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Volatile oils as Alternative Medicine to Treat Abscess Infection Caused by Multidrug-Resistant bacteria

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ABSTRACT

Background and Objective: Volatile oils (1- Eucalyptus, 2- Chamomile, 3- Peppermint, 4- Marjoram, 5- Fennel, 6- Spearmint) have antimicrobial activity against bacteria were used. This study aim to find alternative natural products and

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increase the effectiveness of antibiotics by combination with some of these products to eliminate resistant bacteria. Spearmint oil, will aid pharmaceutical companies in creating new antibiotics that are used to treat illnesses brought on by bacteria that are resistant to pathogens.

Methods: Seventy two swabs from clinical samples of abscesses. All isolates were determined by Bergey's Manual of Determinative Bacteriology and various antibiotics were utilized during the experiment. Volatile oils were used and tested against clinical bacterial isolates.

Results: 72.2% of the isolates of abscesses are *S. aureus*, while 27.8% of the Gram-negative isolates include *P. vulgaris*, *P. aeruginosae*, and *E. coli*. Norfloxacin has a 61.2% sensitivity to isolated pathogenic bacteria, followed by chloramphenicol and amikacin. However, the pathogenic bacteria that have been isolated are completely resistant to cefoperazone and penicillin, The antibiotics norfloxacin (31.25 µg/ml) and chloramphenicol (31.25 µg/ml) were shown to have the lowest minimum inhibitory concentrations (MIC) against *S. aureus* 18, 59, and 70, and *S. aureus* 58, respectively, no significant difference was observed between norfloxacin and chloramphenicol ($p > 0.05$). Fennel and spearmint oils showed significant antagonistic activity and chamomile oil have no antagonistic effect, significant difference was observed between spearmint and fennel ($p < 0.05$) Combination between norfloxacin and spearmint oil were found to have a synergistic impact against *E. coli* number 9 and *S. aureus* numbers 5, 18, and 47.

Conclusion: In addition to offering ongoing protection against bacteria resistant to antibiotics, the variety of volatile oils and their efficacy offer fresh hope for the treatment of abscesses

Introduction

The human body's largest organ and primary barrier, the skin acts as the body's first line of defence against bacterial infections. In addition to preventing environmental diseases from entering the body, it offers a vast ecological niche for a variety of microorganisms (1).

A microbial invasion of the skin layers and underlying soft tissues is the hallmark of skin and soft tissue infections (SSTIs), which are a leading cause of morbidity and mortality. The infections can range in severity from mild to fatal, and they can manifest as anything from simple cellulitis to abscesses, deep tissue necrosis, and necrotising fasciitis (2,3).

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Gram-positive bacteria such as Staphylococcus aureus and β-hemolytic Streptococci, as well as several coagulase-negative bacteria, are the primary cause of bacterial SSTIs. Additionally, non-fermentative bacteria such Pseudomonas species and Acinetobacter bumaniin, as well as Gram-negative Enterobacteriaceae and Staphylococcus spp., can induce (3,4). Numerous bacterial skin diseases have been linked to necrotising fasciitis, scarlet fever, erysipelas, erythrasma, abscesses, folliculitis, furunculosis, and impetigo (5). The Gram-negative bacteria Pseudomonas aeruginosa and Enterobacteriaceae group, together with S. aureus and group A streptococci, are the most frequently implicated in cellulitis (6,7).

There are four types of antimicrobial resistance: microbial resistance, which can be ascribed to either genotypic or phenotypic factors; clinical resistance, which is believed to arise when no response is seen even to higher treatment doses; cross-resistance, which occurs when pathogens develop resistance to antibiotics known to have a similar mechanism of action, such as strains resistant to both ciprofloxacin and levofloxacin; and, finally, parallel or co-resistance, which is seen in antibiotic resistances that are unrelated in their mode of action, such as methicillin and gentamicin-resistant *S. aureus* (8,9).

