

Antibacterial Activity of Essential Oils and in Combination with Some Standard Antimicrobials against Different Pathogens Isolated from Some Clinical Specimens

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Abstract The clinical effectiveness of most of the marketed antimicrobials is found to be threatened by the rapid emergence of multidrug resistant pathogens which increase the need to find alternatives. Hundred years ago, essential oils have been known for their biologic activities in the folkloric medicine in many countries. The objective of our study was to investigate the antibacterial activity of some essential oils against different microorganisms and to study the possible effects between the tested oils and some standard antimicrobials. The antibacterial activity of 11 essential oils was evaluated against *Staphylococcus aureus*, *E. coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and 50 clinical strains isolated from different infections each alone and in combination with some standard antimicrobials using well diffusion method. Minimum inhibitory concentrations were determined using linear regression analysis. Results showed that all tested essential oils have good antimicrobial activity. As Coriander oil showed the highest antimicrobial activity against *Staphylococcus aureus* and *E.coli* followed by Origanum and Ivy oil. Cumin oil showed the highest activity against *E. coli* followed by Origanum oil while Chamomile and Onion oil showed the highest activity against *Pseudomonas aeruginosa*. In-vitro interaction between the tested antimicrobials and oils showed variable results against the tested bacteria. The results are of significance in health care system and microbial diseases treatment. As our study showed that essential oils possessed good antimicrobial activity against the tested strains. Most of essential oils/antimicrobials combinations showed synergistic effects. Essential oils can be used as adjuvant to antibiotic therapy.

Keywords: chamomile, ivy, cumin, onion, essential oils, antibacterial, SEM

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1. Introduction

Infectious diseases represent a critical problem to health and they are one of the main causes of morbidity and mortality worldwide [1]. Emergence of multidrug resistance of these microorganisms is a major medical problem [2]. Despite of the medical need for new antimicrobials, the number of antibacterial compounds present in clinical development is limited and most of the antibiotics developed in the past decade are derivatives or modifications of existing antibiotic classes [3].

Recently, it is important to search for new antimicrobials, or new natural alternatives having antimicrobial activity such as plant extracts and essential oils [4]. The known success of traditional medicine has guided the search for new chemotherapeutic alternatives to eliminate the infections caused by drug-resistant microbes and to reduce the harm caused by antibiotic [5].

Essential oils (EOs) are known for their antibacterial, antifungal, antiviral, insecticidal and antioxidant properties [6]. They are widely used in Folk medicine and food industry for these purposes. Essential oils, also known as volatile oils, are complex mixtures of volatile constituents biosynthesized by plants [7]. EOs contain 2 biosynthetically related groups. These main groups include terpenes, terpenoids and aromatic, aliphatic constituents and some hydrocarbons also exhibit antimicrobial effects [8]. Studies have shown that EO bacterial cell targets include the cell wall and membrane, thereby disturbing ATP production and pH homeostasis [9].

In this study, we investigated the in-vitro antibacterial effects of 11 essential oils of the commonly used herbs in the folkloric medicine in Egypt against *S. aureus* (ATCC 6538), *K. pneumoniae* (ATCC 10031), *Ps. aeruginosa* (ATCC 10145), *E. coli* (ATCC 8739) and 50 clinical strains isolated from different infections and their in-vitro possible effects with antibiotics.

2. Materials and Methods:

2.1. Microorganisms

Standard strains of *S. aureus* (ATCC 6538), *K. pneumoniae* (ATCC 10031), *Ps. aeruginosa* (ATCC 10145), *E. coli* (ATCC 8739) were used as indicator microorganisms for detection of the antimicrobial activity. Fifty clinical strains obtained from patients suffering from different infections were used for screening tests. *S. aureus* (8 strains), *E. coli* (12 strains), *K. pneumoniae* (21 strains) and *Ps. aeruginosa* (9 strains).

2.2. Essential Oils

Essential oils were provided by a commercial company (Extraction and processing of oils are done in agriculture research center (ARC)), Egypt under supervision of Dr. Abd Elfattah Mohamed Hassani El Zahwey (Prof. of medicinal and aromatic dep., ARC).

Oils were cumin (*Cuminum cyminum*, Family Apiaceae), Parsely (*Petroselinum crispum*, Family Apiaceae), Origanum (*Origanum majorana*, Family Lamiaceae), Ivy (*Dolichos lablab*, Family Leguminosae), Chamomile (*Anthemis nobilis*, Family Compositae), Onion (*Allium cepa*, Family. Lilaceae), Basil (*Ocimum basilicum*, Family Lamiaceae), Leek (*Allium ampeloprasum* L., Family Amaryllidaceae), Peppermint (*Mentha piperita* Family Lamiaceae), Spearmint (*Mentha spicata* Family Lamiaceae), Coriandar (*Coriandrum sativum* Family Apiaceae). Analysis of oils are done in The Arabian company for Preserving and Manufacturing Agricultural Crops, APMAC, Egypt). Oils were stored at 4°C in dark glass bottles.

2.3. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing for Amoxicillin/Clavulanic, Amoxicillin/Fluxacillin, Cephalexin, Cefotaxime, Ceftriaxone, Amikacin, ciprofloxacin using disc agar diffusion method according to CLSI [10].

2.4. Evaluation of Antibacterial Activity Using Well Diffusion Technique

Microorganism (0.5 ml) of 1×10^6 CFU/ml (0.5 Mcfarland turbidity) were plated in sterile petri dishes then 20 ml of sterile, molten and cooled (45°C) Muller Hinton agar media was added to all petri dishes. The plates then were rotated slowly to ensure uniform distribution of the microorganisms and then allowed to solidify on a flat surface. After solidification, four equidistant and circular wells of 10 mm diameter were carefully punched using a sterile cork borer. Each sample (5mg/ml) was applied as triplicate. The plates were allowed to stand for one hour for prediffusion of the extract to occur then, incubated overnight at 37°C. All plates were examined and zones of inhibition were recorded [11].

