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ORIGINAL ARTICLE

Effect of Plant Extracts and Volatile Oils on Antibiotic Resistant Bacterial Isolates Causing Infections in Various Parts of the Body

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ABSTRACT

Background: *In order to cure newly emerging human illnesses, plant extracts and volatile oils with antimicrobial action against bacteria were employed. This is because medically important plants have substantial phytochemical classes with therapeutic characteristics.* ***Objectives:*** *This study aims to enhance antibiotic efficacy by combining substitute natural compounds to eradicate resistant bacteria, reducing disease transmission and making treatment difficult.* ***Methodology:*** *The investigation involved 72 clinical samples, including blood, abscesses, wounds, and bedsores. Clinical isolates were identified using Bergey's Manual of Determinative Bacteriology, and antibiotic sensitivity was assessed using NCCLS guidelines. SDS PAGE profiles were used to analyze cell protein profiles.* ***Results:*** *The majority of clinical bacterial isolates (52.77%) are Staphylococcus aureus, which is followed by Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, and Staphylococcus epidermidis. Norfloxacin has a 59.72% sensitivity against clinical bacterial isolates, followed by amikacin and chloramphenicol, whereas penicillin and cefoperazone have no effect on these isolates. The difference between norfloxacin's MIC and MBC was highly significant ($p < 0.01$ HS). The most potent antibacterial action was shown by clove extract and clove volatile oil. Clove extract and norfloxacin together exhibit synergistic suppression of clinical bacterial isolates. Protein bands of the bacteria before and after treatment were different for the control and norfloxacin clove extract-stressed bacterial isolates.* ***Conclusions:*** *This study investigates antibacterial properties of medicinal plants, aiming to prevent drug resistance and develop strategies for future antibiotic synthesis through structural clarification.*

INTRODUCTION

Antibiotics have saved millions of lives from bacterial diseases, but increased antibiotic usage has led to antimicrobial resistance (AMR), which increases the risk of serious disease and death due to the bacteria's ability to survive and proliferate[1]. The extensive use of antibiotics, especially in developing countries, provides bacteria with enough opportunity to acquire antimicrobial resistance (AMR), which can have detrimental effects such as markedly elevated morbidity and death[2]. Antibiotic-resistant bacteria are posing a global health threat, with the World Health Organisation recognizing them as one of the top three dangers, second only to cardiovascular disorders[3].

Staphylococcus aureus and *Escherichia coli* are the primary causes of many human and animal diseases. The first causes infections of soft tissues, skin, and surgical site. Hospital-acquired bacteremia is often caused by *Staphylococcus aureus*, which is often associated with respiratory tract infections[4]. Depending on the organism producing the infection, wound infections can be either non-purulent or purulent (pus-forming). Most aerobic organisms seen in wounds are Gram-positive cocci, such as *Enterococci spp, S. epidermis, S. aureus*, and *Streptococcus pyogenes*. Also Gram-negative bacilli, such as *P. aeruginosa, K. pneumoniae, E. coli*, and *P. vulgaris,* can cause wound infections[5]. Gram-positive bacteria like *S. aureus* and coagulase-negative staphylococci like *S. epidermidis* and *S. saprophyticus* are responsible for the majority of bacterial eye infections[6,7].

Antimicrobial resistance caused by antibiotic misuse lead to significant discoveries for modern medical science and prevented premature deaths[8]. According to the World Health Organisation, as over 80% of the world's population relies on traditional medicine[9,10]. Numerous phytochemical classes found in medically significant plants have therapeutic properties that can be used to treat newly discovered human illnesses[11]. The volatile molecules can penetrate the lipids of the bacterial cell membrane because they are hydrophobic, which causes the cell wall's structure to be disrupted and its permeability to increase[12]. This study aime to investigate the antibacterial activity of specific plant extracts and volatile oils against bacterial isolates in an effort to find possible supplements to traditional antibiotics that could help fight resistance.

METHODOLOGY

Seventy two clinical samples (34 males and 38 females) were taken from the laboratory of the Hilla General Teaching Hospital in Hilla city, Babylon Governorate, Iraq, representing a variety of illnesses (such as ear infections, eye infections, bed sores, abscesses, wounds, and blood). The participants age in the experimental trial ranged from 1 to 72 years. Samples were collected between September 2023 and June 2024.