Aromatic plant essential oils have drawn attention from researchers due to their ability to improve food taste and consumption, promote the release of digestive enzymes, improve gastrointestinal motility, and have antimicrobial, antiviral, antiparasitic, antifungal, immunomodulatory, antioxidant, and anti-inflammatory properties (10). Pharmaceutical companies produce more and more medications in the form of capsules, syrups, ointments, lotions, and sprays that include specific essential oil compositions (11). Numerous illnesses, including digestive issues, headaches, muscle and joint pains, respiratory tract infections, wounds, burns, hair loss, eczema, and other skin conditions, as well as psychological issues like anxiety, depression, difficulty focussing and paying attention, and insomnia, can be treated with essential oils in different combinations (12).

The volatile oils under study like spearmint showed antibacterial activity, and this study looks at ways to stop the organism that causes infections from developing antibiotic resistance. Thus, more structural elucidation of the above-mentioned plants' microbial growth-inhibiting bioactivity could be used as building blocks for the synthesis of new antibiotics in the future.

Material And Method

Study design and samples

72 clinical samples were collected from Zagazig University Hospitals and Clinics. The samples were collected between January 2024 and June 2024, and the patients' ages ranged from 3 to 52. For these investigations, samples from 40 male and 32 female were used.

Macroscopic, microscopic and biochemical tests

Samples were collected using sterile cotton swabs, MacConkey medium, nutrient agar, Mannitol salt agar, and blood agar. To identify bacteria, Bergey's Manual of Determinative Bacteriology (13) was used.

Antibiotic susceptibility test (AST)

Disc diffusion was used to assess the antibiotic sensitivity of each isolated bacterium (14). One colony of each isolated bacterial isolate should be injected via the sterile loop into five millilitres of sterile nutritional broth, incubated for twenty-four hours at 37 degrees Celsius, and its turbidity adjusted to 0.5 McFarland standard saline (15). Using sterile swabs, broth inocula was applied to two Muller-Hinton agar plates per isolate, with antibiotic discs spaced consistently apart. Plates were incubated at 37 °C for 24 hours, and all inhibitory zone diameters, including the disc, were measured in millimetres. Various antibiotics were used in the experiment, including penicillin (P) (10µg), ampicillin (AM) (10µg), erythromycin (E) (15µg), norfloxacin (NOR) (10µg), tobramycin (TOB) (10µg), amikacin (AK) (30µg), chloramphenicol (C) (30µg), and cefoperazone (CFP) (75µg). The plates were maintain at 37 °C for 24 hours.. The reading aimed for total inhibition of development in the unaided eye (16). Norfloxcin and chlormphenicol were examined for MIC and MBC against bacterial isolates based on their sensitivity (17).

Volatile oils susceptibility test (AST)

Eucalyptus, chamomile, peppermint, marjoram, fennel, and spearmint oils are among the six prepared medicinal plant essential oils that were acquired from the El-Captin company for extracting natural oils, plants, and cosmetics, located in AL Obour city, Cairo, Egypt.

Statistical Analysis

The statistical analysis was carried out using SPSS v.29.0.10 (IBM, USA). Data were recorded as significant when the prevalence values were calculated at 0.05 or less ($P<0.05$).

Results

Samples of abscesses were gathered from male and female of all ages. There were 32 female and 40 male, ages 3 to 50 and 5 to 52, respectively. The vast majority of bacterial isolates are gram-positive. *S. aureus* accounts for 72.2% of the isolates, whereas *P. vulgaris*, *P. aeruginosae*, and *E. coli* comprise 27.8% of the Gram-negative isolates, as illustrated in fig. (1).

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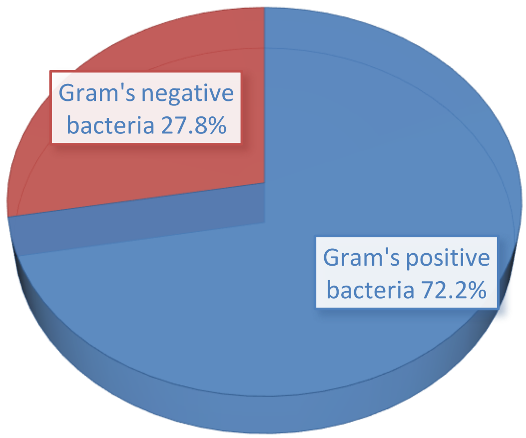

Figure 1: The distribution number of the pathogenic bacterial isolate from the collected samples of abscesses.

