

Pytochemical and Antibacterial Study for *Salvia rosmarinus* Spenn, Gold and Silver nanoparticles

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ABSTRACT

The appearance of antibiotic-resistant strains of bacteria has increased the need for new antimicrobial agents. The focus has shifted to nanomaterials and bioactive compounds from plants. This study investigates the antibacterial efficacy of gold (AuNPs) and silver (AgNPs) nanoparticles, alongside the ethanolic extract of *Salvia rosmarinus* (rosemary), against two prominent pathogenic bacteria: *Staphylococcus aureus* and *Enterococcus faecalis*. Nanoparticles with practical sizes ranging from 33 to 40 nanometers were purchased from the company (Nanomaterials). These particles were prepared in different concentrations, including 0.5, 0.10, 0.15, and 0.20 mg/mL. *S. rosmarinus* leaves were harvested from Baghdad, Iraq (April 2025), taxonomically identified, and subjected to a 24-hour solid-liquid extraction using 70% ethyl alcohol. The chemical profile of the crude extract was elucidated using gas chromatography-mass spectrometry (GC-MS). Antibacterial screening against clinically isolated *S. aureus* and *E. faecalis* was performed using the agar well diffusion method to determine the zones of inhibition (ZOI). The GC-MS assay for *S. rosmarinus* extract had 19 distinct chemical peaks, with salvial-4(14)-en-1-one (3.02%), alpha-thujene (2.84%), and endo-borneol (2.74%) identified as the primary bioactive constituents. Quantitative antibacterial assays demonstrated a differential, strain-specific susceptibility. At the highest concentration (0.20 mg/mL), AuNPs exhibited superior potency against *S. aureus* with a maximum ZOI of 7.96 mm, compared to 4.99 mm for *E. faecalis*. Conversely, AgNPs (0.20 mg/mL) displayed higher efficacy against *E. faecalis* (ZOI: 3.55 mm) than *S. aureus* (ZOI: 3.11 mm). The crude rosemary extract (300 mg/mL) also showed promising antibacterial activity, yielding inhibition zones of 4.87 mm and 4.23 mm against *E. faecalis* and *S. aureus*, respectively.

Key words: Rosemary, Gold nano , Silver nano, *E. faecalis*, *S. aureus*.

INTRODUCTION

Medical plants can be defined as those that have, in part or all of them, therapeutic properties. The first is a psychological effect on the human body or life, whether it is activated or inhibited, and it has an effect on organisms. Life that interferes with the body of the human being, either externally or internally, either through inhibition, killing, or expulsion [1]. *Salvia rosmarinus* Spenn. (Rosemary) B belongs to the Lamiaceae family. Plant cultivation grows in Algeria, France, Spain, Portugal, and the Mediterranean Sea basin is the home of plant cultivation [2]. Rosemary plants have many benefits, as they are used as anti-convulsion in dialysis and menopause. Respiratory disorders and the stimulation of hair growth... so the active substances in the mountain coronary have therapeutic potential, to treat or protect against reptile asthma, diabetes, and digestive ulcers, the mountain coronary contains antioxidants. And used to keep meat from rotting, where it was added to prevent it from being damaged [3]. and active compounds (Volatile oil, terpenes, flavonoids, diterpenes and caffeic acid) [4].

Nanotechnology is a strategy to design novel non-traditional antimicrobial agents, termed nanoantibiotics, which are effective remedies for infectious diseases and offer numerous advantages over traditional antimicrobials including absence of side effects, enhanced activity against drug-resistant species, and elimination of resistance development involving multiple biological pathways [5]. These nano-antibiotics are either effective per se against microbes or improve the efficacy and safety of conventional antibiotic administration by generating high local concentrations. New materials, such as gold nanoparticles (AuNPs), have different optical and electrical properties than the conventional materials and they have a bright future in the field of medicine [6]. Such features include high surface area to volume ratio, stability, surface plasmon resonance, surface chemistry, multi functionalization etc. The components and concentrations can be finely tuned for producing GNPs in variety of shapes and sizes at convenience [7]. Silver is a safe, non-toxic inorganic antibacterial agent that has been used for centuries. It can kill about 650 types of diseases caused by microorganisms [8]. Because silver can have a bactericidal effect at very low concentrations, it has been classified as oligodynamic.