Identification of organisms was made by the size and shape of the colony, whether it is opaque or translucent, mucoid or dry, the production of a characteristic colony color or any change in the color of the agar (such as a greenish discoloration of the medium caused by *P. aeruginosa* exopigment), the swarming growth of *Proteus* species, the presence of hemolysis on blood agar, lactose fermentation on MacConkey's agar medium, and whether not.

Gram staining is a method used to distinguish between Gram-negative and Gram-positive bacteria. The stained slides are then viewed under a microscope using an oil immersion lens[13]. Positve catalase test is detected by bubbles or froth in tested samples[14]. Bacteria were identified using Bergey's Manual of Determinative Bacteriology[15]. Indole test, Methyl red – Voges proskauer test, Triple suger iron agar test, citrate test, urease test, oxidase test were biochemical test for Gram negative bacilli, while coagulase test, mannitol salt agar and novobiocin susceptibility test were for Gram positive cocci.

Using disc diffusion, the antibiotic sensitivity of the isolated bacteria was assessed in accordance with the guidelines of the NCCLS antimicrobial disc susceptibility test[16]. The single colony from each isolated strain should be cultured for 24 hours at 37°C in 5 mL of sterile nutritive broth in order to bring the turbidity down to 0.5 McFerland standard salin. For each strain, sterile swabs were used to give a broth inocula to one or two Muller Hinton agar plates with antibiotic discs affixed at regular intervals. Cefoperazone (CFP) (75µg), amikacin (AK) (30µg), chloramphenicol (C) (30µg), ampicillin (AM) (10µg), erythromycin (E) (15µg), norfloxacin (NOR) (10µg), and tobramycin (TOB) (10µg) were among the antibiotics used in the experiment. In order to achieve total growth inhibition visible to the naked eye, plates were incubated at 37°C for 24 hours while inhibitory zone diameters and discs were measured[17].

The MIC and MBC were ascertained by altering the dilution process. In test tubes, sterile nutritional broth was used to dilute norfloxacin at various concentrations: 500, 250, 125, 62.5, and 31.25 µg/ml. A standard wire loop (Merck) was used to inoculate test tubes containing 1 ml of the various dosages of norfloxacin in nutritive broth with a loopful (10 µl) of bacterial isolates culture, 0.5 McFarland standard[18], following an incubation period of 18 to 24 hours at 37°C, the tubes were examined for growth or turbidity. As an inoculant, a loopful of broth from each test tube that showed no signs of growth was subsequently placed to a nutritional agar plate.

Mentha spicata, Origanum majorana, Thymus vulgaris, Allium sativum, Mentha piperita, Cinnamomum verum, Syzygium aromaticum, Foeniculum vulgare, Zingiber officinale, and *Matricaria recutita* plant extracts were made along with control samples using alcohol, hot water, and cold water. To make the medicinal plant extracts, the active components in the plant samples were ground into a fine powder in a grinder after being completely dried. After that, each powder sample was stored in an airtight jar. An ethanolic extract, a cold water extract, and a hot water extract were made from each medicinal plant. This was accomplished by dissolving 10 grams of finely ground plant material in 50 milliliters of ethanol, hot water, then cold water. The contents are kept for 48 hours in a rotating shaker. The extracts were filtered and dried in a 40°C hot air oven. After that, the extracts were stored for further study at 4°C in a refrigerator[19,20]. The volatile oils (*Mentha piperita, Mentha spicata, Origanum majorana, Syzygium aromaticum, Foeniculum vulgare,* and *Matricaria recutita*) of the Sekem group company, Egypt, were produced by steam distillation and kept at 4°C in the dark prior to usage.

The protein content of both treatment (a combination of *Syzygium aromaticum* extract and norfloxacin MIC for each tested organism) and control (non-treated) bacteria was extracted and assessed[21].

The criteria for inclusion and exclusion. The patient groups included those with blood infections, wounds, abscesses, bed sores, eye infections, and ear infections. People who did not have these infections were excluded.

