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Abstract

This study investigated the chemical composition and antibacterial activities of lime *Citrus aurantifolia* (Cc) and lemon grass *Cymbopogon citratus* (Ca) essential oils (EOs). Standard methods were used to determine their minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC), and gas chromatography–mass spectrometry was used to determine their composition. Each EO and their combination showed promising results in treating test bacterial infections. Cc EO showed the largest inhibition zone diameter (43 mm) against *Staphylococcus aureus* and *Serratia marcescens*, and Ca EO showed the largest (30 mm) and smallest (12 mm) inhibition zone diameters against *Bacillus stearothermophilus* and *S. marcescens*, respectively. The combination of Cc EO and Ca EO (1:1) resulted in the largest (45 mm) and smallest (8 mm) inhibition zone diameters against *Klebsiella pneumoniae* and *Salmonella typhimurium*, respectively. The MIC of Cc EO ranged from 0.78% to 6.25%, and its MBC ranged from 3.13% to 12.50%. The MIC and MBC of combined Cc EO and Ca EO ranged from 0.78% to 6.25% and from 1.56% to 12.5%, respectively. The major components of Cc EO and Ca EO were neral (44.98%) and D-limonene (56.02%). Overall, the combination of lime and lemon grass EOs performed well compared to streptomycin, making them suitable for treating test bacterial infections.

Keywords: antibacterial activity, essential oils, lemon grass, lime, neral, D-limonene

Introduction

Essential oils (EOs) are extracts from different plants. The global number of plant orders is about 300,000, of which 30,000 contain EOs that can be harnessed by man [1]. EOs are plant-derived aromatic liquids. Among the 3000 known EOs, 300 are commercially valued fragrance [2]. EOs chemically originate from terpenes and are a cocktail of diverse compounds, which boost the benefits of EOs [3]. EO-producing plants include lemongrass (*Cymbopogon citratus*, Cc), sweet orange (*Citrus sinenesis*), and lime (*Citrus aurantifolia*, Ca). Lemon grass is globally cultivated for its EOs, particularly in tropics and subtropics [4]. Cc EO is endowed with antibacterial, antibiofilm, antifungal, and antioxidant bioactivities [5–8] and has been used in cosmetics, flavor, fragrances, detergents, pharmaceuticals, perfumery, and soaps [9, 10]. Citrus contains minerals and other essential nutrients that are useful to man.

Fruits possess antimicrobial, anticancer, antiulcer, antioxidant, insecticidal, and liver protective traits [11]. Limonoids are the major compounds in citrus fruit peels that cause bitter taste and zest aroma [12, 13]. Citrus peel EOs from lime, lemon, tangerine, orange, mandarin and grapefruit are produced in high quantity [14] and are rich in volatile oils, flavonoid, and glycerol [15]. Lime contains EOs and has various applications [16]. EOs are extracted by two main methods, namely, azeotropic distillation (i.e., steam distillation, hydrodistillation, and hydrodiffusion) and solvent extraction [17]. The former guarantees EOs of higher purity and yield but requires higher start-up capital than the latter, which is simpler to perform. Compared with steam distillation and hydrodiffusion, hydrodistillation requires inexpensive equipment [18]. In general, EOs can be obtained from different plant plants as a cocktail of chemical compounds with diverse potent activities [3, 19–21] and are used in food preservation, aromatherapy, and perfume production.

Plants contain high amounts of potent phytochemicals including carotenoids, polyphenols, and biologically active EOs [22, 23] with a wide range of antimicrobial activity [24]. Ca possess antifungal, antibacterial, anticancer, and antiflatoxigenic activities [16, 25–29]. The assays used in determining the antimicrobial activities of EOs include disk diffusion, agar-well diffusion, whole-plate diffusion, and optical density [2]. The increase in antimicrobial resistance and the occurrence of superbugs have led to the search for EOs with bioactive potentials as a safe and cost-effective alternative to synthetic drugs. Therefore, this study investigated the bioactive components, *in vitro* antibacterial potential, and synergistic interaction of *C. sinenesis*, Cc, and Ca EOs against selected bacteria.

Materials and Methods

Bacteria strains used in this study. The bacterial strains used in this study were *Bacillus stearothermophilus* NCIB 8222, *Pseudomonas aeruginosa* NCIB 950, *Clostridium sporogenes* NCIB 532, *Proteus vulgaris* NCIB 67, *Bacillus cereus* NCIB 6349, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* NCIB 3610, *Klebsiella pneumoniae* NCIB 418, *Serratia marcescens* NCIB 1377, *Salmonella typhimurium* ATCC 14028, and *Micrococcus luteus* NCIB 196. The strains were cultured in nutrient broth (NB) at 37 °C (note: *B. stearothermophilus* NCIB 8222 was cultured at 55 °C, and *C. sporogenes* NCIB 532 was incubated in anaerobic jar with CO_2 gas packs), for 18–24 h, streaked on sterile nutrient agar slants for 18–24 h, and stored at 4 °C in the refrigerator.

Plant materials. Fresh lime and sweet orange fruits were purchased at Sabo market, Ile-Ife, Osun State, Nigeria. Lemon grass leaves were obtained from Moremi Estate, Ile-Ife, Osun State, Nigeria.

EO extraction. EOs were extracted from fresh lemon grass and lime and sweet orange peels by hydrodistillation using a Clevenger extractor. After the samples were cut into small pieces, 900 g of each plant material was transferred into the distillation flask (5 L) and added with 3 L of water. The mixture was heated on a heating mantle at 85 °C for 3 h while ensuring the chiller was functional by adding methanol when needed [30]. Precautions were taken to prevent the reversal (i.e the reabsorption of the distilled essential oil) of the oil at the hinge point into the flask, and N-hexane was added into the collecting pipe to trap the oil and prevent it from escaping before being collected into amber bottles. The EOs were refrigerated at 4 °C until further analysis.

Antibacterial susceptibility testing of the EOs on selected bacteria strains. The susceptibility of the EOs against the test bacteria were tested using the agar-well diffusion method described by Valgas *et al*. [31] with some modifications. The test bacteria were cultured in NB for 18–24 h and standardized to 0.5 McFarland standard (10^6 CFU/mL). Approximately 100 µL of the standardized bacteria was seeded on solidified sterile Mueller–Hinton agar (MHA) in Petri dishes with sterile swab stick. Wells were then bored into these MHA Petri dishes using a sterile 6 mm cork borer and filled up with 200 µL of the appropriate EOs, i.e., lime, sweet orange, or lemon grass, with the aid of a micropipette. Streptomycin was used as the control. The Petri dishes were left stationary on the laboratory bench for 1 h to allow the proper diffusion of the EOs into the medium before being incubated at 37 °C (note: *B. stearothermophilus* NCIB 8222 was cultured at 55 °C, and *C. sporogenes* NCIB 532 was incubated in anaerobic jar with CO_2 gas packs) for 24 h. The Petri dishes were observed for inhibition zones. The same procedure was repeated for the synergistic activity testing of the EOs with slight variation, i.e., 100 µL each of Cc and Ca EOs were combined at 1:1 v/v.

