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Antibacterial Efficacy of Origanum majorana and Salvia officinalis Extracts Against Escherichia coli and Staphylococcus aureus

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ABSTRACT

This study evaluated the antibacterial activity of aqueous, ethanol, and essential oil extracts of Origanum majorana and Salvia officinalis against two human pathogens Escherichia coli and Staphylococcus aureus and determined their minimum inhibitory concentrations MICs. The findings indicated that the essential oil extracts were more effective in inhibiting bacterial growth compared to the aqueous and ethanolic extracts. O. majorana essential oil produced the largest inhibition zones: 2.38 cm against Escherichia coli and 2.98 cm against Staphylococcus aureus. Salvia officinalis essential oil showed moderate activity, with inhibition zones of 1.45 cm against E. coli and 1.40 cm against S. aureus. The ethanolic extracts produced limited inhibition (for example, S. officinalis ethanolic extract: 0.57 cm against E. coli; S. officinalis ethanolic extract: 2.17 cm against S. aureus; O. majorana ethanolic extract: 0.45 cm against S. aureus. The aqueous extracts generally exhibited weak activity, except for the O. majorana aqueous extract against E. coli inhibition 2.20 cm and S. officinalis aqueous extract against S. aureus inhibition 1.15 cm. Compared with standard antibiotics tetracycline , chloramphenicol some essential-oil treatments especially marjoram showed comparable activity. The results showed that the essential oils exhibited the strongest antibacterial effect. Among them, O. majorana oil was the most active. Based on these observations, the essential oils of O. majorana and S. officinal might serve as promising natural antibacterial agents and could potentially be applied in combination with standard antibiotics.

KEYWORDS: Origanum majorana, Salvia officinalis, essential oils, aqueous extract, alcoholic extract, Escherichia coli, Staphylococcus aureus, antibacterial activity, and minimum inhibitory concentration.

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1.INTRODUCTION

The use of plants as source of remedies for the treatment of many diseases dated back to prehistory and people of all continents have this old tradition. The search for agents to cure infectious diseases began long before people were aware of the existence of microbes. These early attempts used natural substances, usually native plants or their extracts and many of these herbal remedies proved successful (Sofowora, 1982). World Health Organization survey indicated that about 70-80% of the world's population rely on nonconventional medicine, mainly of herbal sources, in their primary healthcare. This is especially the case in developing countries where the cost of consulting a western style doctor and the price of medication are beyond the means of most people (Chan, 2000). Origanum majorana and Salvia officinalis, both members of the Lamiaceae family, are aromatic herbs traditionally used in cooking, folk medicine, and the pharmaceutical industry (Viuda-Martos et al., 2010; Hussain et al., 2010).

Their essential oils are particularly rich in monoterpenes such as thymol, carvacrol, and 1,8-cineole, which have been associated with strong antimicrobial and antioxidant activities (Burt, 2004; Teixeira et al., 2013).

The rapid emergence of antibiotic-resistant bacteria has become a major global health problem (Ventola, 2015; WHO, 2020). Consequently, identifying new and effective antibacterial agents from natural origins has become a priority. The present study focuses on the antibacterial potential of O. majorana and S. officinalis extracts specifically their aqueous, ethanolic, and essential oil forms against two clinically important bacterial species: E. coli and S. aureus.

The primary goal of this research was to compare the inhibitory effects and minimum inhibitory concentrations of the different extracts, and to determine which type of extract demonstrates the strongest antibacterial activity.

2.MATERIALS AND METHODS

2.1. Media preparation

a. Nutrient agar: Prepared according to the manufacturer's instructions (Condalab): 23 g per 1 L distilled water, sterilized at 121 °C for 15 minutes, stored until use.

b. Mueller–Hinton agar: Prepared by dissolving 38 g per 1 L distilled water and sterilized as above. Used for antibacterial testing.

2.2. Bacterial isolates included in the study

Pure bacterial isolates (mentioned in Table 1 below) which was obtained from your doctor's Laboratory in Almarj city.

Table (1): Bacterial isolates included in the study

Gram positive	Gram negative
Staphylococcus aureus	Escherichia coli

2.3 Plant material

Leaves of O. majorana and S. officinalis were purchased from local markets, Washed with tap water followed by distilled water, air-dried at room temperature, Ground to fine powder, and stored in sealed containers until extraction.

