



Antioxidant and Antibacterial Activities of the Essential Oils of *Cymbopogon citratus* and *Citrus sinensis*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors COO and MEO designed the study. Author COO supervised the procedures while author MEO worked on the literature searches, carried out the laboratory studies and wrote the first draft of the manuscript. Statistical analyses were perfected by author KOK. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The use of essential oils from plants as natural antioxidant and antimicrobial is a field of growing interest. The aim of this study was to investigate the antioxidant and antibacterial properties of the essential oils of *Cymbopogon citratus* L. (lemongrass) and *Citrus sinensis* (orange peels) independently.

Methodology: Essential oils extracted by hydrodistillation from *Cymbopogon citratus* and *Citrus sinensis* were individually assayed for 1,1 -diphenyl- 2 picrylhydrazyl (DPPH) radical scavenging activity, hydrogen peroxide scavenging, reducing power and metal chelating activity in relation to the total phenolic and total flavonoid content of each essential oil. Antimicrobial analyses were carried out using pour plate method for the determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the oils on pathogenic bacteria *Staphylococcus epidermidis* and *Staphylococcus aureus* and non-pathogenic bacteria *Lactobacillus casei* and *Lactobacillus brevis*.

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Results: The two essential oils possessed antioxidant and antibacterial activities. However, the essential oil of *C. citratus* showed a higher level of total phenolic ($372.81 \pm 1.36 \mu\text{g/ml}$) and total flavonoid ($377.88 \pm 1.52 \mu\text{g/ml}$) content when compared to the essential oil of *C. sinensis* which showed a lower total phenolic ($262.81 \pm 2.66 \mu\text{g/ml}$) and flavonoid ($79.65 \pm 1.44 \mu\text{g/ml}$) contents. These resulted in a higher level of DPPH radical scavenging and metal chelating activity of *C. citratus* compared to *C. sinensis*. MIC of 212.5 mg/ml oil concentration was obtained for *C. citratus* against *S. epidermidis* and less than 106.25 mg/ml against *S. aureus* while 210 mg/ml oil concentration was obtained for *C. sinensis* against *S. aureus* and 850 mg/ml oil concentration against *S. epidermidis*. MBC was observed for *C. citratus* at 425 mg/ml while *C. sinensis* showed MBC at 840 mg/ml oil concentration. None of the oils showed a significant inhibition against the non-pathogenic bacteria compared to the (Gentamycin) negative control.

Conclusion: The essential oils of *C. citratus* and *C. sinensis* have potential for use as natural antioxidants and antibacterial agents.

Keywords: Antioxidant; antibacterial; *Cymbopogon citratus*; *Citrus sinensis*; essential oils.

1. INTRODUCTION

Essential oils (EOs) are aromatic, oily, volatile and coloured liquids, obtained from plant parts (flowers, buds, seeds, leaves, twigs, bark, herbs, woods, fruits and roots). They are soluble in lipids and organic solvents that have a lower density than water. EOs are generally stored by the plant in secretory cells, cavities, canals or epidermic cells [1]. EOs are commonly extracted using the method of steam distillation for commercial production [2]. They contain numerous secondary metabolites that can slow down or inhibit the growth of bacteria and other pathogens. Microbial activity is a primary mode of deterioration of foods and many microorganisms are responsible for such occurrences that contribute to loss of food quality and safety. Antimicrobial chemicals have been largely used as foods preservatives, but unfortunately the uncontrolled concentrations applied increase the risk of toxic residues in the products [3]. Hence the need for natural, health and environment friendly alternatives. Many EOs have antioxidant properties which help to mop up free radicals which include nitric oxide (NO), singlet oxygen ($^1\text{O}_2$), superoxide (O_2^-), hydroxyl ion (OH $^-$) and hydrogen peroxide (H_2O_2). These are toxic molecules generated in cells under normal metabolic activities as well as in excess depending on the level of exposure to synthetic chemicals, ionizing radiations, pollutants, smoke and so on [4]. *Cymbopogon citratus* (DC.) Stapf. (lemongrass) is an economically important aromatic perennial plant from which essential oil could be extracted. The essential oil of lemongrass has been used in the perfume and flavour industries due to its lemon smell. *C. citratus* has also been used in aromatherapy as a result of

