



Antimicrobial activity of Silver Nanoparticles, Synthesis by green method from *Artemisia monosperma*

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ABSTRACT

Nowadays there is world concentration with synthesis of nanoparticles especially silver and gold nanoparticles, by green method for use in many application, exporting antimicrobial effect, *Artemisia monosperma* is medicinal plant have potent, anticancer, antispasmodic activity. The study aim to synthesis AgNPs from aqueous plant extract by green method to avoid the chemical toxicity biosynthesis and assay the antibacterial activity of synthesis AgNPs. The nanoparticles produced were further characterized using, UV, IR, and Transmission Electron Microscopy (TEM). TEM analysis confirm the synthesis of silver nanoparticles, (AgNPs) which have spherical shape and an average size of 17 nm. Antibacterial assay of capped AgNPs against *Gram-positive* and *Gram-negative* bacteria, revealed that AgNPs had good antibacterial activity than aqueous extract under condition prepared, each nanoparticle and aqueous extract haven't activity toward *Candida albicans*. GC-MS of plant extract investigate many important compounds, flavonoid (Kumatakenin), alkaloid (Aspidospermidin-17-ol) and other organic compounds (methyl-jasmonate and verbenol). These results showed that AgNPs can efficacy used in biotechnological and biomedical applications.

Keywords: *Gram-positive*, *Gram-negative* bacteria, Silver nanoparticles, *Artemisia monosperma*.

INTRODUCTION

From very dangerous *Gram negative* bacteria *Pseudomonas aeruginosa* cause infection due to longer stay in hospital and it is resistance to many antimicrobial because it have ability to survive under drastic condition, *Pseudomonas aeruginosa* is most suitable known agent used for study control for nosocomial hospital infection^{1,2}.

Among great health challenge antimicrobial of green synthesis of nanoparticles from natural source,³ the high ratio of volume to surface and its chemical and physical properties of nanoparticles have important role of excellent antimicrobial activity,⁴ silver nanoparticles are characteristic with good antibacterial, antifungal and antiviral^{5,6}.

The synthesis of silver nanoparticles by green method is preferred due to less expensive



and could be suitable for large scale of synthesis nanoparticles. Many plant extracts can be used for synthesis nanoparticles as they possess many antioxidants which act as reducing agents. Plant extracts possess many antioxidants, which act as reducing agents⁷.

Genus *Artemisia* family asteraceae, comprises about 300 species distributed over all world the genus represented by five species in northern region Saudi Arabia (*A. monosperma*, *A. scoparia*, *A. judica* and *A. sibirica*). *A. monosperma* is green shrub let 50-70-cm high, flowering from spring season and extended to September⁸.

Secondary metabolite of *Artemisia* species and essential oil are widely medicinally used for many years. Studied was aimed to use the medicinal plant *Artemisia monosperma* for green synthesis of nanoparticles and study the antimicrobial activity of synthesis nanoparticles against *Gram positive* and *Gram-negative* bacteria.

MATERIAL AND METHODS

Plant material

Fresh plant *Artemisia monosperma* were collected in spring 2017 year from Tabok, North region in Saudi Arabia, and identified in Biology department, faculty of science, Northern Border University, the samples were kept in Herbarium of faculty of science girl section.

Preparation of plant extract

10 gram of powdered plant sample (Aerial part) of *Artemisia monosperma* was boiling with 100 ml deionized water for 15 min. then filtered and complete the filtrate to 100 ml deionized water and kept at 0 °C for synthesis nanoparticles.

Synthesis of silver nanoparticles AgNPs

Synthesis of the AgNPs, take 0.2, 0.4, 0.6, 0.8, 0.5, 1.0 and 1.5 ml of plant extract and added to the 5 ml, (10⁻³ M) AgNO₃ to each of them the volume was adjusted to 10 ml by de-ionized water. Final concentration of Ag⁺ was 1x10⁻⁴ M, the Ag⁺ ion was reduced to Ag⁰ and color of the solution changed from yellow, brownish-yellow to dark brown, within 2 h at room temp 37 °C and neutral pH condition.

Characterization of Silver Nanoparticles Synthesis

The characterization and confirmation of AgNPs synthesis was conducted at Advanced Materials and Nano scale Research Laboratory, Ain Shams University by UV-Vis Spectrophotometer. UV-Vis spectral analysis was conducted using a Perkin Elmer-Lambda 950–UV-Visible spectrometer. UV-vis spectra of AgNPs and Ag ions were recorded in range from 300 to 500 nm⁹.



