



Article

Chemical Composition, Antibacterial and Combinatorial Effects of the Essential Oils from *Cymbopogon* spp. and *Mentha arvensis* with Conventional Antibiotics

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Abstract: This work aimed to evaluate the chemical composition and antibacterial activity of essential oils of *Cymbopogon citratus* (CCEO), *Cymbopogon khasianus* (CKEO), and *Mentha arvensis* (MAEO) against two Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*) and three Gram-positive (*Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus subtilis*) microbial strains and their combination with antibiotics (chloramphenicol, ampicillin, erythromycin) to observe the synergistic behavior between them. The essential oils (EOs) were investigated by the GC-MS (gas chromatography mass spectrometry) method. The synergistic effect between EOs and antimicrobial agents was analyzed by broth dilution assay. (-)-carvone (52.48%), geraniol (57.66%), and citral (37.83%) were the major components identified in EOs of MAEO, CKEO, and CCEO, respectively. According to the antibacterial activity, EOs demonstrated strong antibacterial activity with MIC values ranging from 0.7 to 18 mg/mL. The interaction between the combination of EOs and antibiotics was determined in terms of FICI (Fractional Inhibitory Concentration Index). Some combinations displayed a partial synergistic effect, and some showed a synergistic and others displayed no effect against bacterial strains. The best synergistic action was shown by the combination of CCEO and Chloramphenicol against *E. coli* with a FICI value of 0.4. Three to four fold reductions in the MIC value of both essential oil and antibiotics were observed. Therefore, this synergistic interaction of the most active EOs with synthetic antibiotics could lead to new combination therapies for combating infections caused by multidrug-resistant microbes at sufficiently low concentrations in the pharmaceutical and food industry.

Keywords: antimicrobial activity; synergistic; *Cymbopogon citratus*; *Cymbopogon khasianus*; *Mentha arvensis*; antibiotics; essential oil



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1. Introduction

Food safety has become an issue of great concern on a global scale as multidrug-resistant bacteria pose one of the main causes of mortality and morbidity [1]. Therefore, there is a pressing need to explore new novel drugs and strategies to respond to the global public health concern.

Due to safety concerns, the trend is shifting toward non-toxic natural antimicrobials as an alternative to synthetic antibiotics to maintain the efficacy of antimicrobials [2].

Among plants, spices, and extracts, EOs have attracted much attention because of their high content of volatile phytochemicals and have been widely used for their antibacterial,

antioxidant, fungicidal, and insecticidal properties in various food packing, food preservatives, and pharmaceuticals industries [3,4]. Additionally, due to the complex structure of EOs and a wide range of bioactive compounds, it can be attributed to the broad range of biological and structural interactions [5,6]. However, despite the wide range of applications, its use in industry is constrained by the following factors: (i) requirement for a high dosage to achieve bactericidal and bacteriostatic effects; (ii) higher costs due to needing a higher dosage; and, (iii) adverse effects after essential oil treatment (changes in the organoleptic properties) [7]. To overcome these issues, new techniques of using EOs are now being researched to address these concerns.

One of the most effective strategies regarding antibacterial complexes is combination therapy to achieve a synergistic interaction against a broad range of the bacterial spectrum. Combinatorial therapy has proven to be an essential feature of antimicrobial treatment due to several important factors: (i) they thwart drug resistance; (ii) increase activity through the use of components with additive or synergistic activity; (iii) reduce the effective doses, reducing both cost/toxic effect, and (iv) increase the spectrum of activity [8]. However, combinations of two or more agents lead to synergistic, additive, partial, and antagonistic effects [9]. Such knowledge could be applied and helpful in developing new, more effective natural antimicrobial agents in food preservation, cosmetics, pharmaceuticals etc [10].