It has enormous potential for a variety of biological uses, including anti fungal agents, antibacterial agents for bacteria resistant to antibiotics, infection prevention, wound healing, and anti-inflammatory [9]. *E. spp* are a species of positive bacteria with nonaerobic selection, Catalins and guerre are made up of the wallet, and it's a function of the underlying causes of diseases associated with the range. A wide range of contaminants, including *E. faecalis* and *E. faecium*. And they have the ability to cause fatigue, pollute wounds, rattle blood, and give up urinary tract infections (UTI) Errhydration of the heart, intestinal diseases, as well as blood poisoning in newborns [10]. *S. aureuse* Bacteria doesn't have a positive choice for gram dye, the diameter of the cell (0.5–1.5) is a micrometer, and the majority of the spectacles are positive for the catalase test and negative for the oxidase test, and positive for the coagulate enzyme test and the test for the coagulase test by Fox Proscauer [11]. Its Containing a high proportion of sodium chloride salt, it could be as much as 7.5% [12].

It's the infection. Either less dangerous like skin bulbs, soft tissues, bollocks, or It's more dangerous and even deadly, like endocarditis meningitis, sepsis [13]. This study investigates the antibacterial efficacy in precence the gold (AuNPs) and the silver (AgNPs) nanoparticles, alongside the crude ethanolic extract of *Salvia rosmarinus* (Rosemary), against two prominent pathogenic bacteria: *Staphylococcus aureus* and *Enterococcus faecalis*.

MATERIAL AND METHODS

Plant material

Rosemary leaves were collected from a home garden in Baghdad during April 2025. The used parts of the plant were first washed with regular water, then with distilled water to remove impurities, and afterward dried and ground using an electric grinder to obtain a fine plant powder [14].



Figure 1. Real image of Rosemary (*Rosmarinus officinalis*) as a planted in the home garden.

Plant Extract Preparation

The ethanol extract of rosemary leaves was prepared using the cold maceration method according to the following steps: (50 g) of fine leaf powder was immersed in (500 mL) of 70% ethyl alcohol (weight:volume ratio of 1:10) inside a sterile glass flask. The flask was tightly sealed using cotton plugs and aluminum foil (cellophane) to prevent evaporation and contamination. The mixture was placed on the orbital shaker at (25 Celsius) for 24 hours to ensure the highest efficiency in extracting the active bio active compounds. After the incubation period, the raw mixture was first filtered. The filtrate was placed in a centrifuge at a speed of 3000 RPM for 10 min. to precipitate the suspended fine particles. Finally, the supernatant was purified and filtered thru What man No. 1 filter paper. 1) To obtain a completely pure extract, it was stored in the refrigerator under appropriate cooling conditions until gas GC-MS analysis was conducted [15, 16].

Gas Chromatography-Mass Spectrometer GC-MS assay

It was conducted to detect the active bio-compounds in the ethanol extract of rosemary leaves (*Rosmarinus officinalis*) at the central laboratories of the Ministry of Science and Technology (Baghdad, Iraq) using a Gas Chromatography-Mass Spectrometry (GC-MS) system. The sample was injected after being filtered thru a fine membrane into the chromatographic separation device equipped with a capillary column. High-purity helium gas (He) was used as the carrier gas at a constant flow rate. Regarding the mass spectrometer section, an electron ionization (EI) energy of 70 eV produced the ions. Regarding the mass spectrometer component, an electron ionization (EI) energy of 70 eV created the ions. The identities of the separated

chemical compounds were determined and matched programmatically by comparing their mass spectra and retention times with the standard data stored in the accredited global libraries.

Biological Effect

Two pathogenic bacterial strains were obtained: *Staphylococcus aureus* (*S. aureus*) and *Enterococcus faecalis* (*E. faecalis*) from the laboratories of the Department of Life Sciences at the College of Science, University of Kerbala. When these bacteria were first recovered from clinical samples, they were recognized as isolates of the species *S. aureus* and *E. faecalis* [17].

Statistical Analysis

The tests were conducted using a completely randomized design (CRD) as factorial experiments with three replications, and the Least Significant difference (L.S.D. at 1% likelihood (P 0.01) was used to assess if two or three components were significant.