Fig. 1. Picture of plant *Artemisia monosperma*

TEM Analysis of AgNPs

Transmission Electron microscopy (TEM) was used to examine the size and morphology of synthesis nanoparticles, the image was done on a JEOL-1200 JEM for the TEM measurements.

Infrared spectra

Infrared spectroscopy were measured on a Perkin-Elmer 1000 fourier transforms infrared spectrometer, silver nanoparticles was centrifuge at 16,330 rev for 20 min. and dried, then mixed with KBr powder for measurement.

Analysis of plant Extract by GC-MS: The constituents of the aqueous extract analyzed by GC-MS as described by Elsharkawy and Shiboop 2018,¹⁰. Compounds identified by comparison of their retention indices (RI), (C9 to C24 n-alkane mixture) and mass spectra with those reported in the literature¹⁰.

Antimicrobial activity assay

Antimicrobial activity assay of the synthesized AgNPs, nanoparticles was studied against the growth of *Gram Positive* bacteria (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923), *Gram negative* bacteria (*E. coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27858) and Fungi (*Candida albicans* ATCC 10231)

in comparison with that of aqueous *Artemisia monosperma* extract by using the standard agar well diffusion technique. as method described by Abdallah *et al.*, 2016¹¹.

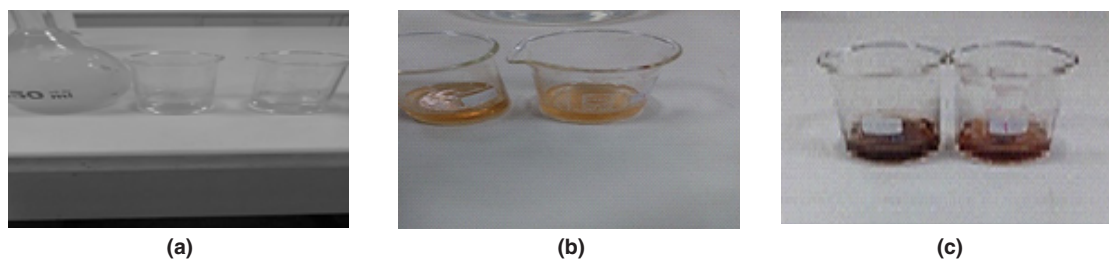
RESULTS AND DISCUSSION

The plant *Artemisia monosperma* was used, to synthesis nanoparticle under facial condition gust by addition of aqueous plant extract to 5 ml of aqueous solution AgNO_3 , at different concentration from 0.2 ml to 1.5 ml of aqueous extract, the solution color change gradually from yellow, brownish-yellow to dark brown gust after 2 h at a normal condition of temperature 37 °C and neutral ph, as shown in Fig 2, indicated the preliminary formation of nanoparticle. The formation of AgNPs was monitored by UV-Visible spectrophotometer, where silver nanoparticles characteristic by absorption under viable range 300-500⁹.

UV-Visible

To confirm the formation and stability of synthesis nanoparticle were assayed by UV-Visible

spectrophotometer, Fig. 3 shows the UV-Visible spectra of AgNPs formed at constant concentration of AgNO_3 (10-3M). The spectra shows the change of color of AgNPs with changing the plant extract concentration. As is clear from the inset in Fig. 3 a the color changed from pale yellow to dark brown depending on the extract concentration. As shown in figure 2, UV -Vis spectra showed that in the range of low amounts of the leaf extract (0.2–1.5 ml in 10 ml solution), the absorption spectra exhibit a gradual increase of the absorbance accompanied with a shift in the λ max of SPR band absorption peak from 450 to 478 nm. Further increasing the concentration of plant extract with constant amount of AgNO_3 , the λ max was shifted to longer wavelengths. The inset UV-Vis spectrum was related to the Ag ions before the bio-reduction event, in which an absorption band appeared at 290 nm. The appearance of the above SPR peak at 460 nm, along with absence of 290 nm absorption band indicate of the successful synthesis of AgNPs under experimental conditions. Such a characteristic SPR peak for AgNPs has been reported to predominantly appear in the range of 300-500 nm¹².