2.5. Determination of the Minimum Inhibitory Concentration (MIC):

Two fold serial dilutions were performed for essential oils. Equal volumes from each were applied separately to

each well. All plates were incubated overnight at 37°C, then collected and zones of inhibition that developed were measured. The average of the zones of inhibition was calculated. The minimum inhibitory concentration (MIC) was calculated by plotting the natural logarithm of the concentration of extract against the square of zones of inhibition. A regression line was drawn through the points. The antilogarithm of the intercept on the logarithm of concentration axis gave the MIC value.

2.6. Testing the Possible Effects between the Tested Essential Oils and Antibiotics.

Sterilized blank paper discs 6 mm in diameter were saturated with 5% DMSO as negative control, 20 µl of essential oil, antibiotic each alone and in combination. Discs were placed onto the agar plates which had previously been inoculated with the above organisms, incubated at $37 \pm 0.1^\circ\text{C}$ for 24 hr [12,13,14]. At the end of the period, the diameter of inhibition zones were measured in mm.

2.7. Scanning Electron Microscopy (SEM) Study of the Bacteria after Oil Exposure:

Overnight culture of the tested standard strains were grown on MH(Muller Hinton) agar at 37°C was put in a saline solution comprising of 0.2% Tween-80. Essential oil at Sub-MIC concentrations were prepared and added into this suspension and was incubated at room temperature. After 24 hours, the bacterial cells were centrifuged at $8000 \times g$ for 15 minutes. The bacterial cells were then washed with 0.1 mol/l Tris-acetate buffer (PH 7.1), fixed in tris-acetate buffer containing 1.5% glutaraldehyde, and then freeze-dried. Each of the bacterial culture was observed by SEM (Hitachi, Japan) at magnifications of 5000, 7500 × g. The bacterial cell suspension in saline with no essential oil treatment served as a negative positive control [15].

3. Results and Discussion

Fifty five samples were collected from patients suffering from GIT infection, UTI, RT infection and wound infections, it was found that 50 (90.9%) samples were positive for growth. All *S. aureus* were isolated from wound infections while all *Ps. aeruginosa* isolates were isolated from respiratory tract infections, *K. pneumoniae* was the most prevalent pathogen isolated from GIT and urinary tract infections followed by *E. coli* (Figure 1).

Ps. aeruginosa, *E. coli* and *S. aureus* were completely resistant to amoxicillin/ flucloxacillin and cefotaxime. In addition, All *Ps. aeruginosa* was resistant to Amoxicillin/clavulanic acid and cephalexin. Amikacin showed the highest activity against the tested isolates followed by ciprofloxacin (Figure 2). For standard strains, Amikacin, cefoperazone and ciprofloxacin were active against all standard strains (Table 1).

Eleven essential oils were screened for their antibacterial activity and their MIC against the tested strains (Table 2 and Figure 3), It was found that coriander, Origanum and Ivy showed the highest antibacterial activity (the lowest MIC) against *S. aureus* and *K. pneumoniae*. Onion oil showed also good antibacterial

activity against *S. aureus* (MIC was 12.082 µg/ml). Chamomile showed the best activity against *Ps. aeruginosa* followed by onion and Origanum oil while

Cumin, Origanum and chamomile showed the highest antibacterial activity (MIC 7.2, 7.5 and 7.7 µg/ml) against *E. coli* (Table 2).

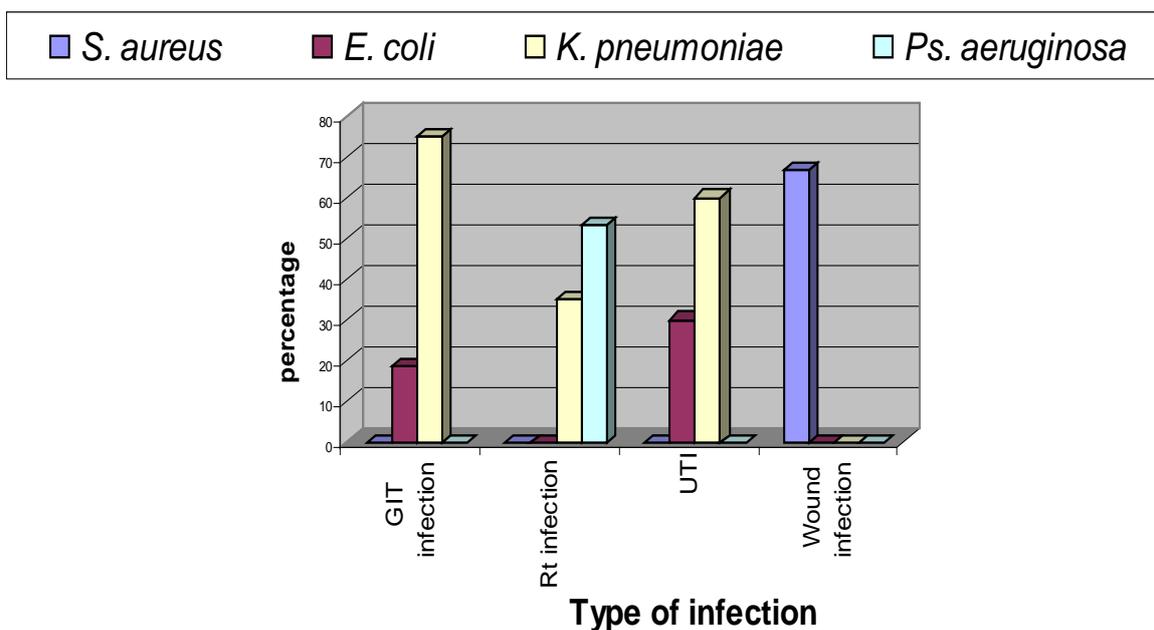


Figure 1. Prevalence of different microorganisms isolated from different types of infection

