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Original article

Antibacterial Activity of Different Solvent Extracts of *Rosmarinus officinalis* and *Artemisia herba-alba* Against Pathogenic Bacteria

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Abstract
This study investigated the antibacterial activities of solvent extracts from *Rosmarinus officinalis* and *Artemisia herba-alba* against four pathogenic bacterial species and three standard antibiotics. The results demonstrated that the antibacterial efficacy of plant extracts was strongly influenced by the type of solvent used. In *R. officinalis*, methanolic and ethanolic extracts exhibited significantly higher inhibition zones against *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Salmonella enterica* (up to 14.10 ± 0.56 mm for *S. aureus*), while *Escherichia coli* showed minimal sensitivity. In contrast, the methanolic extract of *A. herba-alba* produced the highest antibacterial activity against all tested strains, particularly *S. enterica* (15.50 ± 0.46 mm), followed by the ethanolic extract, whereas the acetone extract showed the least activity. Methanolic extracts suggest that methanol is the most efficient solvent for extracting bioactive compounds, including phenolics, flavonoids, tannins, and saponins, which are known to contribute to antimicrobial activity. The weaker performance of acetone may be attributed to its limited ability to dissolve polar phytochemicals. The results also indicated that Gram-positive bacteria were generally more susceptible to the extracts than Gram-negative species, likely due to structural differences in their cell walls. Antibiotics, such as amoxicillin, showed no inhibitory effect against any of the tested bacteria, indicating complete resistance, while ciprofloxacin exhibited the strongest activity across most strains, followed by doxycycline, which showed moderate effectiveness. The statistical analysis (p = 0.00) confirmed significant differences among treatments
Keywords. *Rosmarinus officinalis*, *Artemisia herba-alba*, Antibacterial Activity, Solvent Extracts.

Introduction

The resistance of harmful microorganisms to conventional antibiotics has been steadily increasing in recent years. In contrast, medicinal plants have long been used across various regions of the world to combat pathogenic microbes. Antibiotic-resistant bacterial strains are known to cause a wide range of diseases, including encephalitis, food poisoning, gastritis, and urinary tract infections [1,2]. Given their effectiveness, availability, and lower cost, there has been growing interest in developing antimicrobial agents derived from natural sources. Medicinal plants play a crucial role in human health due to their bioactive compounds.

Artemisia herba-alba, commonly known as white wormwood or desert wormwood, is a grayish dwarf shrub with a distinctive aroma. It is native to North Africa, the Arabian Peninsula, Southwestern Europe, and Western Asia. According to [3], species of *Artemisia* are important sources of bioactive compounds with antibacterial, allelopathic, insecticidal, and fungicidal properties. Similarly, rosemary (*Rosmarinus officinalis*), a woody perennial herb belonging to the Lamiaceae family, is widely distributed in different regions and is well known for its pleasant fragrance [4].

Artemisia herba-alba belongs to the Asteraceae (Compositae) family, which includes more than 400species. It grows naturally in desert regions of the Middle East, Sinai, northwestern India (Himalayas), Egypt, Spain, and North Africa (particularly Libya, Morocco, and Algeria). This small shrub thrives in warm, dry, and sometimes muddy environments and has demonstrated a broad range of pharmacological activities.

Plants are rich in diverse antioxidant compounds, including phenolic acids, flavonoids, and other natural antioxidants. *A. herba-alba* is known to produce aromatic essential oils rich in monoterpenes and sesquiterpene lactones, which exhibit anti-inflammatory and anticarcinogenic properties [5,6]. Traditionally, this plant has been widely used in folk medicine for treating and preventing numerous ailments. Over the past two decades, several pharmacological studies from different regions have confirmed its therapeutic significance, including anti-helminthic, antispasmodic, antibacterial, and wound-healing effects. It has also been used to treat colds, coughs, gastrointestinal disorders, diarrhea, and abdominal cramps in both humans and livestock [7].

There are several bioactive phytochemicals with pharmacological potential in R. officinalis essential oils and extracts. Caffeic acid, carnosic acid, chlorogenic acid, oleanolic acid, rosmarinic acid, ursolic acid, camphor, camphor, carnosol, eucalyptol, rosmanol, rosmaquinones A and B, and luteolin derivatives are among the most frequently reported chemicals [8–11]. Numerous bacterial and fungal species, such as Candida albicans and dermatophytes, are susceptible to rosemary's antibacterial properties. Strong antibacterial properties are exhibited by its essential oils, especially against *Shigella sonnei*, *Salmonella typhi*, *Escherichia coli*, and *S. enteritidis* [12]. Although the antibacterial efficacy of rosemary's essential oils varies based on the target medium's composition, the plant's cytotoxic qualities are also useful for protecting marine and agricultural products. The medical potential of *Artemisia herba-alba* and *Rosmarinus officinalis* is highlighted

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in this review, along with their potential as useful therapeutic resources in contemporary medicine and as natural substitutes for synthetic antibacterial agents.