(a) aqueous Ext before adding AgNO_3 (b) aqueous Ext after adding AgNO_3 (c) Synthesis of nanoparticles after 2 hours

Fig. 2. Color change of Ag NPs synthesis from *Artemisia monosperma*

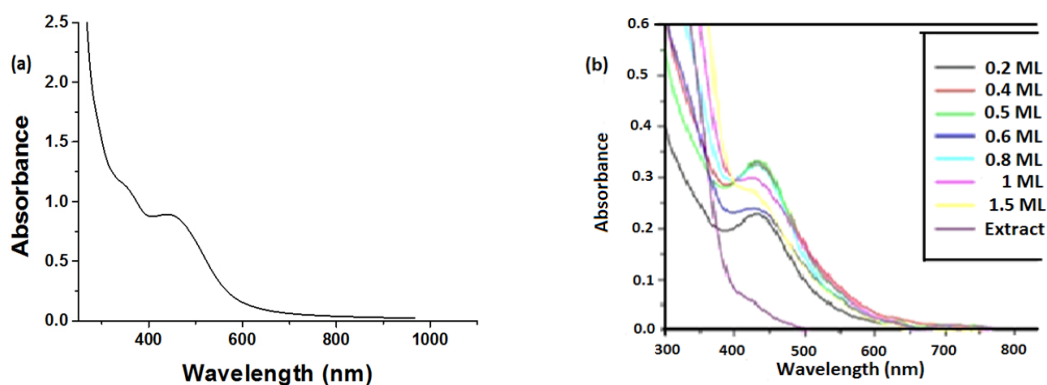


Fig. 3. UV -Vis spectra of AgNPs using different concentration of Plant Extract

FT IR Spectrum

FTIR spectrum was used to identify the possible function groups of biomolecules in the plant extract that might be responsible for bioreduction and coating of AgNPs. The spectrum of the FTIR spectra of plant extract showed a broad band at 3408 cm^{-1} . This band attributed to the OH groups in the biomolecules. The IR bands at 2920 and 2852 cm^{-1} due to C–H stretching vibration modes in hydrocarbon chains. The IR bands at 1181 and 1725 cm^{-1} were characterized as C–O and C=O stretching of the carbonyl functional group. The stronger band at 1642 cm^{-1} was characterized as

C=O of the amide groups of protein, 1516 cm^{-1} due to stretching vibrations of –C–C– in aromatic rings. In the case of Ag NPs, a large shift in the absorbance peak with decreased band intensity was observed from 3408.4 to 3404.7 cm^{-1} and 1382.2 to 1320 cm^{-1} . The spectra also illustrate a prominent shift in the wave numbers corresponding to amide (1652.5 – 1600 cm^{-1}), indicate the presence of free amino group which may be interacted with AgNPs surface maintain the stability of nanoparticles. the IR spectrum, at Fig 4, indicating the capping of the nanoparticles with the extract constituents.

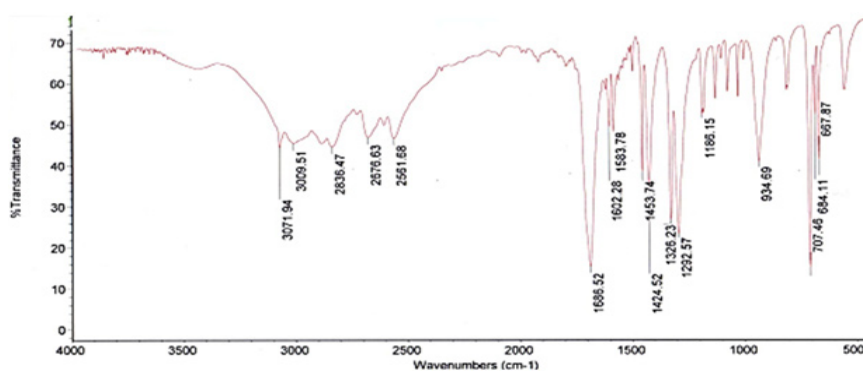


Fig. 4. IR Spectra of silver nanoparticles AgNPs

TEM Analysis of AgNPs

The formation of nanoparticles was confirmed by TEM (Transmission electron microscopy), the image of TEM of silver NPs depicted in Fig. 3, indicate the shape, morphology and size of synthesis AgNPs, the size was in the range of 13-18 nm, and the shape was spherical and irregular.

Antimicrobial activity

The results of the antibacterial evaluation are presented in Table 1. In general, silver Nanoparticles synthesis from *Artemisia monosperma* aqueous extract showed higher antibacterial against both of *Gram positive* and *Gram negative* than plant aqueous extract.

