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Antimicrobial and Antioxidant Activities of Lemongrass Oil (*Cymbopogon citratus*), in Preservation of Fresh Orange Juice

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ABSRACT

In this study illustrated that LGEO (*Cymbopogon citratus*) had antimicrobial activity influenced of G–ve and G+ve bacteria like *E. coli, S. Typhi, B. subtilis* and *Staph. aureus.* In context, it was observed inhibition of fungi and yeast by CCEO especially *A. flavus, A. niger,* and *S. cervisiae*. Our study elicited that CCEO had antioxidant capacity which was DPPH scavenging activity (%) of essential oils. In addition, findings showed that antioxidant activity of essential oils (ferric reducing assay), hence, there was an incremental trend of ferric reducing assay with increasing concentration of oil. The chemical composition of *Cymbopogon citratus* essential oil was included 34 compounds, hence, the main components were α-citral (18.1%), Neral (14.08%). The results showed that increased of CCEO concentration even 1.25 µl/ml, was reinforced of antimicrobial and antioxidant activities. The incorporation of three concentrations of CCEO with cooling at 4 °C was natural preservation of fresh orange juice. Hence, it greatly improved the natural properties and microbiological properties of the fresh juice. It can be recommended that using of LGEO as a natural additive preservative in fresh orange juice with cold storage against the spoilage flora of fresh orange juice, because it has antimicrobial and antioxidants activity, in the same time did not find more of side effects on product and human health that may be occur when use chemical preservatives.

Keywords: *Cymbopogon citratus,* α-citral, antimicrobial activity, *S. cervisiae,* DPPH, antioxidant activity, orange juice

INTRODUCTION

The genus *Cymbopogon* includes about 140 species, more than 52 occurs in Africa, 45 in India, six each in Australia and South America, four in Europe, two in North America and the remaining are distributed in South Asia as mentioned (Jagadish Chandra, 1975). In context, Akhila, (2010) reported that international demand for the EO *Cymbopogon citratus* (DC.) Stapf (Poaceae family), commonly known as lemongrass, is one of the main medicinal and aromatic plants. Who mentioned that cultivated mostly for its essential oil (EO) in tropical and subtropical regions of the world. Lemongrass essential oil (LGEO) is extracted by steam distillation from the dried or fresh leaves of the plant, it produces EO plus aromatic waters, which are often used against inflammatory diseases and microbial infectious as reported by Tiwari *et al*., (2010). Due to its application in the production of fragrances, flavors, perfumery, cosmetics, detergents, and pharmaceuticals, LGEO has a significant economic impact as mentioned (Tyagi and Malik 2012).

In context, Majewska *et al*., (2019) demonstrated that citral is the primary constituent of lemongrass essential oil that two geometric isomers combined in it. Citral A or geranial is the name given to the E-isomer, and citral B or neral is the name given to the Z-isomer. They stated that lemongrass essential oil quality is generally evaluated by its citral content. Where, lemongrass oil should contain at least 75% of citral was *C. citratus* essential oil to be regarded as a high-quality product as stated (Barbosa *et al*., 2008). As marker chemicals in lemongrass essential oil, geranial and neral, limonene, citronellal, myrcene, and geraniol were identified as stated (Schaneberg and Khan, 2002). Marker compounds are chemical components found in medicinal plants that can be utilized to confirm their identity or efficacy. The essential oil composition of lemongrass differs significantly at various harvesting stages reported by Tajidin *et al*., (2012). Because the composition and content of the essential oil are linked to the developmental stage of the entire plant, plant organs, and plant cells, the quality and amount of lemongrass essential oil are strongly reliant on the timing of the plant's harvest as reported (Verma *et al*., 2015). While, Soares *et al*., (2020) revealed that, the major constituents are α-citral, β-citral, myrcene and geraniol, they showed that the presence of 10 different compounds accounting for 83.86% of total peak area *of C. citratus* EO. On the other hand, Císarová *et al*., (2020) reported that the chemical analysis of the lemongrass oil led to the identification of 34 components, characterized by Geranial, Neral. They are harmony with, Yan *et al*., (2021) who recommended that lemongrass essential oil has 18 compounds, includs terpenes or terpenoids. Whereas, Valková *et al*., (2022) reported that 43 compounds were identified, which account for 99.7% of the total volatiles of LGEO, and the main component of the EO was citral. Also, Abdel-Gwada *et al*., (2022) found that 41 constituents from lemongrass EO and represented 98.21% of the EO, where the main components were neral, citral, and minor compounds were 1.79% such as D-limonene and 6-Methyl 5-Hepten-z-

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one, thus 41 compounds perhaps give the EOs bioactivity such as antioxidant, antibacterial, or antifungal. Lemongrass oil may be utilized as a preservative due to the decrease in microbial load caused by the increase in the amount of these natural antioxidant components as stated (Hartatie *et al*., 2019).