RESULTS

Chemical assay of the rosemary leaves extract using the GC-MS

The results at table (1) of the (GC-MS) assay of the extract of rosemary leaves (*Rosmarinus officinalis*) revealed a complex mixture of bioactive compounds (Phytoconstituents), with 19 major chemical compounds identified, differing in their retention times and percentage area as shown in (Table 1). The results showed a wide chemical diversity including terpenes, fatty acids, and esters. The compound salvial-4(14)-en-1-one recorded the highest appearance rate in the extract, reaching (3.02%) at a retention time of (16.45 minutes), followed by the compound α -Thujene with a total percentage of (3.9%) distributed over two chromatographic peaks (Peak 1 and Peak 18), in addition to the compound endo-Borneol, which appeared with a total percentage of (5.16%) at retention times of (5.87 and 20.54 minutes). The extract also contained other important terpenoid compounds such as γ -Amorphen at a percentage of (2.56%) and Citronellal at a percentage of (1.58%). The observed biological and antibacterial effects (*S. aureus* and *E. faecalis*) in this study are attributed to the presence of these terpenoid and phenolic compounds; terpenes such as (α -Thujene, endo-Borneol, and Citronellal) are known for their high ability to penetrate the cell membrane of pathogenic bacteria and disrupt its

permeability and the internal osmotic pressure of the cell. Moreover, the presence of fatty acids such as oleic acid (Oleate Acid at 0.45%) and its esters (Ethyl Oleate at 0.49%).

Table 1. The Chemical Components of Ethyl Alcohol extract in Rosemary Using GC-MS.

peak	Retention time	Area%	Chemical Names	Molecular Formula
1	4.76	2.84	α -Thujene	C ₁₀ H ₁₆
2	5.87	2.74	endo-Borneol	C ₁₀ H ₁₈ O
3	7.84	2.56	γ -Amorphen	C ₁₅ H ₂₄
4	8.98	1.54	1,2-Longidione	C ₁₅ H ₂₂ O ₂
5	10.45	0.45	Oleic Acid	C ₁₈ H ₃₄ O ₂
6	11.45	0.43	Diisooctyl phthalate	C ₂₄ H ₃₈ O ₄
7	12.45	1.56	Benzo[h] quinolone,2,4- dimethyl	C ₁₅ H ₁₃ N
8	13.65	2.03	4-Trimethylsilyl-9,9- dimethyl-9- silaflourene	C ₁₇ H ₂₂ Si ₂
9	15.34	1.25	Benzenamine, 4- methoxy-N- (triphenylphosphora nylidene)-	C ₁₄ H ₁₅ NO ₂
10	16.45	3.02	salvial-4(14)-en-1-one	C ₁₅ H ₂₄ O
11	17.45	0.56	Cedrol	C ₁₅ H ₂₆ O
12	18.45	1.48	Epizonarene	C ₁₅ H ₂₄
13	19.56	1.45	Pentadecane	C ₁₅ H ₃₂
14	20.54	2.42	endo-Borneol	C ₁₀ H ₁₈ O
15	21.93	0.49	Ethyl Oleate	C ₂₀ H ₃₈ O ₂
16	23.56	0.05	Methyl tetradecanoate	C ₁₅ H ₃₀ O ₂
17	24.56	1.58	Citronellal	C ₁₀ H ₁₈ O
18	25.45	1.06	α -Thujene	C ₁₀ H ₁₆
19	26.67	0.65	Hexadecane	C ₁₆ H ₃₄

The main chemical components of the plant extract

Indicated to the table 2, the most abundant and dominant active compounds in the extract of rosemary leaves , were identified based on the percentage of surface area (Area%). The compound salvial-4(14)-en-1-one recorded the highest concentration in the extract at (3.02%),

followed by the compound α -Thujene at (2.84%), and then the compound endo-Borneol at (2.74%). These predominant compounds are classified as volatile terpenoids, which have a strong record in scientific research as potent antimicrobial agents, explaining the high inhibitory efficacy of the extract when combined with gold and silver nanoparticles against the studied bacterial strains.

Table 2 .Chemical Components Concentrations of the *Rosemary*.