The data was analyzed using an IBM (PC/AL) compatible computer and the statistical analysis tool package, Initiate software, version 3.03 (Graph Pad, USA). The probability level that corresponds to the varied grades of statistical significance is shown below: $P<0.05$ indicates non-significant, $P>0.05$ indicates significant, and $P>0.01$ indicates extremely significant.

RESULTS

Clinical samples were obtained from patients with a range of ailments, including blood, wounds, abscesses, bed sores, eye infections, and ear infections. The age range of the patients were 1–62 years for males and 1–72 years for females. The sample consisted of 38 females and 34 males. Blood and abscesses make up the majority of clinical samples (25% and 25%, respectively), followed by wounds (23.62%), ear (13.89%), eye (6.94%), and bed sores (5.55%). Gram-positive cocci make up 58.34% of the isolates, followed by Gram-negative bacilli (41.66%) and Enterobacteriaceae (29.16%) (Table 1).

Table 1: The distribution of isolates of bacteria

Pathogenic Bacterial isolates	Positive samples	Percentage of distribution (%)
S. aureus	38	52.78
P. aeruginosa	9	12.50
E. coli	7	9.72
K. pneumonia	7	9.72
Proteus vulgaris	7	9.72
S. epidermidis	4	5.56
Total	72	100.0

59.72% of pathogenic bacteria are responsive to norfloxacin, followed by amikacin 44.4% and chloramphenicol 43.05%, whereas 91.67% are resistant to ampicillin. However, penicillin and cefoperazone had no effect on them, as illustrated in Figure 1.

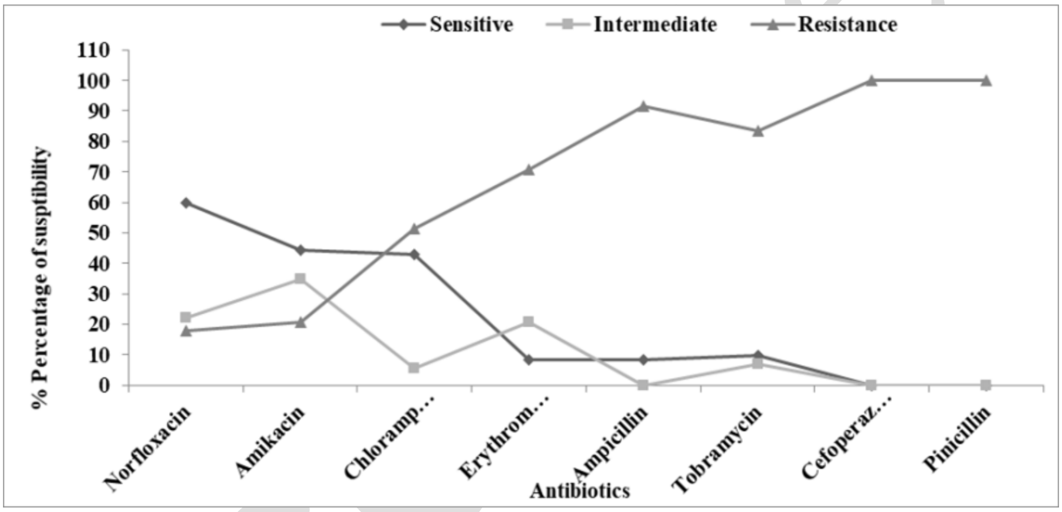


Fig. 1: The sensitivity test findings of the pathogenic isolates to a number of antibiotics

The antibiotic norfloxacin (500 µg/mL) produced the highest MIC against *K. pneumoniae* 27 and *S. aureus* 33, while the antibiotic norfloxacin (500 µg/mL) produced the highest MBC against *S. aureus* 33 and *K. pneumoniae* 4 and 27. The difference between norfloxacin's MIC and MBC was highly significant ($p < 0.01$ HS) (Table 2).

Clove, cinnamon, thyme, and fennel extracts were the most efficient against infections. The extracts of peppermint and chamomile that worked best were those made with alcohol and cold water. In boiling and cold water extracts, ginger and spearmint extracts performed the worst. Garlic performed the worst in alcohol extracts, cold and boiling water, whereas marjoram extract performed the best in boiling water and alcoholic extracts. The bacterial isolates were unaffected by alcohol, hot water, or cold water control samples (Figure 2,3).