Antimicrobial effects using the essential oil of *C. citratus*, was found by Onawunmi (1989), who observed diameter inhibition zone for *E. coli* was 15 mm, and for *B. subtilis* and *S. aureus* was 32 mm, indicating that even at low doses, the C. citratus essential oil had good antibacterial activity. On the other hand, Abdel-Gwada *et al*., (2022) found that inhibition zones that recorded of Lemongrass essential oil against *E.coli, S. Typhimurium*, and *S.aureus* were 35mm, 33.5mm, and 25mm, respectively. On the high concentration of EO plates, distinct zones of inhibition were seen as stated (Singh *et al.,* 2017). They reported there is a correlation between the total phenolic content and the measured activities, these activities have been linked to the phenolic content that also reported by Bahri-Sahloul *et al*., (2014). On the other hand, Silva *et al*., (2008) discussed that both compounds neral and geranial can inhibit spore germination in *Aspergillus* species. Where, the wall and membrane of an *A. flavus* spore were reportedly injected with citral. resulting in a decrease in its elasticity. Also, Císarová *et al*., (2020) indicated that the LEO was highly effective against tested toxigenic *Aspergillus* species in vapor phase, and lemongrass EO does have negative effect on the sensory properties of the breads. In addition, Soares *et al*., (2020) reported that all three yeast strains studied *Candida albicans*, *C. parapsilosis*, and *C. tropicalis* were inhibited by the *C. citratus* EO. In context, Valková *et al*., (2022) indicated that *Candida krusei* had the highest inhibition zone (18.00 mm), according to an in *vitro* antimicrobial study, for *C. albicans*, the values for the minimum inhibitory concentration were found to be the highest.

Mansour *et al*., (2015) reported that Egyptian lemongrass essential oil, with an IC_{50} (1.0 mg/mL), was shown to have better antioxidant activity than Saudi Arabian lemongrass volatile oil, with an IC_{50} (6.9 mg/mL). Its unsaturated alcohols and phenolic components, such as linalool, geraniol, terpin-4-ol, and eugenol, were thought to be responsible for the Egyptian oil's potent DPPH-scavenging abilities. On the other hand, Kumar *et al*., (2017) reported that as oil content rose, there was a gradual trend toward radical scavenging. Since, the presence of active principles, such as citral in lemongrass, may be the cause of these oils' increased capacity to scavenge free radicals. Also, Soares *et al*., (2020) reported that the 2,2'-diphenyl-1-picrylhydrazyl stable free radical may be neutralized by the *C. citratus* EO with an IC_{50} (41.7 g/ml), which is comparable to the value of the synthetic antioxidant (37.7 g/ml). According to their findings (DPPH IC50 of 41.7 g/ml), the antioxidant potential of essential oils was statistically identical to that of synthetic antioxidants. They credit the synergistic impact of monoterpenoid chemicals including geranial, neral, and myrcene for the strong antioxidant activity displayed by the EO as stated by Ruberto and Baratta, (2000). In addition, Valková *et al*., (2022) reported that Citral, geraniol, and 1,8-cineole were the primary components of the LGEO, which demonstrated high antioxidant activity.

Since there is a growing demand for natural rather than synthetic additives, EOs are highly valued for use in many commercial food and beverage items since customers perceive them as "natural" components. However, they create challenges for incorporation into food items because to their low water solubility and strong fragrance, especially at high quantities that are microbiologically effective (Salvia-Trujillo *et al*., 2014). Additionally, consumers have called for foods that have undergone minimum processing and contain little to no synthetic preservatives as reported (Román *et al*., 2017).

The objective of this research is determined the acceptability and safety of LGEO in fresh orange juice, as antimicrobial and antioxidant activity factor, and protected physicochemical properties of orange juice which preserved at 4 °C.

MATERALS AND METHODS

Materials:

Spices: lemongrass (*Cymbopogom citratuse* L.) was obtained at 2023 from AMD *verde* company, Cairo, Egypt.

Orange fruits: was procured from local vegetable market of Mansoura city, El Dakahlia Governorate, Egypt.

Microbial strains: The LGEO was individually tested against a panel of microorganisms including

1- (*Escherichia coli*, and *Salmonella typhi*) as Gram-negative bacteria.
2- (*Bacillus subtilis and Staphylococcus aureus)* as Gram-positive bacteria.
3- (Saccharomyces cerevisiae) as yeasts.
4- (*Aspergillus niger and Aspergillus flavus*) as fungi.

These strains were obtained from (MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Chemicals and reagents:

Without additional purification, all compounds were of reagent grade. Folin-Ciocalteau reagent, 1,1-diphyenyl-picrylhdrazyl (DPPH), Phenolphthaline reagent, Potassium ferricyanide, and Sodium hydroxide were purchased from El-Gomhoria company for chemicals Mansoura, Egypt.