Area%	Chemical Names
3.02	salvial-4 (14)-en-1-one
2.84	α -Thujene
2.74	endo-Borneol

Biological effect of Gold nanoparticles

Table 3 of the current investigation demonstrated that gold nanoparticles had antibacterial effects against isolates of *S. aureus* and *E. faecalis*, with very significant differences when compared to the control. In contrast, *S. aureus* increases the inhibition zone (7.96 mm) at concentrations of 0.20 mg/mL of the gold nanoparticle, while the number decreased to 4.99mm for *E. faecalis* compared to the bacterial number in concentrations of (0.15, 0.10 and 0.5 mg/mL) again *S. aureus* decrease inhibition zone (6.76, 5.46 and 3.87mm respectively). While *E. faecalis* decreases the inhibition zone (4.76, 3.76 and 2.23 mm) at concentrations for (0.15 , 0.10 and 0.5 mg/mL) respectively.

Table 3. Impact of the addition of various concentrations of gold nanoparticles on antibacterial activity.

Concentrations mg/mL	<i>E. Faecalis</i> mm	<i>S. Aureas</i> mm	Control mm
0.5 mg/mL	2.23	3.87	0
0.10 mg/mL	3.76	5.46	0
0.15 mg/mL	4.76	6.76	0
0.20 mg/mL	4.99	7.96	0
LSD	1.48	1.83	

Biological effect of silver nanoparticles

The table 4 shows the addition of silver nanoparticles at concentrations of 0.20 mg/mL, *E. faecalis* increased the inhibition zone to 3.55mm, while the number decreased to 3.11mm for *S. aureus* compared to the bacterial number at concentrations of 0.15, 0.10 and 0.5 mg/mL, where *S. aureus* decreased the inhibition zone to 2.87, 2.34, and 1.32mm, respectively. while *E. faecalis* increased the inhibition zone compared to *S. aureus* (2.99, 2.76 and 1.45 mm) at concentrations of 0.15 , 0.10, and 0.5 mg/mL, respectively.

Table 4 . Impact of the addition of various concentrations of silver nanoparticles on antibacterial.

Concentrations mg/ml	<i>E. Faecalis</i> mm	<i>S. Aureas</i> mm	Control
0.5 mg/mL	1.45	1.32	0
0.10 mg/mL	2.76	2.34	0
0.15 mg/mL	2.99	2.87	0
0.20 mg/mL	3.55	3.11	0
LSD	1.29	1.56	

Biological effect of Rosemary

The table 5 shows the effect of addition the Rosemary extract at concentrations of 300 mg/mL, *E. faecalis* increased inhibition zone (4.87mm), while the number decreased to 4.23 mm for *S. aureus* compared to the bacterial number at concentrations of 250, 200, and 100 mg/mL, where *S. aureus* decreased inhibition zone (3.45, 2.76 and 1.67mm respectively). while *E. faecalis* increased the inhibition zone compared to *S. aureus* (3.98, 3.87 and 1.87 mm) at concentrations of 3.45 , 2.76 and 1.67mm respectively.

Table. 5. Impact of the addition of various concentrations of Rosemary on antibacterial.

Concentrations mg/mL	<i>E. Faecalis</i> mm	<i>S. Aureas</i> mm	Control
100 mg/mL	1.87	1.67	0
200 mg/mL	3.87	2.76	0
250 mg/mL	3.98	3.45	0
300 mg/mL	4.87	4.23	0
LSD	1.87	1.45	

