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RESEARCH ARTICLE

ANTIBACTERIAL EFFICACY OF FIVE NATURAL ESSENTIAL OILS USED IN YEMEN: A COMPARATIVE STUDY

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Abstract

Background and objective: Due to the increasing resistance to synthetic antibiotics and preservatives, there is growing interest in exploring natural alternatives. This study aimed to evaluate the antibacterial potential of five commercially available essential oils in the Yemeni market *Azadirachta indica* (Neem), *Moringa oleifera*, *Rosmarinus officinalis* (Rosemary), *Salvia officinalis*, and *Origanum majorana* against selected Gram-positive and Gram-negative bacterial strains.
Method: Agar diffusion was used to investigate the antibacterial activity of essential oils from five plants: *Azadirachta indica* (neem), *Moringa oleifera*, *Rosmarinus officinalis* (rosemary), *Salvia officinalis*, and *Origanum majorana*.
Results: Significant differences in the inhibitory effects of the plant oils of neem, *Moringa oleifera*, *Rosmarinus officinalis*, *Salvia officinalis*, and *Origanum majorana* against Gram-positive and Gram-negative bacteria were found during the antimicrobial screening process. Nearing the effectiveness of gentamicin (25 mm), *Salvia officinalis* and neem oils demonstrated the highest action against Gram-positive *Staphylococcus aureus* (22 mm and 20 mm inhibition zones, respectively). *Rosmarinus officinalis*, on the other hand, demonstrated a low level of efficacy (10 mm). Most oils were less effective against Gram-negative organisms, and *E. coli* showed resistance to all save *Rosmarinus officinalis* (12 mm).
Conclusions: These results demonstrate the potential of oils produced from plants, especially *Origanum majorana*, neem, and *Salvia officinalis*, as antibacterial agents against Gram-negative bacteria highlights the need for more research into maximizing their use, either alone or in combination with other antibiotics, despite their encouraging efficacy against Gram-positive bacteria.
Keywords: Antimicrobial activity, *Azadirachta indica*, essential oils, *Moringa oleifera*, *Origanum majorana*, *Rosmarinus officinalis*, *Salvia officinalis.*

INTRODUCTION

Aromatic and medicinal plants are rich in natural active compounds that have biological effects, making them a valuable source of substances that can improve health, prevent sickness, and defend against a variety of harmful pathogens[1,2]. Many types of bacteria cause food to decay and result in significant financial losses for companies; additionally, 20% of the world's food production is harmed by food spoilage[3]. Because of their use, chemical preservatives are frequently utilized to stop food from spoiling and causing food poisoning. Microorganisms have become resistant to chemical preservatives due to their continued usage in food preservation, which raises health hazards and the potential for food contamination. Therefore, it became necessary to identify natural and alternative materials that are safer for human health and effective at preventing food from spoiling[4]. When the right conditions are present, food can swiftly deteriorate due to the proliferation of microorganisms within it, which causes it to spoil[5]. Numerous researches have demonstrated the antibacterial, antifungal, antiviral, and anti-oxidative qualities of plant oils. Additionally, it serves as an antimicrobial agent in food preservation, extending the shelf life and safety of food items[6].

The chemical makeup and constituents of plant oil extracts determine their efficacy, and these are influenced by a variety of factors, including the plants' genetic origin, geographic location, and the environmental and agricultural circumstances that impact their growth[7]. Carotenoids, terpenoids, curcumins, coumarins, and terpenes are just a few of the many significant and potent compounds found in plant oils. These compounds have antimicrobial qualities and can also be used as natural food preservatives, which makes them crucial to the food industry[8]. These natural oils' sustainability as a renewable resource and lack of adverse effects, along with their anti-microbial qualities, make them an antibacterial agent and a natural preservative substitute for synthetic chemicals[9].

