85(2): 162-170.
17. Binupriya AR, Sathishkumar M, Yun S-I. Myco-crystallization of Silver Ions to Nanosized Particles by Live and Dead Cell Filtrates of Aspergillus oryzae var.viridisand Its Bactericidal Activity toward Staphylococcus aureus KCCM 12256. Industrial & Engineering Chemistry Research.

2010;49(2):852-8.
18. Shankar SS, Rai A, Ahmad A, Sastry M. Controlling the Optical Properties of Lemongrass Extract Synthesized Gold Nanotriangles and Potential Application in Infrared-Absorbing Optical Coatings. Chemistry of Materials. 2005;17(3):566-72.
19. Shankar SS, Ahmad A, Sastry M. Geranium Leaf Assisted Biosynthesis of Silver Nanoparticles. Biotechnology Progress. 2003;19(6):1627-31.
20. Huang J, Li Q, Sun D, Lu Y, Su Y, Yang X, et al. Biosynthesis of silver and gold nanoparticles by novel sundriedCinnamomum camphoraleaf. Nanotechnology. 2007;18(10):105104.
21. Elavazhagan T, Elavazhagan T. Memecylon edule leaf extract mediated green synthesis of silver and gold nanoparticles. International Journal of Nanomedicine. 2011:1265.
22. Gibson MR. *Glycyrrhiza* in old and new perspectives. Lioydia, 1978; 41: 348.
23. Korbekandi H, Iravani S, Abbasi S. Production of nanoparticles using organisms. Critical Reviews in Biotechnology. 2009;29(4):279-306.
24. Zinatloo-Ajabshir S, Mortazavi-Derazkola S, Salavati-Niasari M. Sono-synthesis and characterization of Ho2O3 nanostructures via a new precipitation way for photocatalytic degradation improvement of erythrosine. International Journal of Hydrogen Energy. 2017;42(22):15178-88.
25. Zinatloo-Ajabshir S, Salavati-Niasari M. Nanocrystalline Pr6O11: synthesis, characterization, optical and photocatalytic properties. New Journal of Chemistry. 2015;39(5):3948-55.
26. Zinatloo-Ajabshir S, Salavati-Niasari M, Zinatloo-Ajabshir Z. Nd 2 Zr 2 O 7 -Nd 2 O 3 nanocomposites: New facile synthesis, characterization and investigation of photocatalytic behaviour. Materials Letters. 2016;180:27-30.
27. Bettaieb Rebey I, Kefi S, Bourgou S, Ouerghemmi I, Ksouri R, Tounsi MS, et al. Ripening Stage and Extraction Method Effects on Physical Properties, Polyphenol Composition and Antioxidant Activities of Cumin (Cuminum cyminum L.) Seeds. Plant Foods for Human Nutrition. 2014;69(4):358-64.
28. Gohari AR, Saeidnia S. A Review on Phytochemistry of Cuminum cyminum seeds and its Standards from Field to Market. Pharmacognosy Journal. 2011;3(25):1-5.
29. Johri RK. Cuminum cyminum and Carum carvi: An update. Pharmacognosy Reviews. 2011;5(9):63.
30. Pandey S, Patel MK, Mishra A, Jha B. In planta Transformed Cumin (Cuminum cyminum L.) Plants, Overexpressing the SbNHX1 Gene Showed Enhanced Salt Endurance. PLOS ONE. 2016;11(7):e0159349.
31. Mnif S, Aifa S. Cumin (Cuminum cyminumL.) from Traditional Uses to Potential Biomedical Applications. Chemistry & Biodiversity. 2015;12(5):733-42.
32. Gagandeep, Dhanalakshmi S, Mendiz E, Rao AR, Kale RK. Chemopreventive Effects of Cuminum cyminum in Chemically Induced Forestomach and Uterine Cervix Tumors in Murine Model Systems. Nutrition and Cancer. 2003;47(2):171-80.
33. Sowbhagya HB. Chemistry, Technology, and Nutraceutical Functions of Cumin (cuminum cyminum L): An Overview. Critical Reviews in Food Science and Nutrition. 2013;53(1):1-10.
34. Sirelkhatim A, Mahmud S, Seeni A, Kaus NHM, Ann LC, Bakhori SKM, et al. Review on Zinc Oxide Nanoparticles: Antibacterial Activity and Toxicity Mechanism. Nano-Micro Letters. 2015;7(3):219-42.
35. Moellering RC. Discovering new antimicrobial agents. International Journal of Antimicrobial Agents. 2011;37(1):2-9.
36. Stanton TB. A call for antibiotic alternatives research. Trends in Microbiology. 2013;21(3):111-3.
37. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. Biotechnology Advances. 2009;27(1):76-83.
38. Guzman M, Dille J, Godet S. Synthesis and antibacterial activity of silver nanoparticles against gram-positive and gram-negative bacteria. Nanomedicine: Nanotechnology, Biology and Medicine. 2012;8(1):37-45.
39. Valentão P, Fernandes E, Carvalho F, Andrade PB, Seabra RM, Bastos ML. Hydroxyl radical and hypochlorous acid scavenging activity of small Centaury (Centaurium erythraea) infusion. A comparative study with green tea (Camellia sinensis). Phytomedicine. 2003;10(6-7):517-22.
