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Preliminary Phytochemical Screening and Antimicrobial Activity of *Quercus infectoria*-Mediated Silver Nanoparticles Compared with Selected Medicinal Plant Extracts

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ABSTRACT

Medicinal plants contain a variety of phytochemicals that provide therapeutic effects, particularly as antimicrobial agents. *Quercus infectoria* galls, *Allium sativum* (Garlic), *Zingiber officinale* (Ginger), *Mentha* species (Mint), and *Salvia* spp. (Sage) have been used historically to treat microbial infections. The purpose of this study was to determine the biological activity and secondary metabolite content of each of these plants and also to assess whether or not the use of silver nanoparticles (AgNPs) synthesized from *Q. infectoria* galls would improve their antimicrobial properties. Aqueous and alcohol extracts of each of the plants were prepared for qualitative analysis of phytochemical constituents. AgNPs were synthesized from an aqueous extract of the galls of *Q. infectoria* and characterized using UV-Vis spectroscopy, Fourier Transform Infrared (FTIR), and Scanning Electron Microscope (SEM). The findings showed that plant extracts contain chemical compounds known as phenolics, tannins, alkaloids, saponins, and glycosides. *Q. infectoria* gall extract exhibited notable antimicrobial activity. The biosynthesized AgNPs exhibited consistent antibacterial efficacy against all tested bacterial strains, with inhibition zone diameters reaching up to 21.1 mm against *Proteus mirabilis*. SEM imaging revealed that the AgNPs formed were spherical, with a size range of 41.82–61.54 nm. Overall, these results indicate that the synthesis of AgNPs using *Q. infectoria* enhances antimicrobial activity, suggesting that phytochemical extracts could provide new, safe, environmentally friendly, and efficient methods for using phytochemicals and nanotechnology to create novel antimicrobial agents for use in future applications in the biomedical field.

1. INTRODUCTION

Medicinal plants have attracted considerable attention since ancient times, as they have been used as sources of both food and medicine in various civilizations and have become integral components of cultural and therapeutic heritage due to their high effectiveness in treating a wide range of diseases. Researchers have extensively studied these plants because of their safety, efficacy, minimal or absent side effects, and low cost [1]. Herbal therapy is considered one of the foundations of traditional medicine and has gained worldwide popularity in the treatment and prevention of numerous diseases [2]. This field has become increasingly important due to the emergence of many infections caused by antibiotic-resistant bacteria, prompting the search for safe, effective, low-cost, and eco-friendly alternatives, where plants remain the most promising option [3].

Medicinal plants contain a wide spectrum of bioactive secondary metabolites, many of which are used either in crude or purified forms as drugs to treat various pathological conditions, thanks to their potent biological activities. These compounds have opened new possibilities for the development and production of alternatives to conventional chemical therapeutics. Among the most biologically active secondary metabolites are phenolics, alkaloids, glycosides, resins, saponins, and tannins, which exert diverse physiological effects on the human body [4, 5].

Secondary plant metabolites also play a crucial role in nanotechnology, functioning as reducing and capping agents during the synthesis of nanoparticles, which ensures their stability (capping agents). Nanotechnology is defined as the science of materials at the nanoscale, where particle sizes range between 1 and 111 nm [6]. There are three main approaches for nanoparticle synthesis: chemical, physical, and biological methods. Among these, green synthesis is preferred because it is environmentally safe, non-toxic, and does not

require harsh conditions of pressure or temperature, unlike chemical or physical methods, while also offering high productivity [7].

Green synthesis using plants is among the most efficient and sustainable strategies because it is eco-friendly, safe, cost-effective, rapid, and easy to perform. Moreover, it produces more stable nanoparticles compared to other biological approaches based on microorganisms such as bacteria, fungi, yeasts, or viruses. Various plant parts, including leaves, stems, fruits, and bark, can be utilized in such processes [8].

Nanotechnology has gained significant research attention owing to its wide range of applications in industry, agriculture, mechanics, biomedicine, electronics, catalysis, energy science, drug delivery, and even space technology. The uniqueness of nanoparticles lies in their small size and large surface area-to-volume ratio compared with bulk materials, which impart novel physical and chemical characteristics [9]. Biosynthesized nanoparticles can be derived from several metals, such as gold, silver, iron, zinc, and palladium [10].

Among these metals, silver nanoparticles (AgNPs) are the most prominent due to their potent antimicrobial properties and broad applications. They are economically feasible, environmentally benign, and relatively non-toxic, making them suitable for biomedical uses [11]. These nanoparticles have demonstrated strong activity against bacteria and fungi, including strains resistant to conventional antibiotics, an escalating global health problem. Therefore, nanotechnology provides a promising solution in nanomedicine by developing nanoscale agents that serve as potential candidates for further investigation as antimicrobial agents to combat multidrug-resistant (MDR) microorganisms [12].

Quercus infectoria Olivier is a small deciduous tree belonging to the family Fagaceae, commonly distributed in Iraq, Syria, Iran, and Turkey, and extending further to Asia Minor, Europe, and North Africa [13]. According to Morell and Balkin [14], the plant extracts of this species are rich in tannins, phenolic compounds, flavonoids, aromatic compounds [15], sterols, volatile organic compounds, fatty acids, alkaloids, and saponins.

This study investigates the biological efficacy and phytochemical composition of extracts obtained from three medicinal plants: *Quercus infectoria* galls, *Allium sativum*, *Zingiber officinale*, *Mentha* spp., and *Salvia officinalis*. Next, the best-performing plant extract will be used to create AgNPs and study whether this process improves their antimicrobial effectiveness compared to that of just the plant extract alone. The ultimate goal is to use a combination of phytochemistry + Green Nanotechnology to develop a more effective and less toxic method for developing effective antimicrobial agents.

2. MATERIALS AND METHODS

2.1 Bacterial isolates

This study made use of bacterial cultures obtained from the Department of Biology - Microbiology Laboratory at the University of Mosul as shown in Table 1.

2.2 Culture media

The enrichment culture medium used in this study was a nutrient agar. This product was purchased from Thermo Fisher Scientific (USA) and prepared according to the manufacturer's recommendations. Nutrient agar was sterilized by autoclaving at 121 ℃ for 15 min, which allowed for complete removal of any potential contaminating organisms.