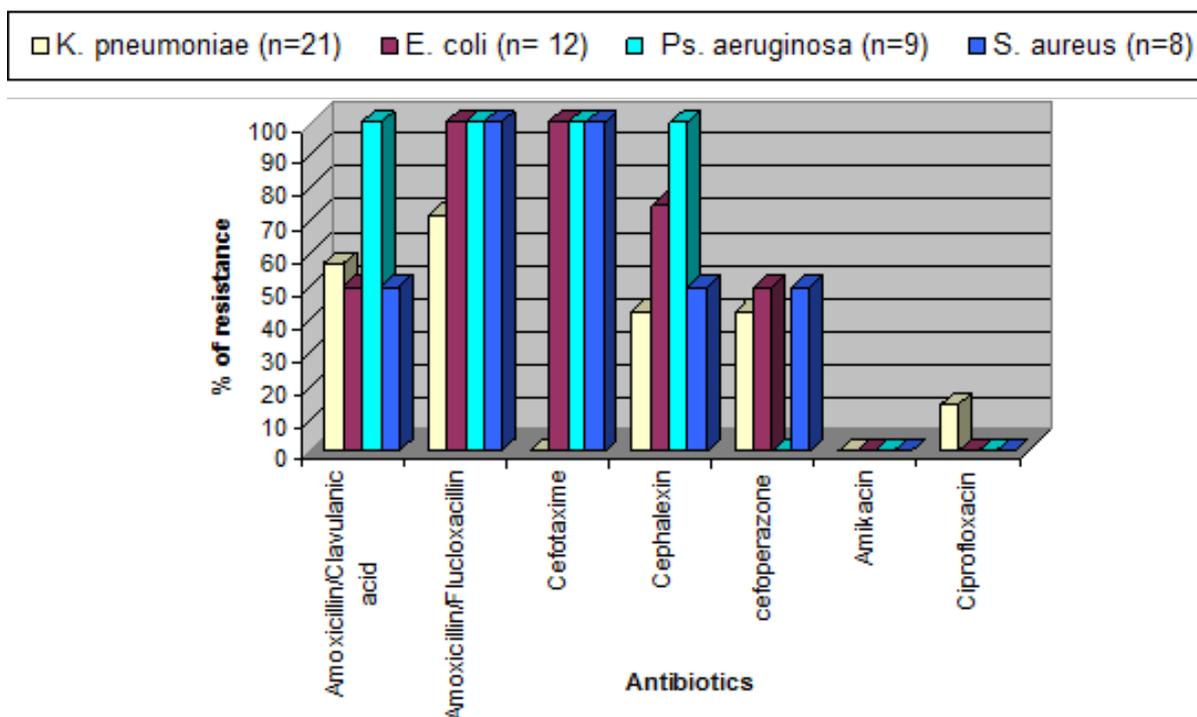


Figure 2. Resistance pattern of the isolated microorganisms

Table 1. Antibiotic susceptibility of the tested standard strains

Antibiotics		Inhibition zones diameter (mm)			
		<i>S. aureus</i>	<i>Ps. aeruginosa</i>	<i>K.pneumoniae</i>	<i>E.coli</i>
AMIKACIN	AK 30	28 (S)	25 (S)	24 (S)	25 (S)
AMOX./CLAV.	AMC 30	48 (S)	6 (R)	25 (S)	22 (S)
AMOX./FLUXACILLIN	AF 10	40 (S)	6 (R)	20 (S)	9 (R)
CEFOPERAZONE	CFP 75	32 (S)	32 (S)	34 (S)	26 (S)
CEFOTAXIME	CTX 30	40 (S)	6 (R)	32 (S)	30 (S)
CEPHALEXIN	CL 30	6 (R)	6 (R)	22 (S)	23 (S)
CIPROFLOXACIN	CIP 5	36 (S)	40 (S)	35 (S)	35 (S)

S: sensitive; R: Resistant according to CLSI 2011.

Table 2. Minimum inhibitory concentration (MIC) of essential oils against the standard strains ($\mu\text{g/ml}$)

Strains	Oils										
	Cum	Parss	Corian	Origan	Ivy	Chamo	Onion	Bas	Leek	Pipp	Spear
<i>S. aureus</i>	1161	8.21	11.1	11.1	11.99	1056	12.082	399.4	16.346	15.99	17.116
<i>Ps. aeruginosa</i>	84.97	1202	8.21	17.3	42.46	5.11	11.91	963.69	572.08	31.92	34.54
<i>K. pneumoniae</i>	204.87	19.53	31.92	6.287	6.596	6.89	105.3	22.54	52.11	9.56	5411.30
<i>E. coli</i>	7.219	6.9	5.99	7.571	83.55	7.72	31.92	225.88	34.55	32.62	394.19

Bold numbers refer to low MIC values (high activity).