40. Krishnaraj C, Jagan EG, Rajasekar S, Selvakumar P, Kalaichelvan PT, Mohan N. Synthesis of silver nanoparticles using Acalypha indica leaf extracts and its antibacterial activity against water borne pathogens. Colloids and Surfaces B: Biointerfaces. 2010;76(1):50-6.
41. Jeyashree R, Revathi S. Synthesis of AgNPs of *Cuminum cyminum* seed extract, characterization and antimicrobial activity. Inter J Eng Sci Comput, 2017; 7(7): 14193-14196.
42. Wolfe K, Wu X, Liu RH. Antioxidant Activity of Apple Peels. Journal of Agricultural and Food Chemistry. 2003;51(3):609-14.
43. Luximon-Ramma A, Bahorun T, Soobrattee MA, Aruoma OI. Antioxidant Activities of Phenolic, Proanthocyanidin, and Flavonoid Components in Extracts ofCassia fistula. Journal of Agricultural and Food Chemistry. 2002;50(18):5042-7.
44. Thayumanavan B, Sadasivam S. Physicohemical basis for the preferential uses of certain rice varieties. Qualitas Plantarum Plant Foods for Human Nutrition. 1984;34(4):253-9.
45. Miller GL. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. Analytical Chemistry. 1959;31(3):426-8.
46. Sadasivam S, Manickam A. *Biochemical Methods*. 3rd ed. New Delhi, India: New Age. Inter, 2008, 274-284.
47. Awoyinka OA, Balogun IO, Ogunnowo AA. Phytochemical screening and *in vitro* bioactivity of *Cnidoscolus aconitifolius* (Euphorbiaceae). J Med Plants Res, 2007; 1(3): 63-65.
48. Shankar SS, Rai A, Ahmad A, Sastry 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. 2004;275(2):496-502.
49. Egorova EM, Revina AA. Synthesis of metallic nanoparticles in reverse micelles in the presence of quercetin. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2000;168(1):87-96.
50. Nadagouda MN, Hoag G, Collins J, Varma RS. Green Synthesis of Au Nanostructures at Room Temperature Using Biodegradable Plant Surfactants. Crystal Growth & Design. 2009;9(11):4979-83.
51. Sharma VK, Yngard RA, Lin Y. Silver nanoparticles: Green synthesis and their antimicrobial activities. Advances in Colloid and Interface Science. 2009;145(1-2):83-96.
52. Chopade B, Ghosh, Patil, Ahire, Kitture, Jabgunde, et al. Synthesis of silver nanoparticles using Dioscorea bulbifera tuber extract and evaluation of its synergistic potential in combination with antimicrobial agents. International Journal of Nanomedicine. 2012:483.
53. Nabikhan A, Kandasamy K, Raj A, Alikunhi NM. Synthesis of antimicrobial silver nanoparticles by callus and leaf extracts from saltmarsh plant, Sesuvium portulacastrum L. Colloids

and Surfaces B: Biointerfaces. 2010;79(2):488-93.
54. Zargar M, Hamid AA, Bakar FA, Shamsudin MN, Shameli K, Jahanshiri F, et al. Green Synthesis and Antibacterial Effect of Silver Nanoparticles Using Vitex Negundo L. Molecules. 2011;16(8):6667-76.
55. Rupiasih NN, Aher A, Gosavi S, Vidyasagar PB. Green synthesis of silver nanoparticles using latex extract ofThevetia peruviana: a novel approach towards poisonous plant utilization. Journal of Physics: Conference Series. 2013;423:012032.
56. Nakkala JR, Mata R, Gupta AK, Sadras SR. Biological activities of green silver nanoparticles synthesized with Acorous calamus rhizome extract. European Journal of Medicinal Chemistry. 2014;85:784-94.
57. Nakkala JR, Mata R, Gupta AK, Sadras SR. Green synthesis and characterization of silver nanoparticles using *Boerhaavia diffusa* plant extract and their antibacterial activity. Ind Crop Prod, 2014; 52: 562-566.
58. Suna Q, Cai X, Li J, Zheng M, Chenb Z, Yu CP. Green synthesis of silver nanoparticles using tea leaf extract and evaluation of their stability and antibacterial activity. Coll Surf A: Physicochem Eng Asp, 2014; 444: 226-231.
59. Gopinath V, MubarakAli D, Priyadarshini S, Priyadharsshini NM, Thajuddin N, Velusamy P. Biosynthesis of silver nanoparticles from Tribulus terrestris and its antimicrobial activity: A novel biological approach. Colloids and Surfaces B: Biointerfaces. 2012;96:69-74.
60. Mariselvam R, Ranjitsingh AJA, Usha Raja Nanthini A, Kalirajan K, Padmalatha C, Mosae Selvakumar P. Green synthesis of silver nanoparticles from the extract of the inflorescence of Cocos nucifera (Family: Arecaceae) for enhanced antibacterial activity. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2014;129:537-41.