Table 1. Pathogenic bacterial isolates used in the study

No.	Bacterial Isolate	Gram Staining
1	*Proteus mirabilis*	Gram-negative(-)
2	*Pseudomonas aeruginosa*	Gram-negative(-)
3	*Enterobacter cloacae*	Gram-negative(-)
4	*Escherichia coli*	Gram-negative(-)
5	*Klebsiella pneumoniae*	Gram-negative(-)
6	*Bacillus cereus*	Gram-positive(+)
7	*Staphylococcus aureus*	Gram-positive(+)

2.3 Preparation of plant samples

In March, 2025, through a certified herbal shop in Mosul, Nineveh Province, Iraq, the dried gall of *Quercus infectoria* Olivier, the bulb of *Allium sativum* L., the rhizome of *Zingiber officinale* Roscoe, the leaves of *Mentha* spp., and the leaves of *Salvia officinalis* L. were obtained. All plants involved were authenticated by the Plant Taxonomist in the Department of Biology, University of Mosul. The authenticated voucher specimens will be kept in the Herbarium Room of the Department of Biology with the following accession numbers: QI-01, AS-02, ZO-03, MS-04, and SO-05 for future reference. Following authentication, all materials were cleaned of foreign matter, air-dried, and finely powdered before extraction.

To prepare the plant materials for extraction, they were thoroughly washed to remove any dirt or other contaminants and then processed into a fine powder using an electric grinder. All extracts were kept in closed, dark containers until required for use in experiments.

2.4 Extraction procedures

2.4.1 Aqueous extraction

The aqueous extracts of the plant materials [16] were created using a modified procedure from the study [16] following the procedures used for aqueous extracts by Ibrahim and his colleagues in 2022. Accordingly, 20 g of powdered plant material was infused with 200 mL of distilled water at 25 ℃ for 30 minutes. After 30 minutes, the plant material and distilled water mixture was blended for 10 minutes in an electric blender. The mixture was then filtered using a piece of medical gauze to obtain the solid material, which resulted in a clear supernatant that was centrifuged at 3000 rpm for 15 minutes, yielding a clear upper solution (supernate) and a lower separated solid (pellet) that was concentrated with a rotary evaporator using a rotary evaporator set at under 40 ℃. The final extract was then either freeze-dried into a powder or freeze-dried into a gummy residue. The weight of the extract was recorded, and all dried extracts were stored in a refrigerator at 4 ℃ for future experiments and analyses [17].

2.4.2 Alcoholic extraction

Following the procedure outlined by Abbas et al. [18], alcoholic extraction was conducted with 20 g of plant powder placed in thimbles and extracted in a Soxhlet extractor using 200 mL of 95% ethanol, heated to 40 degrees Celsius, for a period of 24 hours. Afterward, the extract was concentrated on a rotary evaporator (40 ℃) and dried in glass Petri dishes at room temperature. At the end of this process, the dried extracts were weighed and stored at 4 ℃ until further use.

2.5 Preliminary screening for the biological activity of crude plant extracts

A stock solution was created at a concentration of 212 mg/mL by dissolving (2.12 g) of dried crude extract in 10 mL of dimethyl sulfoxide (DMSO). The antimicrobial activities of extracts against pathogenic bacteria were evaluated using the Agar Well Diffusion method as described in the study [19] and the Clinical and Laboratory Standards Institute (CLSI). Mueller-Hinton agar plates were made, and six-millimetre (6 mm) holes were punched out of them with the aid of sterile corn borers. To create a bacterial inoculum, bacterial colonies were suspended in 0.9% saline to achieve a concentration similar to that of the McFarland standard number 0.5 (~1.5×10^8 CFU/mL). Bacterial inoculum, which was prepared at a concentration of 0.1 mL in saline and allowed to soak up any liquid for 2 minutes, was used to evenly coat the surface of the agar plate with a layer of 122 µL of the extract. The agar plates were then incubated at 37 ℃ for a period of 24 hours, and after incubation, the diameter of the zones of bacterial inhibition around the holes was measured and recorded.

2.6 Extraction of secondary compounds from *Quercus infectoria*

2.6.1 Extraction of phenolic compounds

Phenolic compounds were obtained using a modified Gayon-Ribereau method [20] whereby a 250 mL glass beaker was filled with 20 grams of ground plant, to which an additional 90 mL of 2% acetic acid solution was then added. The combination was then placed in a water bath where it was subjected to acid reflux for a minimum of six hours at 25 degrees Celsius or less. Following the completion of reflux, the extracted plant material was filtered through a Whatman No. 1 filter paper, after which it was mixed with an equal amount of ethyl acetate to form two distinct layers (one containing the extracted phenolic compounds). The top layer containing the phenolic compounds was collected using a separatory funnel. The concentrated (dried) phenolic fraction obtained from the separating funnel was dried with a rotary evaporator, weighed, and stored in the refrigerator at four degrees Celsius until required for future use.

2.6.2 Extraction of alkaloid compounds

The extraction method for alkaloids utilized by Ibrahim et al. [21], with slight modifications, included a substrate ratio of 10 grams of powdered plant material (ten grams of plant powder) and ethanol, as well as a filtration process through paper (Whatman 1), and the use of concentrated ammonia to achieve a pH level of 8. To extract alkaloids from plants using this extraction method, the pH level must range from 8 to 11. In this study, alkaloids were extracted from 10 grams of powdered plant material using three methods of extraction. The extraction process was performed using three separate extractions of the same sample material.

2.7 Determination of the minimum concentration showing detectable inhibition of *Quercus infectoria* fruit peel extracts

The agar well diffusion method was used to determine the minimum concentration showing detectable inhibition for *Quercus infectoria* fruit peel extracts (crude, phenolic, and alkaloid fractions). A stock solution was prepared from the crude extract at 212 mg/mL in DMSO and then used to create a series of dilutions that produced different concentrations of extract. An aliquot (122 µL) of each concentration was transferred to the appropriate well in an agar plate, and a DMSO negative control was also included. The agar plates were incubated at 39 ℃ for 24 hours, after which the size of the inhibition zones was measured to find out the minimum concentration showing detectable inhibition for each extract.