Table (2): Plant used in the study

User Part	Plant Family	The Scientific Name
Leaves	Lamiaceae	Origanum Majorana
Leaves	Lamiaceae	Salvia Officinalis

3.4 Preparation of plant extracts

Figure (1): Salvia officinalis

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Figure (2): Origanum Majorana

3.4.1. Preparation of aqueous extract:

The method (Ahmed et al., 1998) was used to prepare aqueous extracts by mixing 20 g of plant power for each plant sample separately with 400 ml of distilled water in a 1000ml volumetric flask, then leaving the suspension in a shaking water bath at a temperature of 40 c for a period. 24 hours, then suspension was filtered using several layers of medical gauze, then sterile glass bottle and placed in a flat glass dish in an electric oven at 40 degrees for 48 hours until the sediment from the filtrate became a powder stuck to the glass, then it was scraped off and collected in an airtight glass container. Close and store the extract after weighing in the refrigerator until use.

3.4.2. Preparation of alcoholic:

Use 96% ethanol alcohol as a solvent to prepare the alcoholic extract (Khanzada, 2006) for the plant sample using the same method used to prepare the aqueous extract

3.4.3. Preparation of volatile oil:

The volatile oil was extracted for each plant sample tested separately using the hydra distillation method according to the standard method (European pharmacopeia, 2008), where 100 grams of dry plant powder was weighed, 750 ml of distilled water was added to it, and it was placed in the distillation system for up to 4 hours, and the distillate was collected. (Volatile oil and water) were filtered through anhydrous sodium sulfate on filter paper no (1) the obtained volatile oil was weighed and stored in dark containers at -18 c.

3.5. Preparing different concentrations of plant extracts: aqueous, alcoholic, and volatile oils

Different concentrations of oils were prepared based on the method (Hadizadeh et al, 2009) by dissolving the required amount in a 0.05% Tween 20 solution to obtain concentrations ranging from…..ppm to….. ppm of the tested oil.

Likewise, the method for alcoholic extracts, or for aqueous extracts, is the same as the previous method, but instead of using tween, use distilled water for dissolution. Concentrations (300, 150, and 75) mg/ml were used for all extracts it is twofold dilution.

3.6. Antibacterial Activity and Determination of Minimum Inhibitory Concentration (MIC)

The antibacterial activity of the extracts was evaluated usingtheagarwelldiffusionmethod(Bloomfield,1991). Muller–Hinton Agar was poured into sterile Petri dishes and inoculated with 100 µL of bacterial suspension evenly spread over the surface. Wells (8 mm in diameter) were made using a sterile cork borer.

Different concentrations of each extract (300, 150 and 75 mg/mL) were prepared:

Essential oils were dissolved in 0.05% Tween 20.

Ethanolic and aqueous extracts were dissolved in ethanol or distilled water, respectively.

A 100-µL aliquot of each concentration was placed into separate wells. Plates were left for 30 min at room temperature to allow diffusion and then incubated at 37 °C for 24 h. The diameter of inhibition zones was measured in millimeters. The lowest concentration that completely inhibited visible bacterial growth was recorded as the MIC.

3.7. Commercial antibiotics

To compare the effectiveness of plant extracts with some common commercial antibiotics two antibiotics were used; Tetracycline (10 ug) and Chloramphenicol (5 ug). The antibiotic disc was placed on the surface of the MHA solid medium in the petri dish, and then incubated for 24 hours at 37 c. After incubation, the inhibition zone around the disc was measured for

each plante and considered as a positive control.

3.RESULTS

3.1 Effect of volatile oils of tested plants against E.coli and comparison with tested antibiotics

The effect of the volatile oil of the tested plants against E.coli was explained in Table (3). Marjoram oil showed the strongest effect against E. coli, with an inhibition zone of 2.38 cm.

The minimum inhibitory concentration for both Salvia and marjoram oils was 75 mg/mL, while Salvia oil required a higher concentration of 300 mg/mL to inhibit E. coli growth.

Table (3): Effect of volatile oils of tested plants against E. col and comparison with tested antibiotics

E. coli				
Plant	300 mg/ml	150 mg/ml	75mg/ml	MIC (mg/ml)
	Inhibition Zone (cm)			
Marjoram	2.383 a	2.117	1.667	75
Salvia officinalis	1.450 d	0.000	0.000	300
Control	0.000 c	0.000	0.000	-
L.S.D	0.2717**			
Antibiotics	Inhibition Zone (cm)			
Chloramphenicol(5ug)	1.117			
Tetracycline(10ug)	3.267			
Control	0.000			

Means followed by different letters (a, b, c, d) within the same column are significantly different at $P \leq 0.05$ according to LSD test.

