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Synthesis and (I.R and TEM) Characterization of leafs and stem nanoparticles of *Artemisia* plant: Comparative study for the evaluation of Anti-bacterial efficiency

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Abstract

New nanoparticles of ***Artemisia*** plant extracts with Silver ions (Ag^+) were synthesized, the particles were characterized by I.R and TEM images. Both of Stems and Leafs were selected in this study. The anti- bacterial investigation included Negative gram (***E. Coli***) and Positive gram (***Staphylococcus***). The results of I.R analysis showed presence different of functional groups of – OH, -C =O and Aromatic which explained of presence of varies compounds as phenols and Flavonoids in the nanoparticles. TEM images of nanoparticles exhibited that the nanoparticles in Nano scale of 0.12 -24 nm. The anti-bacterial studies showed that nanoparticles gave high inhibition zones comparing with plant extracts on the both ***E. coli*** and ***Staphylococcus*** species. But the effect nanoparticles gave more effective on ***E. Coli*** (Negative gram) bacteria comparing to ***Staphylococcus*** (positive gram), this result is harmony with some previous studies.

Keywords: Nanoparticles, Artemisia, TEM, I.R, Antibacterial

Introduction

There are distinct methods chemical, biological, and physical have been used to synthesize nanoparticles. Using a tube furnace and atmospheric pressure, evaporation-condensation is the physical approach used to create nanoparticles (Gurav *et al*., 1994) [3]. AgNPs were synthesized by conventional physical procedures such as pyrolysis and spark discharge (Tien *et al*., 2008) [11]. Physical techniques have the benefits of speed, radiation as a reducing agent, and no harmful chemicals; nevertheless, they also have drawbacks, such as high energy consumption and low yield, solvent contamination, and uneven distribution. Chemical techniques create the silver nanoparticles using organic solvents or water. (Wiley *et al*., 2005) [12], (Tao *et al*., 2006) [10]. Three primary ingredients are often used in this process: metal precursors, reducing agents, and stabilizing/capping agents. In general, there are two steps involved in the reduction of silver salts: (1) nucleation and (2) subsequent growth. There are two general ways to obtain silver nanomaterial's: The synthesis of nanoparticles may be roughly categorized into two stages: the "Bottom-up" and the "Top-down" methods. This study aims to evaluate the efficiency of antibacterial effective of plant extract comparing with their nanoparticles on two different species of bacteria including (***E. coli*** and ***Staphylococcus***).

Experimental Part

Collection and Preparation the plants:

Fresh stems and leaves of ***Artemisia*** were collected from Khulan region at the southern of Al Gabal al Akhdar Mountain, Libya, during spring 2023. Ensuring they are free from pesticides and other contaminants. The samples were kept at dry place until become completely dry. The leaves and stems were separated. Then the samples were cut into small pieces to increase the surface area for extraction.Use a blender or mortar and pestle to grind the leaves and stems into a fine paste. Then samples were kept in polyethylene bags until using.

Extraction Process:

Five grams of the ground plant samples transferred into a heat-resistant container. Then 200 ml of distilled water was added to the container. Gently heat the mixture using a water bath for 30 minutes at a temperature of 70- 80°C. By Stirring the

mixture occasionally. The mixture was allowed to cool to room temperature. Use filter paper or cloth to filter the mixture. Pour the mixture through the filter into a clean container to separate the liquid extract from the solid plant residue. Repeat the filtration process if necessary to ensure a clear extracts. Cool the extract to 4°C to complete the synthesis process. By following these steps, you can effectively prepare a plant extract from dried ***Artemisia*** leaves and stems.

Preparation of Silver Nitrate Solution:
Dissolve a specific amount of silver nitrate ($AgNO_3$ mM) in deionized water to prepare a 1 mM solution. The solution was stirred until the silver nitrate is completely dissolved, then the solution was kept in dark glass bottles.

Synthesis of Ag Nanoparticles:
A mixture was prepared in a 10:1 ratio and a 50 ml solution is made. The solution is covered with aluminum foil and left at room temperature until the color of the Artemisia changes from yellow to dark brown, Fig 1. The samples are mixed in a centrifuge for 20 minutes until the solution is suspended .5 ml of distilled water is added to the precipitate, mixed again, and the process is repeated.