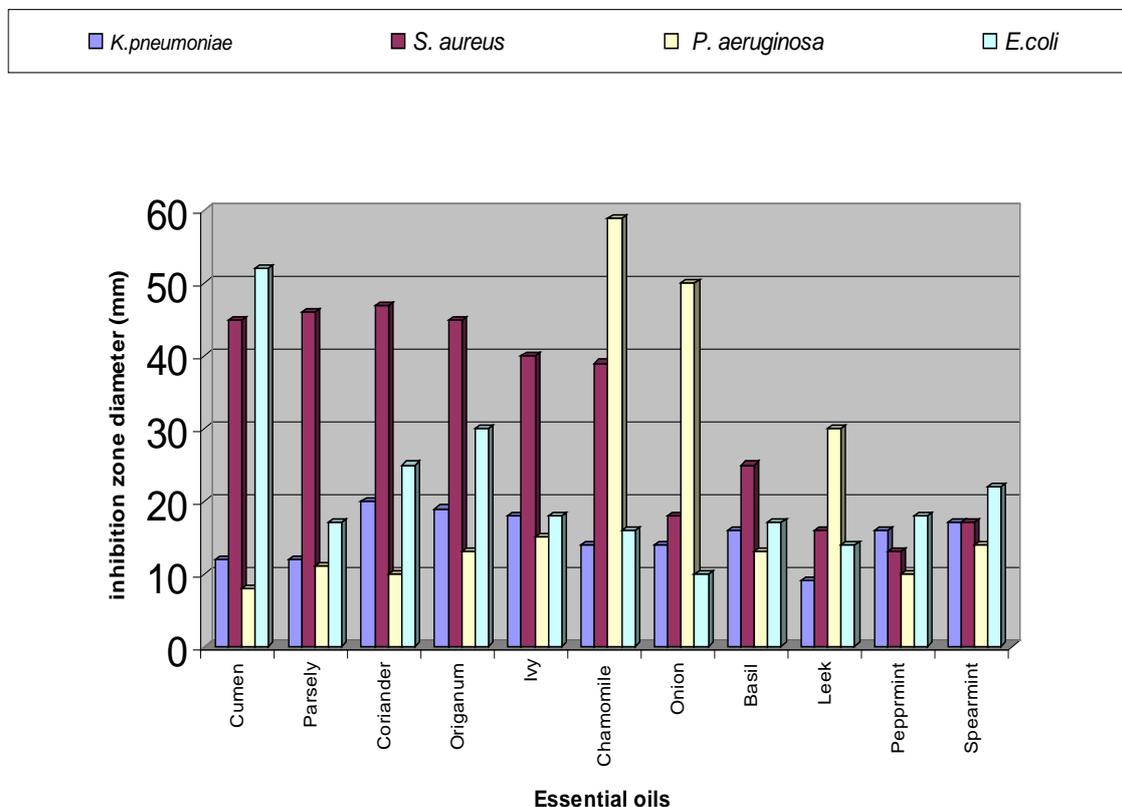


Figure 3. Antimicrobial activity of essential oils against the tested standard strains

Studies on the antimicrobial activity of dipropyl disulfide and dipropyl trisulfide, which are the main components in onion, leek, shallot and chive EO, are few. However, it was noted that dipropyl trisulfide demonstrated antimicrobial activity against *Staphylococcus aureus* [16]. The antibacterial activity of these EO may be related to the propyl derivatives.

Antimicrobial activity of origanum essential oil was reported in many studies [17,18,19]. Until now its potential therapeutic roles such as diaphoretic, carminative, antispasmodic, antiseptic, and tonic properties have been recognized [20]. In addition, it has been used widely in China as a kind of food additive because it has a broad spectrum of action against bacteria, a rapid effect, and little residue, and now its antibacterial effect has been researched in vitro [20-25] and in vivo [26] throughout the world. In our study, Origanum oil showed high activity against all tested strains. Sienkiewicz *et al.*, [27] showed that Origanum oil was active against all clinical strains of *E. coli* and *Ps. aeruginosa* but strains of *E. coli* were more sensitive to the tested oil. Essential oil from *Origanum heracleoticum* L. inhibited the growth of *Escherichia coli* and *Pseudomonas aeruginosa* clinical strains with different patterns of resistance. Also, Chaudhry *et al.*, [28] reported that Origanum oil have activity against all tested bacteria (*K. pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *S. paratyphi* B, *Serratia*

marcescens and *Shigella dysenteriae*). In addition, Bouhdid *et al.*, [29] found that treatment of *Ps. aeruginosa* and *S. aureus* with *Origanum compactum* EO resulted in a more significant injury to *Ps. aeruginosa* cells than to *S. aureus*. At the minimum inhibitory concentration (MIC) and at 1.5 X MIC. *Ps. aeruginosa* cells were unable to grow, cell permeability was disturbed, and membrane potential was disrupted.

Table 2 and Figure 3 showed that chamomile showed high activity against the tested standard gram negative bacteria in comparison to its effect on Standard *S. aureus* strain. The antibacterial and antiviral effects of chamomile have been well documented in many studies [30,31]. Compounds in the essential oil of chamomile were effective against *Staphylococcus* and *Candida* [31]. Of chamomile's essential oil components, α -bisabolol had the strongest activity against Gram-positive and Gram-negative bacteria. Chamazulene also had strong antimicrobial activity. Spiroethers had weak activity against Gram-positive bacteria but were inactive against Gram-negative bacteria [32]. German chamomile esters and lactones showed activity against *Mycobacterium tuberculosis* and *M. avium* [33].

The main constituents of *Cuminum cyminum*, EO are cumin aldehyde (25%), g-terpinene (19%), p-mentha-1,4-dien-7-al (16.6%), p-mentha-1,3-dien-7-al (13%), b-pinene (10.4%), and p-cymene (7.2%). In our study, It was

found that Cumin oil showed the highest activity against *E. coli*. Derakhshan *et al.*, [34] found that treatment with *C. cyminum* EO reduced capsule formation and induced the formation of filamentous *Klebsiella pneumoniae* cells but in this study, Cumin oil showed high MIC value against *K. pneumoniae*. Salman *et al.*, [35] found that black cumin oil inhibited the growth of *S. aureus* and *P. aeruginosa* while *E. coli* was resistant [36].