Therefore, the present study has been designed to verify an antibacterial synergistic interaction between essential oils (*Cymbopogon citratus*, *Cymbopogon khasianus*, and *Mentha arvensis*) and conventional antibiotics. The Genus *Cymbopogon* (family: Gramineae) is a tall, densely tufted perennial grass with profuse tillering known for its medicinal use [11]. *Cymbopogon citratus* is one of the most mentioned species with various medical applications. This plant is known for its calmative, anti-depressant, blood depurative, sedative, analgesic, antifungal, antimicrobial, anti-inflammatory, and diuretic effects [12]. Essential oil of Lemongrass has been shown to effectively inhibit the growth of many different bacteria, including *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis* (MRSE), and Gram-negative bacteria [13]. *Cymbopogon khasianus*, also known as Himrosa, is a perennial grass native to India and subtropical Asia. The essential oil of *Cymbopogon khasianus* is reported to have strong antimicrobial, antioxidant, and anti-inflammatory properties [11,14]. The herbaceous perennial plant, *Mentha arvensis*, also known as corn mint or wild mint, belongs to the lamiaceae family. It is widely distributed in the temperate regions of Europe and western and central Asia, east to the Himalayas and eastern Siberia and North America. The essential oil of *Mentha arvensis* has attracted much interest from researchers due to its worldwide occurrence and several biological activities, including antibacterial, antifungal, antiviral, and cytotoxic activities [15].

2. Experimental Material Methods

2.1. Material

Essential oils of *Cymbopogon citratus*, *Cymbopogon khasianus*, and *Mentha arvensis* were procured from the Council of Scientific & Industrial Research, Indian Institute of Integrative Medicine (CSIR-IIIM) Canal Road, Jammu. Two Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*) and three Gram-positive (*Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus subtilis*) microbial strains used in the study were obtained from IMTECH, Chandigarh, India. Antimicrobial agents (chloramphenicol, ampicillin and erythromycin) were purchased from Hi-media, Mumbai, India.

2.2. Chemical Profiling of EOs

The EOs and bioactive components separation were analyzed using gas chromatography and mass spectrometry. A GC/MS 4000 Varian system (Varian Inc., Palo Alto, CA, USA) with HP-5 MS Agilent column (Agilent Technologies, Santa Clara, CA, USA) (30 × 0.25 mm id, film thickness 0.25 µm) was used. Working conditions of setup-the injector temperature was set at 280 °C, the oven temperature was set at 50 °C, programmed to rise to 300 °C every 3 °C rise/min. Helium was used as a carrier gas at a constant

flow of 1.0 mL/min. 0.2 µL of each essential oil solution was injected into the column and analyzed. The mass spectra were recorded with electron impact voltage and the mass range at 70 eV and 40–500 m/z , respectively, at the speed of one scan per second. The volatile organic compounds were identified by comparing each component's obtained mass spectra with the authentic reference compound present in the NIST database or with the literature [16,17].

2.3. Determination of Minimum Inhibitory Concentration (MIC)

The broth dilution method was employed to assess the MIC of essential oils and antibiotics with minor modifications [18]. The fresh inoculum was prepared in Muller Hinton broth (MHB) and adjusted to 0.5 Mc Farland standards. In brief, each essential oil was dissolved in DMSO (1:1 v/v ratio) for the preparation of stock solution and then the final volume raised to 1 mL. In a similar way, MHB was also used to dilute the EOs. Similarly, dilutions for antibiotics were performed. Next, 100 µL of each diluted necessary oil/antibiotics were put into sterilized tubes containing 90 µL of MHB and 10 µL of microbial suspension (10^6 CFU/mL), resulting in a total final volume of 200 µL. Appropriate positive control (broth and microbial suspension) and negative control (no inoculum) were used to test the bacterial growth. The sterilized tubes were then kept at 37 °C for 24 h. After incubation, 40 µL of the *p*-iodonitrotetrazolium violet solution was added to examine the bacterial growth for another 20 min. Development of a pink color was denoted as bacterial growth, and the tubes in which there was no change in color were defined as MIC for particular oil/antibiotics.