Material and Methods

Plant Material and Extraction

Fresh leaves of *R. officinalis* and *A. herba-alba* were collected, thoroughly washed, and air-dried at room temperature. The dried leaves were then ground into a fine powder. For the extraction process, 50 g of the powdered material was soaked in 500 mL of 70% ethanol, 96% methanol, and 90% acetone for 72 hours, with occasional shaking to enhance extraction. Afterward, the mixtures were filtered, and the solvents were evaporated at 40°C using a rotary evaporator to obtain concentrated extracts.

The bacterial species used in this study

Included both Gram-positive and Gram-negative bacteria.

Gram-positive bacteria

Staphylococcus aureus and *Streptococcus pyogenes* were obtained from El-Bayda Hospital.

Gram-negative bacteria

Escherichia coli and *Salmonella enterica* were also obtained from El-Bayda Hospital.

Agar Well Diffusion Methods

The agar well diffusion method was employed to assess the antimicrobial activity of the extracts. Nutrient agar (NA) plates were inoculated with bacterial cultures using sterile cotton swabs, and wells were created in each plate with a sterile borer. Approximately 100 µL of each plant extract was carefully introduced into the wells using a sterile syringe. The plates were then incubated at 37°C for 18–24 hours. Following incubation, the zones of inhibition around each well were measured in millimeters using a ruler to evaluate the antibacterial activity, as described by [13].

Antibiotic Sensitivity Tests

The in vitro antimicrobial susceptibility of the tested microorganisms to three antibiotics, Amoxycillin, Ciprofloxacin, and Doxycycline, was evaluated. Inocula were prepared by transferring isolated colonies from an overnight nutrient agar culture into 2 mL of tryptone soya broth (TSB). A sterile cotton swab was then dipped into the standardized suspension, rotated several times, and pressed against the inner wall of the tube above the fluid level to remove excess inoculum. The swab was evenly streaked across the surface of a sterile Mueller-Hinton Agar (MHA) plate. This streaking process was repeated twice, rotating the plate each time to ensure uniform distribution of the inoculum. After allowing the plates to dry for 5 minutes, antibacterial discs were placed on the agar surface using a disc dispenser. The plates were then incubated at 37°C for 18–22 hours. Following incubation, the diameters of the inhibition zones were measured to the nearest millimeter using a vernier caliper (junior) [14].

Statistical analysis

Statistical analysis was performed using Minitab software version 17. Data were first tested for normal distribution, and statistical significance was evaluated using one-way ANOVA. A p-value of less than 0.05 ($P < 0.05$) was considered statistically significant, as described by [15].

Results and Discussion

This study aimed to evaluate the antibacterial activity of alcoholic extracts from *Rosmarinus officinalis* (rosemary) and *Artemisia herba*-alba (white wormwood) against selected bacterial species.

Table 1. Mean inhibition zones (mm) of solvent extracts of Rosmarinus officinalis against selected bacteria

Solvent extracts	***Escherichia coli***	***Staphylococcus aureus***	***Streptococcus pyogenes***	***Salmonella enterica***
Acetone	3.80 ± 0.62 A	8.40 ± 0.72 B	6.33 ± 0.60 C	5.17 ± 0.57 B
Methanol	3.27 ± 0.40 A	14.1 ± 0.56 A	9.13 ± 0.61 B	6.13 ± 0.71 B
Ethanol	0.00 ± 0.00 B	13.03 ± 0.35 A	11.03 ± 0.35 A	8.17 ± 0.38 A
P. Value	0.000	0.000	0.000	0.002

The results revealed that the antibacterial activity of *Rosmarinus officinalis* extracts varied depending on the solvent used. Methanol and acetone extracts showed slight activity against *Escherichia coli* (3.27–3.80 mm), whereas the ethanol extract exhibited no inhibitory effect (0.00 mm). In contrast, the methanol and ethanol extracts demonstrated markedly higher inhibition zones against *Staphylococcus aureus*, *Streptococcus*

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pyogenes, and *Salmonella enterica*, reaching up to 14.10 ± 0.56 mm for *S. aureus*. All measurements were based on the mean values obtained from three replicates performed per treatment group. This is consistent with earlier research indicating that polar solvents like ethanol and methanol are more effective at extracting phenolic and flavonoid compounds responsible for antimicrobial activity. Gram-positive bacteria (such as *S. aureus*) are typically more vulnerable to these compounds than Gram-negative bacteria (like *E. coli*), owing to the presence of an outer membrane in Gram-negative species that restricts the penetration of active agents. [16,17]. The variation among solvents can also be attributed to differences in their polarity and solubility properties, influencing the extraction of bioactive components such as rosmarinic acid, carnosic acid, and carnosol compounds known for their antibacterial efficacy [18, 19].
Similar observations have been reported [20] and [21], who discovered that ethanol extracts of rosemary demonstrated significant effects against *Staphylococcus* spp. and *Salmonella*, while *E. coli* exhibited reduced sensitivity. Variations in bacterial strains, extraction conditions (such as solvent concentration, temperature, and duration), and testing methods (disc vs. well diffusion) may also contribute to the differences observed among studies.