2.8 Antibiotic susceptibility test of bacterial isolates

The disk diffusion method was performed according to the classical method described by research [22] and in accordance with CLSI guidelines [23]. Muller-Hinton agar plates were prepared, and the target bacterial isolates were spread at equal density on the surface of the medium. Five standard antibiotic discs were placed on the surface of the plates, and the plates were incubated under conditions appropriate to the isolate type. After the specified incubation period, the diameters of the inhibition zones around the discs were measured and compared with the standard sensitivity criteria to classify susceptibility (susceptible, intermediate, resistant) according to CLSI Table 2. The diameters of the inhibition zones for each of these antibiotics were measured using the same method used to measure the inhibition zone of extracts as in the previous paragraph. Each assay consisted of 122 µL of extract solution in a 6-mm diameter well, and DMSO was used as a negative control (the same concentration of DMSO was used to prepare the extracts), while ciprofloxacin (5 µg/disc) was used as a positive control. The antibacterial activity of the extracts was assessed by measuring the diameter (in mm) of the inhibition zone after incubating the plates at 37 ℃ for 24 h, using a digital caliper to measure the diameter of each inhibition zone. The data were collected from three independent experiments and presented as mean ± standard deviation.

Table 2. Antibiotics used in the study

No.	Antibiotics	Symbol	Disk Content (µg/disc)
1	Ciprofloxacin	CIP	5
2	Ampicillin	AMP	25
3	Tetracycline	TET	31
4	Gentamicin	GEN	10
5	Streptomycin	STR	10

2.9 Preparation of silver nanoparticles from the aqueous extract of Aleppo oak fruit bark (*Q. infecttoria*)

AgNPs were developed using a protocol established by Waen-Ngoen et al. [24], whereby an initial solution of 1mM silver nitrate ($AgNO_3$) was combined with an aqueous extracts from Aleppo oak fruit bark (*Quercus infectoria*) at a ratio of 1:8. The resulting composite materials were placed in an incubator that shook at 120 rpm in darkness for six hours, followed by twenty-four hours of standing which allowed the characteristic colour change indicative of nanoparticle formation to occur. After completion of this incubation period, samples were centrifuged at 6222 rpm for 22 minutes. The resulting supernatants were discarded, and the nanoparticle pellets underwent three washes with 10mL of distilled water to ensure that remaining impurities were removed. Following successful washing, AgNPs were dried in an oven at 40 ℃ and saved for future testing.

The treatments studied used a common quantity of the stock

solutions, but the actual amount of silver and the actual amount of phytochemicals in each treatment were different. Therefore, comparisons made between treatments should be viewed as a preliminary assessment of antibacterial activity and not a direct assessment of effectiveness.

2.10 Characterization of silver nanoparticles

2.10.1 UV-Vis spectrophotometry

Filtration of the biosynthesized AgNPs suspension was performed using Whatman No. 1 filter paper before its analysis via UV-Vis spectrophotometry [25]. For confirmation of the presence of AgNPs through surface plasmon resonance (SPR), absorbance spectra were measured in the wavelength range of 300 to 700 nm.

2.10.2 Fourier Transform Infrared spectroscopy

Spectra were recorded using a Fourier Transform Infrared (FTIR) spectrometer in the range 400–4000 cm^{-1} using a KBr lens. Non-aggregates in the original extract were identified using a silver nitrate ($AgNO_3$) reduction technique and independent coverage stability [26]. Analysis was conducted at the Scientific Center/University of Basra.

2.10.3 Scanning Electron Microscope

The morphological characteristics of the prepared nanoparticles were determined in terms of shape and size using a Scanning Electron Microscope (SEM) at the Electron Microscopy Unit, University of Tehran, Iran.

2.11 Biological activity of silver nanoparticles

The biological activity of the nanoparticles was evaluated using the agar well diffusion method, as described in section 11-2. A stock solution of 1000 µg/mL was prepared (dissolving 0.001 g in 1 mL of DMSO) for AgNPs, silver salts, and crude aqueous and alcoholic plant extracts of *Q. infectoria* fruit peels. From this solution, the remaining concentrations (12, 120, 212, 500, and 912 µg/mL) were prepared.

2.12 Statistical design and analysis

Data from evaluations were statistically analyzed using SPSS [27]. The design of the experiment was a completely randomized design (CRD), and as such, the mean values of any treatment groups were compared by calculating the least significant difference (LSD) at a level of significance of 0.01.

3. RESULTS

3.1 Percentage of weights of crude plant extracts (aqueous and alcoholic)

The crude yields of the plant extracts (shown in Table 3) were evaluated to ascertain the difference between aqueous and alcoholic solvents. *Quercus infectoria* Olivier had the greatest aqueous yield (60.10%), followed by *Zingiber officinale* (45.20%) and *Allium sativum* (34.14%). In relation to the alcoholic yield, *Quercus infectoria* Olivier and *Zingiber officinale* displayed similar yields of 49.02%.

3.2 Specific chemical detections of medicinal plants

Specific chemical tests were conducted on the crude aqueous and alcoholic extracts of the three medicinal plants under study to identify their main active compounds (Table 4). The results showed that the aqueous extract of the fruit bark of the Aleppo oak (*Quercus infectoria* Olivier) contained a wide range of active compounds, including tannins, alkaloids, phenols, resins, saponins, glycosides, and flavonoids, while coumarins were not recorded. The alcoholic extract of the plant showed similar results in terms of the quality of the active compounds. The aqueous and alcoholic extracts of garlic also contained all the active compounds except coumarins.

Table 3. Yield percentages of aqueous and alcoholic crude medicinal plant extracts

Medicinal Plant Species	Alcoholic Extract (%)	Aqueous Extract (%)
Quercus infectoria Olivier	49.02	60.10
Allium sativum	36.09	34.14
Zingiber officinale	49.02	45.20

Table 4. Phytochemical screening of active secondary metabolites in the studied medicinal plant extracts

Phytochemical Compounds	Alcoholic Extract			Aqueous Extract		
Alkaloids	Zingiber	Allium	Q. inf.	Zingiber	Allium	Q. inf.
Phenols	-	+	+	+	+	+
Tannins	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+
Coumarins	-	+	+	+	+	+
Saponins	+	+	+	+	+	+
Flavonoids	-	+	+	+	+	+

3.3 Biological activity of crude aqueous plant extracts

The results showed highly significant differences between the concentrations of most aqueous extracts, as well as between the diameters of inhibition of bacterial isolates at a probability level of $p \leq 0.01$ (Table 5). Aleppo oak (*Quercus infectoria*) bark extract was found to be the most effective in inhibiting the growth of all bacterial isolates, recording the highest diameter of inhibition of 30.00 mm against *Proteus mirabilis*. Meanwhile, garlic showed significant inhibitory activity against four bacterial isolates: *Staphylococcus aureus*, *P. mirabilis*, *Enterobacter cloacae*, and *Bacillus cereus*.