As seen in Table (3), the oils of clove, spearmint, peppermint, and fennel have antagonistic effects on pathogenic bacteria, whereas marjoram oil has a moderate effect and chamomile oil has no effect on bacterial isolates.

Table 2: Norfloxacin's bactericidal concentrations (MBCs) and minimal inhibitory concentrations (MICs)

Bacterial isolates	Serial No.	Norfloxacin	
		(MIC) (µg/ml)	(MBC) (µg /ml)
K. pneumoniae	4	125	500
K. pneumoniae	7	31.25	125
P. aeruginosa	17	125	250
S. aureus	20	62.5	125
K. pneumoniae	27	500	500
S. aureus	32	31.25	62.5
S. aureus	33	500	500
Proteus vulgaris	38	31.25	62.5
S. aureus	40	62.5	62.5
S. aureus	41	31.25	31.25
S. aureus	54	31.25	62.5
Proteus vulgaris	58	125	250
P. aeruginosa	59	31.25	62.5
E. coli	65	31.25	125
E. coli	66	62.5	125
E. coli	67	31.25	62.5
$\bar{X}$		113.28	181.64
SD		155.15	170.10
t		-2.97	
P		0.0096 HS	

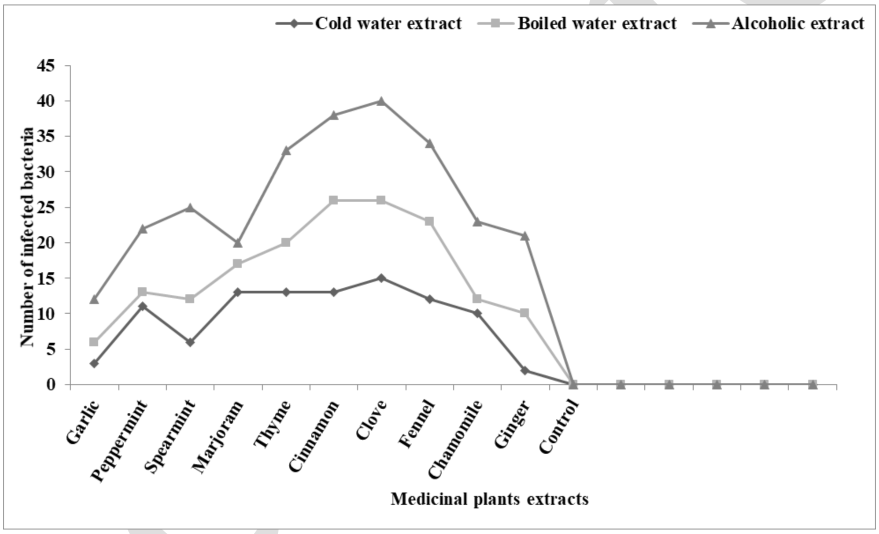
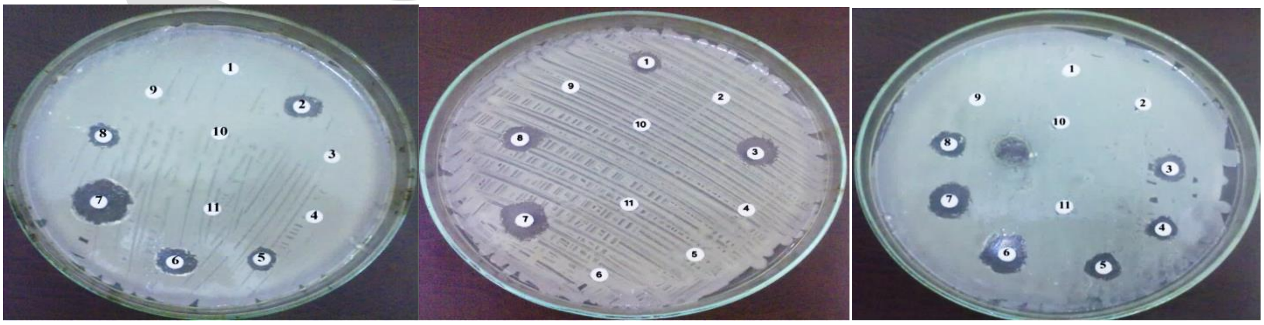