Research has demonstrated that the antibacterial action of plant oils is mostly caused by the presence of active chemicals in their basic composition[10]. With about 1400 plant species and about 3000 active compounds that have been identified and might be utilized as natural preservatives in the food sector, plants represent a permanent natural resource rich in potent antibacterial chemicals[11]. The pungent aroma and acupressure-shaped leaves of the rosemary plant (*R. officinalis*), which is a member of the *Lamiaceae* family, set it apart. The Mediterranean basin is where this plant is indigenous. Numerous beneficial substances, including carnosic and rosmarinic acid, are present in the plant's oil. Enhances the human digestive system and has anti-inflammatory, antioxidant, and immune-boosting properties. It is used as a food flavoring and in the cosmetics industry because it contains chemicals that are protective against bacteria. Different ratios of oils, such as camphor (22.3%), limonene (7.64%), linalool (11.58%), and myrcene (2.14%), are found in rosemary leaves[12].

One of the therapeutic plants in the *Moringaceae* family, the moringa plant (*M. oleifera*) is grown in India as well as a number of other Asian, African, and South American nations. The sector of good nutritional supplements, infertility therapy, cardiovascular disease treatment, blood pressure reduction, and diabetes prevention are just a few of its numerous applications[13]. Moringa seed oils are utilized in a variety of industries, including food and medicine, and can also be utilized as natural antimicrobial and antioxidant materials[14]. The herbal plant known as neem (*A. indica*) is indigenous to India and its surrounding areas. Because of its therapeutic and medical qualities, extracts from different portions of neem have been employed historically as insecticides and health tonics. The antibacterial properties of neem oil, which include limonoids, azadirachtin, nimbin, nimbidin, and nimbolide, give it a broad range of protection against harmful bacteria, both positive and negative gram[15,16].

Formerly known as *Majorana hortensis Moench*, *Origanum majoranaL.* is a delicate perennial herb belonging to the genus *Origanum*[17]. Known by most as sweet marjoram, it is indigenous to Cyprus and Antolia (Turkey) and has spread throughout the Mediterranean region, particularly Egypt[18].

The main goal of this study is to evaluate the antimicrobial activity of the five nature oils available in Yemen market.

MATERIAL AND METHODS

The five medicinal essential oils were purchased from Yassin spices, Sana'a, Yemen and authenticated in the institute laboratory.

Antibacterial screening:

Antibiotic sensitivity testing was conducted using a number of bacterial strains that were accessible in the institute laboratories' stock culture, including: Gram-positive *Enterococci and Staphylococcus aureus* are bacteria. Gram-negative *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae* are bacteria. Mueller Hinton agar medium injected with the test organisms' bacterium was used to apply the agar diffusion method. Discs having a diameter of 5 mm were impregnated with either the control or the oils. The discs were then inserted into the culture medium's surface. Standard antibacterial discs of ofloxacin and gentamycin were utilized, respectively. To check for bacteria, the plates were incubated for 24 hours at 35–37°C[19-22]. Following incubation, the inhibitory zone diameters were measured in millimeters, and the findings were assembled into a table. The minimum inhibitory concentrations (MIC) of four essential oils against the tested microorganisms were also determined by micro dilution method

Statistical analysis

The data were analyzed using SPSS version 21. The results are provided as mean ± SEM. One-way analysis of variance (ANOVA) followed by Turkey's HSD post hoc test was used to compare results across and within the groups. The results were considered significant when p<0.05.

Table1: Antimicrobial activity of plants oils as inhibition zone diameter [a,b].

Bacterial strain disc diffusion assay	*S. aureus*	*Enterococci*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*
Neem	20±0.37	10±0.11	8±0.10	12±0.63	15±0.25
M. oleifera	17±0.44	10±0.14	8 ±0.12	12±0.37	12±0.37
R. officinalis	10±0.25	13±0.13	12±1.2	10±0.37	8±0.58
S. officinalis	22±0.12	10±0.10	8±0.12	13±0.07	12±0.12
O.majorana	10±0.12	11±0.09	8±0.12	10±0.08	27±0.12
Positive control					
Gentamycin	25±0.44	25±0.44			
Ofloxacin			20±0.1	20±0.34	20±0.34

Inhibition zone diameter (mm); a- Results are Mean±SD of triplicate values.
b- 6-10 mm: no activity; 12-15 mm: low activity; 16-19 mm: good activity; above19 mm: significant activity.