Determination of the minimum inhibitory concentrations (MICs) of the Eos. The MIC of Cc and Ca EOs was determined following the broth dilution method of Oluduro *et al.* [32]. Twofold dilutions of the EOs were prepared aseptically. Afterward, 100 µL of each EO was added to 900 µL of sterile NB in microtubes and inoculated with 10 µL of standardized bacteria (0.5 McFarland standard). The control tube contained 1000 µL of sterile NB and the standardized inoculum. The mixture was incubated at 37 °C (note: *B. stearothermophilus* NCIB 8222 was cultured at 55 °C, and *C. sporogenes* NCIB 532 was incubated in anaerobic jar with CO_2 gas packs), for 24 h after which each mixture was observed using the optical density in comparison to the control tubes. MIC was recorded as the least concentration of the EOs that prevented bacterial growth in the broth mixture. This procedure was repeated for the combination of Cc and Ca EOs.

Determination of the minimum bactericidal concentrations (MBCs) of the Eos. The MBCs of the EOs were investigated as described by Spencer and Ragout de Spencer [33]. The 24-hour-old broth mixture for MIC determination was streaked out on sterile nutrient agar plates, incubated at 37 °C (note: *B. stearothermophilus* NCIB 8222 was cultured at 55 °C, and *C. sporogenes* NCIB 532 was incubated in anaerobic jar with CO_2 gas packs), for 72 h, and checked for the presence or absence of growth. MBC was taken as the least concentration of the EOs that completely inhibited bacterial growth at the end of incubation. This procedure was repeated for the combination of Cc and Ca EOs.

Gas chromatography–mass spectrometry (GC–MS) of Eos. The GC–MS of Ca and Cc EOs was performed on Agilent Technologies Intuvo 9000 GC System and Agilent Technologies 5977B Mass Selective Detector (MSD) attached to 4513A Automatic Liquid Sampler.

The instrument was furnished with a HP-5MS capillary column (length 30 m, inner diameter 0.25 mm and film thickness 0.25 µm). Helium was used as the carrier gas at a flow rate of 1.2 mL/min, and 1 µL of each sample was injected into the inlet maintained at 300°C. The oven temperature was maintained at 40 °C for 2 min and then raised to 200 °C at 5 °C/min for 2 min, 275 °C at 15°C/min for 2 min, and 315°C at 20 °C/min for 2 min. The MSD transfer line was kept at 250 °C. The source temperature was 230 °C, and the MS Quad was 150 °C. The ionization mode was electron ionization at 70 eV. The constituents of different EOs were determined by comparing the relative retention indices and mass spectra against the Wiley and NIST library for GC/MS using the probability merge search software and the NIST MS spectra search program. The relative amount (% composition) of an individual oil component represents the percentages of the peak area.

Statistical data analysis. Data were analyzed with SPSS 26 software. Comparison across treatments was performed with univariate ANOVA, followed by Tukey's posthoc tests. Results were regarded as statistically different when $p < 0.05$.

Results

Inhibitory effect of the EOs on the bacterial strains. The sweet orange peel EO was not active against all the test bacteria (result not shown). Figure 1 shows the sensitivity patterns exhibited by the EOs against the bacteria. The results indicated that 82% of the bacteria were susceptible to Cc EO, and the inhibition zone diameter ranged from 11 mm to 43 mm. Cc EO displayed the largest inhibition zone diameter of 43 mm against *S. aureus* subsp. *aureus* ATCC 25923, implying that its activity was significantly higher than that of conventional antibiotic streptomycin ($p < 0.05$). Meanwhile, the smallest inhibition zone diameter of 11 mm was recorded against *S. marcescens* NCIB 1377. The order of decreasing antibacterial activity against the test bacteria was as follows: *S. aureus* subsp. *aureus* ATCC 25923 (43 mm) > *C. sporogenes* NCIB 532 (40 mm), *B. stearothermophilus* NCIB 8222 (40 mm) and *B. cereus* NCIB 6349 (40 mm) > *B. subtilis* NCIB 3610 (37 mm) > *M. luteus* NCIB 196 (30 mm) > *P. vulgaris* NCIB 67 (26 mm) > *K. pneumonia* NCIB 418 (24 mm) > *Serratia marcenscens* NCIB 1377 (11 mm) (Figure 1). Cc EO had no activity against *P. aeruginosa* NCIB 950 and *S. typhimurium* ATCC 14028. Approximately 91% of the bacterial isolates were susceptible to Ca EO, and the inhibition zone diameter ranged from 12 mm to 30 mm (Figure 2). Ca EO displayed the largest inhibition zone diameter of 30 mm against *B. stearothermophilus* NCIB 8222, a value significantly higher than that of streptomycin ($p < 0.05$), and the smallest inhibition zone diameter of 12 mm against *K. pneumoniae* NCIB 418. The activity of Ca EO activity as indicated by its inhibition zone diameters was in the following order: *B. stearothermophilus* NCIB 8222 (30 mm) > *B. cereus* NCIB 6349 (19 mm) > *C. sporogenes* NCIB 532, *P. vulgaris* NCIB 67 and *B. subtilis* NCIB 3610 (17 mm) > *S. aureus* subsp. *aureus* ATCC 25923 (15 mm) > *S. typhimurium* ATCC 14028 (14 mm) > *S. marcenscens* NCIB 1377 and *P. aeruginosa* NCIB 950 (13 mm) > *K. pneumoniae* NCIB 418 (12 mm) Figure 2. Ca EO had no activity against *M. luteus* NCIB 196. All the organisms tested against 1 mg/mL streptomycin showed varying susceptibility. The results for the antibacterial activities of combined Cc EO and Ca EO against the test bacteria are presented in Figure 3. All the bacterial strains were sensitive to the combined EOs, with the largest inhibition zone diameter observed for *K. pneumoniae* NCIB 418 at 45 mm, which was significantly higher than that of streptomycin ($p < 0.05$), followed by *S. aureus* subsp. *aureus* ATCC 25923 with the inhibition zone diameter of 36 mm. The smallest inhibition zone diameter of 8 mm was observed against *S. typhimurium* ATCC 14028.

MICs and MBCs of Cc and Ca Eos. The MICs and MBCs of Cc and Ca EOs are as shown in Table 1. The MIC of Cc EO on the test bacteria was between 0.78% and 6.25%. The least MIC of 0.78% was observed for *C. sporogenes* NCIB 532, *B. stearothermophilus* NCIB 8222, and *S. aureus* subsp. *aureus* ATCC 25923; and the highest MIC of 6.25% was observed for *S. marcescens* NCIB 1377 and *P. vulgaris* NCIB 67. The MBC ranged between 1.56% and 12.50% (Table 1). The MIC of Ca EO against the test bacteria ranged between 1.56% and 12.50%. The least MIC of 1.56% was obtained for *B. stearothermophilus* NCIB 8222 and *B. cereus* NCIB 6349, and the highest MIC of 12.50% was observed for *S. marcescens* NCIB 1377. The MBC ranged from 3.13% to 25.00% (Table 1).

MICs and MBCs of the combined Cc and Ca Eos. The MICs and MBCs of the combined Cc EO and Ca EO are presented in Table 2. The MIC of the EO combination against the susceptible bacteria was between 0.78% and 6.25%. The least MIC of 0.78% was obtained for *K. pneumoniae* NCIB 418 and *M. luteus* NCIB 196, and the highest MIC of 6.25% was reported for *S. marcescens* NCIB 1377, *S. typhimurium* ATCC 14028, and *S. aureus* subsp. *aureus* ATCC 25923. The MBC of the EO combination ranged between 3.13% and 12.50%.