3.2. Effect of alcohol extracts of tested plants against E.coli and comparison with tested antibiotics

The results revealed that E.coli growth inhibited by only the ethanol extract of Salvia 0.5667 cm whereas no effects were reported for the rest of plants Table(4).

When the alcoholic extracts of plants were tested against E.coli with concentrations ranged from 75 to 300 mg/ml, only Salvia plant exhibited an inhibitory effect at a concentration of 300 mg/ml. Regarding the MIC of the alcoholic extract of Salvia value of 300mg/ml.

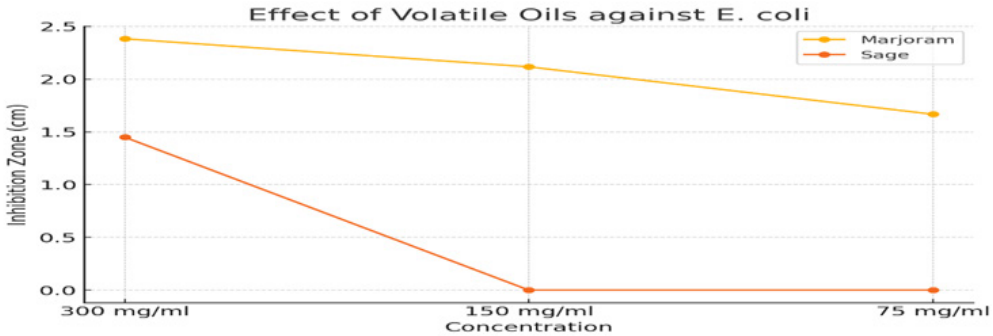

Figure (3): Effect of Origanum majorana and Salvia officinalis essential oils on E. coli at different concentrations

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Table (4): Effect of alcoholic extracts of tested plants against E. coli and comparison with tested antibiotics

Escherichia coli				
Plant	300 mg/ml	150 mg/ml	75mg/ml	MIC (mg/ml)
	Inhibition Zone (cm)			
Marjoram	0.000	0.000	0.000	-
Salvia officinalis	0.5667	0.000	0.000	300
Control	0.000	0.000	0.000	-
L.S.D	0.05418**			
Antibiotics	Inhibition Zone (cm)			
Chloramphenicol(5ug)	3.267			
Tetracycline (10ug)	1.117			
Control	0.000			

Means followed by different letters (a, b, c, d) within the same column are significantly different at $P \leq 0.05$ according to LSD test.

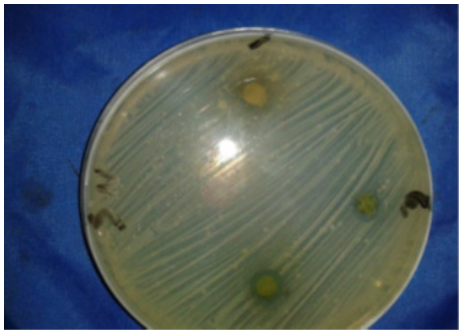

Figure (4): The alcoholic extract of O. majorana showed no inhibitory activity against E. coli at all tested concentrations (75–300 mg/mL).

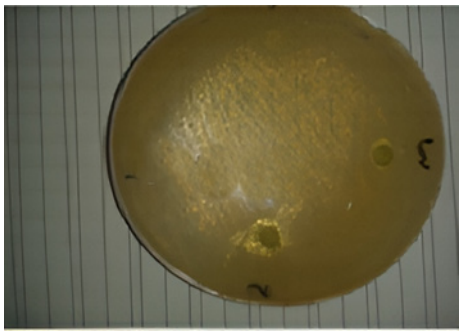

Figure (5): The alcoholic extract of S. officinalis exhibited limited antibacterial activity, with an inhibition zone of 0.56 cm at 300 mg/mL only.

3.3. Effect of aqueous extracts of tested plants against E.coli and comparison with tested antibiotics

The aqueous extracts of the tested plants were generally less active against E. coli, as shown in Table (5). With exception of marjoram aqueous extract at the concentration of 300 mg/ml, which exhibited an inhibitory zone of 2.200 cm.