Table 5. Arithmetic mean diameters of inhibition zones of crude aqueous plant extracts against bacterial isolates (in mm)

Bacteria	*Quercus infectoria* Olivier	*Allium sativum*
Proteus mirabilis	30.0 ± 0.6	18.0 ± 0.8
Pseudomonas aeruginosa	31.0 ± 0.5	16.0 ± 0.9
Enterobacter cloacae	25.0 ± 0.7	0.4 ± 0.1
Escherichia coli	25.0 ± 0.6	14.0 ± 0.8
Klebsiella pneumoniae	27.0 ± 0.7	1.9 ± 0.2
Bacillus cereus	26.0 ± 0.5	15.0 ± 0.7
Staphylococcus aureus	28.0 ± 0.6	0.9 ± 0.2

Note: N = 3, LSD = least significant difference (Bacteria = 1.07, Extract = 0.90), $p \leq 0.01$.

3.4 Biological efficacy of crude alcoholic plant extracts

The antibacterial activity of the crude ethanol extracts of the three test plants displayed a statistically significant difference when comparing them against the bacterial isolates (see Table 6). *Quercus infectoria* showed the greatest antibacterial effect on all bacteria tested, with inhibition zones ranging from 27–33 mm, with maximum effects against *Pseudomonas aeruginosa* (33 mm), *Staphylococcus aureus* (31 mm), and *Proteus mirabilis* (32 mm). *Allium sativum* and *Zingiber officinale* exhibited moderate efficacy, as detailed in Table 6.

Table 6. Arithmetic mean diameters of inhibition zones of crude alcoholic plant extracts against bacterial isolates (in mm)

Bacteria	*Quercus Infectoria* Olivier	*Allium sativum*	*Zingiber officinale*
Proteus mirabilis	32.0 ± 0.5	21.0 ± 0.8	14.0 ± 0.7
Pseudomonas aeruginosa	33.0 ± 0.4	19.0 ± 0.7	16.0 ± 0.6
Enterobacter cloacae	28.0 ± 0.6	6.0 ± 0.3	5.0 ± 0.3
Escherichia coli	27.0 ± 0.6	16.0 ± 0.7	6.0 ± 0.3
Klebsiella pneumoniae	29.0 ± 0.6	7.0 ± 0.4	5.5 ± 0.3
Bacillus cereus	30.0 ± 0.5	18.0 ± 0.7	19.0 ± 0.6
Staphylococcus aureus	31.0 ± 0.5	6.5 ± 0.3	6.0 ± 0.3

Note: N = 3, LSD = least significant difference (Bacteria = 1.07, Extract = 0.90), $p \leq 0.01$.

3.5 Determination of the concentration-dependent antibacterial activity of phenolic and alkaloid fractions isolated from the peels of *Quercus infectoria* fruits

Distinct variations were found in the concentration of the majority of phenolic extracts in comparison to the level of inhibition observed by the bacterial isolates $p \leq 0.01$. The Minimum concentration showing detectable inhibition of phenolic extracts differed significantly by bacterial species, with the lowest Minimum concentration showing detectable inhibition being 1.0 mg/mL for *Klebsiella pneumoniae* and 11.0 mg/mL for *Pseudomonas aeruginosa*, *Enterobacter cloacae*, and *Escherichia coli*. The highest Minimum concentration of 26.0 mg/mL was observed for *Bacillus cereus*, which reflects the variable efficacy of phenolic compounds against bacterial species. Similar to the phenolic extract, significant differences were also noted between the concentration of the alkaloid extract and its minimum concentration, showing detectable inhibition against each bacterial isolate ($p \leq 0.01$); however, its activity was restricted to specific bacterial isolates, showing notable efficacy against *Bacillus cereus* while *Staphylococcus aureus* exhibited no detectable susceptibility. These results indicate that phenolic compounds represent a more effective and comprehensive option for inhibiting a wide range of bacteria compared to alkaloid extracts. This highlights the importance of studying the concentrated bioactivity of plant extracts rich in secondary compounds to develop antibacterial treatments for drug-resistant bacteria, as detailed in Table 7 and Table 8, respectively.

Table 7. Minimum concentration showing detectable inhibition of phenolic extracts of *Quercus infectoria* peels against the studied bacterial isolates

Bacteria	Min. Conc	Phenol Concentrations (mg/mL)				
		5	10	25	50	75
Proteus mirabilis	3.0	20.0	25.0	27.0	29.0	31.0
Pseudomonas aeruginosa	11.0	0.0	0.0	11.0	18.0	20.0
Enterobacter cloacae	11.0	0.0	0.0	12.0	19.0	21.0
Escherichia coli	11.0	0.0	0.0	11.0	16.0	18.0
Klebsiella pneumoniae	1.0	0.2	0.0	0.4	0.5	0.0
Bacillus cereus	26.0	0.0	0.0	0.0	13.0	15.0
Staphylococcus aureus	25.0	0.2	0.4	0.6	13.0	13.0

Note: N = 3, LSD = least significant difference (Bacteria = 1.07, Extract = 0.90), $p \leq 0.01$, min. = minimum, conc = concentration.

Table 8. Minimum concentration showing detectable inhibition of Alkaloid extracts of *Quercus infectoria* peels against the studied bacterial isolates

Bacteria	Min. Conc	Alkaloid Concentrations (mg/mL)				
		5	10	25	50	75
Proteus mirabilis	5.0	13.0	13.0	17.0	17.0	22.0
Pseudomonas aeruginosa	3.0	0.0	0.0	1.3	1.7	0.0
Enterobacter cloacae	0.0	0.0	0.0	0.0	0.0	0.0
Escherichia coli	0.0	0.0	0.0	0.0	0.0	0.0
Klebsiella pneumoniae	6.0	0.0	2.0	4.0	0.0	0.0
Bacillus cereus	26.0	0.0	0.0	0.0	11.0	22.0
Staphylococcus aureus	0.0	0.0	0.0	0.0	0.0	0.0

Note: N = 3, LSD = least significant difference (Bacteria = 1.07, Extract = 0.90), $p \leq 0.01$, min. = minimum, conc = concentration.