61. Ashokkumar S, Ravi S, Kathiravan V, Velmurugan S. RETRACTED: Synthesis of silver nanoparticles using A. indicum leaf extract and their antibacterial activity. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2015;134:34-9.
62. Sadeghi B, Rostami A, Momeni SS. 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. 2015;134:326-32.
63. Sadeghi B, Gholamhoseinpoor F. A study on the stability and green synthesis of silver nanoparticles using Ziziphora tenuior (Zt) extract at room temperature. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2015;134:310-5.
64. Kumar S, Daimary RM, Swargiary M, Brahma A, Kumar S, Singh M. Biosynthesis of silver nanoparticles using *Premna herbacea* leaf extract and evaluation of its antimicrobial activity against bacteria causing dysentery. Inter J Pharm Biosci, 2013; 4(4): 378-384.
65. Gondwal M, Pant GJN. Biological evaluation and green synthesis of silver nanoparticles using aqueous extract of *Calotropis procera*. Intern J Pharm Biosci, 2013; 4(4): 635-643.
66. Rout A, Jena PK, Parida UK, Bindhani BK. Green synthesis of silver nanoparticles using leaves extract of *Centella asiatica* L. For studies against human pathogens. Intern J Pharm Biosci, 2013; 4(4): 661-674.
67. Elavazhagan T, Elavazhagan T. Memecylon edule leaf extract mediated green synthesis of silver and gold nanoparticles. International Journal of Nanomedicine. 2011:1265.
68. Santhoshkumar T, Rahuman AA, Rajakumar G, Marimuthu S, Bagavan A, Jayaseelan C, et al. Synthesis of silver nanoparticles using Nelumbo nucifera leaf extract and its larvicidal activity against malaria and filariasis vectors. Parasitology Research. 2010;108(3):693-702.
69. Kesharwani J, Yoon KY, Hwang J, Rai M. Phytofabrication of Silver Nanoparticles by Leaf Extract of *Datura metel*: Hypothetical Mechanism Involved in Synthesis. Journal of Bionanoscience. 2009;3(1):39-44.
70. Shahverdi AR, Fakhimi A, Shahverdi HR, Minaian S. Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against Staphylococcus aureus and Escherichia coli. Nanomedicine: Nanotechnology, Biology and Medicine. 2007;3(2):168-71.
71. Deshpande L, Chopade B. Plasmid mediated silver resistance in Acinetobacter baumannii. Biometals. 1994;7(1).
72. Liau SY, Read DC, Pugh WJ, Furr JR, Russell AD. Interaction of silver nitrate with readily identifiable groups: relationship to the antibacterialaction of silver ions. Letters in Applied Microbiology. 1997;25(4):279-83.
73. Wright JB, Lam K, Burrell RE. Wound management in an era of increasing bacterial antibiotic resistance: A role for topical silver treatment. American Journal of Infection Control. 1998;26(6):572-7.
74. Burt S. Essential oils: their antibacterial properties and potential applications in foods—a review. International Journal of Food Microbiology. 2004;94(3):223-53.
75. Gray JE, Norton PR, Alnouno R, Marolda CL, Valvano MA, Griffiths K. Biological efficacy of electroless-deposited silver on plasma activated polyurethane. Biomaterials. 2003;24(16):2759-65.
76. Agrios GN. Plant Pathology. 3rd ed. Academic Press, Inc. New York. 1988; 803.
77. Larkin RP, Fravel DR. Efficacy of Various Fungal and Bacterial Biocontrol Organisms for Control of Fusarium Wilt of Tomato. Plant Disease. 1998;82(9):1022-8.
78. Singh K, Panghal M, Kadyan S, Chaudhary U, Yadav JP. Green silver nanoparticles of Phyllanthus amarus: as an antibacterial agent against multi drug resistant clinical isolates of Pseudomonas aeruginosa. Journal of Nanobiotechnology. 2014;12(1).
79. Karamian R, Asadbegy M, Pakazad R. Essential Oil Compositions, Antioxidant and Antibacterial Activities of TwoSalviaSpecies (S. grossheimiiBioss. andS. syriacaL.) Growing in Iran. Journal of Essential Oil Bearing Plants. 2014;17(2):331-45.
80. Alkhathlan H, Khan M, Khan ST, Khan M, Adil SF, Musarrat J, et al. Antibacterial properties of silver nanoparticles synthesized using Pulicaria glutinosa plant extract as a green bioreductant. International Journal of Nanomedicine. 2014:3551.
81. Gopinath V, Velusamy P. Extracellular biosynthesis of silver nanoparticles using Bacillus sp. GP-23 and evaluation of their antifungal activity towards Fusarium oxysporum. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2013;106:170-4.
82. Ramy SY, Osama FA. In vitro study of the antifungal efficacy of zinc oxide nanoparticles against Fusarium oxysporum and Penicilium expansum. African Journal of Microbiology Research. 2013;7(19):1917-23.
83. Krishnaraj C, Jagan EG, Ramachandran R, Abirami SM, Mohan N, Kalaichelvan PT. Effect of biologically synthesized silver nanoparticles on Bacopa monnieri (Linn.) Wettst. plant growth metabolism. Process Biochemistry. 2012;47(4):651-8.