And the MIC at the same concentration, the rest of plants showed no effect on E.coli at all concentrations used.

As for the minimum inhibitory concentration (MIC) for the extract against E.coli, the lowest inhibitory concentration for the marjoram plant was 300mg/ml.

Table (5): Effect of aqueous extracts of tested plants against E.coli and comparison with tested antibiotics

E.coli				
Plant	300 mg/ml	150 mg/ml	75mg/ml	MIC (mg/ml)
	Inhibition Zone (cm)			
Marjoram	2.200 b	0.000	0.000	300
Salvia officinalis	0.000 a	0.000	0.000	-
Control	0.000 a	0.000	0.000	-
L.S.D	0.07762**			
Antibiotics	Inhibition Zone (cm)			
Chloramphenicol(5ug)	3.267			
Tetracycline (10ug)	1.117			
Control	0.000			

Means followed by different letters (a, b, c, d) within the same column are significantly different at $P \leq 0.05$ according to LSD test.

3.4. Effect of plant extracts used against S.aureus and comparison with tested antibiotic

Effect of volatile oils of tested plants against S.aureus and comparison with tested antibiotics Table (6) shows the results of the volatile oils of the tested plants against S. aureus, again the marjoram oil highest inhibitory effect, as the inhibition zone reached a value of 2.983cm, Whereas the least effect was reported for the Salvia extract (1.400cm).

The results showed significant differences between the tested plants in their effects on S. aureus at the 0.05 significance level. Where concentrations ranged from 75 to 300 mg/ml, the MIC test showed that S.aureus was more sensitive to all concentration except to sage as a higher MIC of 150 mg/ml was recorded in comparison 75 mg/ml for the rest.

Table (6): Effect of volatile oils of tested plants against S.aureus and comparison with tested antibiotics

S. aureus				
Plant	300 mg/ml	150 mg/ml	75mg/ml	MIC (mg/ml)
	Inhibition Zone (cm)			
Marjoram	2.983 a	2.400	2.300	75
Salvia officinalis	1.400 d	0.550	0.000	150
Control	0.000 e	0.000	0.000	
L.S.D	0.6571**			
Antibiotics	Inhibition Zone (cm)			
Chloramphenicol(5ug)	2.683			
Tetracycline(10ug)	2.550			
Control	0.000			

Means followed by different letters (a, b, c, d) within the same column are significantly different at $P \leq 0.05$ according to LSD test.

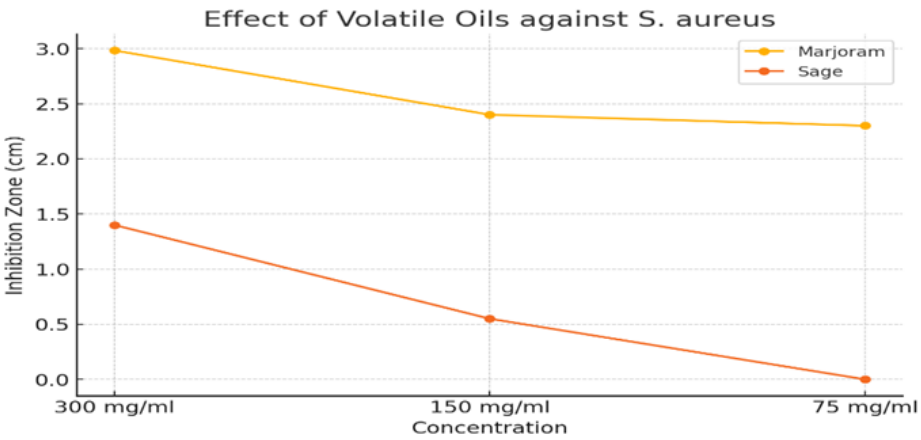

Figure (6): Effect of Origanum majorana and Salvia officinalis essential oils on S. aureus at different concentrations.