Methods:

Preparation of fruit juices:

Fresh, consistent, completely ripe Valencia orange (*Citrus sinensis*), called summer orange were hand-peeled, deseeded, and rinsed under running water in a clean laboratory environment before the pulp was mixed. Orange juice that had been further extracted was divided into four equal batches of 400 mL each after being filtered through a clean muslin cloth as reported (Kapoor et al., 2014). Following that, LGEO was added to four batches of fresh orange juice at four different concentrations (0.5, 0.75, 1, and 1.25 l/ml), with the control batch remaining LGEO-free. All batches divided into glass bottles and are stored in refrigerator at 4 °C, then analyses were carried out at 0 time and each four days.

Extraction of LGEO:

The air-dried and finely grounded plants were submitted to water distillation for 3 hours using a Clevenger-type apparatus. The obtained essential oils were dried over anhydrous sodium sulfate and, after decanted stored at -18 ^{o}C in glass vials in the dark until used (Tepe *et al*., 2005).

Analytical methods:

Chemical analysis:

The pH values of orange juice were determined using a digital potentiometer, and the Total Acidity of orange juice was determined using phenolphthalein as an indicator with 0.1N NaOH (equivalents of citric acid) (AOAC 2012), and TSS °Brix were determined according to the AOAC (2012). To determine the chemical components of LGEO was utilized Gas Chromatography/ Mass Spectrometry (GC/MS) technique (Adams, 1995).

Refractive index:

Using an Abbe refractometer, the essential oil's refractive index value was measured at room temperature (Abbement 3200, Germany), according to Guenther (1961).

Evaluation of antimicrobial activity (disc diffusion method) of LGEO:

The size of the zone of inhibition surrounding each disc, taking into consideration the disc diameter, was used to measure the antibacterial activity according to (Helal *et al*., 2006, and Al Haiali *et al*., 2012).

Evaluation of antioxidant activity of CCEO:

Antioxidant activity assessment (DPPH test): It was employed to assess the LGEO's capacity to scavenge free radicals according to Rekha *et al*. (2012).

Ferric reducing antioxidant power (FRAP): It was employed to determine the LGEO's ferric reducing assay according to Rekha *et al*. (2012).

Microbiological analysis:

- Plate agar count was used to determine the aerobic bacterial count following a 48 ± 2 hrs incubation at 35± 1ºC °C, and the potato dextrose agar medium was used to count the number of yeasts and molds present (APHA, 1992)
- *Salmonella Typhimurium* detection and count each sample was combined aseptically (in quantities of 25 g or ml) with 225 ml of sterile buffer peptone water, which was then incubated for 24 hours at 35 °C according to (Bridson, 2006).
- *Escherichia coli* in samples were counted by spreading 0.l ml of each sufficient (expected) dilution onto plates of sorbitol MacConkey agar medium, followed by a 24-hour incubation period at 35° C (Bridson , 2006).
- *Staphylococcus aureus* detection and counting using Baird Parker media augmented with egg yolk and potassium tellurite solution. 48 hours were spent incubating plates at 37 °C (Bridson, 2006).

Organoleptic tests (sensory evalution of fruit juces):

The 9 point hedonic test was used to measure the overall acceptability on a scale as described by (Bisla et al., 2014).

Statistical Analysis:

Two ways ANOVA analyze the experimental data, and the least significant difference test (LSD) was then used to further investigate the means as using CoStat program, version 6.311 (2005). To compare treatment means, least significant difference tests with a 0.05 level of significance were run. Results are presented as mean ± standard error. All determinations were repeated three times.

RESULTS AND DISCUSSION

Our results illustrated that refractive index of lemongrass essential oil at ambient (room temperature) was 1.4890. Hence, refractive index is used mainly to measure the change in unsaturation as the oil is hydrogenated. It is a measure of how fast light travels through a substance and it is used to identify, confirm purity and measure concentration of the substance as mentioned (Codex standard, 2001). The value obtained is within the range of the literature (1.469-1.479) of *Cymbopogon citratues* according to Codex standard (2001). This finding is agreed with Kumar *et al*. (2017); Olayemi *et al*. (2018) they reported that the refractive index 1.483-1.489 of lemongrass essential oil. However, this study had data higher than reported by Codex standard, (2001), they found that the refractive index was 1.431 ± 0.030. These differences could also be attributed to the region from which the cultivars were obtained which reported by Attokaran (2011).