The main constituent of Coriander (*Coriandrum sativum* L.) Linalool (40.9–79.9%), neryl acetate (2.3–14.2%), γ -terpinene

(0.1–13.6%) and α -pinene (1.2–7.1%) were identified as main components in the oil of Coriander. Coriander EO exhibited the highest activity against *S. aureus* and *K. pneumoniae*. Coriander EO antibacterial activity was documented in many studies. As Orhan *et al.*, [37] reported that Coriander EO was effective against ESBL producing *K. pneumoniae* strains. Also, Opalchenova and Obreshkova, [38] reported that Coriander EO showed high activity against multidrug resistant *E. faecalis*, *Ps. aeruginosa* and *S. aureus*.

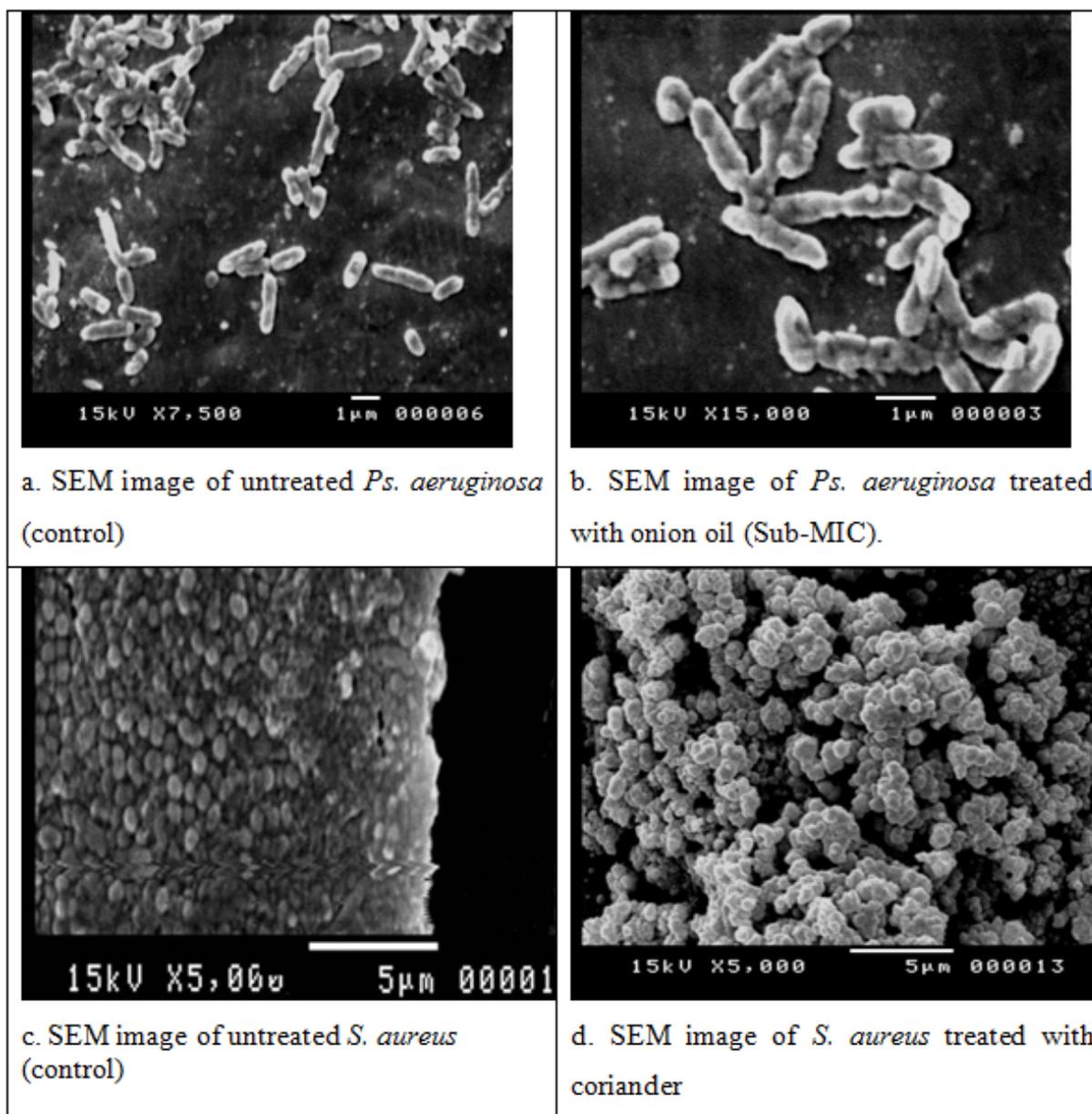


Figure 4. Scanning electron micrographs showed the morphological response of *S. aureus* and *Ps. aeruginosa* to the effect of essential oils

The effect of the tested oils on the tested isolates were verified using Scanning electron microscope (SEM). Figure 4b showed the effect of Onion oil on *Ps. aeruginosa*. The image showed the presence of cell wall deformities, irregularity and holes. Figure 4d showed that *S. aureus* treated with coriander appeared swallowing with irregular cell wall and aggregated.

Many researchers studied the EO on the microbial cells. Ultrastructural analysis by atomic force microscopy, scanning electron microscopy, and transmission electron microscopy (TEM) has shown severe-to-moderate

changes in the surface of several bacterial pathogens including *Aeromonas hydrophila* exposed to *Origanum vulgare* and *Rosmarinus officinalis* Eos [39] and both Gram-positive and Gram-negative foodborne pathogens treated with carvacrol (3.3 mM) [40]. Also, treating Gram-positive and Gram-negative bacteria with origanum and clove oil display different types of injuries on their cell surface [41,42]. *E. coli* cells exposed to origanum and clove EOs showed the formation of holes in the cell surface, in contrast to *Bacillus subtilis*, which merely exhibited cell surface malformation [41].