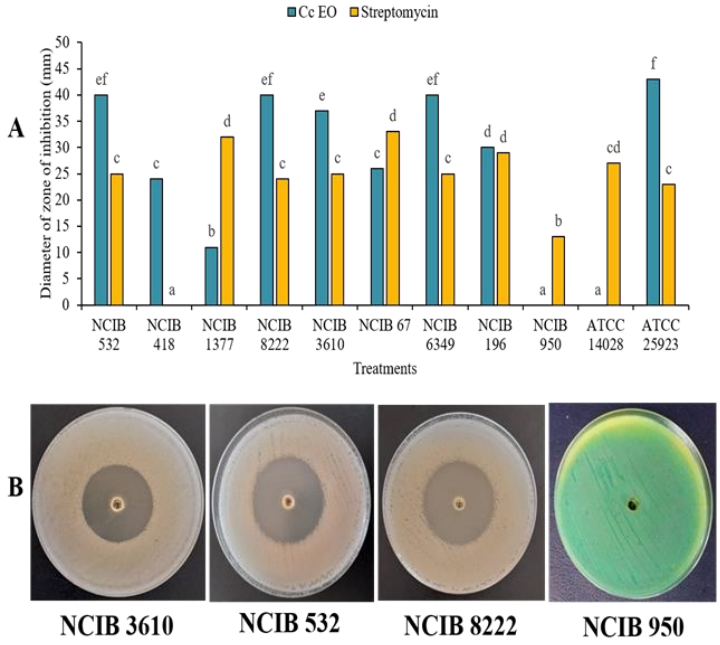

Figure 1. Antibacterial Activities of *Cymbopogon Citratus* Essential Oil Against the Test Bacteria

A. Graphical presentation of *Cymbopogon citratus* essential oil activities against the test bacteria; B. Selected pictorial representation of *Cymbopogon citratus* essential oil activities against the test bacteria; a, b, c, d, e, and f: the bars with the same alphabet are not significantly different from one another, and bars with different alphabets are significantly different from one another. NCIB 8222: *Bacillus stearothermophilus*, NCIB 950: *Pseudomonas aeruginosa*, NCIB 532: *Clostridium sporogenes*, NCIB 67: *Proteus vulgaris* NCIB 67, NCIB 6349: *Bacillus cereus*, ATCC 25923: *Staphylococcus aureus*, NCIB 3610: *Bacillus subtilis*, NCIB 418: *Klebsiella pneumoniae*, NCIB 1377: *Serratia marcescens*, ATCC 14028: *Salmonella typhimurium*, and NCIB 196: *Micrococcus luteus.*

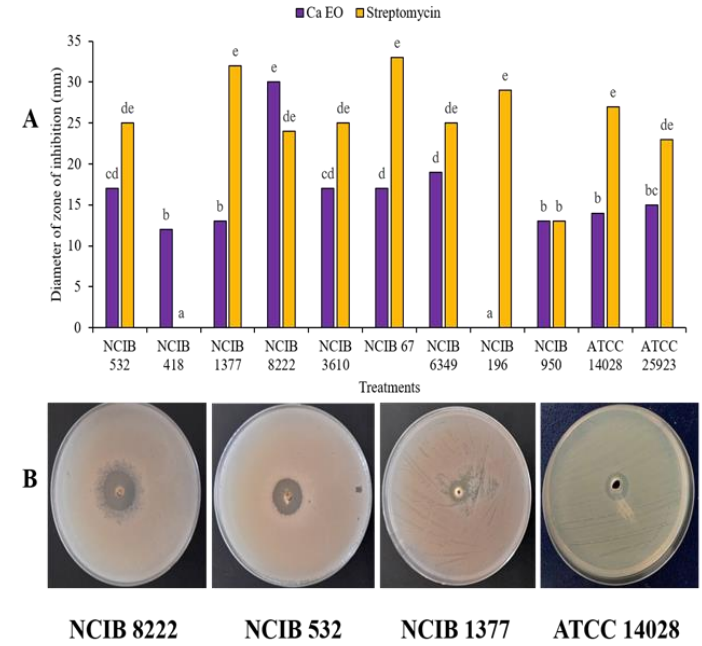

Figure 2. Antibacterial Activity of *Citrus aurantifolia* Essential Oils Against the test Bacteria

A. Graphical presentation of *Cymbopogon citratus* essential oil activities against the test bacteria; B. Selected pictorial representation of *Cymbopogon citratus* essential oil activities against the test bacteria; a, b, c, d, e, and f: the bars with the same alphabet are not significantly different from one another, and bars with different alphabets are significantly different from one another. NCIB 8222: *Bacillus stearothermophilus*, NCIB 950: *Pseudomonas aeruginosa*, NCIB 532: *Clostridium sporogenes*, NCIB 67: *Proteus vulgaris* NCIB 67, NCIB 6349: *Bacillus cereus*, ATCC 25923: *Staphylococcus aureus*, NCIB 3610: *Bacillus subtilis*, NCIB 418: *Klebsiella pneumonia*, NCIB 1377: *Serratia marcescens*, ATCC 14028: *Salmonella typhimurium*, and NCIB 196: *Micrococcus luteus.*

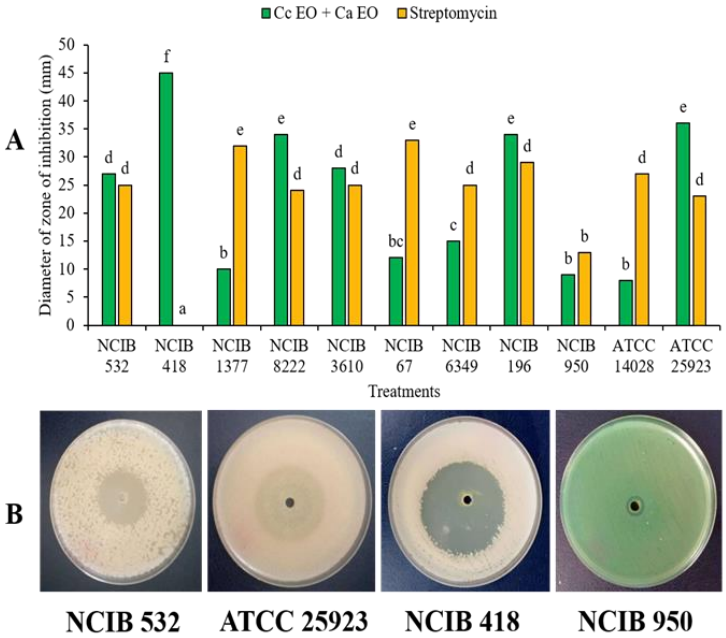

Figure 3. Synergistic Antibacterial Activities of Combined *Cymbopogon citratus* and *Citrus aurantifolia* Essential Oils (Cc EO + Ca EO) Against the Test Bacteria