its long record of extensive therapeutic applications in traditional medicine in a number of countries [5,6]. According to Tzortzakis [7], lemongrass essential oil showed antifungal activity through the significant reduction of spore germination and germ tube length in selected microorganisms. Additional studies have also been carried out to demonstrate the antibacterial effect of *C. citratus* essential oil [8,9]. *Citrus sinensis* L. Osbeck (sweet orange) peels can be used for the extraction of orange essential oil [10]. It has been shown to contain mainly limonene [11] which can be extracted from the oil by distillation. Limonene gives citrus fruits their familiar aroma. It is therefore used in perfume and household cleaners for its fragrance. It is also an effective environmentally friendly and relatively safe solvent which makes it a desirable active ingredient in adhesives, stain removers and strippers [12]. *C. sinensis* essential oil has been shown to possess antifungal, anti-aflatoxigenic and antioxidant activities [13,14]. This research aimed at investigating the free radical scavenging activity of the EOs of *C. citratus* and *C. sinensis* as well as their antibacterial effects on two pathogenic food borne bacteria; *Staphylococcus aureus* and *Staphylococcus epidermidis* and two non-pathogenic food borne bacteria; *Lactobacillus brevis* and *Lactobacillus casei*.

2. MATERIALS AND METHODS

2.1 Chemicals

All chemicals were of analytical grade. DPPH, EDTA, quercetin, were purchased from Sigma Aldrich (St. Louis, MO).

2.2 Plant Materials and Bacterial Agents

Lemongrass was cultivated in a screen house that belongs to the Department of Biochemistry, University of Ibadan and harvested on demand while the orange peels were obtained from oranges bought at Bodija market. Identification of samples was done at the herbarium in the Department of Botany, University of Ibadan, Nigeria. The lemongrass leaves were harvested fresh, chopped and kept in the refrigerator prior to extraction. The orange peels were also obtained fresh as thin slices and kept refrigerated until when needed for extraction. For this study, four bacterial agents were used; *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Lactobacillus casei* and *Lactobacillus brevis*. The microbes were isolated from food samples in the Department of Food Technology, University of Ibadan.

2.3 Extraction of Essential Oils

A total of 1700 g of fresh orange peels were subjected to hydrodistillation in an all glass set-up consisting of a round bottom flask (which contained weighed fresh orange peels and distilled water) heated on a heating mantle for about 3 hours for each round of the process which was connected to a condenser. The eluent was a mixture of EO and distilled water. This was separated using a separating funnel. The distillate that is, the oil was collected in amber coloured bottles and kept in the refrigerator at 4°C until use for analysis. The volume of orange peel EO obtained was 42 ml. The same procedure was followed for the extraction of EO from lemongrass. However, 3500 g of lemongrass gave 50 ml of EO.

2.4 Antioxidant Activities

Essential oils were dissolved in analytical grade ethanol throughout antioxidant assays.

2.4.1 Total phenolic content

The total phenolic content of the EOs of *C. citratus* and *C. sinensis* was determined by spectrophotometric method [15]. Each oil (0.5 ml) was mixed with 1 ml of Folin-Ciocalteu phenol reagent. After 5 minutes, 10 ml of 7% Na₂CO₃ solution was added to the mixture followed by addition of 13 ml of distilled water. This was mixed thoroughly. The mixture was kept in the dark for 90 minutes at 25°C after which the absorbance was read at 750 nm. The total

phenolic content was evaluated from a Gallic acid standard curve and expressed as µg of acid gallic equivalent (µg GAE/mg).

2.4.2 Total flavonoid content

Total flavonoid content was estimated spectrophotometrically using the method of Zhisten et al. [16] as modified by Talukdar, [17]. 0.5 ml of 2% ethanolic AlCl₃ (aluminium chloride) solution was added to 0.5 ml of sample. After 45 minutes incubation at room temperature, the absorbance of the reaction mixture was measured at 420 nm. Quercetin was used as standard flavonoid and the total flavonoid content was calculated from Quercetin and expressed as Quercetin Equivalent Antioxidant (QUE) in µg/mg.