Table 9. Average diameters of antibiotic inhibition zones against pathogenic bacterial isolates (in mm)

Bacteria	Antibiotics				
	Ciprofloxacin	Ampicillin	Tetracycline	Gentamicin	Streptomycin
	5	25	30	10	10
Proteus mirabilis	25.0	31.0	28.0	24.0	11.0
Pseudomonas aeruginosa	31.0	8.0	6.0	9.0	7.0
Enterobacter cloacae	35.0	7.0	8.0	10.0	10.0
Escherichia coli	33.0	10.0	9.1	11.0	10.0

Note: N = 3, LSD = least significant difference (Bacteria = 1.07, Extract = 0.90), $p \leq 0.01$.

3.6 Antibiotic sensitivity of clinical bacterial isolates

The results showed highly significant differences between most antibiotics and the diameters of inhibition of bacterial isolates ($p \geq 0.01$). Among the tested pathogens, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, and *Escherichia coli* exhibited distinct susceptibility patterns, as detailed in Table 9. Proteus mirabilis demonstrated high susceptibility to most of the antibiotics used, particularly showing exceptional antibacterial activity against Gentamicin and Ampicillin,

while remaining susceptible to Ciprofloxacin and Tetracycline. These results indicate a marked variability in the response of clinical bacterial isolates to conventional antibiotics, reflecting the importance of exploring effective therapeutic alternatives, such as plant extracts and nanocomposites, to combat the phenomenon of multiple antibiotic resistance.

3.7 Characterization of silver nanoparticles

3.7.1 UV-visible spectrophotometer analysis

The results of sample analysis using a UV-Visible Spectrophotometer confirmed the formation of AgNPs. A distinct absorption peak was observed at a wavelength of 457 nm when the sample was examined within the wavelength range (300–800 nm), as shown in Figure 1. This absorption peak, which corresponds to the SPR phenomenon, is a characteristic feature of the formation of AgNPs. Its appearance is attributed to the collective vibrations of free electrons on the particle surface when exposed to radiation, confirming the successful formation of the nanoparticles.

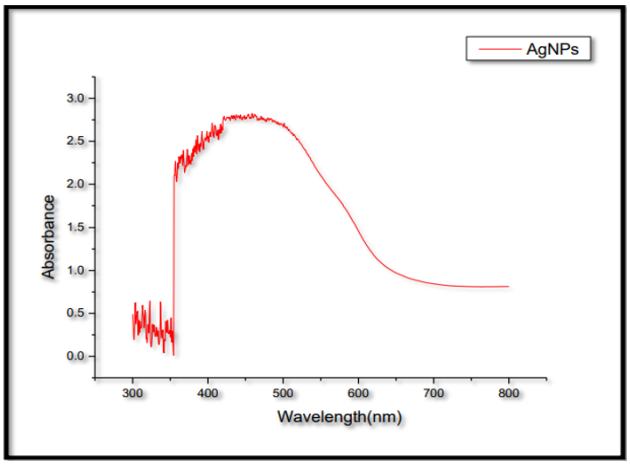

Figure 1. Ultraviolet spectrophotometer analysis of biosynthesis of silver nanoparticles (AgNPs) at a wavelength of 457 nm

3.7.2 Fourier Transform Infrared Spectroscopy

The FTIR results revealed the presence of active functional groups in the aqueous extract of *Quercus infectoria* peels, which contributed to the reduction of silver ions (Ag^{+}) and the stabilization of AgNPs (Figures 2 and 3). The shift and appearance of specific bands at 3362 and 1656 cm^{-1} indicate the involvement of groups such as -NH, C-H, and C = O in the biosynthesis process. The identified functional groups and their corresponding peak positions are summarized in Table 10.

Table 10. Functional groups associated with the synthesis of silver nanoparticles (AgNPs) from the Aleppo oak bark extract (*Q. infectoria*)

No.	Functional Groups	Chemical Bond / Vibration	Peak Intensity	Frequency (cm^{-1})
1	Secondary amine	N–H stretching	Medium	3362
2	Alkyne	C–H stretching	Sharp	3272
3	Aliphatic ketone	C=O stretching	Strong	1727
4	Unsaturated ketone-*aβ*	C=C stretching	Strong	1611
5	Nitro compound	N–O stretching	Strong	1523
6	Alkane	C–H bending	Medium	1448
7	Sulfonic acid	S=O stretching	Strong	1347
8	Ester	C–O stretching	Strong	1193
9	1,4-disubstituted aromatic ring	C–H bending	Strong	815

3.7.3 Scanning Electron Microscope results

SEM results showed that AgNPs prepared from the aqueous extract of *Quercus infectoria* peel exhibited spherical and sub-spherical shapes, with partial aggregates resulting from evaporation during sample preparation. The particle sizes ranged between 41.82 and 61.54 nm, as shown in Figure 4. This nanoscale size distribution supports the observed antibacterial potential, as these dimensions are favorable for efficient interaction with bacterial cell walls. The prepared particles fall within the true nanoscale range.