A. Graphical presentation of *Cymbopogon citratus* essential oil activities against the test bacteria; B. Selected pictorial representation of *Cymbopogon citratus* essential oil activities against the test bacteria; a, b, c, d, e, and f: the bars with the same alphabet are not significantly different from one another, and bars with different alphabets are significantly different from one another; NCIB 8222: *Bacillus stearothermophilus*, NCIB 950: *Pseudomonas aeruginosa*, NCIB 532: *Clostridium sporogenes*, NCIB 67: *Proteus vulgaris* NCIB 67, NCIB 6349: *Bacillus cereus*, ATCC 25923: *Staphylococcus aureus*, NCIB 3610: *Bacillus subtilis*, NCIB 418: *Klebsiella pneumonia*, NCIB 1377: *Serratia marcescens*, ATCC 14028: *Salmonella typhimurium*, and NCIB 196: *Micrococcus luteus.*

Table 1. Minimum Inhibitory Concentrations and Minimum Bactericidal Concentrations of *Cymbopogon citratus* and *Citrus aurantifolia* Essential Oils

Bacterial strains	Cc EO		Ca EO	
	MIC (%)	MBC (%)	MIC (%)	MBC (%)
Clostridium sporogenes NCIB 532	0.78	1.56	3.13	6.25
Klebsiella pneumoniae NCIB 418	3.13	6.25	6.25	12.50
Serratia marcescens NCIB 1377	6.25	12.50	12.50	25.00
Bacillus stearothermophilus NCIB 8222	0.78	1.56	1.56	3.13
Bacillus subtilis NCIB 3610	1.56	3.13	3.13	6.25
Proteus vulgaris NCIB 67	6.25	12.50	3.13	6.25
Bacillus cereus NCIB 6349	1.56	3.13	1.56	3.13
Micrococcus luteus NCIB 196	1.56	3.13	ND	ND
Pseudomonas aeruginosa NCIB 950	ND	ND	6.25	12.50
Salmonella typhimurium ATCC 14028	ND	ND	6.25	12.50
Staphylococcus aureus subsp. *aureus* ATCC 25923	0.78	1.56	3.13	3.13

Keys: NCIB = National Collection of Industrial Bacterial; Cc EO = *Cymbopogon citratus* essential oil; Ca EO = *Citrus aurantifolia* essential oil; MIC = Minimum Inhibitory Concentration; MBC = Minimum Bactericidal Concentration; ND = Not Determined

Table 2. Minimum Inhibitory Concentrations and Minimum Bactericidal Concentrations of Combined *Cymbopogon citratus* and *Citrus aurantifolia* Essential Oils

Bacterial strains	Cc EO + Ca EO	
	MIC (%)	MBC (%)
Clostridium sporogenes NCIB 532	1.56	3.13
Klebsiella pneumoniae NCIB 418	0.78	1.56
Serratia marcenscens NCIB 1377	6.25	12.50
Bacillus stearothermophilus NCIB 8222	1.56	3.13
Bacillus subtilis NCIB 3610	1.56	3.13
Proteus vulgaris NCIB 67	3.13	6.25
Bacillus cereus NCIB 6349	3.13	6.25
Micrococcus luteus NCIB 196	0.78	1.56
Pseudomonas aeruginosa NCIB 950	3.13	6.25
Salmonella typhimurium ATCC 14028	6.25	12.50
Staphylococcus aureus subsp. *aureus* ATCC 25923	6.25	12.50

Keys: NCIB = National Collection of Industrial Bacterial; Cc EO = *Cymbopogon citratus* essential oil; Ca EO = *Citrus aurantifolia* essential oil; MIC = Minimum Inhibitory Concentration; MBC = Minimum Bactericidal Concentration

Table 3. Chemical Constituents of *Cymbopogon citratus* Essential Oil

S/N	Name	% Composition	Retention Time	Quality
1	Trans-1-ethyl-3-methylcyclopentane	0.72	5.33	87
2	1-ethyl-3-methylcyclopentane	1.45	5.39	81
3	Trans-1,2-dimethylcyclohexane	0.93	5.55	94
4	Trans-1,3-dimethylcyclohexane	1.59	5.73	91
5	Trans-1,2-dimethylcyclohexane	0.41	5.88	50
6	Cis-1,2-dimethylcyclohexane	0.13	6.34	94
7	Ethylcyclohexane	0.32	6.47	90
8	α-pinene	0.07	9.45	93
9	(1S)-6,6-dimethyl-2-methylene-bicyclo[3.1.1]heptane	0.13	10.79	95
10	6-methyl-5-heptene-2-one	1.08	11.18	97
11	β-myrcene	19.71	11.36	97
12	1,3,8-ρ-menthatriene	2.53	12.35	96
13	D-limonene	9.20	12.49	99
14	(Z)-3,7-dimethyl-1,3,6-octatriene	0.82	13.10	97
15	ρ-(1-propenyl)-toluene	0.29	14.39	96
16	3-methyl-2-(2-methyl-2-butenyl)-furan	0.07	14.61	76
17	Linalool	1.69	14.75	92
18	(E,Z)-2,6-dimethyl-2,4,6-octatriene	0.38	15.58	96
19	7-methyl-3-methylene-6-octenal	1.98	16.09	78
20	Isomeral	3.13	16.67	97
21	3,7-dimethyl-3,6-octadienal	7.90	17.24	98
22	Neral	44.98	19.23	90
	Total	99.51		

Table 4. Chemical Constituents of *Citrus aurantifolia* Essential Oil

S/N	Name	% Composition	Retention Time	Quality
1	Trans-1-ethyl-3-methylcyclopentane	0.45	5.33	81
2	Trans-1,2-dimethylcyclohexane	0.18	5.55	94
3	1,4-dimethylcyclohexane	0.32	5.73	91
4	Ethylcyclohexane	0.07	6.47	90
5	2-methyl-bicyclo(3.1.0)hex-2-ene	0.48	9.26	97
6	(1S)-2,6,6-trimethylbicyclo(3.1.1)hept-2-ene	3.12	9.47	97
7	Camphene	0.46	9.91	97
8	β-pinene	23.58	11.01	91
9	β-myrcene	1.77	11.34	95
10	α-phellandrene	0.40	11.74	83
11	1-methyl-4-(1-methylethyl)-1,3-cyclohexadiene	0.46	12.14	98
12	D-limonene	56.02	12.84	99
13	β-ocimene	0.99	13.16	95
14	γ-Terpineme	5.63	13.54	95
15	1-methyl-4-(1-methylethylidene)-cyclohexane	1.32	14.37	98
16	Linalool	1.51	14.82	96
17	5-methyl-3-(1-methylethylidene)-1,4-hexadiene	0.17	15.58	97
18	Neral	0.07	16.07	50
19	Terpinen-4-ol	2.90	17.22	87
	Total	99.90		

Chemical composition of Cc and Ca Eos. GC–MS revealed 22 different compounds Cc EO. The most abundant compounds were neral (44.98%) and β-myrcene (19.71%). D-limonene (9.20%), 3, 7-dimethyl-3,6-octadienal (7.90%), isomeral (3.13%), and 1,3,8-ρ-menthatriene were the minor constituents as presented in Table 3.

Ca EO was made up of 19 distinct compounds. The most abundant compounds were D-limonene (56.02%) and β-pinene (23.58%). Other minor components such as γ-terpinene (5.63%), (1S)-2,6,6-trimethylbicyclo(3.1.1) hept-2-ene(3.12%), and terpinen-4-ol (2.90%) were also identified (Table 4).