2.4.3 Reducing power

The reducing power was estimated according to the method of Oyaizu et al. [18]. 0.1 ml of essential oil (5 µg/ml -40 µg/ml) was mixed with 0.5 ml phosphate buffer (0.2 M, pH 6.6) and 0.5 ml potassium ferricyanide (K₃[Fe(CN)₆] 0.1%), followed by incubation at 50°C for 20 minutes. After which 0.5 ml of 10% Trichloroacetic acid (Cl₃CCOOH) was added to terminate the reaction. Upper portion of the solution (1 ml) was mixed with 1 ml distilled water and 0.1 ml of Iron(III)chloride (FeCl₃) solution (0.01%) was added. The reaction mixture was allowed to stand 10 minutes at room temperature before the absorbance was read at 700 nm. Ascorbic acid was used as the standard. A higher absorbance of the reaction mixture indicated greater reducing power.

2.4.4 DPPH radical scavenging activity

DPPH (2,2-diphenyl -1- picryl hydrazyl) radical scavenging activity was estimated according to the method of Gyamfi et al. [19]. 0.1 ml of essential oil (10 µg/ml – 40 µg/ml) was added to 3.9 ml of DPPH (0.025 g/l prepared in methanol). The samples were shaken and allowed to stand in dark room for 35 minutes and absorbance was read at 520 nm. Ascorbic acid was used as the standard. DPPH scavenging ability was calculated using:

DPPH radical scavenging activity=

$$\frac{(\text{Absorbance of control} - \text{Absorbance of sample}) \times 100}{(\text{Absorbance of control})}$$

DPPH solution without sample served as control.

2.4.5 Metal chelating activity

The chelation of ferrous ions was estimated using the method of Dinis et al. [20] with slight modification. 0.5 ml ferrous chloride (0.2 mM) was added to 0.2 ml ferrozine (5 mM). The reaction was started by the addition of 0.1 ml (5 µg/ml -40 µg/ml) of the sample and incubated at room temperature for 10 minutes and the absorbance was measured at 562 nm. EDTA was used as positive control.

2.4.6 Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging ability of the EOs was estimated according to the method of Ruch et al. [21]. 0.1 ml of sample (5 µg/ml – 40 µg/ml) was added to 3.0 ml hydrogen peroxide (40 mM) prepared in phosphate buffer (50 mM, pH 7.4) and the absorbance was measured at 230 nm after 10 minutes of incubation at room temperature against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as the standard. The percentage of hydrogen peroxide scavenging ability was calculated using:

Hydrogen peroxide scavenging activity=

$$\frac{(\text{Absorbance of control} - \text{Absorbance of sample}) \times 100}{\text{Absorbance of control}}$$

2.5 Antimicrobial Tests

2.5.1 Preparation of graded concentration of each oil sample by serial dilution

2 ml of the EOs of lemon grass and orange peels were pipetted into different test tubes labelled 1 in each case (100%). 1 ml of Tween 20 (solvent for dissolving essential oils) was pipetted into five test tubes labelled 2 to 6. This was done in two categories since two EOs were involved. 1 ml of pure essential oil was pipetted into tube 2 from tube 1 (50%). 1 ml was then pipetted from tube 2 into tube 3 (25%). After which 1 ml from tube 3 was pipetted into tube 4 (12.5%). From this, 1 ml was pipetted into tube 5 (6.25%) and 1 ml from tube 5 into tube 6 (3.12%). Test tubes 7 and 8 were for negative and positive control respectively. Test tube 7 contained 1 ml Tween 20 (solvent used) while test tube 8 contained 1 ml (10 µg/ml) gentamycin (an antibiotic).

2.5.2 Pour plate method

An overnight culture of each organism was done by taking a loop full of the organism from stock. This was inoculated each into the sterile nutrient

broth of 5 ml each and incubated for 18-24 hours at 37°C. 0.1 ml of each organism was obtained from the overnight culture and put into 9.9 ml of sterile distilled water to get 1: 100 of the dilution of the organisms. 0.2 ml from the diluted organisms was prepared into sterile nutrient agar at 45°C which was aseptically poured into sterile petri dishes. This was allowed to solidify for about 45-60 minutes. Wells were made using a sterile cork borer of 8 mm according to the number of graded concentration of the samples. In each well, the graded concentrations of the samples were poured in duplicate and the plates were allowed to stay on the bench for about 2 hours to allow for pre-diffusion. The plates were incubated uprightly for 18-24 hours at 37°C. Bacteria plates were observed for growth after 24 hours of incubation.