3.5. Effect of alcohol extracts of tested plants against S.aureus and comparison with tested antibiotics

Table (7) shows the results of the effect of plant extracts on S.aureus it was clear that salvia were the most effective and showed the same effect, as the inhibition zone reached a value of 2.167, then marjoram extract with the least inhibitory effect (0.450cm). MICs results against S.aureus, are illustrated in the same table, where all extracts showed the same MIC values of 150mg/ml

Table (7): Effect of alcohol extracts of tested plants against S.aureus and comparison with tested antibiotics

S.aureus				
Plant	300 mg/ml	150 mg/ml	75mg/ml	MIC (mg/ml)
	Inhibition Zone (cm)			
Marjoram	0.450 a	0.300	0.000	150
Salvia officinalis	2.167 c	1.817	0.000	150
Control	0.000 d	0.000	0.000	-
L.S.D	0.1417**			
Antibiotic	Inhibition Zone (cm)			
Chloramphenicol(5ug)	2.683			
Tetracycline(10ug)	2.550			
Control	0.000			

Means followed by different letters (a, b, c, d) within the same column are significantly different at $P \leq 0.05$ according to LSD test.

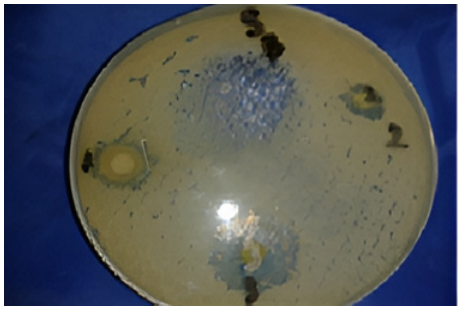

Figure (7): The alcoholic extract of O. majorana displayed weak antibacterial activity against S. aureus, with a maximum inhibition zone of 0.45 cm at 300 mg/mL

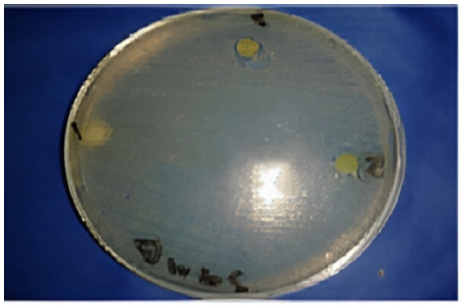

Figure (8): The alcoholic extract of S. officinalis demonstrated relatively strong antibacterial activity against S. aureus, producing inhibition zones up to 2.17 cm at 300 mg/mL.

3.6. Effect of aqueous extracts of tested plants against S.aureus and comparison with tested antibiotics

Table (8) shows the results of the effect of the aqueous extracts of the tested plants against S.aureus. Salvia extracted more effect against this bacterium as the inhibitory zone reached a value of 1.150cm.

While the marjoram extracts showed no effect against S.aureus at all concentrations. The Salvia extract exhibited an effect at two concentrations, 300mg/ml and 150mg/ml, and showed no effect at a concentration of 75mg/ml. As for the marjoram extracts, they showed no effect at all concentrations.

Table (8): Effect of aqueous extracts of tested plants against s.aureus and comparison with tested antibiotics

S.aureus				
Plant	300 mg/ml	150 mg/ml	75mg/ml	MIC (mg/ml)
	Inhibition Zone (cm)			
Marjoram	0.000	0.000	0.000	-
Salvia officinalis	1.150	0.917	0.000	300
Control	0.000	0.000	0.000	-
L.S.D	0.1390**			
Antibiotics	Inhibition Zone (cm)			
Chloramphenicol(5ug)	2.683			
Tetracycline(10ug)	2.550			
Control	0.000			

Means followed by different letters (a, b, c, d) within the same column are significantly different at $P \leq 0.05$ according to LSD test.

Table (9): Effect of commonly used commercial antibiotics against tested bacteria.

S.A*	L.M*	E.C*	S.T*	Antibiotics
2.550	2.500	1.117	0.967	Tetracycline
2.683	3.250	3.267	2.967	Chloramphenicol
0.5062***	L.S.D			

Means followed by different letters (a, b, c, d) within the same column are significantly different at $P \leq 0.05$ according to LSD test.

Table (10): Comparison of the effect of Salvia officinalis plant extracts with antibiotics against tested bacteria.

Tetracycline	Chloramphenicol	Aqueous	Alcoholic	Volatile oils	Bacteria
1.117	3.267	0.000	0.000	2.056	E.coli
2.550	2.683	0.611	0.250	2.561	S. aureus

Means followed by different letters (a, b, c, d) within the same column are significantly different at $P \leq 0.05$ according to LSD test.

Table (11): Comparison of the effect of Marjoram plant extracts with antibiotics against tested bacteria.