Discussion

The antibacterial activity of Cc, Ca, and *C. sinensis* EOs and the combination of Cc EO and Ca EO against selected bacteria were investigated. *C. sinensis* EO showed no activity against all the test bacteria in this study (data not shown). This observation is similar to the work of Javed *et al.* [34], who reported no activity for *C. sinensis* var. Malta EO against *K. pneumoniae*. However, this report was contrary to the work of some researchers [35–37]. The difference in observation may be due to various factors, such as the types of test bacteria exposed to *C. sinensis* EO and the geographical source of the *C. sinensis* used in the different studies.

Cc EO possesses bioactivity against several pathogenic microbes [38], including antibacterial activity [39–41]. In this study, Cc EO inhibited all the test bacteria. This observation agreed with the work of Zulfa *et al.* [42], who reported the antibacterial activity of Cc EO against *B. cereus, K. pneumoniae,* and *S. aureus.* The inhibition zone diameter of 40 mm obtained for Cc EO against *S. aureus* subsp. *aureus* ATCC 25923 is close to the 49 mm reported for *S. aureus* ATCC 6538 by Tadtonga *et al.* [43]. Gram-positive organisms show higher susceptibility than gram-negative bacteria, which is in concordance with the report of Ewa *et al.* [44]. Cc EO disrupts bacterial biofilms and inhibits bacterial multiplication by destroying the lipid bilayer bonds and disintegrating the cell membrane [45, 46]. A low concentration of Cc EO leads to the temporary inhibition of microbes, and a high concentration induces bactericidal, fungicidal, and virucidal activities [47, 48]. The bioactivities of Cc EO majorly depend on the test

bacteria and EO concentration [49, 50]. However, the composition, extraction method, plant age, and temperature can also affect the EO efficacy. As such, Cc EO from different plants may exhibit varying nature and intensity of activity. The host's morphophysiological attributes can also influence oil effectiveness [51].

Ca EO inhibited the majority of the test bacteria in this study in varying degrees. Ca EO possesses antioxidant, antimicrobial, and insecticidal activities [52]. The largest inhibition zone diameter of 30 mm exhibited by Ca EO was observed against *B. stearothermophilus* NCIB 8222, a gram-positive bacterium. This finding is contrary to the observation of Galovicová *et al*. [52], who posited that the most pronounced inhibition of Ca EO was recorded against gram-negative bacteria. The activity of Ca EO against *Bacillus* spp., *Salmonella* spp. and *S. aureus* in the present work is somewhat similar to the report of Onyeagba *et al*. [53], who observed Ca EO inhibition zone diameters of 17, 17, and 13 mm against *S. aureus, Bacillus* spp., and *Salmonella* spp., respectively. Chi *et al.* [54] also reported slightly similar activities with Ca EO inhibition zone diameters of 20.1, 21.1, and 20.1 mm against *S. aureus, B. cereus*, and *S. typhi,* respectively. A recent study further reiterated the antibacterial activities of *Citrus* peels against multidrug-resistant bacteria [55].

The combination of Cc and Ca EOs resulted in an antagonistic interaction due to the reduction in activity against *S. marcescens* NCIB 1377 and *B. cereus* NCIB 6349 with the inhibition zone diameters of 10 and 15 mm, respectively. Synergism was observed in the antibacterial activities of Cc EO + Ca EO against *K. pneumoniae* NCIB 418 and *M. luteus* NCIB 196 with inhibition zone diameters of 45 and 34 mm, respectively. The EO combination also showed the following activities: 9 and 8 mm inhibition zone diameters for *P. aeruginosa* NCIB 950 and *S. typhimurium* ATCC 14028, respectively. This value indicates a reduction in activity compared with Ca EO alone. The same trend was observed for *C. sporogenes* NCIB 532, *B. stearothermophilus* NCIB 8222, *B. subtilis* NCIB 3610, and *P. vulgaris* (NCIB 67) with inhibition zone diameters of 27, 34, 28, and 12 mm, respectively. This reduction in Cc EO activity may be due to the inhibition caused by one or more components of the EOs; hence, their combination can promote or hinder their synergistic activity compared with that of their individual activities. Streptomycin at a concentration of 1 mg/mL inhibited most of the test bacteria except *K. pneumoniae* (NCIB 418). Furthermore, Cc EO was 64% more active than streptomycin, indicating its strong antibacterial property that can be harnessed for therapeutic applications. The antibacterial activities of the EOs against gram-positive and -negative bacteria may differ due to their cell wall composition [49, 56]. The broad-spectrum activities of the EOs observed in this study could be an insight into their use as promising, cheap, reliable, and environmentally friendly alternative to conventional antibiotics.

GC–MS revealed that the most abundant compounds in Cc EO were neral (44.98%) and β-myrcene (19.71%). This observation is in agreement with the findings of Inouye [23], who reported that citral, citronellal, and limonene are the major constituents of Cc EO and could be responsible for its antimicrobial activity. Citral is one of the bioactive components in Cc EO responsible for its antibacterial activity [48, 57–60]. Scanning electron microscopy confirmed that citral damages *C. sakazakii* cell membranes [61]. In addition to citral, other minor components play active roles in Cc EO's antibacterial activities [58, 62, 63].

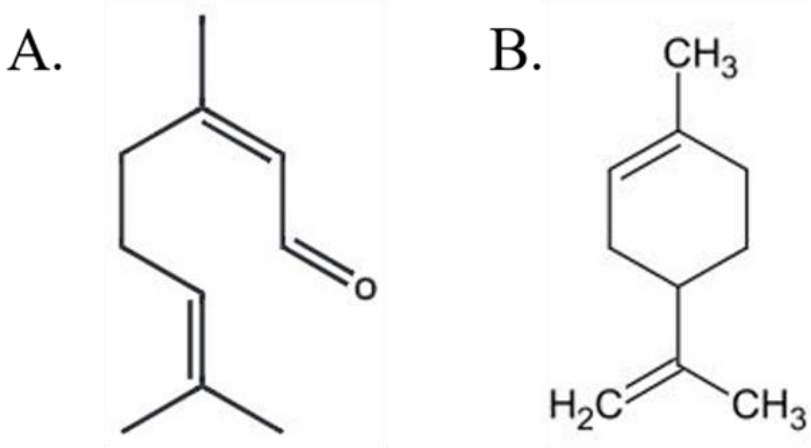

Figure 4. Chemical structure of the major components of *Cymbopogon citratus* and *Citrus aurantifolia* essential oils. A. Neral from *Cymbopogon citratus* essential oil [69]; B. D-Limonene from *Citrus aurantifolia* essential oils [70]

The most abundant compounds in Ca EO were D-limonene (56.02%) and β-pinene (23.58%). This observation is in consonance with other studies [27, 64–67] except for the work of Galovicová *et al.* [52], who reported α-phellandrene (48.5%) and p-cymene (16.5%) as Ca EO's major components. In addition to its antibacterial activity, D-limonene possesses multifunctional activities including antioxidant, antiinflammatory, anticancer, antidiabetic, and gastroprotective effects [68]. The well-established chemical structure of neral [69] and D-limonene [70], the most abundant compounds in Cc EO and Ca EO, respectively, are presented in Figure 4.