2.4. Synergistic Studies of EOs with Synthetic Antibiotics

The broth dilution method was used to determine the interaction between EOs and synthetic drugs (chloramphenicol, ampicillin, and erythromycin). In sterile tubes, 50 µL of each drug dilution, 90 µL of Muller Hinton broth, and 10 µL of microbial suspension were added and kept at 37 °C for 24 h. After incubation, 40 µL of 0.4 mg/mL of *p*-iodonitrotetrazolium violet solution was added to examine the bacterial growth for another 20 min. The appearance of pink color indicated the growth of bacteria. The FIC Index was used to determine the interaction between two drugs to calculate the fractional inhibitory concentration index (FICI). Fractional inhibitory concentration indices (FICI) were determined as follows:

$$(\text{FICI} = \text{FIC of agent I} + \text{FIC of agent II})$$

$\text{FIC of agent I} = \text{MIC of agent I in combination with agent II} / \text{MIC of agent I alone}$, and $\text{FIC of agent II} = \text{MIC of agent II in combination with the agent I} / \text{MIC of agent II alone}$. Where agent I and agent II are two distinct constituents (EOs/or antibiotics) and the results were elucidated as ≤ 0.5 FICI synergy, $0.5 < \text{FICI} \leq 0.75$ partial synergy; $0.75 < \text{FICI} \leq 2$ no effect; >2 antagonism [19].

3. Results and Discussion

3.1. Chemical Composition of Plant EOs

The chemical composition of hydro-distilled essential oils was determined by GC-MS analysis. The percentage content and Kovats index of each compound of essential oil CKEO and MAEO are presented in Table 1. The phytochemical constraints with their area percent of CCEO were presented in our previous study [20]. The volatile composition of EOs of CKEO and MAEO, a total of 43 primary compounds, were identified, which accounted for 92.32–95.48% of the total volatile oils. GC-MS analysis revealed that the principal component present in the essential of CKEO were geraniol (57.66%) followed by geranyl acetate (8.24%), *cis*-*p*-menth-2-en-1-ol (4.68%), and β -ocimene (3.55%) with some minor constituents. Regarding MAEO, there was a prevalence of monoterpenes (84.64%), bicyclic monoterpene (4.67%), and a lower concentration of diterpene alcohol (2.45%) and sesquiterpene (0.55%). The most abundant bioactive components were (-)-carvone (52.48%), limonene (12.83%), and menthol (7.96%). The major constituent found in the essential oil of CKEO was similar to that reported for the essential oil of *C. khasianus*, with variation

in the percentages [11,21]. In another study, carvone (60.25%) was identified as a major constituent in the essential oil of *M. arvensis* [22]. Some relevant studies on plant essential oil of *M. arvensis* found menthol as the main constituent, which may be explained by the fact that the composition of essential oil is influenced by numerous factors such as genetic variations, the occurrence of different chemotypes, geographical origin, maturity stage of the plant, harvesting season, and method of essential oil extraction [23]. The ratio of phytochemical constituents present in the essential oils and the interactions between the various bioactive components considerably impact the biological activity of EOs [24,25].

Table 1. Chemical composition of essential oils obtained from fresh leaves of plants.