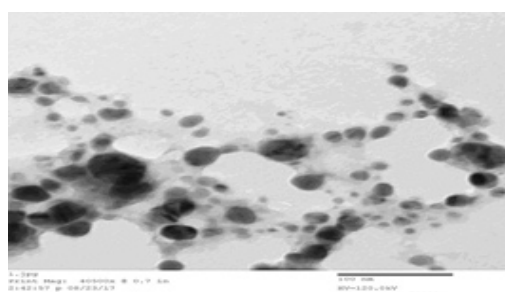
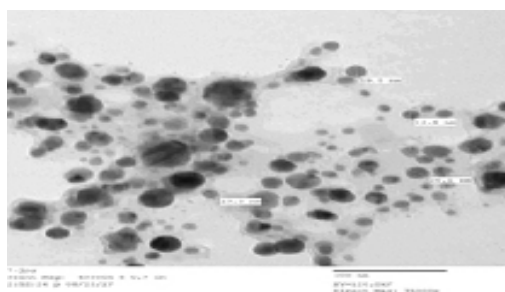


Fig. 5. TEM image of AgNPs synthesis from plant *Artemisia monosperma*

The mean zone of inhibition of of *Artemisia monosperma* ranged between $2.5.0 \pm 0.0$ to $3.5 \pm 0.5\text{ mm}$, for *Gram-positives*, and 1.5 ± 0.5 to 2.0 ± 1.0 for *Gram-negatives* for aqueous extract

which is week as compared with nanoparticles which shows the mean inhibition zone ranged between 7.0 ± 0.5 to 8.0 ± 0.0 for *Gram negative*, among all tested bacteria the most susceptible bacteria was

pseudomonas aeruginosa. Elsharkawy *et al.*,¹⁴ reported that Mean zone of inhibition of the essential oils of *Artemisia monosperma* ranged between 20.5±0.0 to 23.0±0.5 mm, for *Gram-positives*, and 16.5±0.5 to 10.0±1.0 for *Gram-negatives*. The most susceptible bacteria was *Staphylococcus aureus*. The antimicrobial activity of silver is well known. In its nanometric form, this characteristic is accentuated. Due to their size, AgNPs can enter cells and inhibit enzymatic systems in the respiratory chain of some bacteria and thereby alter their DNA synthesis

The solubility of NPs in aqueous medium was enhance antibacterial activity where the a plant aqueous extract show moderate antibacterial activity . The antimicrobial activity of the prepared nanoparticles were studied and the activity was found to depend on the concentration of nanoparticles and their sizes. Other studies confirm our result and recommended by use AgNPs as alternative control of microorganism with less risk of toxicity to mammalian cells¹⁵.

Table 1: Antimicrobial activity of the tested plant's against some microorganism

Tested compound	Mean zone of inhibition (mm)				
	<i>G-bacteria bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>G+bacteria Pesudomonas aeruginosa</i>	<i>Echerichia coli</i>	<i>Fungi Candida albicans</i>
Ag NPs	8.0 ±0.0	7.0±0.5	8.5±1.5	8.0±0.5	0
Aqueous plant extract	1.5±0.5	2.0±1.0	3.5±0.5	2.5±0.5	0
Erythromycin (15 µg/disc)	6.0±0.0	18.0±1.0	7.5±0.5	6.0±0.0	2.2±0.0

Phytochemical analysis

Preliminary phytochemical analysis of aqueous extract showed the plant contain more active constituents, flavonoid, terpene, steroid, coumarin and alkaloid compound, so further analysis of extract was studied to investigate and identified on the chemical compounds in the plant

which responsible on antimicrobial activity, aqueous extract was fractionated between methanol and water, methanol fraction was analysis by GC-MS and illustrated the presence of 13 compounds shown in Table 2, while water fraction show the presence of protein and glucoside¹⁶.