2.5.3 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC for each EO was determined using the first four concentrations by modifying the method of Braz J [22] and Weigand I [23]. Each of the plates used was divided into four parts representing the four microorganisms after solidification of the nutrient agar. Since four concentrations of the initial six were considered for MIC, a total of four plates were used in this case. The growth of microbes in each of the plates was checked for after 24 hours. The MIC showed the minimum inhibition concentration of the oils. Divisions that showed no growth were then transferred into another set of plates for Minimum Bactericidal Concentration (MBC). MBC showed the minimum concentration at which the antibacterial agent had completely killed the bacteria. After 24 hours of MBC, organisms that showed no growth were considered dead.

2.6 Statistical Analysis

Data were expressed as mean ± standard deviation and were analysed using student's t-test. Values of p<0.05 were considered to be statistically significant.

3. RESULTS AND DISCUSSION

3.1 Antioxidant Properties of the Essential Oils

3.1.1 Total phenolic content

Phenolic compounds of plants have been found to be very important due to their diverse

antioxidant activities. According to Table 1, phenolic compounds were present in the EOs of *C. citratus* and *C. sinensis* based on the amount of total phenolics present in each. However, the phenolic content of the essential oil of *C. citratus* was much higher than that of *C. sinensis*. Total phenolic content of essential oils has been shown to contribute to the free radical scavenging activity of these oils [24,25].

3.1.2 Total flavonoid content

Phenolic compounds of plants fall into several categories; prominent among these are the flavonoids which have potent antioxidant activities [26], they have been shown to be highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various free radicals [27]. According to Table 1 below, flavonoids were present in the EOs of *C. citratus* and *C. sinensis* based on the amount of total flavonoids present in each. However, the flavonoid content of the EO of *C. citratus* was much higher than that of *C. sinensis*. According to Lin CW et al. [24], high flavonoid content of plant essential oils contributes to their free radical scavenging activity.

Table 1. Total phenolic and flavonoid content of the EOs of *C. citratus* and *C. sinensis*

Essential oil	Total phenolic content (µg GAE/mg)	Total flavonoid content (µg QUE/mg)
<i>C. citratus</i>	372.81±1.36 ^a	377.88±1.52 ^b
<i>C. sinensis</i>	262.81±2.66 ^a	79.65±1.44 ^b

Values represent mean ± standard deviation of triplicate readings, n=3. Values with the same subscript letter are significantly ($P<0.05$) different

3.1.3 Reducing power

This reaction is based on the principle of increase in the absorbance which indicates an increase in the antioxidant activity. The colour of the test solution changes to green due to the reduction of the Fe^{3+} /ferricyanide complex to the ferrous form depending on reducing power of the test solution. Reducing power is a potent antioxidant defence mechanism which is based on either electron transfer or/ and hydrogen atom transfer by antioxidant molecules. Ferric-to-ferrous iron reduction occurs rapidly with all reductants with half reaction reduction potential above that of Fe^{3+}/Fe^{2+} [28]. According to Fig. 1, the EO of *C. sinensis* showed a higher level of

reducing power in a concentration dependent manner compared to the EO of *C. citratus* and the standard antioxidant ascorbic acid.

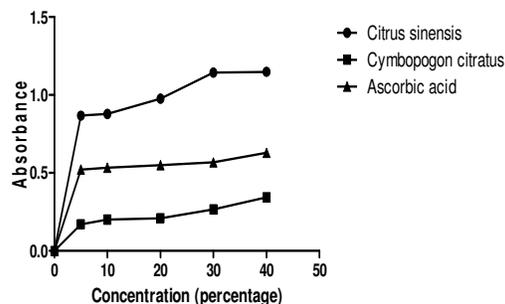


Fig. 1. Reducing power of the essential oils of *C. citratus*, *C. sinensis* and ascorbic acid

3.1.4 DPPH radical scavenging activity

The stable organic radical DPPH has been widely employed in studies of the antioxidant capacity of EOs [29]. DPPH is a free radical donor that accepts an electron or hydrogen to become a stable diamagnetic molecule [19]. The DPPH (1,1 -diphenyl- 2 picrylhydrazyl) test measures the hydrogen atom or electron donor capacity of the EOs to the stable radical DPPH formed in solution [30]. It also measures the ability of the EOs to scavenge free radicals in solution. In this assay, antioxidant compounds reduce the purple radical (picryl hydrazyl) to the corresponding pale yellow hydrazine (picryl hydrazine). The discoloration indicates the free radical scavenging activity of the tested samples. Both oils showed appreciable free radical scavenging activity in a concentration dependent manner when compared with the standard Ascorbic acid though the EO of *C. citratus* had a higher effect compared to that of *C. sinensis* as shown in Fig. 2. Several studies have demonstrated the antioxidant potential of *C.citratus* [31,32] and *C.sinensis* [33,34] and a number of plant essential oils have been shown to possess DPPH free radical scavenging activities [24].