Table 2. Mean inhibition zones (mm) of solvent extracts of Artemisia herba alba against selected bacteria

Solvent extracts	*Escherichia coli*	*Staphylococcus aureus*	*Streptococcus pyogenes*	*Salmonella enterica*
Acetone	6.10 ± 0.26 A	9.1 ± 0.46 B	8.27 ± 0.35 B	7.767 ± 0.15 C
Methanol	6.73 ± 0.60 A	12.2 ± 0.45 A	10.53 ± 0.40 A	15.50 ± 0.46 A
Ethanol	1.30 ± 0.20 B	10.03 ± 0.25 B	8.07 ± 0.21 B	11.97 ± 0.35 B
P. value	0.00	0.00	0.00	0.00

The data indicate that the methanol extract of *Artemisia herba alba* exhibited the highest antibacterial activity against all tested bacterial strains, followed by the ethanolic extract, while the acetone extract showed the lowest inhibitory effect. Methanol extract produced inhibition zones ranging from 6.73 ± 0.60 mm (*E. coli*) to 15.50 ± 0.46 mm (*S. enterica*), which were significantly higher ($p = 0.00$) than those of the other solvents. The structural distinctions between Gram-positive and Gram-negative bacteria could be the cause of this variation. Although *S. enterica* seems more vulnerable than *E. coli* in this instance, the outer membrane of Gram-negative bacteria, including *E. coli* and *S. enterica*, can function as a barrier, decreasing the penetration of antimicrobial drugs. A more recent investigation of Silybum marianum also reported that methanolic extracts exhibited significantly stronger antibacterial effects against both Gram-positive and Gram-negative bacteria than other solvents [22]. This suggests that methanol is the most efficient solvent for extracting bioactive compounds responsible for antibacterial properties in this sample .
The superior activity of the methanol extract can be attributed to its ability to dissolve a wide range of compounds, such as phenolics, flavonoids, tannins, and saponins. These compounds are well known for their antimicrobial potential. Ethanol, being slightly less polar, extracted fewer or less active compounds, resulting in moderate inhibition zones. Acetone was less effective, possibly due to its limited solubility for certain polar phytoconstituents. The essential oils of *A. herba-alba* were found to have significant microbiological activity against strains of *Salmonella typhimurium*, *Escherichia coli*, *Micrococcus luteus*, *Staphylococcus aureus*, *Bacillus cereus*, and *Enterococcus faecalis*. Additionally, *A. herba-alba*'s antibacterial activity has been verified in several Candida yeast strains[23]

Table 3. The antibacterial efficacy of three standard antibiotics against four pathogenic bacterial strains

Antibiotic	Symbol	Concentration mg/ml	Pathogenic bacterial			
			Escherichia coli	Staphylococcus aureus	Streptococcus pyogenes	Salmonella enterica
Amoxycillin	AM	20	0	0	0	0
Ciprofloxacin	CIP	30	± 0.361 A 11.20	14.033 ± 0.208 A	6.433 ± 0.551 B	14.23 ±0.416 A
Doxycycline	D0X	30	8.200 ± 0.361 B	10.133 ± 0.208 B	7.600 ± 0.100 A	5.200 ± 0.458 D
P. value			0.00	0.00	0.00	0.00

Table 7 showed that the sensitivity of Amoxycillin exhibited no inhibition zone against all tested bacteria, suggesting complete resistance .Such resistance has been widely reported in clinical isolates of *E. coli* and *S. aureus* [24,25]. Ciprofloxacin demonstrated the highest antibacterial activity across most bacterial strains, with inhibition zones of 11.20 ± 0.361 mm for *E. coli*, 14.03 ± 0.208 mm for *S. aureus*, and 14.23 ± 0.416 mm for *S. enterica* .The relatively smaller zone (6.43 ± 0.551 mm) against *S. pyogenes* suggests a lower susceptibility .Similar results were observed by [26], who found ciprofloxacin to be significantly more effective than β-lactam and tetracycline antibiotics against multidrug-resistant Gram-negative bacteria.

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Doxycycline showed moderate activity, with inhibition zones ranging from 5.20 ± 0.458 mm (*S. enterica*) to 10.13 ± 0.208 mm (*S. aureus*). This indicates variable bacterial sensitivity. But resistance often occurs through ribosomal protection proteins or efflux mechanisms [27]. The P-value for all comparisons was 0.00, confirming statistically significant differences ($P < 0.05$) in the antibacterial effects among antibiotics. This supports the conclusion that ciprofloxacin is the most effective antibiotic against both Gram-positive and Gram-negative strains in this study.

Conclusion

The present study demonstrated that both *Rosmarinus officinalis* and *Artemisia herba-alba*, especially their methanolic extracts, possess considerable antibacterial potential that may complement or enhance conventional antibiotic therapy, particularly against resistant bacterial strains. that the antibacterial activity of *R.officinalis* and *A. herba-alba* extracts varies considerably with the type of extraction solvent and bacterial strain tested. When compared with standard antibiotics, ciprofloxacin exhibited the highest antibacterial activity, while amoxicillin showed complete resistance among the tested strains. Encourage further phytochemical and pharmacological studies to identify and characterize the specific compounds responsible for their bioactivity.

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