Table 2: compounds identified by GC-MS from methanol fraction

Compounds	RI	RRI	%
Dasycarpidan-methanol, acetate	650	671	0.05
Verbenol	812	813	7.54
Azafrin	501	514	0.05
Olean1-2-ene-3,15,16,21,22,28-hexol	439	472	0.02
Ursodeoxycholic acid	439	472	0.55
Nerolidyl acetate	807	819	0.50
Xanthoxilin	854	860	3.95
Methyl jasmonate	857	871	0.64
Aspidospermidin-17-ol	652	675	0.02
psi.,psi.Carotene,	433	436	0.04
Pterin -6-carboxylic acid	669	695	0.07
5,4'-dihydroxy-3,7-dimethoxyflavone (Kumatakenin)	651	666	2.7

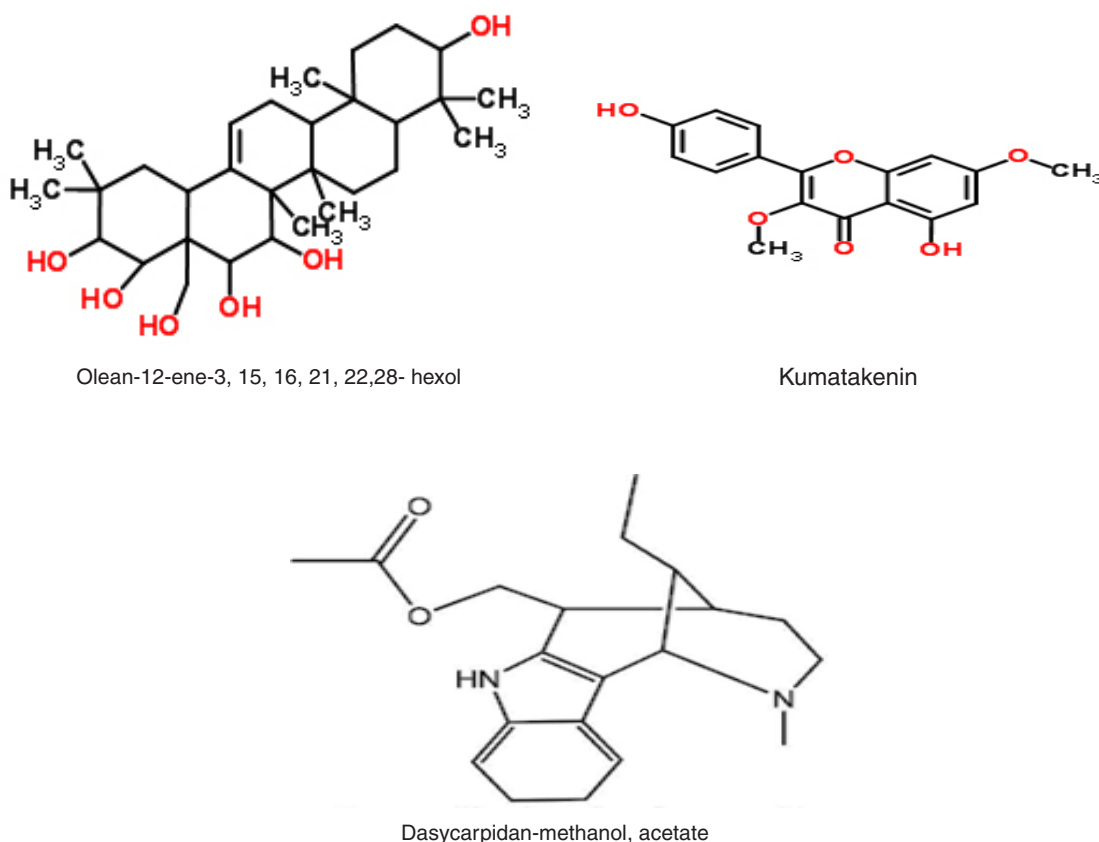


Fig. 4. Structure of compounds of Methanol Extract of *Artemisia monosperma* identified by GC-MS

CONCLUSION

This work presented synthesis consisting of a reduction of the silver ions using a *Artemisia monosperma* extract for the first time. The silver nanoparticles synthesis were confirmed using different techniques. In present study, antimicrobial of synthesized AgNPs by green methods from aqueous extract of plant *A. monosperma*, as well as plant extract alone were assayed against *Gram positive*, *Gram negative* bacteria and *Candida albicans*, the results indicate the higher activity against *Gram positive* and *Gram negative* bacteria of synthesis AgNPs than aqueous plant extract, where the solution acting as capping ligand. GC-MS of

plant extract shown many important flavonoid (Kumatakenin), alkaloid (Aspidospermidin-17-ol) and other organic compounds (methyl-jasmonate and verbenol). The results support the importance of further study to confirm our result to use these AgNPs as antibiotic and investigate the combination of silver nanoparticles with antibiotic against resistance.

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