3.1.5 Metal chelating activity

The ability of antioxidants to chelate transition metals and prevent such metals from participating in the initiation of lipid peroxidation and oxidative stress through metal catalyzed reaction is an important antioxidant mechanism [35,36]. Iron can become dangerous when Fe^{2+} reacts with hydrogen peroxide (H_2O_2) to produce

hydroxyl radical (OH*), hence the need for possible removal of iron by chelation. The results of the determined Fe²⁺ chelating ability of the EOs are presented in Fig. 3. Both oils showed appreciable metal chelating ability in a concentration dependent manner. However, the EO of *C. citratus* showed a higher metal chelating ability compared to *C. sinensis* EO as shown in Fig. 3. The metal chelating ability of *C. citratus* was higher compared to that of *C. sinensis*. This further buttresses the effect of the higher level of total phenolic and flavonoid content of the EO of the former compared to the later. *C. citratus* essential oil has been shown to possess a high level of metal chelating activity [37].

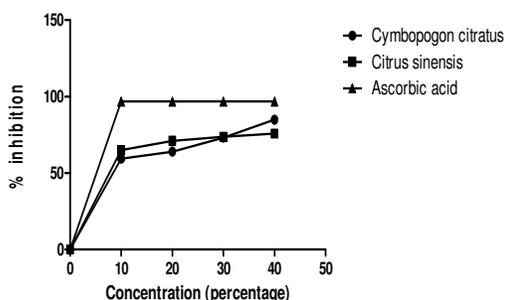


Fig. 2. DPPH radical scavenging ability of the EOs of *C. citratus*, *C. sinensis* and ascorbic acid (standard)

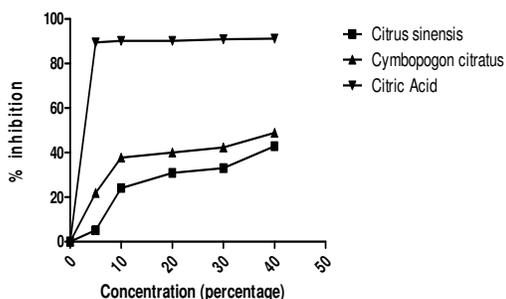


Fig. 3. Metal chelating ability of the EOs of *C. citratus*, *C. sinensis* and citric acid (standard)

3.1.6 Hydrogen peroxide scavenging ability

Iron can become dangerous when Fe (II) reacts with hydrogen peroxide (H₂O₂) to produce hydroxyl radical (OH*), hence the need for possible removal of hydrogen peroxide to prevent its reaction with iron by scavenging with antioxidants. According to Fig. 4, there is a slight difference in the hydrogen peroxide scavenging ability of the EO of *C. citratus* and *C. sinensis*

compared with the standard antioxidant ascorbic acid.

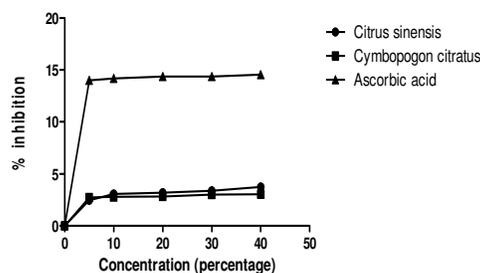


Fig. 4. Hydrogen peroxide scavenging activity of the EO of *C. citratus*, *C. sinensis* and ascorbic acid (standard)