Fig. 2: Bacterial isolates' sensitivity to alcoholic, cold water and boiling water plant extracts



Cold water extracts Boiling water extracts Alcoholic extracts

Fig. 3: Using the disc diffusion method, the effects of cold, boiling water, and alcoholic plant extracts on *K. pneumonia* number 4. While the chamomile, ginger, and control extracts are unsuccessful against *K. pneumonia* number 4, the clove extract in the figure is effective against the pathogen.
1- Garlic, 2- Peppermint, 3- Spearmint, 4- Marjoram, 5- Thyme, 6- Cinnamon, 7- Clove, 8- Fennel, 9- Chamomile, 10- Ginger, 11- Control.

Table 3: The inhibitory zone diameter (mm) of different essential oils against pathogenic bacterial isolates.

Bacterial isolates	Serial No.	Diameter of inhibition zones (mm)					
		Clove	Chamomile	Peppermint	Marjoram	Fennel	Spearmint
K. pneumoniae	4	12	ND	8	8	15	10
K. pneumoniae	7	10	ND	14	10	18	12
P. aeruginosa	17	13	ND	12	10	15	12
S. aureus	20	13	ND	15	10	15	15
K. pneumoniae	27	15	ND	12	9	18	13
S. aureus	32	15	ND	15	12	17	12
S. aureus	33	22	ND	14	11	15	20
Proteus vulgaris	38	18	ND	15	11	13	15
S. aureus	40	15	ND	13	8	17	19
S. aureus	41	12	ND	15	10	15	12
S. aureus	54	20	ND	15	11	15	15
Proteus vulgaris	58	15	ND	10	11	14	12
P. aeruginosa	59	13	ND	12	12	13	10
E. coli	65	13	ND	10	10	12	15
E. coli	66	15	ND	10	12	15	13
E. coli	67	15	ND	9	9	15	15

ND: No detection / Zero

The results of the combination effect, showing that the MIC of norfloxacin antibiotics and plant extract clove had a significant synergistic effect against *K. pneumoniae* numbers (4, 7, and 27), *S. aureus* numbers (20, 33, 40, 41, and 54), *E. coli* numbers (66 and 67), *P. aeruginosa* numbers (17 and 59), and *P. vulgaris* numbers (38 and 58) more than when norfloxacin was used alone. When norfloxacin and spearmint volatile oil were combined, they showed a greater synergistic impact against *K. pneumoniae* number (4), *S. aureus* numbers (20, 40, and 41), *E. coli* numbers (66 and 67), *P. aeruginosa* numbers (17 and 59), and *P. vulgaris* number (58) than when norfloxacin was used alone. Highly significant difference was observed between MIC of norfloxacin and clove extract ($p<0.01$ HS), while non significant difference was observed between MIC of norfloxacin and cinnamon extract, fennel oil, spearmint oil respectively ($p>0.05$) (Table 4).

Table 4: The diameter of the inhibition zone (mm) of the combination of the norfloxacin MICs and other antibacterial agents

Bacterial isolates	Serial No.	Norfloxacin MICs		Diameter of inhibition zones (mm)			
				Plant extract		Volatile oil	
				Clove	Cinnamon	Fennel	Spearmint
		µg/ml	IZ	IZ	IZ	IZ	IZ
K. pneumoniae	4	125	8	18	10	ND	10
K. pneumoniae	7	31.25	10	15	11	8	10
P. aeruginosa	17	125	12	15	13	8	13
S. aureus	20	62.5	12	13	14	15	15
K. pneumoniae	27	500	12	16	10	ND	ND
S. aureus	32	31.25	10	10	ND	ND	ND
S. aureus	33	500	15	19	11	ND	ND
Proteus vulgaris	38	31.25	10	15	15	10	10
S. aureus	40	62.5	15	20	15	ND	18
S. aureus	41	31.25	10	15	ND	8	12
S. aureus	54	31.25	10	12	12	ND	10
Proteus vulgaris	58	125	12	15	12	12	14
P. aeruginosa	59	31.25	10	15	13	10	15
E. coli	65	31.25	16	12	ND	ND	10
E. coli	66	62.5	10	18	12	15	20
E. coli	67	31.25	10	14	ND	8	13
$\bar{X}$			11.37	15.12	9.25	10.44	13.07
SD			2.24	2.68	5.70	5.76	6.03
t				-4.29	-1.27	0.22	-1.75
P				0.00018 HS	0.216 NS	0.833 NS	0.095 NS