Fig 1: *Artemisia* extract before and after the addition of Ag+

Characterization of silver nanoparticles:
Typically involves several analytical techniques to assess their physical, chemical, and optical properties. Here are some common methods used for characterization**:**

Fourier Transform Infrared Spectroscopy (FTIR)
FTIR is used to analyze the chemical composition and functional groups present on the surface of silver nanoparticles. By measuring the absorption of infrared radiation, FTIR can identify the presence of organic molecules, stabilizing agents, or surface coatings on the nanoparticles. By employing these characterization techniques, researchers can gain a comprehensive understanding of the properties and behavior of silver nanoparticles, which is essential for their application in various fields such as catalysis, biomedicine, sensing, and nanotechnology.

Antibacterial activity
This study investigates the antibacterial activity of plant extracts from *Artemisia* (plant leaves and stems). The extracts were tested both before and after treatment with silver nitrate. The bacterial strains used for testing were ***Escherichia coli*** (a Gram-negative bacterium) and ***Staphylococcus*** (a Gram-positive bacterium). The method involved spreading the extracts at different concentrations and measuring the inhibition zones to determine antibacterial efficacy.

Extracts' Efficacy:
Both untreated and silver nitrate-treated extracts exhibited antibacterial activity against ***E. coli*** and ***Staphylococcus***. The presence of silver nanoparticles enhanced the antibacterial effect, showing significant inhibition zones for both types of bacteria.

Results & Discussion
I.R analysis:
FTIR **Spectra of *Artemisia* leafs**. The C-H Stretching: A peak observed at 3333 cm^{-1} can be attributed to the C-H stretching vibrations **.**C≡C Triple Bond: A peak observed at 2119 cm^{-1} indicates the presence of carbon-carbon triple bonds (C≡C), -C=O Stretching: A peak observed at 1631 cm^{-1} is due to conjugated carbonyl (C=O) stretching vibrations. The C-H Peak: The peak at 3333 cm^{-1} indicates the presence of C-H stretching vibrations, which are typically associated with aliphatic compounds C≡C Peak: The peak at 2119 cm^{-1} corresponds to carbon-carbon triple bonds (C≡C), suggesting the presence of alkyne. **-**C=O Peak: The peak at 1631 cm^{-1} is due to the stretching vibrations of conjugated carbonyl groups (C=O), indicating the presence of ketones, aldehydes, or carboxylic acids, Fig 2.

For ***Artemisia*** **stems:** The Hydroxyl Group (O-H): was a broad peak observed at 3339 cm^{-1} can be attributed to the hydroxyl group, indicating strong hydrogen bonding. Carbonyl Group (C=O): Peaks observed at 1630 cm^{-1} and 1611 cm^{-1} are due to conjugated carbonyl stretching. Broad O-H Peak: The peaks at 3239 cm^{-1} for leafs and 3339 cm^{-1} for ***Artemisia*** stems indicate the presence of hydroxyl groups. These broad peaks correspond to the stretching vibrations of the hydroxyl group, indicating strong hydrogen bonds. C =O Peak: The peaks at 1630 cm^{-1} and 1611 cm^{-1} in both samples are attributed to carbonyl stretching, indicating the presence of carbonyl groups in conjugated compounds. Fig 3.

FTIR measurement was used to determine the presence of bioactive molecules that could be responsible for AgNP stabilization by acting as capping agents. The absorption spikes at 3,349.75, 2,917.77, 1,537.95, 1,385.60, and 1,076.08 cm^{-1} were determined for C. fragrant leaf extract, while AgNPs shows absorption spikes at 3,326.47, 2,913.91, 1,526.28, 1,329.43, and 1,014.37 cm^{-1}. Higher peaks at 3,349.75 and 3,326.47 cm^{-1} can be attributed to bounded hydroxyl (–OH) in alcohols in the C. fragrans leaf extract. The presence of smaller bands at 2,917.77 and 2,913.91 cm^{-1} for extract and AgNPs was due to the –CH stretching in alkanes. Peaks at 1,537.95 cm^{-1} (extract) and 1,526.28 cm^{-1} (AgNPs) correspond to the C=O group. The bands at 1,385.60 cm^{-1} (extract) and 1,329.43 cm−1 (AgNPs) correspond to the C–O–C group. The peaks at 1,076 cm^{-1} (extract) and 1,014 cm^{-1} (AgNPs) are attributed to the C–O stretching in esters that were found in the leaf extract. The spectrum of AgNPs was observed regarding the sites of the functional groups. Therefore, these groups found in plant leaf extracts were responsible for silver nitrate reduction to AgNPs and capping nanoparticles for stabilization and preventing their aggregation in the medium.