3.2 Antimicrobial Activity

Phytochemicals such as phenolic compounds have been shown to contribute immensely to the protective potential and aromatherapeutic effects of EOs and the tendency of essential oils to boost the nutritional quality of food and food products and also serve as preservatives against food borne pathogens [38-40]. Clear zones of inhibition were observed on plates with higher concentration of essential oil while shorter or no zone at all for culture plates with lower concentrations. The zone of inhibition was measured in mm as shown in Tables 3 and 4. Based on the results of this study, the EO of *C. citratus* showed a higher inhibitory effect against the tested microorganisms. It is interesting to note that the EOs of *C. citratus* and *C. sinensis* did not show a significant inhibition against *Lactobacillus casei* and *Lactobacillus brevis* compared to the negative control. These microbes possess probiotic properties [41,42]. *L. casei* has industrial application especially in dairy production as well as medical applications [42]. *C. citratus* inhibited *Staphylococcus aureus* to a higher extent compared to *Staphylococcus epidermidis* based on the MIC of less than 106.25 mg/ml of the former compared to 212.5 mg/ml MIC of the later as shown in Table 5. *C. sinensis* however, showed a MIC of 210 mg/ml oil against *S. aureus* while 840 mg/ml oil concentration was required for *S. epidermidis* as shown in Table 6. The MBC for *C. citratus* was 425 mg/ml oil concentration for *S. aureus* and *S. epidermidis* while that of *C. sinensis* was 840 mg/ml concentration for only *S. aureus* as shown in Table 7. *C. citratus* has been shown to possess strong antibacterial activity [37] and has been demonstrated to be a potent preservative against *S. aureus* and *S. epidermidis* [43].

Table 2. EC50 values (%) of radical scavenging of *C. citratus* and *C. sinensis*

	<i>C. citratus</i> (%)	<i>C. sinensis</i> (%)	Standard (%)
DPPH scavenging activity	10	10.6	4
H ₂ O ₂ scavenging activity	420	350	206
Metal chelating activity	51	55	3

Table 3. Inhibition zone of the EO of *C. citratus* indicating the inhibition of the growth of the four microorganisms used

Concentration (mg/ml)	<i>S. epidermidis</i> (mm)	<i>S. aureus</i> (mm)	<i>L. casei</i> (mm)	<i>L. brevis</i> (mm)
850	20	24	14	14
425	18	20	12	12
212	14	18	10	10
106.25	12	16	-	-
53.13	10	14	-	-
26.67	-	10	-	-
(Tween 20)	10	12	10	10
(Gentamycin) 10 µg/ml	38	40	36	38

‘-’ means no zone of inhibition

Table 4. Inhibition zone of the EO of *C. sinensis* indicating growth inhibition of the four microorganisms used

Concentration (mg/ml)	<i>S. epidermidis</i> (mm)	<i>S. aureus</i> (mm)	<i>L. casei</i> (mm)	<i>L. brevis</i> (mm)
840	14	20	14	14
420	12	18	12	12
210	10	16	10	10
105	-	14	-	-
52.5	-	12	-	-
26.13	-	10	-	-
(Tween 20)	10	12	10	10
Gentamycin (10 µg/ml)	38	40	36	38

‘-’ means no zone of inhibition

Table 5. Minimum inhibition concentration (MIC) of *C. citratus* EO

Concentration (mg/ml)	<i>S. epidermidis</i>	<i>S. aureus</i>	<i>L. casei</i>	<i>L. brevis</i>
850	-	-	±	±
425	-	-	+	+
212.5	-	-	+	+
106.25	+	-	+	+

‘-’ means no growth while ‘+’ means growth observed and ‘±’ means unclear growth

Table 6. Minimum inhibitory concentration (MIC) of *C. sinensis* EO

Concentration (mg/ml)	<i>S. epidermidis</i>	<i>S. aureus</i>	<i>L. casei</i>	<i>L. brevis</i>
840	-	-	-	-
420	+	-	+	+
210	+	-	+	+
105	+	+	+	+

‘-’ means no growth while ‘+’ means growth observed

Table 7. Minimum bactericidal concentration (MBC) of the EOs used

Concentration (mg/ml)	<i>S. epidermidis</i>	<i>S. aureus</i>	<i>L. casei</i>	<i>L. brevis</i>	Essential oil
840	+	-	+	+	<i>C. sinensis</i>
420	+	+	+	+	<i>C. sinensis</i>
850	-	-	+	+	<i>C. citratus</i>
425	-	-	+	+	<i>C. citratus</i>
212.5	+	+	+	+	<i>C. citratus</i>

'-' means no growth while '+' means growth observed.

4. CONCLUSION

Based on this study, the EOs of *C. citratus* and *C. sinensis* possess free radical scavenging activity which makes them potent natural antioxidants. The oils have also shown bacteriostatic and bactericidal properties at varying concentrations. Hence, the essential oils of *C. citratus* and *C. sinensis* can be used as potent natural antibacterial agents against the investigated pathogenic bacteria.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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