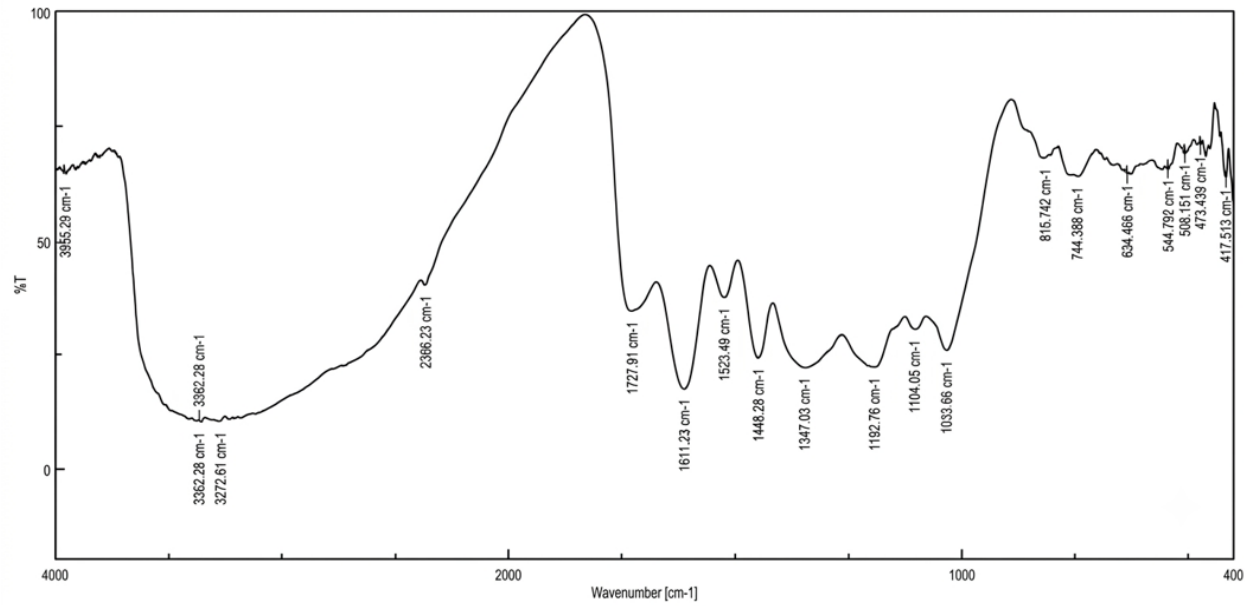

Figure 2. Fourier Transform Infrared (FTIR) spectrum of biosynthesized silver nanoparticles (AgNPs) prepared using aqueous *Quercus infectoria* gall extract

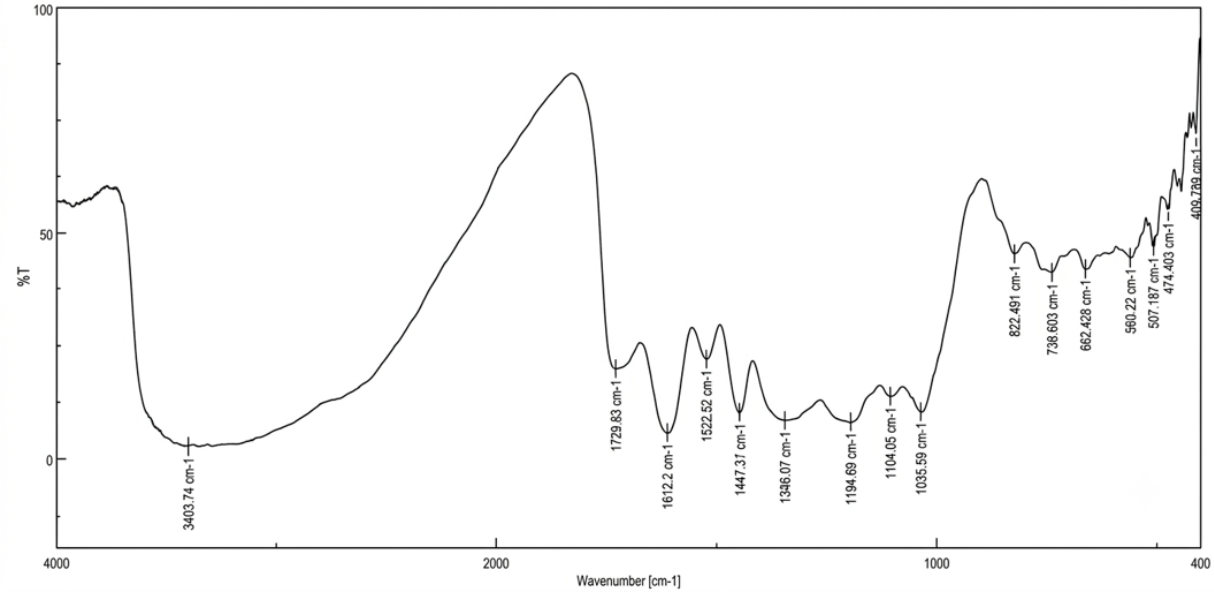

Figure 3. Fourier Transform Infrared (FTIR) spectrum of *Quercus infectoria* crude extract, displaying the primary functional groups involved in the capping and stabilization process

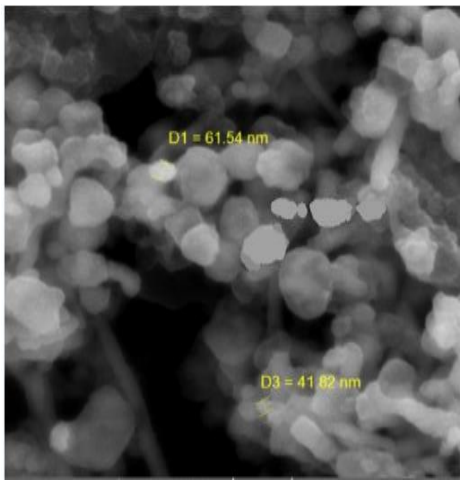

Figure 4. Morphology and size of silver nanoparticles (AgNPs) from *Quercus infectoria* peel extract using Scanning Electron Microscope (SEM)

Table 11. Average inhibition zone diameters (mm) of biosynthesized silver nanoparticles (AgNPs) against pathogenic bacterial isolates at various concentrations

Bacteria	AgNPs Concentrations (µg/mL)				
	75	150	300	600	900
Proteus mirabilis	13.0	13.0	17.0	15.0	21.0
Pseudomonas aeruginosa	11.0	12.0	18.0	16.0	25.0
Enterobacter cloacae	12.0	13.0	17.0	16.0	22.0
Escherichia coli	11.0	15.0	12.0	15.0	20.0
Klebsiella pneumoniae	12.0	12.0	4.0	14.0	19.0
Bacillus cereus	13.0	14.0	12.0	12.0	22.0
Staphylococcus aureus	14.0	13.0	14.0	19.0	18.0

Note: N = 3, LSD = least significant difference (Bacteria = 1.07, Extract = 0.90), $p \leq 0.01$.

3.8 Biological activity of silver nanoparticles

The results showed significant differences ($p \leq 0.01$) in the inhibition zone diameters across different AgNPs concentrations and bacterial species. The antibacterial activity was concentration-dependent, ranging from 75 to 900 µg/mL. The maximum inhibition zone diameter of 25 mm was observed against *Pseudomonas aeruginosa* at 900 µg/mL, while the lowest inhibition zone of 11 mm was recorded against the same isolate at 75 µg/mL. Similar trends were observed for *Escherichia coli* at these concentrations, as detailed in Table 11.

3.9 Biological activity of *Quercus infectoria* extracts and silver nanoparticles

The results showed significant differences ($p \leq 0.01$) in the antibacterial activity of the tested agents against clinical bacterial isolates. The synthesized AgNPs exhibited effective antibacterial activity, with inhibition zone diameters reaching 21.1 mm against the Gram-negative bacterium *Proteus mirabilis*. Comparative analysis (Table 12) indicates that while AgNPs showed consistent activity across all tested isolates, the crude extracts of *Q. infectoria* also demonstrated notable inhibitory potential under the experimental conditions.