RESULTS

Neem, *M. oleifera, R. officinalis, S. officinalis,* and *Origanum majorana* were among the undiluted plant oils whose antimicrobial test findings showed unique patterns of inhibition against both Gram-positive and Gram-negative bacteria. *S. officinalis* oil showed the highest activity against Gram-positive pathogens, generating an inhibitory zone against *Staphylococcus aureus* of 22 mm, which was very near to the effectiveness of the common antibiotic gentamicin (25 mm). *R. officinalis* had the least amount of effect (10 mm), while Neem oil was the most potent (20 mm), followed by *M. oleifera* (17 mm). All oils showed moderate to weak efficacy against *Enterococci*, with inhibition zones between 10 and 11 mm, which is much smaller than the 25 mm of gentamicin. The outcomes varied considerably among Gram-negative bacteria. Only *R. officinalis* had moderate activity (12 mm) in comparison to ofloxacin's 20 mm, indicating that E. coli was resistant to the majority of oils. *M. oleifera* and *R. officinalis* oils were less effective (10–12 mm) against *K. pneumoniae*, whilst neem and *S. officinalis* oils showed moderate suppression (12–13 mm). Notably, *O. majorana* oil and neem oil (15 mm) significantly suppressed *P. aeruginosa*, outperforming all other treatments with a remarkable 27 mm zone that even surpassed that of ofloxacin (20 mm). *R. officinalis* oil was the least effective (8 mm), whereas oils from *S. officinalis* and *M. oleifera* also shown modest effects (12 mm a piece). Because Gram-positive bacteria have simpler cell walls, the oils were generally more effective against them. While *O. majorana* showed significant action against *P. aeruginosa*, indicating its potential as a specialist antibacterial agent, *S. officinalis* and neem oils were shown to be the most widely effective.

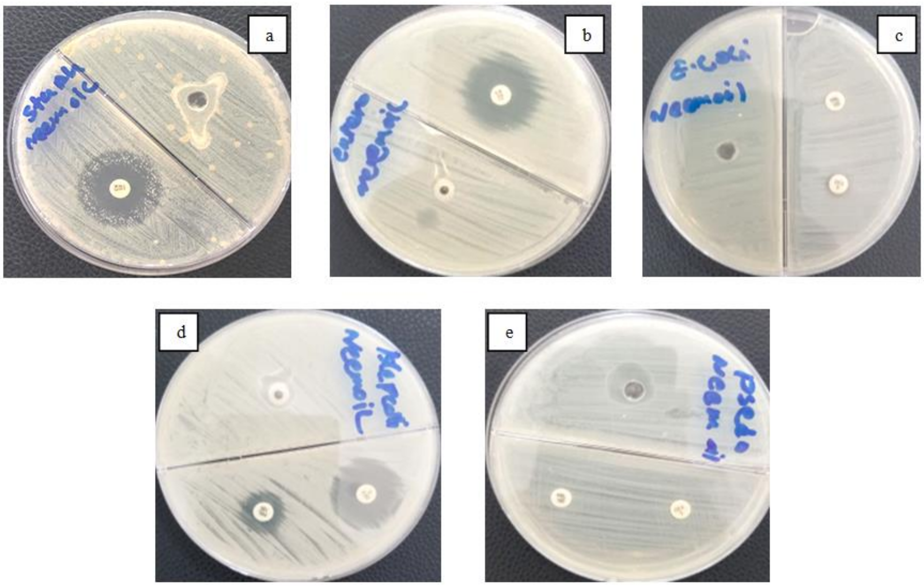

Figure 1: Antimicrobial effect of Neem oil on *S. aureus, Enterococci, E. coli, K. pneumonia,* and *P. aeruginosa.*