Sometimes the use of single antibiotic does not produce the desired effective inhibitory effects and to overcome this, a combination of drugs often exercises their synergistic effect which surpasses their individual performance [43]. Combined antimicrobial therapy was found to delay the emergence of bacterial resistance and may also produce good synergistic effect in the treatment of sever infections. Synergy between antimicrobial agents and plant extracts is a novel concept. Although displaying many researches in this field, none of the plant extract tested have been successfully used for clinical use as an antimicrobial agent [44]. The synergistic effect may be due to certain complex formation which becomes more effective in the inhibition

of a particular species of microorganisms either by inhibiting the cell wall synthesis or by causing its lysis or death [43].

Our results showed that synergistic effect was verified from the combination of different antibiotics with the tested essential oils. Although *K. pneumoniae* showed high resistance to Amoxicillin/clavulanic acid, amoxicillin/flucxacillin and cefoperazon, synergistic activity was shown by these antibiotics with the all tested essential oils. As Synergism shown by coriander oil with all tested antibiotics except with amoxicillin/clavulanic acid (synergism was shown on 85.7 % of the tested isolates) (Table 3) (Figure 5).

Table 3. The effect of antibiotic combination with the tested essential oils on *K. pneumoniae* isolates

% of clinical <i>Klebsiella pneumoniae</i> isolates inhibited by Ab/ essential oil combination (% of total no.=21)							
AB \ Oils	Amoxicillin/Clavulanic acid	Ciprofloxacin	Amoxicillin/Flucloxacillin	Amikacin	Cefotaxime	Cephalexin	Cefoperazone
Cumin	85.7	85.7	85.7	100	85.7	100	85.7
Parsely	71.4	85.7	100	85.7	116.6	73.4	85.7
Coriander	85.7	100	100	100	100	100	100
Origanum	95.2	85.7	100	85.7	100	73.4	100
Ivy	100	100	100	100	100	100	80.9
Chamomile	71.4	100	85.7	100	73.5	100	28.6
Onion	71.4	100	85.7	100	73.5	100	85.7
Basil	95.2	90.5	100	90.5	100	81.9	90.5
Leek	95.2	85.7	100	85.7	100	73.5	85.7
Peppermint	100	57.1	100	57.1	100	32.6	85.7
Spearmint	100	95.2	100	95.2	100	90.7	85.7

AB: antibiotics.

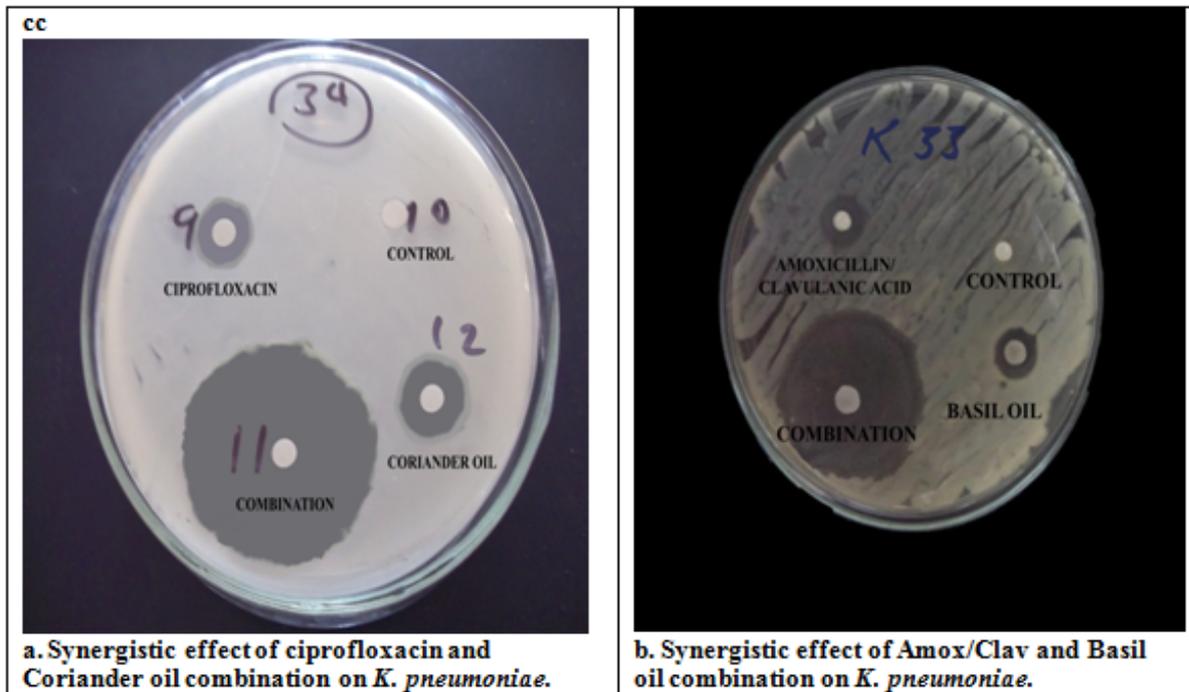


Figure 5. Effect of essential oil and antibiotic combinations on the growth of *K. pneumoniae*