Table 12. Comparative analysis of average inhibition zone diameters (mm) of silver salts ($AgNO_3$), raw *Quercus infectoria* extracts, and biosynthesized silver nanoparticles (AgNPs) against pathogenic bacterial isolates

Bacteria	Silver Salts ($AgNO_3$)	Aqueous Extract	Alcoholic Extract	Biosynthesized AgNPs
Proteus mirabilis	16.0	16.0	13.0	21.1
Pseudomonas aeruginosa	22.0	14.0	15.0	24.3
Enterobacter cloacae	17.0	5.0	2.0	22.0
Escherichia coli	15.0	15.0	12.0	20.0
Klebsiella pneumoniae	13.0	2.0	4.0	19.0
Bacillus cereus	14.0	0.0	3.0	22.0
Staphylococcus aureus	13.0	2.0	2.0	18.0

Note: N = 3, LSD = least significant difference (Bacteria = 1.69, Extract = 1.30), $p \leq 0.01$.

Due to growing concerns about the emergence of multidrug-resistant organisms (MDROs), there is increasing interest in using plant-derived products and metallic nanoparticles as alternative antimicrobial therapies. In particular, there are several studies that have investigated the therapeutic properties of *Quercus infectoria* extracts and Ag-based preparations, specifically AgNPs, for their antibacterial potential based on their unique physicochemical characteristics and broad spectrum of activity. Characterizing their antibacterial efficacy against clinically relevant pathogens will help to evaluate their potential as promising in vitro antimicrobial agents.

Based on extraction tests with *Quercus infectoria* (also called Aleppo oak) galls, the yield obtained through water extraction was 60.10% and through alcohol extraction was 48.8%. This is comparable to the results of another study, which showed that extraction using water yielded an average yield of ~44.85%, and extraction using ethanol yielded ~46.5% [28]. An investigation showed that the highest yield from methanol extraction was ~51.6%. The extract of methanol of *Q. infectoria* gall powder contained the driest powder yield (51.64%, w/v) of all the extracts, from 100 g of the gall powder, followed by acetone (50.85%, w/v) [27]. This finding supports the belief that galls of *Q. infectoria* are one of the most readily extractable plant materials with polar solvents, because of their very high tannin content as well as the many phenolic compounds and other plant phytochemicals that are polar. In fact, the galls contain extremely high levels (50-70% estimated) of natural tannins along with other polyphenols [29].

In comparison, the root-extraction yields of *Mentha* spp. (13.2% with an aqueous solvent and 15.5% with an alcoholic solvent), although lower than those of other plants, follow the general trend that extraction yield depends on the plant species, the plant part used, and the solvent type. For instance, when extracting leaves of *Acacia ferruginea*, the lowest yield was approximately 2.5% when using specific solvents, while the maximum yield was approximately 16.3% for another solvent used [30]. A recent study of wood extracted from *Boehmeria rugulosa* reported a higher yield with water (31.97%) than with any of the other solvents tested (ethanol, methanol, or acetone), which emphasizes that the type of solvent used during an extraction will determine the efficiency of the extraction process [31].

Quercus infectoria's aqueous and alcohol extracts had the most extensive phytochemical profiles, containing tannins, alkaloids, phenolics, saponins, glycosides, flavonoids, and resins, which confirms previous reports that *Q. infectoria* galls are the most abundant and best-studied natural source of hydrolysable tannins (50-70%), galloylated flavonoids, and flavonoids with antimicrobial and other therapeutic activities [32]. Studies support that garlic extracts show extensive and varied phytochemical constituents when extracted using both water and alcohol, which correspond with published studies indicating that aqueous and alcohol extracts of *Allium sativum* contain flavonoids, phenolic compounds, saponins, alkaloids, and glycosides [33].

The results of the study on the biological activity of crude alcoholic plant extracts are consistent with the results of studies [34, 35] which concluded that *Q. infectoria* was one of the richest sources of phenolic compounds found in the natural environment with a strong potential to inhibit bacterial pathogens however, further toxicological assessments and in vivo studies are required to evaluate its safety and efficacy before any consideration for human use.

Contrary to this evidence, *Allium sativum* exhibited a much greater range and stronger level of antibacterial activity against Gram-positive and Gram-negative species; this was attributed to the numerous organosulfur compounds that are present in its extracts. One of the most well-studied organosulfur compounds present in these extracts, namely allicin, penetrated and damaged the bacterial cell membrane while disrupting the enzymatic and nucleic acid synthesis processes of the organism, ultimately resulting in bacterial cell death [36]. In contrast, the alcoholic extract of *Quercus infectoria* exhibited a significantly greater level of antibacterial activity than all of the previously discussed plant extracts. The increased effectiveness of *Quercus infectoria* is likely due to the fact that many of the phenolics, flavonoids, alkaloids, and tannins found in the extract of *Quercus infectoria* are highly soluble in the alcoholic solvent [37-39]. AgNPs biosynthesis through a polyester peak that showed at 457 nm SPR. SPR is when a sphere has oscillating electrons, and this peak is also the first sign of AgNPs' creation. During AgNPs formation, the absorption peaks can range between 400-450 nm but vary based on particle size and shape in terms of their environment (solvent). This peak indicates spherical nanoparticles (nanoscale range = 10 - 100 nm) and demonstrates nanoscale uniformity and stability. Ajmal et al. [40] documented similar findings of an SPR peak specific to AgNPs made from plant sources that displayed similar absorption peaks due to being made from reduced phytochemicals. In other words, during synthesis, phytochemicals (meaning phenolics and flavonoids, etc.) most likely promoted the Ag^{+} ion's reduction and agglomeration prevention while promoting biological activity by holding the particles in suspension.

The analysis of FTIR demonstrated many functional groups that could play a role in the reduction of AgNPs and also in their stabilization. The broad peak of the studied FTIR at 3362 cm^{-1} is attributed to O–H stretching and N–H stretching vibrations of the phenolic compounds and proteins. The peak at 2970 cm^{-1} corresponds to the stretching of C–H aliphatic (63/65). The peaks at 1727 cm^{-1} and 1611 cm^{-1} correspond with the presence of carbonyl (C=O) and aromatic C=C functional groups. These functional groups are commonly found in phytochemicals such as tannins, flavonoids, and phenolic compounds from the *Quercus infectoria* extracts. The shift in the FTIR spectra also indicates the role of the biomolecules as reducing and capping agents for the formation of the AgNPs, and this is similar to other studies' findings, which demonstrated shifting and showed the role of plant metabolites in the reduction and stabilization of Silver NPs [41]. Similar findings were observed by Disaanayake et al. [42] in their study on *Ocimum sanctum* AgNPs.