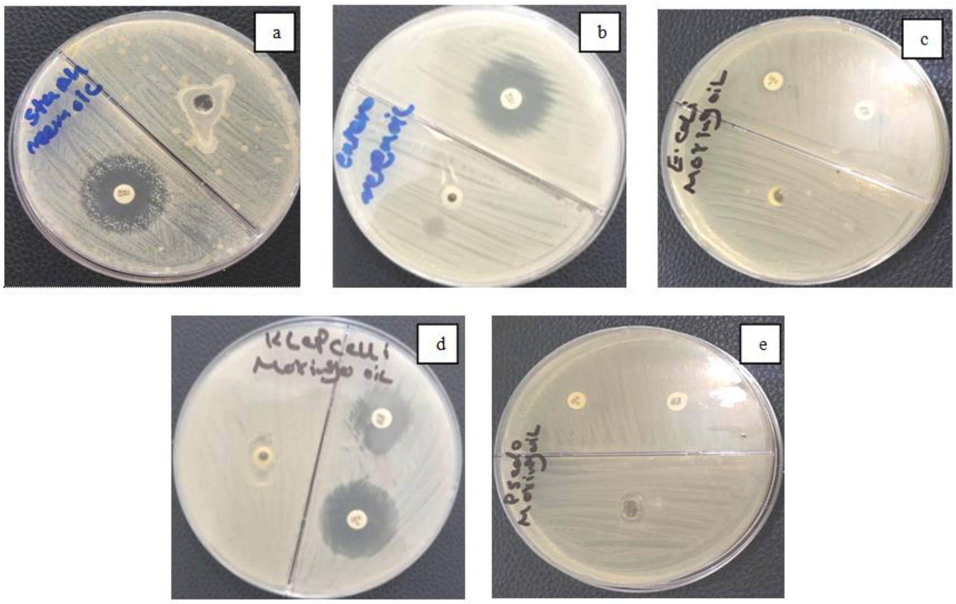

Figure 2: Antimicrobial effect of *M. oleifera* oil on *S. aureus, Enterococci, E. coli, K. pneumonia,* and *P. aeruginosa.*

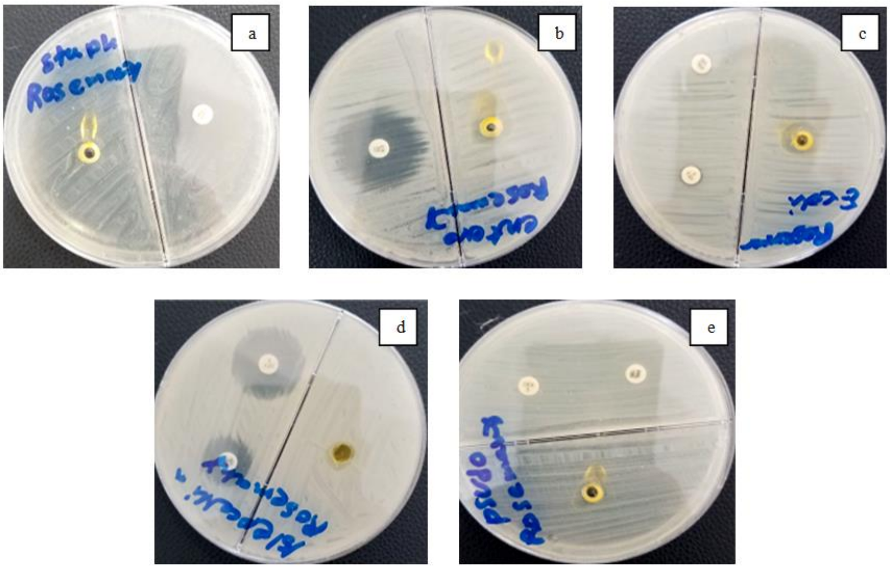

Figure 3: Antimicrobial effect of *R. officinalis* oil on *S. aureus, Enterococci, E. coli, K. pneumonia,* and *P. aeruginosa.*