Tetracycline	Chloramphenicol	Aqueous	Alcoholic	Volatile oils	Bacteria
1.117	3.267	0.733	0.000	1.194	E.coli
2.550	2.683	0.000	0.700	1.372	S.aureus

Means followed by different letters (a, b, c, d) within the same column are significantly different at $P \leq 0.05$ according to LSD test.

4.DISUSSION

The results of this study showed clear differences in the antibacterial effects of the plant extracts tested. Overall, the essential oils demonstrated stronger inhibitory activity than both the alcoholic and aqueous extracts. This agrees with the findings of (Abdalla and Abdelgadir 2016).

The essential oil and aqueous extract of majorana in particular showed clear inhibition of bacterial growth, similar to what was reported by (Busatta et al. 2008). The weak antibacterial activity of the ethanolic and aqueous extracts may be due to the low levels of volatile compounds or the absence of important hydrophobic molecules that are usually concentrated in essential oils. Water, in particular, was not an effective solvent for extracting such compounds, which may explain the limited inhibitory activity observed (Merillon & Riviere, 2018).

When compared with the commercial antibiotics used in this study tetracycline and chloramphenicol the essential oils, especially which of O. majorana, showed similar or slightly lower inhibition. This suggested that these oils had real antibacterial potential, but cannot fully replace conventional antibiotics.

Unlike antibiotics, which act through defined biochemical mechanisms (for example, tetracycline inhibits protein synthesis), essential oils usually act through broader mechanisms such as damaging the bacterial membrane, denaturing proteins, and inducing oxidative stress (Burt, 2004).

The antibacterial activity of essential oils is likely related to multiple simultaneous effects on bacterial cells. Because of this multi-target action, bacteria may find it more difficult to develop resistance compared to when exposed to a single-mechanism synthetic antibiotic. (Merillon & Riviere, 2018).

Therefore, the findings of this study indicate that plant extracts, particularly the essential oils, may be useful as supportive antimicrobial agents. However, they should not be considered a substitute for conventional antibiotics, but rather a possible addition to current treatment approaches.

The higher antibacterial activity of O. majorana essential oil compared to S. officinalis

may be related to its chemical composition. Marjoram oil contains higher amounts of phenolic monoterpenes such as thymol, carvacrol, and terpinen-4-ol, which are known for their strong antibacterial properties (Lambert et al., 2001; Burt, 2004).

By contrast, S. officinalis essential oil has more 1,8-cineole and camphor, which are less potent antibacterial agents, explaining its lower inhibitory zones. Overall, the findings support earlier studies that highlighted the potential of O. majorana and S. officinalis essential oils as natural antimicrobial agents (Burt, 2004; Özcan & Erkmen, 2001; Teixeira et al., 2013).

These results reinforce the importance of continued research on essential oils as promising natural products to combat bacterial infections, especially in light of the growing problem of antibiotic resistance worldwide.

5. CONCLUSION

This study demonstrated that O.majorana and S.officinalis possess noticeable antibacterial activity, especially in their essential oil forms. The essential oils showed much stronger inhibition of bacterial growth than the ethanolic and aqueous extracts. Among all treatments, the essential oil of O. majorana was the most active, producing inhibition zones of 2.38 cm against E. coli and 2.98 cm against S. aureus, with a minimum inhibitory concentration of 75 mg/mL. In comparison, S. officinalis essential oil showed moderate antibacterial action, with inhibition zones of 1.45 cm for E. coli and 1.40 cm for S. aureus, and higher MIC values (300 mg/mL and 150 mg/mL, respectively).

The ethanolic and aqueous extracts were generally weak in their antibacterial effects, suggesting that volatile components such as thymol, carvacrol, and 1,8-cineole are mainly responsible for the strong antibacterial properties observed in the essential oils.

The findings also showed that S. aureus was more sensitive to the essential oils than E. coli, which may be related to differences in the structural composition of their cell walls. Although the essential oils did not exceed the activity of the commercial antibiotics used for comparison, the inhibition zones produced by O. majorana oil were sufficiently notable to suggest possible practical applications.

Further work is needed to identify the specific bioactive compounds responsible for the antibacterial activity and to evaluate the safety and effectiveness of these oils in vivo. Investigating their combined use with existing antibiotics may also help determine whether they can enhance antibacterial efficacy or reduce required antibiotic dosages.

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