KI	Compound	MAEO	CKEO
985	α -pinene	2.07	-
993	Camphene	0.05	0.07
1003	Sabinene	0.67	-
1005	β -pinene	1.88	-
1010	β -myrcene	0.36	-
1017	α -phellandrene	-	1.49
1024	o-cymene	0.28	0.73
1026	Limonene	12.83	1.45
1027	Eucalyptol	2.37	-
1032	β -ocimene	-	3.55
1037	γ -terpinene	-	0.26
1041	α -citronellol	0.11	-
1051	Linalool	0.15	2.16
1060	cis-p-menth-2-en-1-ol	-	4.68
1065	Alloocimene	-	0.16
1071	2-Hexen-1-al	3.88	-
1075	p-mentha-1,5-dien-8-ol	-	0.23
1079	Terpinen-4-ol	-	0.14
1082	α -terpineol	0.66	-
1083	γ -terpineol	1.26	-
1084	cis-piperitol	-	0.72
1085	(E)-Isopiperitenol	0.18	-
1095	Neral	-	0.39
1096	Pulegone	0.43	-
1098	Geraniol	-	57.66
1099	(-)-carvone	52.48	-
1100	Isopulegol	1.70	-
1101	Piperitone	0.83	1.89
1173	Menthol	7.96	-
1206	Menthyl acetate	3.04	-
1231	Geranyl acetate	-	8.24
1279	Hexadec-7-yn-1-ol	1.48	-
1404	Caryophyllene	-	1.61
1409	Calarene	-	0.18
1420	Geranyl butyrate	-	2.37
1421	Humulene	-	0.10
1435	GermacreneD	0.28	0.08
1455	B-cadinene	-	0.49
1464	Kessane	-	0.21
1488	Spathulenol	0.12	-
1490	Caryophyllene oxide	0.43	0.12
1699	Neryl hexanoate	-	0.96
1724	Diisobutylphthalate	-	2.38
Total		95.48%	92.32%

3.2. Antibacterial Activity (MIC) of EOs

The results of the individual antibacterial effects of EOs and antibiotics against a wide range of bacterial strains are presented in Table 2. The essential oil of *C. khasianus* and *C. citratus* was found to be effective at low concentrations against all tested bacterial strains with MIC values lying within the range 700–1400 µg/mL, followed by *M. arvensis* with MIC values lying between 2500–5000 µg/mL against all the microbial strains. The different EOs exhibited varied antibacterial activity, which may be due to different volatile constituents present in them as well as the nature of the test organism [26]. The stronger potency of EO of *C. khasianus* and *C. citratus* may be due to the presence of a high level of principal components geraniol and citral, respectively, against all the microbial strains, which are described to possess magnificent antimicrobial properties [27–32]. The antibacterial activity of *M. arvensis* may be attributed to its predominant constituents, (-)-carvone, limonene and menthol, which are reported to possess good antibacterial potential [33–35]. All these compounds have a monoterpenes class, which has been shown to display antibacterial activity by damaging the cell organelle, inhibiting important processes like ion transport and respiration [36]. It is believed that essential oils containing monoterpenes can accumulate in the bacterial cell membrane, causing loss of integrity, and leakage of intracellular content, leading to cell lysis and death [37]. In addition to major compounds, minor components also contribute to the antibacterial activity of EO as the whole essential oil contains a complex mixture of bioactive compounds, and it is difficult to possess antimicrobial efficacy with a single component alone. This may be due to synergistic interaction between dominant and minor features that enhance the whole EO's activity [38].

Table 2. Minimum inhibitory concentration of essential oils against different bacterial strains.

Component	Minimum Inhibitory Concentration (µg/mL)				
	<i>B. subtilis</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>M. luteus</i>	<i>S. aureus</i>
CCEO	800	1300	900	1150	1200
CKEO	700	900	800	1000	1400
MAEO	2500	3500	2500	5000	5000
Chloramphenicol	2	2	2	1.5	1.5
Ampicillin	0.50	0.50	0.50	0.40	0.40
Erythromycin	1	1.5	1	1	1

3.3. Synergistic Studies of EOs with Synthetic Antibiotics

Combinatorial therapy is a promising strategy to overcome antibiotic resistance by combining synthetic antibiotics and natural products such as essential oils [39,40]. Such combinations of antimicrobial agents may exhibit different interactions, i.e., synergism, additive, antagonistic, and no effect. Synergistic interactions, in particular, show increased efficiency and lower toxicity due to their multi-target action, which may avoid antibiotic resistance and be effective against multidrug-resistant bacteria strains [41,42]. The results of the combined effect of EOs and chloramphenicol are displayed in Table 3. Synergism was observed against *S. aureus*, *K. pneumoniae*, and *E. coli* in the combination of chloramphenicol with the EOs of *C. khasianus* ($1/4 \text{ MIC}_{C. khasianus} + 1/4 \text{ MIC}_{\text{chloramphenicol}}$) and *C. citratus* ($1/4 \text{ MIC}_{C. citratus} + 1/4 \text{ MIC}_{\text{chloramphenicol}}$), showing a FIC index lying between 0.40 and 0.50. Regarding *M. luteus*, all the combinations showed no effect. Partial synergistic interaction was noted when *M. arvensis* was combined with chloramphenicol against all five microbial strains (FICI = 0.63) except *M. luteus*. Also, *C. citratus* showed partial synergism against *S. aureus*. A total of three to four fold reductions were observed in the MIC of essential oils and antibiotics in partial synergistic and synergistic interactions, respectively. Out of 15 combinations tested, 3 combinations showed synergism, 5 combinations displayed partial synergism, and the remaining 7 binary mixtures showed no effect.