Conclusion

The antibacterial potential of Cc EO and Ca EO was favorably comparable with that of streptomycin. As such, each EO and their combinations can be used as resource to synthesize new plant-based drugs for the management of infections caused by the pathogenic bacteria investigated in this study. The combination of the EOs had an exceptional antibacterial activity against *K. pneumoniae* compared with the conventional antibiotic—streptomycin. This study revealed that Cc and Ca EOs possess potent antibacterial activity, justifying their use in traditional medicine.

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Consent for Publication

All the authors have consented to the submission of this article for peer review and to publish the findings of this investigation.

Availability of Data and Material

All data generated or analyzed during this study are included in this published article.

Competing Interest

The authors declared no conflicts of interest.

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References

[1] Can Baser, K.H., Gerhard, B. 2010. Handbook of Essential Oils: Science, Technology and Applications, 1st ed. CRC Press. Taylor and Francis Group.

[2] Burt, S. 2004. Essential oils: their antibacterial properties and potential applications in foods–A review. Int. J. Food Micro. 94(3): 223–253, https://doi.org/10.1016/j.ijfoodmicro.2004.03.022.

[3] Prabuseenivasan, S., Jayakumar, M., Ignacimuthu, S. 2006. *In vitro* antibacterial activity of some plant essential oils. BMC Complem. Altern. M. 6: 39, https://doi.org/10.1186/1472-6882-6-39.

[4] Chanthal, S., Prachakoli, S., Ruangviriyachai, C., Luthria, D.L. 2012. Influence of extraction methodologies on the analysis of five major volatile aromatic compounds of Citronella Grass (*Cymbopogon nardus*) and Lemongrass (*Cymbopogon citratus*) grown in Thailand. J. AOAC Int. 95(3): 763–772, https://doi.org/10.5 740/jaoacint.11-335.

[5] Ekpenyong, C.E., Akpan, E.E. 2017. Use of *Cymbopogon citratus* essential oil in food preservation: Recent advances and future perspectives. Crit. Rev. Food Sci. 57(12): 2541–2559, https://doi.org/10.1080/10408398.2015.1016140.

[6] Widelska, G., Stelmasiewicz, M., Skalicka-Wozniak, K., Oniszczuk, A., Ludwiczuk, A. 2018. PP68. Antioxidant activity of lemongrass essential oil and its constituents. Facta Un. Ser. Phys. Chem. Technol. 16(1): 132.

[7] Martinazzo, A.P., de Oliveira, F.D.S., de Souza Teodoro, C.E. 2019. Antifungal activity of *Cymbopogon citratus* essential oil against *Aspergillus flavus*. Ciência E Nat. 41: e20, https://doi.org/10.5902/2179460X36055.

[8] Shendurse, A.M., Sangwan, R.B., Kumar, A., Venkateshappa, R., Patel, A.C., Gopikrishna, G. *et al*. 2021. Phytochemical screening and antibacterial activity of lemongrass (*Cymbopogon citratus*) leaves essential oil. J. Pharmacogn. Phytochem. 10(2): 445–449.

[9] Preedy, V.R. 2016. Essential Oils in Food Preservation, Flavor and Safety. Elsevier. USA.

[10] Kuete, V. 2017. African Medicinal Spices and Vegetables and Their Potential in the Management of Metabolic Syndrome. In Kuete, V. (eds.), Medicinal Spices and Vegetables from Africa. Academic Press. London. pp. 315–327.

[11] Ballistreri, G., Fabroni, S., Romeo, F.V, Timpanaro, N., Amenta, M., Rapisarda. P. 2019. Anthocyanins and Other Polyphenols in *Citrus* Genus: Biosynthesis, Chemical Profile, and Biological Activity. In Watson, R.R. (eds.), Polyphenols in Plants: Isolation, Purification and Extract Preparation, 2nd ed. Academic Press. USA. pp. 191–215.

[12] Roy, A., Saraf, S. 2006. Limonoids: Overview of significant bioactive triterpenes distributed in plants kingdom. Biol. Pharm. Bull. 29(2): 191–201, https://doi.org/10.1248/bpb.29.191.

[13] Gualdani, R., Cavalluzzi, M.M., Lentini, G., Habtemariam, S. 2016. The chemistry and