The sensitivity of norfloxacin against isolated pathogenic bacteria is 61.2%, followed by chloramphenicol, and amikacin. But the pathogenic bacteria that have been isolated are 100% resistant to cefoperazone and penicillin, and then tobramycin and ampicillin, as illustrated in Table (1), and fig. (2).

Table 1: Pathogenic isolates' sensitivity to various antibiotics is tested.

Tested antibiotics	Sensitive isolates		Intermediate isolates		Resistance isolates	
	No.	%	No.	%	No.	%
Norfloxacin (NOR)	44	61.2	12	16.6	16	22.2
Cefoperazone (CFP)	0	0.0	0	0.0	72	100.0
Erythromycin (E)	8	11.2	16	22.2	48	66.6
Tobramycin (TOB)	4	5.6	0	0.0	68	94.4
Ampicillin (AM)	12	16.6	0	0.0	60	83.4
Penicillin (P)	0	0.0	0	0.0	72	100.0
Amikacin (AK)	28	38.9	28	38.9	16	22.2
Chloramphenicol (C)	36	50.0	4	5.6	32	44.4

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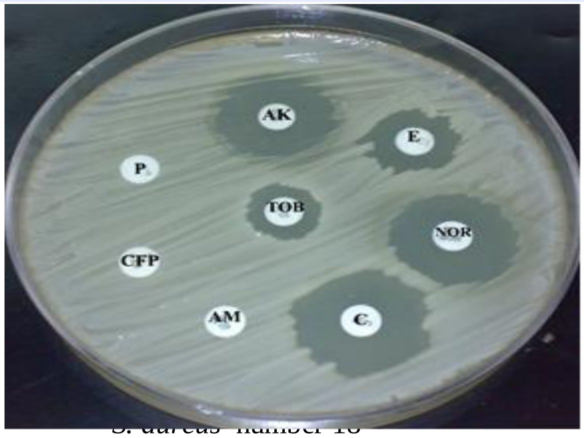

Figure 2: Bacterial isolates' sensitivity to antibiotics using the disc diffusion method

AK = amikacin, E = erythromycin, NOR = norfloxacin, C = chloramphenicol, AM = ampicillin, CFP = cefoperazone, P = penicillin & TOB = tobramycin.

The minimum MIC was obtained with norfloxacin antibiotic (31.25 µg/ml) against *S. aureus* 18, 59 & 70, and the minimum MIC was obtained with chloramphenicol antibiotic (31.25 µg/ml) against *S. aureus* 58, as illustrated in Table 2. No significant difference was observed between norfloxacin and chloramphenicol ($p > 0.05$).

Table 2: MICs (µg/ml) and MBCs of norfloxacin and chloramphenicol antibiotics against bacterial isolates.

a) Antibiotic norfloxacin

Bacterial isolates	No.	(MIC) (µg /ml)	(MBC) (µg /ml)
P. aeruginosa	2	125	250
S. aureus	5	62.5	125
E. coli	9	125	250
S. aureus	18	31.25	62.5
S. aureus	47	500	500
S. aureus	58	62.5	62.5
S. aureus	59	31.25	31.25
S. aureus	70	31.25	62.5
Proteus vulgaris	72	125	250

b) Antibiotic chloramphenicol

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Bacterial isolates	No.	Diameter of inhibition zones (mm)

Bacterial isolates No.

Bacterial isolates	No.	(MIC) (µg /ml)	(MBC) (µg /ml)
P. aeruginosa	2	125	250
S. aureus	5	125	125
E. coli	9	62.5	250
S. aureus	18	62.5	250
S. aureus	47	62.5	125
S. aureus	58	31.25	125
S. aureus	59	125	250
S. aureus	70	125	250
Proteus vulgaris	72	250	250

$p > 0.05$

Marjoram oil exhibited moderate antagonistic activity, while fennel oil and spearmint oil exhibited considerable antagonistic activity. When it comes to bacterial isolates, eucalyptus oil have no antagonistic effects on the majority of them, chamomile oil have no antagonistic effect, as illustrated in Table 3, and fig. 3.