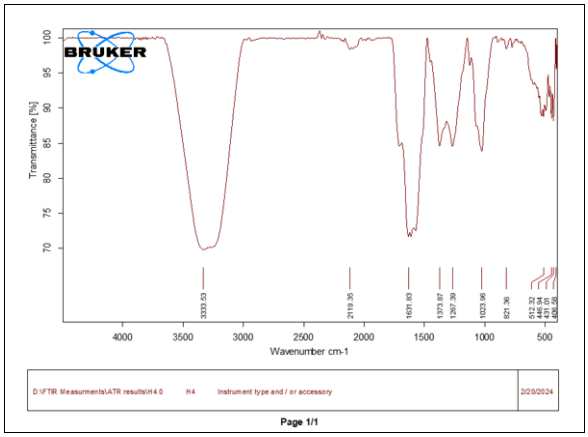


Fig 2: I.R Spectra of ***Artemisia*** leaf nanoparticles

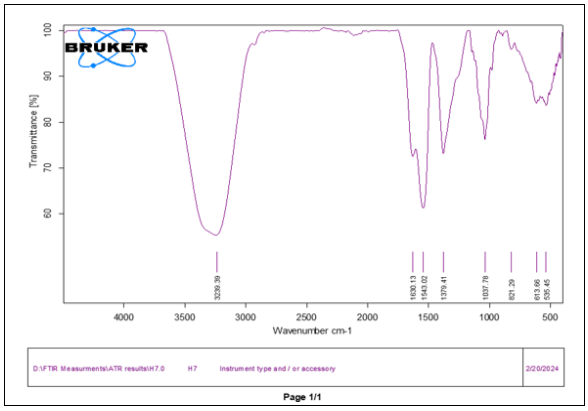
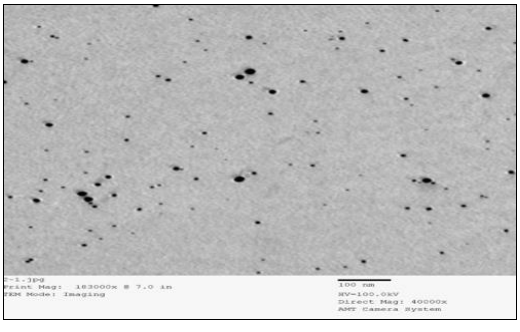


Fig 3: I.R Spectra of ***Artemisia*** stem nanoparticles

TEM Images analysis:

In this study, silver nanoparticles (AgNPs) synthesized using plant extracts were analyzed. The detailed morphology and size of the nanoparticles were examined, particularly those synthesized using (***Artemisia)*** leafs and Stems, Advanced imaging techniques such as Transmission Electron Microscopy (TEM) was employed to visualize the nanoparticles.

Artemisia Leafs**:** Shape and Size: The AgNPs synthesized from ***Artemisia*** leafs were observed to be nearly spherical in shape. Particle Size: The size of the nanoparticles ranged between 0.12 and 13 nm**-**Detection: The nanoparticles were successfully detected and characterized, confirming their spherical morphology and nano scale dimensions. ***Artemisia*** Stems: Shape and Size: The AgNPs synthesized from stems also exhibited a nearly spherical shape. Particle Size: The size of the nanoparticles was found to range between 0.54 and 24 nm. The nanoparticles were successfully detected and characterized, showcasing their spherical shape and consistent size. Figures (4 & 5).