Table 3. Effect of combinations between essential oils and chloramphenicol.

Microorganisms	Combinations	MIC (µg/mL)	MIC _c (µg/mL)	FIC	FICI	Interaction
<i>S. aureus</i>	<i>M. arvensis</i>	5000	1666.66	0.33	0.63	Partial synergistic
	Chloramphenicol	1.5	0.5	0.3		
	<i>C. citratus</i>	1200	400	0.33	0.63	Partial synergistic
	Chloramphenicol	1.5	0.5	0.3		
	<i>C. khasianus</i>	1400	350	0.25	0.50	Synergistic
	Chloramphenicol	1.5	0.3	0.25		
<i>M. luteus</i>	<i>M. arvensis</i>	5000	2500	0.5	1	No effect
	Chloramphenicol	1.5	0.75	0.5		
	<i>C. citratus</i>	1150	575	0.5	1	No effect
	Chloramphenicol	1.5	0.75	0.5		
	<i>C. khasianus</i>	1000	500	0.5	1	No effect
	Chloramphenicol	1.5	0.75	0.5		
<i>E. coli</i>	<i>M. arvensis</i>	3500	1166.66	0.33	0.63	Partial synergistic
	Chloramphenicol	2	0.6	0.3		
	<i>C. citratus</i>	1300	325	0.2	0.4	Synergistic
	Chloramphenicol	2	0.4	0.2		
	<i>C. khasianus</i>	900	450	0.5	1	No effect
	Chloramphenicol	2	1	0.5		
<i>B. subtilis</i>	<i>M. arvensis</i>	2500	833.33	0.33	0.63	Partial synergistic
	Chloramphenicol	2	0.6	0.3		
	<i>C. citratus</i>	800	400	0.5	1	No effect
	Chloramphenicol	2	1	0.5		
	<i>C. khasianus</i>	700	350	0.5	1	No effect
	Chloramphenicol	2	1	0.5		
<i>K. pneumoniae</i>	<i>M. arvensis</i>	2500	833.33	0.33	0.63	Partial synergistic
	Chloramphenicol	2	0.6	0.3		
	<i>C. citratus</i>	900	450	0.5	1	No effect
	Chloramphenicol	2	1	0.5		
	<i>C. khasianus</i>	800	200	0.25	0.45	Synergistic
	Chloramphenicol	2	0.4	0.2		

Furthermore, synergistic behavior was shown against *S. aureus*, *M. luteus* and *E. coli* in the combination of ampicillin with the EO of *M. arvensis* (1/4 MIC *M. arvensis* + 1/4 MIC ampicillin). Regarding the combined effect against *S. aureus* and *B. subtilis*, CKEO and CCEO displayed synergistic interaction with FICI lying between 0.49 and 0.50. Nonetheless, a partial synergy was observed for the combination of EO of *M. arvensis* against *K. pneumoniae* and *B. subtilis*, EO of *C. citratus* against *E. coli*, and EO of *C. khasianus* against *M. luteus* with ampicillin (FICI = 0.66). Notably, no combination of EOs and ampicillin showed antagonistic behavior (Table 4).