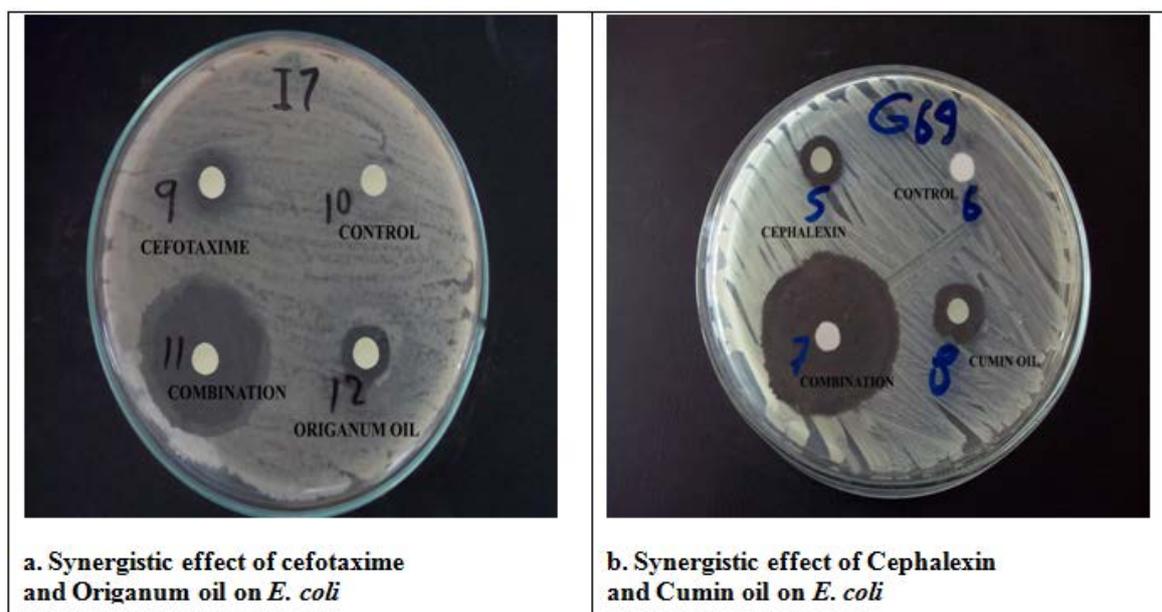
Table 4 and Figure 6 Showed that combination of the tested oils with antibiotics gave synergistic activity with most of the tested *E. coli* isolates especially combinations containing Cumin, origanum and coriander EO. In a study done by Si *et al.*, [44] it was found that Origanum oil showed good activity when used in combination with fluoroquinolones, doxycycline, lincomycin, and mequindox florfenicol against MDR strains of extended-spectrum b-

lactamase producing *E. coli*. Also, Origanum EO showed synergistic activity against ESBL *E. coli* when used in combination with ceftriaxone, doxycycline, florfenicol, kanamycin, levofloxacin, lincomycin, maquindox, polymyxin, sarafloxacin. Van Vuuren *et al.*, [45] found that Pippermint oil showed synergistic activity against *S. aureus* and *K. pneumoniae* when combined with ciprofloxacin.

Table 4. The effect of antibiotic combination with the tested essential oils on *E. coli*

Oils	% of clinical <i>Escherichia coli</i> isolates (% from total no.=12)						
	Amoxicillin/ Clavulanic acid	Ciprofloxacin	Amoxicillin/ Flucloxacillin	Amikacin	Cefotaxime	Cephalexin	Cefoperazone
Cumin	91.6	100	83.3	100	91.6	75	100
Parsely	75	75	75	50	50	50	50
Coriander	75	66.6	100	100	75	100	91.6
Origanum	83.3	91.6	100	83.3	91	83	91.6
Ivy	75	100	100	75	75	100	75
Chamomile	66.6	100	91.6	100	100	75	75
Onion	75	100	72	100	75	50	75
Basil	75	100	100	100	75	75	75
Leek	75	75	75	100	100	75	75
Peppermint	100	100	100	83.3	75	83.3	75
Spearmint	100	83.3	100	75	66	100	75

AB: antibiotics.

**Figure 6.** Effect of essential oil and antibiotic combinations on the growth of *E. coli***Table 5. The effect of antibiotic combination with the tested essential oils on *Ps. aeruginosa***

Oils	% of clinical <i>Pseudomonas aeruginosa</i> isolates (% from total no.=9)						
	Amoxicillin/ Clavulanic acid	Ciprofloxacin	Amoxicillin/ Flucloxacillin	Amikacin	Cefotaxime	Cephalexin	Cefoperazone
Cumin	33.3	100	66.6	100	100	33.3	100
Parsely	55.5	55.5	100	66.6	66.6	66.6	100
Coriander	33.3	100	66.6	100	66.6	33.3	100
Origanum	55.5	77.7	100	55.6	88.8	77.7	100
Ivy	66.6	100	100	0.0	0.0	100	100
Chamomile	88.8	66.6	100	100	55.5	77.7	100
Onion	77.7	100	88.8	100	100	0.0	100
Basil	66.6	88.89	100	77.7	77.7	33.3	100
Leek	33.3	100	66.6	100	100	33.3	100
Peppermint	66.6	55.5	100	55.5	77.7	55.5	100
Spearmint	66.6	55.5	100	77.7	77.7	55.5	100

AB: antibiotics.

Table 5 and Figure 7 showed that the tested oils increased the activity of antibiotics against *Ps. aeruginosa* especially when oils combined with cefoperazone. So, they may be used as adjunctive therapy in the treatment of respiratory infections in combination with antibiotics. The Synergistic activity of different EO were studied by many researchers [46,47].

All *S. aureus* isolates were isolated from skin infections. It was found that combination of Coriander with

antibiotics showed synergistic activity against most of the tested *S. aureus* isolates. Also, combination of the tested oils with cefotaxime exhibited synergistic activity against all tested isolates except for Ivy and onion showed synergism with cefotaxime against 87.5% of *S. aureus* isolates (Table 6 and Figure 8). Many studies showed the synergistic activity of different EO with antibiotics against *S. aureus* [45-50].