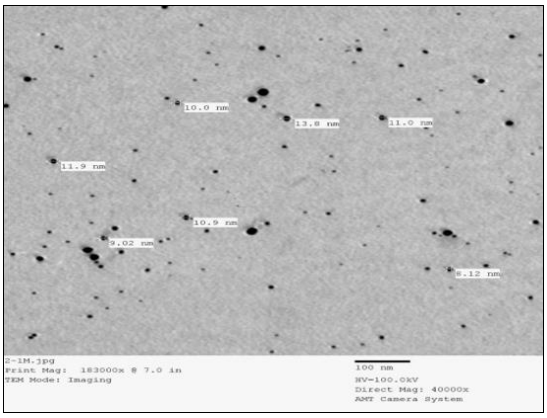


Fig 4: TEM analysis of *Artemisia* Leafs nanoparticles

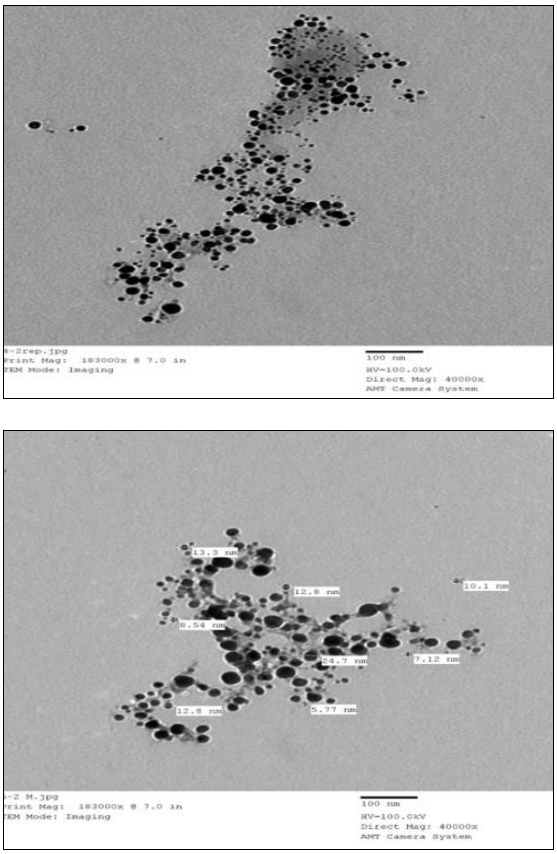


Fig 5: TEM analysis of Artemisia Stem nanoparticles

Antibacterial investigation:

Anti-Bacterial activity of extracts (Before adding Ag nanoparticles):

The extract of ***Artemisia*** plant leafs was also tested on two types of bacteria used in the study. It showed an inhibition of 11 mm for the growth of Gram-positive bacteria and 10 mm for the growth of Gram-negative bacteria, as shown in Table 1 and Fig 6.

Table 1: The effect of *Artemisia* plant leafs extract before adding nanoparticles

Concentration (mg/mL)	Inhibition Zone (mm) E. coli	Inhibition Zone (mm) Staphylococcus
25	7	6
50	9	7
75	10	6
100	11	10

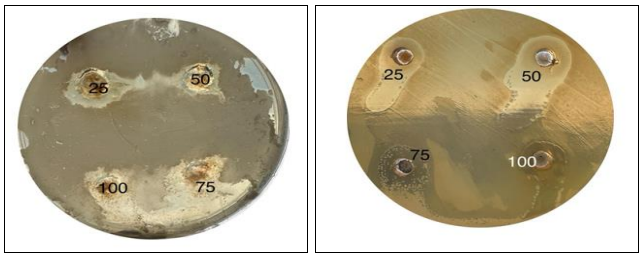


Fig 6: The effect of ***Artemisia*** plant leafs extracts

Also the extract of Artemisia stems was tested on two types of bacteria used in the study. It showed an inhibition of 7 mm for the growth of Gram-positive bacteria and 10 mm for the growth of Gram-negative bacteria, as shown in Table 2

Table 2: The effect of ***Artemisia*** Stems extract before adding nanoparticles

Concentration (mg/mL)	Inhibition Zone (mm) E. coli	Inhibition Zone (mm) Staphylococcus
25	7	10
50	5	7
75	4	6
100	3	4

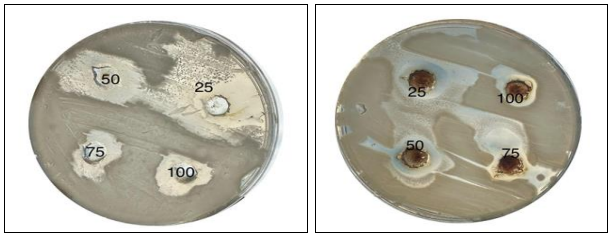


Fig 7: The effect of ***Artemisia*** Stems plant extract on ***Staphylococcus*** and ***E. coli bacteria***

Anti -bacterial investigation of synthesis nanoparticles:
The nanoparticles of ***Artemisia*** **Leaf showed** inhibition zone of 13 mm for the growth of staph bacteria and13 mm for the growth of E -coli bacteria. Table 3 and Fig 8. On the other side *Artemisia* stem extract nanoparticles gave inhibition zone of 9 mm against ***Staphylococcus*** bacteria and 12 mm against ***E. Coli*** bacteria. Also these zones are higher than the zone values recorded the plants without addition nanoparticles. Table 3 and Fig 8.