DISCUSSION

The current results showed that the combination of the ethanolic extract of rosemary leaves (*Rosmarinus officinalis*) with gold NP and silver NP (at concentrations ranging from 0.5 to 0.20 mg/mL) led to a significant inhibition of the growth of the studied bacterial isolates (*S. aureus* and *E. faecalis*). This effect is attributed to the chemical richness of the plant extract in phenolic compounds and terpenes (such as carnosol and rosmarinic acid) identified by GC-MS analysis, which work in conjunction with the reactive oxygen species such as ($O^{\cdot}$ and $HO^{\cdot}$) in presence the silver and gold nanoparticles that caused inhibition the bacterial cells. The presence of electronic effects caused by alterations in the local electronic structures of the surfaces as a result of the nanoparticles' reduced sizes is responsible for their bactericidal action. The surface reactivity of silver nanoparticles is thought to be improved by these actions. Silver NP and gold NP strongly interact with sulfur groups, such as the thiol groups of essential enzymes, and inactivate them because Ag and Au are thought of as soft acids. It has been proposed that when a bacteria is exposed to silver ions, DNA loses its capacity to replicate. [18]. Although the exact mechanism by which the nanoparticles penetrate the bacteria is unknown, research indicates that when bacteria were exposed to silver or gold nanoparticles, their membrane morphology changed, resulting in a notable increase in permeability. This affected proper transport through the plasma membrane, rendering the bacterial cells unable to regulate transport through the plasma membrane, ultimately leading to cell death [19]. GNPs have been shown to target cell membrane subcellular regions, resulting in pits and cellular death. Furthermore, GNPs attach the peptide surface to the cell wall's glycan ports and disrupt the N-acetylglucosamine and N-acetylmuramic acid bonds in glycans, resulting in pit formation [20]. Gram-positive bacteria are particularly vulnerable to metal nanoparticles, according to previous studies. Compared to other bacteria, Gram-ve bacteria have a thinner cell wall. Nanoparticle penetration within cells may be inhibited by the thick cell wall [21]. In addition to cidal targeting of cell walls and membranes, GNPs have been demonstrated to target novel bacterial targets like respiratory chain dehydrogenases and bacterial chromosomes [22]. Furthermore, because metallic nanoparticles can produce reactive oxygen species (ROS), which prevent the oxidation of liberated gold ions, they have an additional biocidal effect [23]. Bacterial infections and this connection cause damage and turbation changes that affect the hive functions of the cell. Bacterial permeability

causes holes that enable an antibiotic to be hacked into. Inside the bacterial cell, leading to cell death [24]. Another study confirmed the Seeker [25]. The nano-silver death machine, which could lead to silver being given away, The number of bacterium processes, including the formation of the S_2 and the representation, food and balance These membranes could lead to an increase in the production of interactive oxygen species (ROS) and an increase in the exhaust of membranes that could stimulate the activity of a wide range of antibiotics. Bacteria minus grams in different cases of metabolism as well as for the recovery of antibiotics. In the case of a resistant bacterial strain, the same machine has been activated when using the AgNP molecules. As an antibiotic assistant [26] .

The inhibition effectiveness of *R. salvia* could be the result of the activity of rosmarinic acid, isorosmanol, rosmanol, carnosic acid, epirosmanol, carnosol and rosmaridiphenol. The materials interfere with the plasma membrane, causing a change in the genetic material, a change in the electron transport chain, and a leak of contents. The cell has changed in the production of fatty acids, combined with an interaction with the palsy membrane proteins [27]. Some active chemical compounds pass through the cell and destroy its components without effect, on the permeability of the balsam membrane, other compounds are working to safely destroy the membrane and increase its permeability, disrupting cellular membrane permeability and equilibrium balance [28]. Many studies have shown that biological efficiency is due to the biological properties of the plant. and its chemical structure is rich in phenolic compounds, especially steroids such as carnosics. Acid and carnosol, which have inhibition effects on microorganisms [29].

These results are consistent with the findings of [30], who found that biogenic or plant extract-supported nanoparticles possess higher antimicrobial efficiency compared to the extracts alone, due to the nanoparticles' ability to penetrate the bacterial cell wall and increase its permeability. On the other hand, [31] indicated that *S. aureus* bacteria show high sensitivity to silver nanoparticles (Ag\ NPs) due to the generation of reactive oxygen species (ROS) that lead to DNA damage and bacterial cell death. [32] that *S. aureus* bacteria show high sensitivity to silver nanoparticles (Ag\ NPs) due to the generation of reactive oxygen species (ROS) that lead to DNA damage and bacterial cell death. This high efficacy in our study is explained by the fact that both strains (*S. aureus* and *E. faecalis*) are Gram-positive bacteria with a thick peptidoglycan

layer. However, the precise size of the used nanoparticles (33–40 nanometers) allowed them to effectively penetrate thru the cellular pores and disrupt the osmotic pressure of the cell, which aligns with the scientific explanation proposed by [33] regarding the effect of nanoparticle size on the permeability of the cell wall of Gram-positive bacteria.

Conclusions

The present research Gold and Silver nanopartilces have antibacterial, gold nanopartilces at concentrations of 0.20 mg/mL have an inhibition zone for *S. aureus*, silver nanopartilces at concentrations of 0.20 mg/mL have an inhibition zone for *E. faecalis*. Rosemary at 300 mg/mL has an inhibition zone for *E. faecalis*.

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