Table 3: Different essential oils' diameter of inhibitory zone (mm) against isolates of pathogenic bacteria

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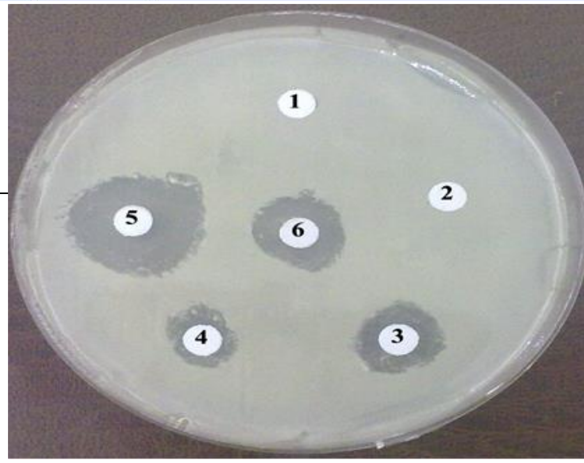

					Marjoram	Fennel	Spearmint
P. aeruginosa					10	12	15
S. aureus					10	15	15
E. coli					12	17	12
S. aureus					12	15	13
S. aureus					11	15	20
S. aureus					11	13	15
S. aureus					8	17	19
S. aureus					11	14	12
Proteus vulgaris	72	9	ND	9	9	15	15

S.

***aureus* number 18**

Figure 3: Essential oils' impact on bacterial isolates using the disc diffusion method.
1- Eucalyptus, 2- Chamomile, 3- Peppermint, 4- Marjoram, 5- Fennel, 6- Spearmint.

The minimum MIC were obtained at spearmint oil which recorded 50 % against *S. aureus* 5, 47, 58, 59, *E. coli* number 9, *Proteus vulgar* is number 72 and fennel oil which recorded 50% against *E. coli* number 9 *and S. aureus* 59, significant difference was observed between spearmint and fennel ($p < 0.05$), as illustrated in fig. (4).

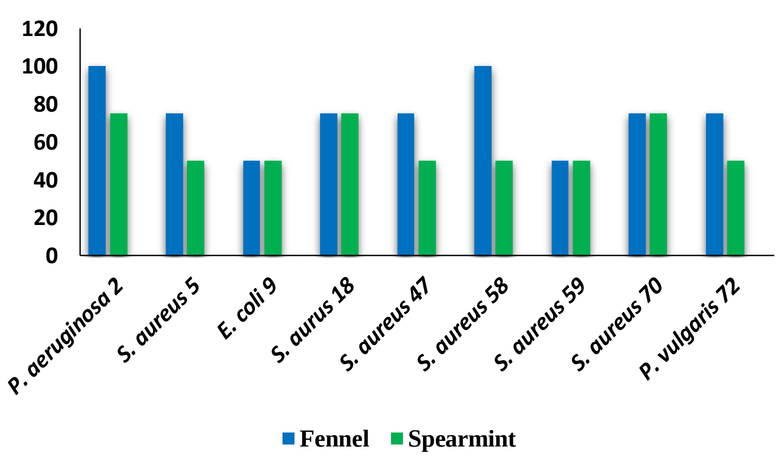

Figure 4: The percentage of various volatile oils' minimum inhibitory concentrations (MICs).

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Norfloxacin and spearmint oil were found to have a synergistic impact against *E. coli* number 9 and *S. aureus* numbers 5, 18, and 47. Fennel oil and norfloxacin together showed a synergistic effect against *S. aureus* numbers 18 and 47, as illustrated in Table 4.