Spherical to sub-spherical AgNPs have mainly been formed from the use of *Quercus infectoria* peel extract, where the average ranges from 41.8 to 61.5 nm, and the slight degree of aggregation is likely due to the dry state of the extracted sample. The morphology was in agreement with previous studies on green synthesised AgNPs using other plant extracts. For example, the AgNPs synthesised from *Azadirachta indica* leaf extract were also reported to be mostly spherical and have diameters between 25 and 55 nm due to the stabilisation of AgNPs through phytochemicals that acted in a capping method [43]. Additionally, Pereira et al. [44] found that plant-mediated AgNPs synthesis will generally yield spherical AgNPs in the range of 10 to 80 nm and are dependent on the

plant source as well as reaction conditions. Another related study showed the use of an Aesculus indica bark extract to synthesise AgNPs with an average crystallite size of 42.96 nm; however, the SEM images suggested a rod-like shape of the AgNPs, indicating the importance of phytochemical variations as mediators of particle shape [45].

A number of studies conducted recently on AgNPs produced using plant extracts reveal that the majority of green synthesis methods have produced spherical or quasi-spherical nanoparticles ranging in size from 25 to 80 nm, as demonstrated by SEM and Transmission Electron Microscope (TEM) analyses [46, 47]. Additionally, there is evidence for a strong dose-dependent antibacterial effect of AgNPs, whereby increasing concentrations of AgNPs correlate with increased inhibition zone diameters [44]. The trend was observed across numerous bacterial species, including Gram-negative pathogens (e.g., *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus* spp.) and Gram-positive bacteria (e.g., *Bacillus*, *Staphylococcus* spp.), supporting the concentration-dependent effectiveness and broad applicability of AgNPs that have been biosynthesized using plant materials. While these results suggest that the substances tested have high antibacterial activity when tested in the laboratory, they are only preliminary and do not include toxicity or pharmacological safety testing. More testing is needed to determine whether or not the results will have clinical relevance; therefore, both in vivo and cytotoxicity studies are needed.

The present investigation provides evidence that AgNPs produced via the aqueous extracts of *Quercus infectoria* (oak) peels have significantly higher levels of antibacterial activity against all clinical isolates evaluated when compared to both crude plant extracts and silver salts. This finding supports previous studies where it was found that when silver is in a nanoscale form, the large surface area to volume ratio increases the antibacterial properties compared with ionic silver because of the continued release of Ag^+ ions that have bioactivity. There has also been a study comparing different forms of silver, including ionic silver (Ag^+) solutions and nanoparticulate silver, against several strains of *Proteus*, and found that all the forms had low minimum inhibitory concentrations and bactericidal concentrations (MBCs) and were capable of inhibiting growth in the pathogenic species *Proteus* spp. Previous work has shown that AgNPs within the 20–80 nm range have significant antimicrobial activities, primarily due to the controllable release of silver ions, while AgNPs that are smaller (~10 nm) possess even greater toxicity towards *E. coli* due to increased penetration and reactivity within bacterial cells [45].

Pseudomonas aeruginosa is inhibited by AgNPs in addition to their use as a potential alternative antimicrobial agent or supplement to conventional antibiotic therapy against resistant pathogens [33]. AgNPs appear to be effective as broad-spectrum bactericides and have shown no significant difference in antibacterial activity against either Gram-positive or Gram-negative bacteria, nor are there differences observed when comparing antibiotic-susceptible versus antibiotic-resistant organisms [28]. The antibacterial action of AgNPs is believed to occur when the nanoparticles bind to the membranes of bacteria, increasing the permeability of the membrane and causing physical and structural damage to the membrane that results in cell lysis [15]. While AgNPs are enhanced in their ability to kill bacteria with plant extracts due to the combination of nanosilver's ability to permeate membranes, release ionic silver, and generate reactive oxygen species, AgNPs formulations that contain phytochemical capping agents, such as phenolics, flavonoids, and proteins, help stabilize the AgNPs against aggregation, thereby increasing the likelihood of obtaining enhanced antibacterial activity. In fact, this combination of the toxic effects of nano silver plus the antimicrobial properties associated with the capping agents can result in dose-dependent and broad-spectrum antibacterial activity.

While these results suggest that the substances tested have high antibacterial activity when tested in the laboratory, they are only preliminary and do not include toxicity or pharmacological safety testing. More testing is needed to determine whether or not the results will have clinical relevance; therefore, both in vivo and cytotoxicity studies are needed.

4. CONCLUSION

The results of this research show that extracts from medicinal plants have significant potential as antimicrobial agents; for example, extracts of *Quercus infectoria* galls demonstrated high activity against pathogenic bacteria. Furthermore, the successful green synthesis of AgNPs from *Q. infectoria* aqueous extract provided significant antibacterial properties, supporting their potential as antimicrobial agents. Characterization confirmed that the nanoparticles produced were spherical and had the desired nanoscale properties. In conclusion, the results of the study support a future direction of integrating phytochemicals with nanotechnology to produce environmentally friendly and effective antimicrobial agents.

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AUTHOR CONTRIBUTIONS

The overall concept of the study was developed by Hamza A. Saadallah and Alaa Saeed Sheet. Hamza A. Saadallah supervised the research project, led the methodology for the biosynthesis and immobilization of AgNPs, interpreted the UV-Vis and IR spectral data, and performed the final review and editing of the manuscript. Alaa Saeed Sheet and Alaa R. Ali Al-Taie isolated and characterized the bacteria and conducted antimicrobial tests. Samar Mahmoud Fathi performed the plant extraction and formal research, while Mohammed Aheb Al-Kardoushi managed the data collection, software application, and statistical analysis. Alaa K. Ibrahim contributed resources and visualizations. All authors read and approved the final manuscript.

ETHICAL ANCHORS AND PARTICIPATION

The plant samples used in this study were collected from non-protected areas and did not include any endangered

species or taxa listed under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). All institutional, national, and international guidelines for the collection and use of plant materials in scientific research were strictly followed.

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