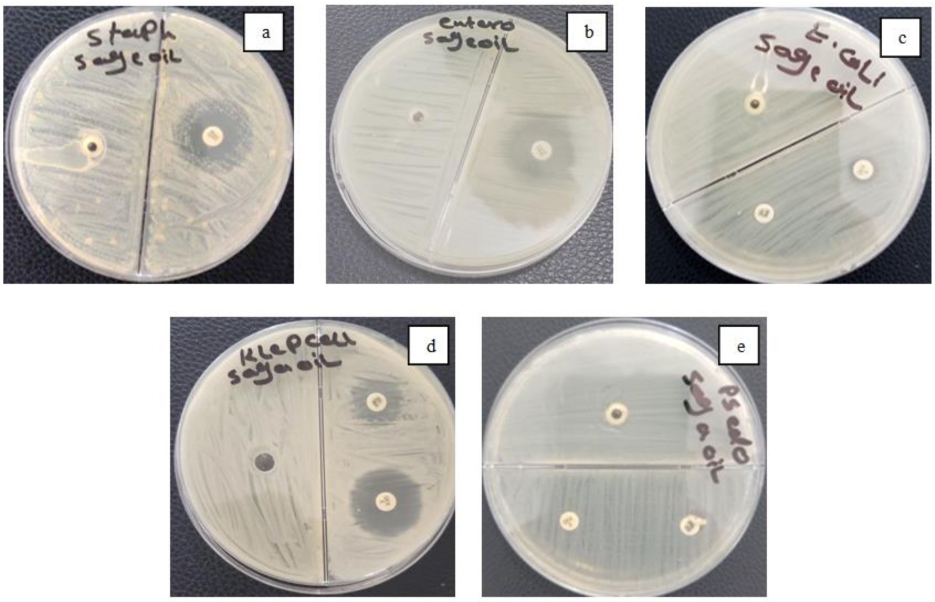

Figure 4: Antimicrobial effect of *S. officinalis* oil on *S. aureus, Enterococci, E. coli, K. pneumonia,* and *P. aeruginosa.*

Although their inconsistent effectiveness against Gram-negative strains emphasizes the need for more study into increasing their activity or combining them with traditional treatments, these findings show the potential of plant-derived oils in the fight against bacterial illnesses.

DISCUSSION

The antimicrobial profiles of the five essential oils tested in this study revealed significant variations in their effectiveness against both Gram-positive and Gram-negative bacteria. *S. officinalis* and Neem oils demonstrated potent inhibition of *S. aureus,* with inhibition zones measuring 22 mm and 20 mm, respectively comparable to gentamicin (25 mm). This is in line with earlier studies by Babatunde *et al.*[15], and Ukaoma *et al.*[16], which attributed the antibacterial action of neem oil to its active components such as azadirachtin and nimbin. On the other hand, *O majorana* displayed remarkable activity against *P. aeruginosa* (27 mm), outperforming the standard antibiotic ofloxacin (20 mm). This corroborates findings by Vagi *et al.*[17], who reported high antibacterial efficacy of *O. majorana* oils rich in monoterpenes like thymol and carvacrol known for disrupting microbial membranes**.** *R. officinalis* showed the weakest antimicrobial performance, particularly against *P. aeruginosa* (8 mm) and *E. coli* (12 mm), consistent with Jawad *et al.*[12], who observed limited spectrum activity for rosemary oils. *M. oleifera* exhibited moderate inhibition, especially against *S. aureus* (17 mm), aligning with prior studies attributing its activity to flavonoids and polyphenols**.** The generally greater susceptibility of Gram-positive bacteria is explained by their simpler peptidoglycanrich cell walls, which allow essential oil components to penetrate more easily. In contrast, the outer membrane of Gram-negative bacteria provides a formidable barrier to hydrophobic compounds, limiting the effectiveness of many oils.