IZ: Inhibition zone

Before and after treatment, the bacteria's protein bands were different. For instance, the protein band molecular weight of 48.092 KD in *S. aureus* number 20 disappeared after treatment, but the molecular weights of each protein band in *E. coli* number 66 were 9.070, 90.767, and 100.800 KD after treatment (Figure 4).

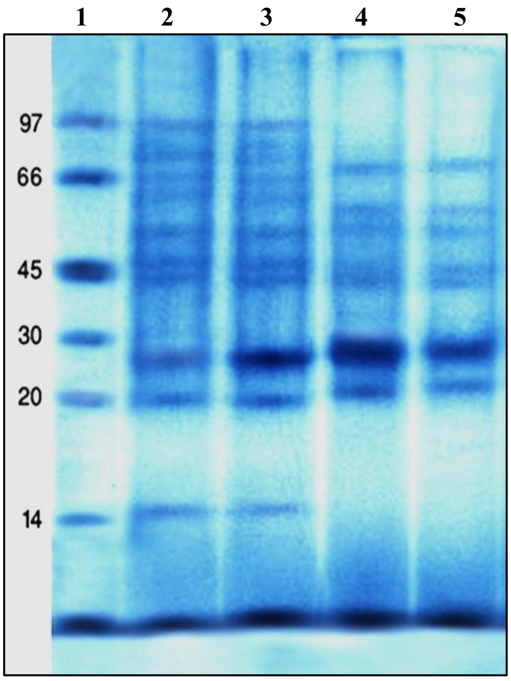


Fig. 4. Protein analysis for clinical bacterial isolates before and after stress.

1- Marker. 2- *S. aureus* number 20 before stress (control). 3- *S. aureus* number 20 after stress. 4- *E. coli* number 66 before stress (control). 5- *E. coli* number 66 after stress.

*Stress = Bacterial isolates number 20, and 66 cultivation with norfloxacin & clove.

DISCUSSION

The aim of this study was to assess how well different plant extracts and essential oils worked against clinical isolates that were resistant to multiple drugs. Our primary findings demonstrated that alcoholic extracts of thyme, clove, fennel, and cinnamonom are more efficient than other plant extracts as antibacterial agents against clinical isolates that are resistant to numerous medications. Clinical isolates that are resistant to numerous medications can be effectively combatted by the volatile oils of peppermint, clove, fennel, and spearmint. Antibiotics will increase the sensitivity of multidrug-resistant clinical isolates when they are combined with plant extracts or volatile oils; for instance, clove extract and norfloxacin will increase the sensitivity of bacterial isolates to the antibiotic.

Blood, wounds, and abscesses were the primary sources of *S. aureus, P. aeruginosa, E. coli, K. pneumonia, P. vulgaris*, and *S. epidermidis* isolates in this study. The bacteria that induce pus formation or wound infections are *Proteus, Neisseria, Actinomyces, S. aureus, Clostridium, Vibrio vulnificus, E. coli*, and *Candida*. In this study, 34.7% of wound swabs contained *S. aureus*. *S. aureus* (38.5%) and *P. aeruginosa* (29.4%) were the most prevalent wound bacteria among the 143 individuals (59.8%) who were community members. Hospitalization was experienced by 96 participants (40.2%). *S. aureus* (36.5%), *E. coli* (15.6%), and *P. aeruginosa* (13.5%) were the most common bacteria in the samples[22].

The bacterial isolates used in this study are 100%, 100%, and 91.66% resistant to ampicillin, cefoperazone, and penicillin, respectively, and 59.72% and 44.40% sensitive to norfloxacin and amikacin. Amikacin is the most efficient antibiotic against Gram-negative bacteria, according to research on drug susceptibility patterns[12]. Certain multidrug-resistant strains of *Citrobacter freundii, E. coli, K. pneumoniae*, and *S. aureus* were resistant to twelve separate antimicrobials. Of the 188 *S. aureus* isolates, 92 (18.9%) had erythromycin resistance, with cefotaxime (77.1%) and ampicillin and penicillin G (86.7%) showing the highest resistance[23].