pharmacology of citrus limonoids. Molecules. 21(11): 1530, https://doi.org/10.3390/molecules21111530.
[14] Schwab, W., Davidovich-Rikanati, R., Lewinsohn, E. 2008. Biosynthesis of plant-derived flavor compounds. Plant J. 54(4): 712–732, https://doi.org/10.1111/j.1365-313X.2008.03446.x.
[15] Shahnah, S., Ali, M., Ansari, S.H., Bagri, P. 2007. A New sesquiterpene derivative from fruit peel of *Citrus limon* (Linn.) Burm. f. Sci. Pharm. 75(4): 165–170, https://doi.org/10.3797/scipharm.2007.75.165.
[16] Poulose, S.M., Harris, E.D., Patil, B.S. 2005. Citrus limonoids induce apoptosis in human neuroblastoma cells and have radical scavenging activity. J. Nutr. 135(4): 870–877, https://doi.org/10.1093/jn/135.4.870.
[17] Elyemni, M., Louaste, B., Nechad, I., Elkamli, T., Bouia, A., Taleb, M., *et al*. 2019. Extraction of essential oils of *Rosmarinus officinalis* L. by two different methods: Hydrodistillation and microwave assisted hydrodistillation. Sci. World J. 2019(1): 3659432, https://doi.org/10.1155/2019/3659432.
[18] Ibrahim, M., Ankwai, G.E. Gungshik, J.R., Taave, P. 2021. Comparative extraction of essential oils of *Mentha piperita* (mint) by steam distillation and enfleurage. Niger. J. Chem. Res. 26(2): 56–63, https://dx.doi.org/10.4314/njcr.v26i2.2.
[19] Perczak, A., Gwiazdowska, D., Marchwinska, K., Jus, K., Gwiazdowski, R. and Waskiewicz, A. 2019. Antifungal activity of selected essential oils against *Fusarium culmorum* and *F. graminearum* and their secondary metabolites in wheat seeds. Arch. Microbiol. 201: 1085–1097, https://doi.org/10.1007/s00203-019-01673-5.
[20] Falleh, H., Jemaa, M.B., Saada, M., Ksouri, R. 2020. Essential oils: A promising eco-friendly food preservative. Food Chem. 330: 127268, https://doi.org/10.1016/j.foodchem.2020.127268.
[21] da Silva, C.D.B., Gutteres, S.S., Weisheimer, V., Schapoval, E.E.S. 2008. Antifungal activity of the lemongrass oil and citral against *Candida* spp. Braz. J. Infect. Dis. 12(1): 63–66, https://doi.org/10.1590/s1413-86702008000100014.
[22] Soni, S., Soni, U.N. 2014. *In vitro* anti-bacterial and anti-fungal activity of selected essential oils. Int. J. Pharm. Pharm. Sci. 6(6): 586–591.
[23] Kunow, M.A. 2003. Maya Medicine: Traditional Healing in Yucatan. UNM Press. New Mexico. pp. 117.
[24] Aibinu, I., Adenipekun, T., Adelowowtan, T., Ogunsanya, T., Odugbemi, T. 2007. Evaluation of the antimicrobial properties of different parts of *Citrus aurantifolia* (lime fruit) as used locally. Afr. J. Tradit. Complement. Altern. Med. 4(2): 185–190.
[25] Narang, N., Jiraungkoorskul, W. 2016. Anticancer Activity of key lime, *Citrus aurantifolia*. Pharmacogn. Rev. 10(20): 118–122, https://doi.org/10.4103/0973-7847.194043.
[26] Intorasoot, A., Chornchoem, P., Sookkhee, S., Intorasoot, S. 2017. Bactericidal activity of herbal volatile oil extracts against multidrug-resistant *Acinetobacter baumannii*. J. Intercult. Ethnopharm., 6(2): 218–222, https://doi.org/10.5455/jice.20170411091159.
[27] Pathirana, H.N.K.S., Wimalasena, S.H.M.P., De Silva, B.C.J., Hossain, S., Heo, G.J. 2018. Antibacterial activity of lime (*Citrus aurantifolia*) essential oil and limonene against fish pathogenic bacteria isolated from cultured olive flounder (*Paralichthys olivaceus*). Fish. Aquat. Life. 26(2): 131–139, https://doi.org/10.2478/aopf-2018-0014.
[28] Enejoh, O.S., Ogunyemi, I.O., Bala, M.S., Oruene, I.S., Suleiman, M.M., Ambali, S.F. 2015. Ethnomedical importance of *Citrus aurantifolia* (Christm) swingle. Pharm. Innov. J. 4(8): 01–06.
[29] Lin, L.Y., Chuang, C.-H., Chen, H.-S., Yang, K.-M. 2019. Lime (*Citrus aurantifolia* (Christm.) Swingle) essential oils: Volatile compounds, antioxidant capacity, and hypolipidemic effect. Foods. 8(9): 398, https://doi.org/10.3390/foods8090398.
[30] Pednekar, P.P., Vakil, B.V., Sane, R.T., Datar, A.G. 2013. Phytochemical profile and antioxidant activity of the essential oil from Blumea eriantha DC. Int. J. Pharm. Pharmacol. Sci. 5: 404–413.
[31] Valgas C., De Souza S.M., Smânia E.F.A. 2007. Screening methods to determine antibacterial activity of natural products. Braz. J. Microbiol. 38(2): 369–380.
[32] Oluduro, A.O., Bakare, M.K., Omoboye, O.O., Dada, C.A., Olatunji, C.I. 2011. Antibacterial effect of extracts of *Acalypha wilkesiana* on gastrointestinal tract pathogens and bacteria causing skin infections in neonates. Ife J. Sci. 13(2): 371–380.
[33] Spencer, J.F.T., de Spencer, A.L.R. 2004. Public Health Microbiology: Methods and Protocols. Humana Press. New York.
[34] Javed, S., Javaid, A., Mahmood, Z., Javaid, A., Nasim, F. 2020. Biocidal activity of citrus peel essential oils against some food spoilage bacteria. Int. J. Enology Viticulture. 7(7): 001–005.
[35] Hasija, H., Ibrahim, G., Wadia, A. 2015. Antimicrobial activity of *Citrus Sinensis* (orange), *Citrus limetta* (sweet lime) and *Citrus limon* (lemon) peel oil on selected food-borne pathogens. Int. J. Life Sci. Res. 3(3): 35–39.
[36] Nwachukwu, B.C., Taiwo, M.O., Olisemeke, J.K., Obero, O.J., Abibu, W.A. 2019. Qualitative properties and antibacterial activity of essential oil obtained from *Citrus sinensis* peel on three selected bacteria. Biomed. J. Sci. Tech. Res. 19(4): 14427–14432, https://doi.org/10.26717/BJSTR.2019.19.003323.
[37] Akarca, G., Sevic, R. 2022. Biological activities of *Citrus limon* L. and *Citrus sinensis* L. peel essential oils. J. Essent.-Oil Bear. Pl. 24(6): 1415–1427, https://doi.org/10.1080/0972060X.2021.2022000.

[38] Mukarram, M., Choudhary, S., Khan, M.A., Poltronieri, P., Khan, M.M.A., Ali, J., *et al*. 2022. Lemongrass essential oil components with antimicrobial and anticancer activities. Antioxidants. 11(1): 20, https://doi.org/10.3390/antiox11010020.

[39] Silva, N.B., Rangel, M.L., Castro, R.D., Lima, J.M., Castellano, L.R.C., Valença, A.M.G., *et al*. 2020. Anti-biofilm and hemolytic effects of cymbopogon citratus (Dc) stapf essential oil. Pesqui. Bras. Odontopediatria Clin. Integr. 19: e5011.

[40] Farias, P.K.S., Silva, J.C.R.L., de Souza, C.N., da Fonseca, F.S.A., Brandi, I.V., Martins, E.R., *et al*. 2019. Antioxidant activity of essential oils from condiment plants and their effect on lactic cultures and pathogenic bacteria. Cienc. Rural. 49(2): e20180140.

[41] Naik, M.I., Fomda, B.A., Jaykumar, E., Bhat, J.A. 2010. Antibacterial activity of lemongrass (*Cymbopogon citratus*) oil against some some selected pathogenic bacterias. Asian Pac. J. Trop. Med. 3(7): 535–538, https://doi.org/10.1016/S1995-7645(10)60129-0.

[42] Zulfa, Z., Chia, C.T., Rukayadi, Y. 2016. *In vitro* antimicrobial activity of *Cymbopogon citratus* (lemongrass) extracts against selected foodborne pathogens. Int. Food Res. J. 23(3): 1262–1267.

[43] Tadtonga, S., Watthanachaiyingcharoena, R., Kamkaen, N. 2014. Antimicrobial constituents and synergism effect of the essential oils from *Cymbopogon citratus* and *Alpinia galangal*. Nat. Prod. Commun. 9(2): 277–280.

[44] Rembiałkowska, E., Wiśniewska, K. 2019. Analysis of laboratory animal health status and fertility in biological evaluation of organically-produced crops-A review. Pol. J. Food Nutr. Sci. 59(1): 11–15.

[45] Moore-Neibel, K., Gerber, C., Patel, J., Friedman, M., Ravishankar, S. 2012. Antimicrobial activity of lemongrass oil against *Salmonella enterica* on organic leafy greens. J. Appl. Microbiol. 112(3): 485–492, https://doi.org/10.1111/j.1365-2672.2011.05222.x.

[46] Kotzekidou, P., Giannakidis, P., Boulamatsis, A. 2008. Antimicrobial activity of some plant extracts and essential oils against foodborne pathogens *in vitro* and on the fate of inoculated pathogens in chocolate. LWT-Food Sci. Technol. 41(1): 119–127, https://doi.org/10.1016/J.LWT.2007.01.016.

[47] Singh, B.R., Singh, V., Singh, R.K., Ebibeni, N. 2011. Antimicrobial activity of lemongrass (*Cymbopogon citratus*) oil against microbes of environmental, clinical and food origin. Int. Res. Pharm. Pharmacol. 1(9): 228–236.

[48] Lu, M., Han, Z., Xu, Y., Yao, L. 2013. *In vitro* and *In vivo* anti-tobacco mosaic virus activities of essential oils and individual compounds. J. Microbiol. Biotechnol. 23(6): 771–778, https://doi.org/10.4014/jmb.1210.10078.