Table 3: Effect of leafs ***Artemisia*** nanoparticles on the studied Bacteria

Concentration (mg/mL)	Inhibition Zone (mm) E. coli	Inhibition Zone (mm) Staphylococcus
25	9	7
50	11	9
75	12	8
100	13	13

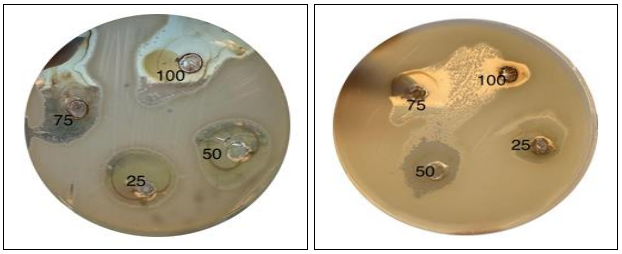


Fig 8: Effect of leafs ***Artemisia*** nanoparticles on the studied Bacteria

Table 4: Effect of stems ***Artemisia*** nanoparticles on the studied Bacteria

Concentration (mg/mL)	Inhibition Zone (mm) E. coli	Inhibition Zone (mm) Staphylococcus
25	9	12
50	7	9
75	6	8
100	5	6

Fig 9: Effect of stems ***Artemisia*** nanoparticles on the studied Bacteria

The antimicrobial activity of the synthesized nanoparticles was evaluated using MIC which is defined as the minimum concentration of the sample required to inhibit growth against the tested microorganisms.
Tables (1-4) and (6-9) showed the results of antimicrobial activity from the silver nanoparticles used against the species. This is due to silver nanoparticles ability to affect both the chemical and physical properties of the cell walls and cell membranes. This is achieved by the nanoparticles attaching themselves onto the cell which is negatively charged, and as a result, disturbs the important functions such as respiration and osmoregulation (Sharma *et al*., 2009) [9]. Further on, the synthesized nanoparticles cause damage within the bacteria cell wall by interacting with proteins and DNA after they permeate the bacteria cell wall (Ahn *et al*., 2018). The synthesized nanoparticles was found to have higher antimicrobial activity against gram-negative ***E. Coli*** compared to the gram-positive Staphylococcus, these results are harmony with that results reported by (Logeswari *et al*., 2015) [6].
Also reported a similar trend when they synthesized silver nanoparticles using the plant extracts; their silver nanoparticles gave higher antimicrobial activity against gram-negative selected bacteria in this study. The high activity that was observed against the gram-negative bacteria was attributed to the cell wall. Gram-negative bacteria are known to have thinner walls as compared to the gram positive bacteria, thus are easier to permeate (Dakshayani *et al*., 2019) [2].
On the other hand, silver nanoparticles cannot easily permeate and interact with gram-positive bacteria is due to the rigid structure that results from the thick peptidoglycan which consists of peptides cross-linked together giving linear and chains of polysaccharides (Rashid *et al*., 2018) [7].
The synthesized silver nanoparticles gave better activity compared to the neomycin that was used as a positive control. The difference in activity was caused by smaller size and high surface area of silver nanoparticles. Therefore, they have a higher adsorption capacity and thus are more likely to interact with biomolecules (Ren *et al*., 2019) [8].
The high antimicrobial activity that was observed with ***Artemisia*** AgNPs was due to the small size of the silver

nanoparticles as shown in the TEM images compared to the other silver nanoparticles that were synthesized using ***Artemisia*** AgNPs and were found to be agglomerated (Hernández-morales *et al*., 2019) [4]. Smaller nanoparticles are known to have high surface area and thus can easily interact with the cell, permeate, and destroy the cell (Jemilugba *et al*., 2019 [5] & Logeswari *et al*., (2015) [6].

Conclusion

Nanoparticles of Artemisia with Silver ions exhibited antibacterial activities, the results of this study showed that, nanoparticles gave antibacterial efficiency higher than Artemisia extracts.

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