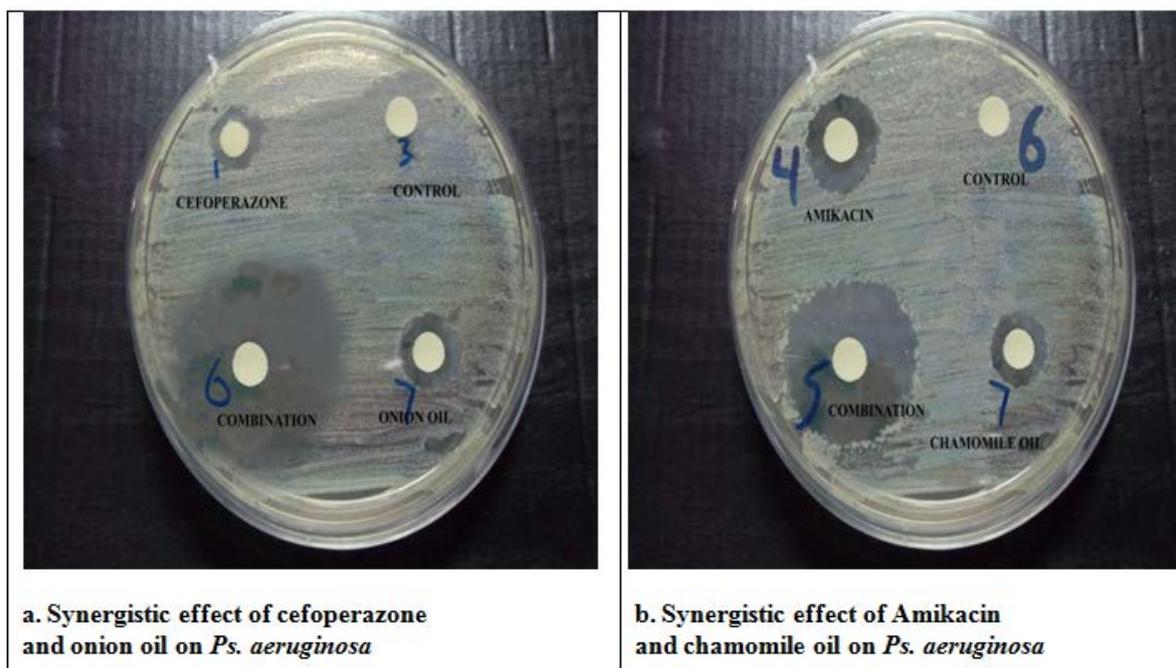


Figure 7. Effect of essential oil and antibiotic combinations on the growth of *Ps. aeruginosa*

Table 6. The effect of antibiotic combination with the tested essential oils on *S. aureus*
% of clinical *Staphylococcus aureus* isolates (% from total no.=8)

AB	Amoxicillin/ Clavulanic acid	Ciprofloxacin	Amoxicillin/ Flucloxacillin	Amikacin	Cefotaxime	Cephalexin	Cefoperazone
Oils							
Cumin	37.5	100	62.5	50	100	50	87.5
Parsely	50	50	87.5	37.5	100	100	62.5
Coriander	75	100	100	100	100	87.5	100
Origanum	75	87.5	100	87.5	100	100	100
Ivy	100	100	87.5	100	87.5	100	62.5
Chamomile	100	100	100	100	100	87.5	25
Onion	87.5	100	50	100	87.5	100	87.5
Basil	50	87.5	50	62.5	100	0.0	100
Leek	37.5	100	50	62.5	100	37.5	50
Peppermint	100	37.5	100	75	100	100	100
Spearmint	100	62.5	100	75	100	87.5	100

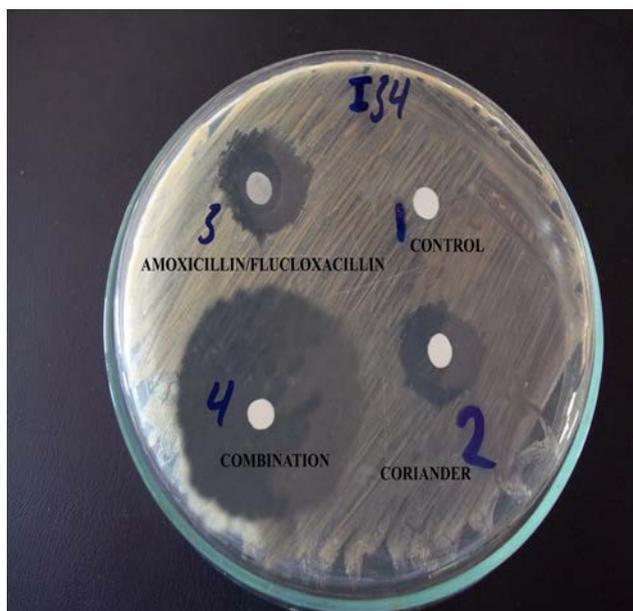


Figure 8. Effect of Amox/Fluclox and Coriander oil combination on the growth of *S. aureus*

4. Conclusion

All tested essential oils possess antimicrobial properties, with greater efficacy when used synergistically, that support their folklore application as medical remedy. All the isolated *S. aureus* strains were obtained from wound infections. So, Coriander, origanum, Ivy & chamomile in combination with an antibiotic can be used in topical preparation for the treatment of skin infections. *Ps. aeruginosa* were obtained from respiratory tract infections. Our results showed the high activity of Chamomile and Onion oil against *Ps. aeruginosa* strains. So, they can be used in preparations for treatment RT infections caused by *Ps. aeruginosa*. Cumin oil showed the highest activity on *E. coli* isolated from GIT & UTI. Resistance pattern of clinical isolates showed most of the tested isolates showed high resistance to the tested antibiotics except for amikacin. When antibiotics were used in combination with the tested oils, synergistic activity was shown in most of isolates. As a result, using antibiotics in combinations with essential oils, leads to the increase of antibiotic efficacy and decrease the incidence of resistance emergence.

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