The results of the combined antibacterial activity of EOs with standard antibiotic (erythromycin) are given in Table 5. Synergism was observed in the combination of erythromycin with EO of *M. arvensis* (1/4 MIC *M. arvensis* + 1/4 MIC erythromycin) against *E. coli*, *S. aureus*, and *K. pneumoniae*. The synergistic combinations correspond to 1/4 MIC *C. khasianus* + 1/4 MIC erythromycin were potent against *M. luteus* and *B. subtilis*. Also, EO from *C. citratus* showed synergism against *E. coli* and *M. luteus* with FICI 0.50. The remaining combinations showed a partial synergism, decreasing the MIC of the antibiotic and essential oils by three-fold. No combinations displayed antagonistic behavior. These results indicated that the essential oils tested could potentiate the effect of antibiotics. Thus, these essential oils could serve as an adjuvant in therapy with ampicillin, chloramphenicol, and erythromycin. Normally, combinations of drugs have been demonstrated as an important feature of antimicrobial therapy because of various essential contemplations like (i) they utilize compounds having synergistic or additive action to elevate the activity, (ii) they impede resistivity of the

drug, (iii) they reduce requisite doses, thereby, decreasing cost and unfavorable/dangerous after effects, and (iv) they elevate the range of activity [43].

Table 4. Effect of combinations between essential oils and ampicillin.

Microorganisms	Combinations	MIC (µg/mL)	MICc (µg/mL)	FIC	FICI	Interaction
<i>S. aureus</i>	<i>C. khasianus</i>	1400	350	0.25	0.5	Synergistic
	Ampicillin	0.40	0.1	0.25		
	<i>C. citratus</i>	1200	600	0.5	1	No effect
	Ampicillin	0.40	0.2	0.5		
	<i>M. arvensis</i>	5000	1250	0.25	0.5	Synergistic
	Ampicillin	0.40	0.1	0.25		
<i>M. luteus</i>	<i>C. khasianus</i>	1000	333	0.3	0.6	Partial synergistic
	Ampicillin	0.40	0.13	0.3		
	<i>C. citratus</i>	1150	575	0.5	1	No effect
	Ampicillin	0.40	0.2	0.5		
	<i>M. arvensis</i>	5000	1250	0.25	0.5	Synergistic
	Ampicillin	0.40	0.1	0.25		
<i>E. coli</i>	<i>C. khasianus</i>	700	352	0.5	1	No effect
	Ampicillin	0.50	0.25	0.5		
	<i>C. citratus</i>	800	266	0.3	0.6	Partial synergistic
	Ampicillin	0.50	0.16	0.3		
	<i>M. arvensis</i>	2500	625	0.25	0.49	Synergistic
	Ampicillin	0.50	0.12	0.24		
<i>B. subtilis</i>	<i>C. khasianus</i>	900	450	0.5	1	No effect
	Ampicillin	0.50	0.25	0.5		
	<i>C. citratus</i>	1300	352	0.25	0.49	Synergistic
	Ampicillin	0.50	0.12	0.24		
	<i>M. arvensis</i>	3500	1166	0.3	0.6	Partial synergistic
	Ampicillin	0.50	0.16	0.3		
<i>K. pneumoniae</i>	<i>C. khasianus</i>	800	400	0.5	1	No effect
	Ampicillin	0.50	0.25	0.5		
	<i>C. citratus</i>	900	450	0.5	1	No effect
	Ampicillin	0.50	0.25	0.5		
	<i>M. arvensis</i>	2500	833	0.33	0.66	Partial synergistic
	Ampicillin	0.50	0.16	0.33		

Essential oils contain a wide range of metabolites with diverse mechanisms of action. However, the fact that crucial oils alter the permeability of the bacterial cell membrane is well known. In combination with antibiotics (which have different mechanisms of action), essential oils can help maximize the therapeutic potential [44,45]. The synergistic interactions observed in the present study could result from the inhibition of a common biochemical pathway and increase in cell permeability resulting in enhanced uptake of the antibiotics by the bacterial cells [46]. The most likely effect may be related to the fact that essential oil constituents act by unblocking the cell membrane channels, facilitating the passage of antimicrobial agents to reach the internal target [47]. All of our findings support the use of essential oils as a biotechnological tool for therapeutic use as tropical formulations, antifouling coatings, food preservations, and other uses, either use one or in combination with antibiotics.