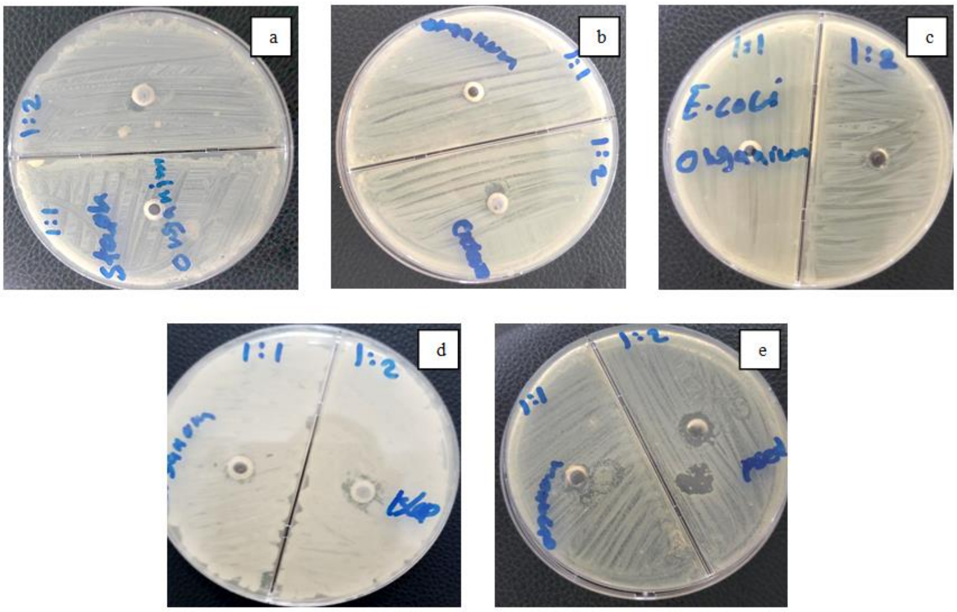

Figure 5: Antimicrobial effect of *O. majorana* oil on *S. aureus, Enterococci, E. coli, K. pneumonia*, and *P. aeruginosa.*

Limitation of study

No tests were conducted to determine how the oil affected human cells. To find safe amounts, cytotoxicity tests should be used in future research. Although the study makes the assumption that it has antibacterial activity, GC-MS analysis was not used to confirm the precise composition. Small variety of microbes only a small number of bacterial strains were investigated for this investigation. Future studies should assess how it affects fungus, such as viruses and *C. albicans*.

CONCLUSIONS

The study analyzed the antimicrobial properties of five essential plant oils against Gram-positive and Gram-negative bacteria. *S. officinalis* showed the strongest inhibitory effect against *S. aureus*, followed by neem oil. *O. majorana* was particularly effective against *P. aeruginosa*, with an exceptional 27 mm inhibition zone. These findings validate traditional medicinal uses and highlight the potential of plant-derived antimicrobials in combating antibiotic-resistant infections. It is also important to address the source and regulatory status of the oils used in this study. Although the essential oils were purchased from local vendors in Yemen, they are imported products. This raises critical concerns about their purity, chemical composition, and authenticity. Without proper regulatory oversight including GC-MS analysis, microbial safety testing, and standardization there is a potential risk of using adulterated or degraded oils. Therefore, national health authorities should implement and enforce stringent guidelines for the importation and distribution of essential oils. Ensuring the safety, efficacy, and quality of these products is essential for protecting public health and ensuring the reliability of future scientific research involving natural bioactive agents.

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AUTHOR'S CONTRIBUTION

Al-Hashmi AAAA: writing original draft. **Marwan HMH:** writing methodology. **Taib MAG:** investigation. **Farea AAA:** formal analysis. **Al-Sabai YSY:** data curation. **Yahya Al-Wadaii AS:** lab preparation. **Jahaf AAA:** conceptualization. **Al-Madwami AYM:** writing, review and editing. **Qushasha HAA:** writing. **Al-Bakri BMY:** review, and editing. **Arrabyee AMS:** review, and editing. Final manuscript was checked and approved by all authors.

DATA AVAILABILITY

Data will be made available on request.

CONFLICT OF INTEREST

None to declare.

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