[49] Dewi, G., Nair, D.V.T., Peichel, C., Johnson, T.J., Noll, S., Johny, A.K. 2021. Effect of lemongrass essential oil against multidrug-resistant *Salmonella* Heidelberg and its attachment to chicken skin and meat. Poultry Sci. 100(7): 101116, https://doi.org/10.1016/j.psj.2021.101116.

[50] Hassoun, A., Çoban, Ö.E. 2017. Essential oils for antimicrobial and antioxidant applications in fish and other seafood products. Trends Food Sci. Technol. 68: 26–36, https://doi.org/10.1016/j.tifs.2017.07.016.

[51] Swamy, M., Akhtar, M.S., Sinniah, U.R. 2016. Antimicrobial properties of plant essential oils against human pathogens and their mode of action: An updated review. Evid.-Based Compl. Alt. 2016(1): 3012462, https://doi.org/10.1155/2016/3012462.

[52] Galovicová, L., Borotová, P., Vukovic, N.L., Vukic, M., Kunová, S., Hanus, P., *et al*. 2022. The potential use of *Citrus aurantifolia* L. essential oils for decay control, quality preservation of agricultural products, and anti-insect activity. Agronomy, 12(3): 735, https://doi.org/10.3390/agronomy12030735.

[53] Onyeagba, R.A., Ugbogu, O.C., Okeke, C.U., Iroakasi, O. 2004. Studies on the antimicrobial effects of garlic (*Allium sativum* Linn), ginger (*Zingiber officinale* Roscoe) and lime (*Citrus aurantifolia* Linn). Afr. J. Biotechnol., 3(10): 552–554, https://doi.org/10.5897/AJB2004.000-2108.

[54] Chi, P.T.L., Van Hung, P., Le Thanh, H., Phi, N.T.L. 2020. Valorization of *Citrus* leaves: Chemical composition, antioxidant and antibacterial activities of essential oils. Waste Biomass Valori. 11: 4849–4857, https://doi.org/10.1007/s12649-019-00815-6.

[55] Azghar, A., Dalli, M., Azizi, S., Benaissa, E.M., Ben Lahlou, Y., Elouennass, M., *et al*. 2023. Chemical composition and antibacterial activity of citrus peels essential oils against multidrug-resistant bacteria: A comparative study. J. Herb. Med. 42: 100799, https://doi.org/10.1016/j.hermed.2023.100799.

[56] Devi, M.A., Sahoo, D., Singh, T.B., Rajashekar, Y. 2020. Toxicity, repellency and chemical composition of essential oils from *Cymbopogon* species against red flour beetle *Tribolium castaneum* herbst (Coleoptera: Tenebrionidae). J. Verbrauch. Lebensm. 15: 181–191, https://doi.org/10.1007/s00003-019-01264-y.

[57] Inouye S. 2003. Laboratory evaluation of gaseous essential oils (Part 2). Int. J. Aromatherapy. 13(4): 95–107, https://doi.org/10.1016/S0962-4562(03)00082-1.

[58] Negrelle, R.R.B., Gomes, E.C. 2007. *Cymbopogon citratus* (DC.) stapf: Chemical composition and biological activities. Rev. Bras. Pl. Med. 9: 80–92.

[59] Adukwu, E.C., Bowles, M., Edwards-Jones, V., Bone, H. 2016. Antimicrobial activity, cytotoxicity and chemical analysis of lemongrass essential oil (*Cymbopogon flexuosus*) and pure citral. Appl.

Microbiol. Biotechnol. 100: 9619–9627, https://doi.org/10.1007/s00253-016-7807-y.

[60] Sharma, S., Habib, S., Sahu, D., Gupta, J. 2019. Chemical properties and therapeutic potential of citral, a monoterpene isolated from lemongrass. Med. Chem. 17(1): 2–12, https://doi.org/10.2174/1573406416666191227111106.

[61] Alagawany, M., El-Saadony, M.T., Elnesr, S.S., Farahat, M., Attia, G., Madkour, M., *et al*. 2021. Use of lemongrass essential oil as a feed additive in quail's nutrition: Its effect on growth, carcass, blood biochemistry, antioxidant and immunological indices, digestive enzymes and intestinal microbiota. Poultry Sci. 100(6): 101172, https://doi.org/10.1016/j.psj.2021.101172.

[62] Shi, C., Song, K., Zhang, X., Sun, Y., Sui, Y., Chen, Y., *et al*. 2016. Antimicrobial activity and possible mechanism of action of citral against *Cronobacter sakazakii*. PLoS ONE, 11(7): e0159006, https://doi.org/10.1371/journal.pone.0159006.

[63] Gill, A.O., Holley, R.A. 2004. Mechanisms of bactericidal action of cinnamaldehyde against *Listeria monocytogenes* and of eugenol against *L. monocytogenes* and *Lactobacillus sakei*. Appl. Environ. Microbiol. 70(10): 5750–5755, https://doi.org/10.1128/AEM.70.10.5750-5755.2004.

[64] Boukhatem, M.N., Kameli, A., Ferhat, M.A., Saidi, F., Tayebi, K. 2014. The food preservative potential of essential oils: Is lemongrass the answer? J. Verbrauch. Lebensm. 9: 13–21, https://doi.org/10.1007/s00003-013-0852-x.

[65] Asnaashari, S., Delazar, A., Habibi, B., Vasfi, R., Nahar, L., Hamedeyazdan, S., *et al*. 2010. Essential oil from *Citrus aurantifolia* prevents ketotifen-induced weight-gain in mice. Phytother. Res. 24(12): 1893–1897, https://doi.org/10.1002/ptr.3227.

[66] Spadaro, F., Costa, R., Circosta, C., Occhiuto, F. 2012. Volatile composition and biological activity of key lime *Citrus aurantifolia* essential oil. Nat. Prod. Commun. 7(11): 1523–1526, https://doi.org/10.1177/1934578X1200701128.

[67] Dao, T.P., Tran, T.H., Nguyen, P.T.N., Ngan, T.T.K., Quyen, N.T.C., Anh, T.T., *et al*. 2019. Chemical composition and evaluation of antibacterial activities of essential oil from lemon (*Citrus aurantifolia* L.) leaves growing Tien Giang Province, Vietnam. Asian J. Chem. 31(10): 2284–2286, https://doi.org/10.14233/ajchem.2019.22144.

[68] Anandakumar, P., Kamaraj, S., Vanitha, M.K. 2020. D-limonene: A multifunctional compound with potent therapeutic effects. J. Food Biochem. 45(1): e13566, https://doi.org/10.1111/jfbc.13566.

[69] Paoli, M., Maroselli, T., Casanova, J., Bighelli, A. 2023. A fast and reliable method to quantify neral and geranial (citral) in essential oils using ^{1}H NMR spectroscopy. Flavour Frag. J. 38(6): 476–482, https://doi.org/10.1002/ffj.3760.

[70] Klimek-Szczykutowicz, M., Szopa, A., Ekiert, H. 2020. *Citrus limon* (lemon) phenomenon—A review of the chemistry, pharmacological properties, applications in the modern pharmaceutical, food, and cosmetics industries, and biotechnological studies. Plants. 9(1): 119, https://doi.org/10.3390%2Fplants9010119.