Commercial antibiotics are used to assess pathogenic isolated UTI-causing bacteria. The urine isolates were susceptible to ofloxacin, nitrofurantoin, and nalidixic acid, but resistant to ampicillin and trimethoprim-sulphamethoxazole[24]. The most vulnerable urine isolates were sensitive to gentamicin (58.33%), amikacin (70.27%), nitrofurantoin (66.60%), norfloxacin (64.28%), and meronem (76.19%)[25]. These results verify that all urine isolates were ampicillin intolerant but susceptible to amikacin, norfloxacin, and ofloxacin.

In the present investigation, ginger extract is less efficient than clove extract against bacterial isolates. Aqueous and organic clove extracts were shown to be more effective (most MIC values fell between 13.33±2.67 and 256±0.00 µg/mL). Clove extracts shown much greater efficiency ($p<0.05$) against the majority of the pathogens studied when compared to other herbal extracts. Remarkably, both extracts exhibited outstanding activity[26]. Among the plant extracts, clove was more effective at inhibiting growth than eucalyptus and ginger against all strains that were tested. The inhibitory zone of clove was significantly bigger against reference strain PA01 (20.06 ± 0.57; $P < 0.05$), clinical isolates 2 (17.6 ± 0.57; $P < 0.01$), and 4 (17.3 ± 0.57; $P < 0.01$)[27]. All tested bacterial strains, including *Salmonella paratyphi, Enterococcus cloacae, S. aureus, E. coli, Citrobacter* spp, and *Candida albicans*, were eliminated by clove extract[28].

Using the disc diffusion method, the antibacterial properties of cardamom, cinnamon, and clove essential oils (EOs) were evaluated against nine strains of Gram-positive bacteria, four strains of Gram-negative bacteria, seven molds, and two yeasts in comparison to phenol.

The antimicrobial spectra (width of inhibition zones) of 10% clove EO were 1.48 times larger than those of 10% phenol, indicating that clove EO exhibited the strongest antibacterial activity[29]. With the exception of *K. pneumoniae* and *P. aeruginosa* for thyme and *K. pneumoniae* for clove, all bacterial species under study were vulnerable to the bactericidal activities of thyme and clove essential oils. As shown by a similar study the essential oil of cinnamon was shown to have bactericidal effect against both Gram-positive and Gram-negative bacteria[30]. The high concentration of carvone (67%) in *Mentha spicata* essential oil showed strong antibacterial action against *Escherichia coli* and *Staphylococcus* aureus similar to other reports[31].

This investigation validates our conclusion that clove extract works well with ofloxacin but not with garlic because the combination of plant extracts and antibiotics can either boost or decrease their efficiency[32]. Mint oil and norfloxacin work together to combat *E. coli, P. aeruginosae, Klebsiella species, Candida albicans*, and *Streptococcus species*[33].

CONCLUSION

Over time, bacteria develop resistance to antibiotics, hence efforts have been made to identify natural and safe substitutes. Essential oils and plant extracts have been utilized to eliminate dangerous germs that are resistant to medicines and to boost the potency of antibiotics by including these organic components. Clinically resistant bacterial isolates were affected by oils of clove, spearmint, peppermint, and fennel as well as extracts of clove, cinnamon, thyme, and fennel. When norfloxacin's MIC is paired with plant extracts and volatile oils, there are synergistic effects; clove extract and norfloxacin worked best together. Therefore, with further structural clarification, the biological activity that prevents the growth of bacteria in the plants indicated above might be used as a precursor for the future development of novel antibiotics.

Ethics Statement

The study was approved by Al-Mustaqbal University, The College of Health and Medical Technologies, Ethical Committee under the number Lab7/2023.

Conflicts Of Interest

No conflicts of interest are disclosed.

Data Availability

Upon request, the corresponding author will provide all the data used to support our study's conclusions.

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