In conclusion, combining natural products with conventional antibiotics has proven to be a promising alternative for lowering the MIC of these drugs (chloramphenicol, ampicillin, and erythromycin) and allowing the use of lower dosages against multidrug-resistant bacteria. The same conclusion has been previously reported for other medicinal plants [40,42,48–51]. As a result, additional research is needed to understand these

oils' mechanism of action and identify the best essential oil/antibiotic combination for medical therapy.

Table 5. Effect of combinations between essential oils and erythromycin.

Microorganisms	Combinations	MIC (µg/mL)	MICc (µg/mL)	FIC	FICI	Interaction
<i>S. aureus</i>	<i>C. khasianus</i>	1400	466	0.33	0.66	Partial synergistic
	Erythromycin	1	0.33	0.33		
	<i>C. citratus</i>	1200	400	0.33	0.66	Partial synergistic
	Erythromycin	1	0.33	0.33		
	<i>M. arvensis</i>	5000	1250	0.25	0.50	Synergistic
	Erythromycin	1	0.25	0.25		
<i>M. luteus</i>	<i>C. khasianus</i>	1000	250	0.25	0.50	Synergistic
	Erythromycin	1	0.25	0.25		
	<i>C. citratus</i>	1150	287.5	0.25	0.50	Synergistic
	Erythromycin	1	0.25	0.25		
	<i>M. arvensis</i>	5000	1666	0.33	0.66	Partial synergistic
	Erythromycin	1	0.33	0.33		
<i>E. coli</i>	<i>C. khasianus</i>	700	233	0.33	0.66	Partial synergistic
	Erythromycin	1.5	0.5	0.33		
	<i>C. citratus</i>	800	200	0.25	0.50	Synergistic
	Erythromycin	1.5	0.37	0.25		
	<i>M. arvensis</i>	2500	625	0.25	0.50	Synergistic
	Erythromycin	1.5	0.37	0.25		
<i>B. subtilis</i>	<i>C. khasianus</i>	900	225	0.25	0.50	Synergistic
	Erythromycin	1	0.25	0.25		
	<i>C. citratus</i>	1300	433	0.33	0.66	Partial synergistic
	Erythromycin	1	0.33	0.33		
	<i>M. arvensis</i>	3500	1166	0.33	0.66	Partial synergistic
	Erythromycin	1	0.33	0.33		
<i>K. pneumoniae</i>	<i>C. khasianus</i>	800	266	0.33	0.66	Partial synergistic
	Erythromycin	1	0.33	0.33		
	<i>C. citratus</i>	900	300	0.33	0.66	Partial synergistic
	Erythromycin	1	0.33	0.33		
	<i>M. arvensis</i>	2500	625	0.25	0.50	Synergistic
	Erythromycin	1	0.25	0.25		

4. Conclusions

In the present investigation, all the tested essential oils (*M. arvensis*, *C. citratus*, and *C. khasianus*) showed antimicrobial activity with greater potency against both Gram-positive and Gram-negative bacterial strains. In combination with antibiotics, these essential oils showed synergistic behavior against some bacterial isolates, with FIC lying between 0.40 and 0.50. They contributed a three to four fold decrease in individual MICs of all standard antibiotics tested. Using essential oils in combination with antibiotics reduced the minimum effective dosage and decreased the probability of selecting resistant strains. Furthermore, using essential oils as adjuvants can reduce side effects and treatment expenses. Our results suggest developing a new class of antimicrobial agents based on a combination of antimicrobial compounds having potential applications in addressing the ever-increasing incidence of antimicrobial resistance. Future research is warranted on further investigations on these plant species for their potential use in combinatorial antibiotic therapy for minimizing the effective dose of drugs and their mechanism of action.

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