Table 4: Diameter of inhibition zone (mm) of combination between different antibacterial agents MICs and MICs of norfloxacin

Bacterial isolates	**Serial No.**	**Norfloxacin MICs**		**Diameter of inhibition zones (mm)**	
				Different volatile oils MICs	
				Fennel	**Spearmint**
		µg/ml	IZ	IZ	IZ
P. aeruginosa	2	125	10	ND	10
S. aureus	5	62.5	8	ND	10
E. coli	9	125	10	8	11
S. aureus	18	31.25	12	25	25
S. aureus	47	500	10	15	20
S. aureus	58	62.5	10	ND	ND
S. aureus	59	31.25	12	ND	ND
S. aureus	70	31.25	15	ND	ND
Proteus vulgaris	72	125	10	10	10

IZ: Inhibition zone

Discussion

A collection of pus in any area of the body that typically results in surrounding edema and inflammation is called an abscess. Abscesses are frequently tactilely detectable, A sensitive, readily compressed lump that is typically encircled by a colored region ranging from deep red to pink is called an abscess. There is pus and debris in the center of an abscess. Abscesses can appear anywhere on your body and are painful and heated to the touch (18).

Gram-positive cocci accounted for 65.4% of the bacterial infections in this study, whilst Gram-negative bacilli made up 34.5% (19). In another investigation, 26 (83.87%) of the bacterial infections were Gram-positive, while 16.13 percent were Gram-negative (20). In line with earlier research, the results indicated that the Staphylococcus genus was the most prevalent, accounting for a high percentage (35.5%) of S. aureus (21)

Among the Gram-positive bacterial isolates, S. aureus (R3) exhibited the widest range of antibiotic resistance; it was more resistant to 12 different classes of antibiotics, including aminoglycosides, penicillins, macrolides, quinolones, lincosamides, tetracyclines, antifolate, and sulphonamides. The results were consistent with earlier research conducted in Botswana

(22).

Essential oils are frequently used to treat a wide range of illnesses, including infectious diseases of the skin. Additionally, it is incorporated as an active ingredient in numerous topical formulations used to treat cutaneous infections in order to reduce herpes, dandruff, acne, lice, and other skin infections (23). According to the results of the antibacterial activity, fennel essential oil has a strong antibacterial effect on strains of A. baumannii, S. aureus, and S. epidermidis. The corresponding inhibition diameters were 26 mm, 20 mm, and 16 mm, in decreasing order. In comparison to the two previously stated bacteria, the three additional strains K. pneumoniae, E. cloacae, and E. coli proved to be sensitive, albeit to a lesser extent. The second group's measured inhibition diameters were 14 mm, 10 mm, and 8 mm, in that order. The fennel EO (0 mm) caused resistance in P. aeruginosa (24).

Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Listeria monocytogenes, Salmonella typhimurium, and Escherichia coli were all susceptible to the mild antibacterial activity of Mentha spicata essential oil, with Listeria monocytogenes being the most vulnerable (25). The antibacterial activity of spearmint oil was the strongest against Escherichia coli and Staphylococcus epidermis, while the antifungal activity against Candida glabrata was the weakest. However, because essential oils are made up of several different components, their antimicrobial action is actually caused by the additive, synergistic, or antagonistic activities of those components (26). The high quantity of carvone (67%) in Mentha spicata essential oil shown strong antibacterial action against Escherichia coli and Staphylococcus aureus (27). The antibiotics norfloxacin and mint oil work in concert to combat Candida albicans, Streptococcus species, P. aeruginosae, Klebsiella species, and E. coli (28)

Conclusion

Multidrug resistant (MDR) isolates of S. aureus, P. vulgaris, P. aeruginosae, and E. coli have been identified. There was significant antagonistic action in the volatile oils of spearmint and fennel. The combination of spearmint oil and norfloxacin was proven to work in concert to. The volatile oils that were evaluated shown antibacterial activity and a promising antimicrobial effect on germs that were resistant to many drugs. Pharmaceutical companies will therefore benefit from the identification and isolation of secondary metabolites of those medicinal plants as they develop novel antibiotics to treat ailments brought on by pathogens, including bacteria that are resistant to polypharmacy. Therefore, additional structural clarity is required to understand the biological activity that inhibits the growth of microorganisms in the volatile oils listed above, particularly spearmint, which may be utilized as a precursor for the development of future antibiotics.

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