Table (1) and Figure (1) showed that composition of *Cymbopogon citratus* essential oil include 34 compounds. The main components were α-citral (18.1%), Neral (14.08%), β-myrcene (11.64%), m-Cymol (5.86%), and Verbenol (5.43%). It had minor components i.e, 1,3,8-p-Menthatriene, 3-Carene, α-Pinene, (+-)-Linalool, Carveol, Isopulegone, Limonene, and Nerol acetate. In addition, compounds average are less than 2% such as p-Cymenene, Citronellal, (-)-cis-Isopiperitenol, and Geraniol, others. This result is harmony with (Ganjewala *et al*. (2008) who reported that α-citral as the major oil constituents which accounted for 80-85% of the total monoterpenes.

Table 1. *Cymbopogen citratus* essential oil composition determined using GC–MS

No	RT (min)	Name	Area sum%
1.	6.245	β-Myrcene	11.64
2.	6.474	1,3,8-p-Menthatriene	4.84
3.	6.765	m-Cymol	5.86
4.	6.884	3-Carene	2.05
5.	7.032	α-Pinene	2.61
6.	7.655	p-Cymenene	1.45
7.	7.754	(+-)-Linalool	2.23
8.	7.963	Carveol	2.34
9.	8.147	Terpinolene	0.38
10.	8.356	Isopulegone	2.96
11.	8.459	Citronellal	1.16
12.	8.606	Limonene	2.36
13.	8.844	Verbenol	5.43
14.	9.226	(-)-cis-Isopiperitenol	1.21
15.	9.373	Narirutin	0.79
16.	9.677	Neral	14.08
17.	9.833	Geraniol	1.39
18.	10.07	α-Citral	18.1
19.	10.858	Ascaridole	0.51
20.	10.977	Humulena	0.39
21.	11.194	Nerol acetate	2.44
22.	11.649	p-Cymen-7-ol	0.31
23.	11.854	α-Longipinene	0.70
24.	12.395	cis-Sequisabinene hydrate	0.33
25.	13.847	Phytol	0.90
26.	14.442	Hexa-hydro-farnesol	1.41
27.	15.057	Dihydrosqualene	1.41
28.	15.397	Heptacosane	1.92
29.	15.688	Geranyl isovalerate	1.35
30.	16.513	Erucic acid	1.54
31.	17.337	Octacosanol	2.02
32.	18.177	1-Tricosanol	1.76
33.	19.166	1-Hexacosanol	1.18
34.	20.38	Nonacosane	0.97
Total			97.68

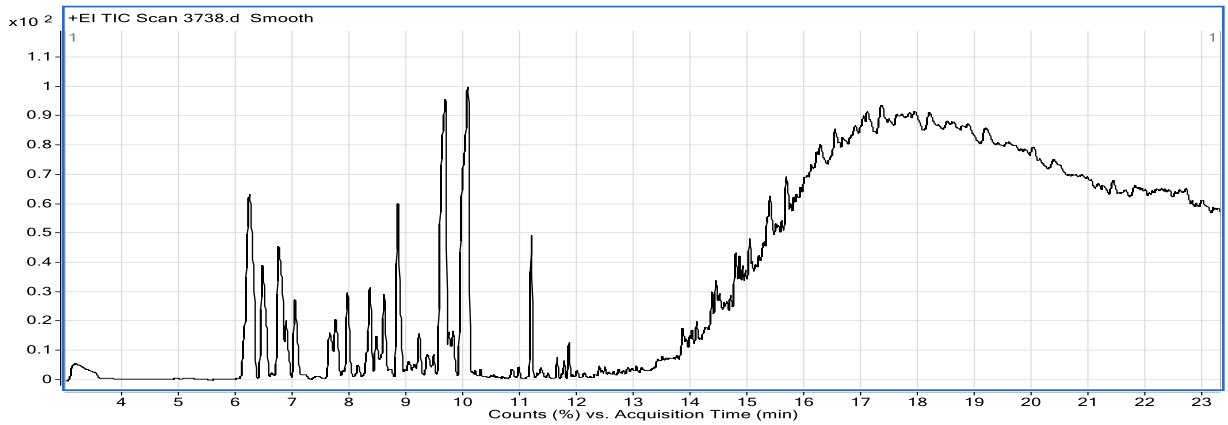

Figure 1. GC–MS chromatogram of lemongrass essential oil

Antimicrobial activity of LGEO:

Table (2) revealed that lemongrass essential oil had antimicrobial activity on microorganisms, where it was carried out used agar disc diffusion method. Where, the agar disc diffusion method is a widely used method for quickly evaluating the antibacterial properties of natural extracts and EO as mentioned (Boukhatem *et al*., 2014). Antimicrobial effects by using the essential oil of *C. citratus*, was found by Onawunmi (1989) as mentioned previous. Results of antimicrobial activity of LGEO by determined diameter inhibition zone illustrated that lemongrass oil was influenced bacteria like *E. coli* and *S. Typhi*, as gram negative bacteria that values about 6.5-11.5 mm, and 7.5-12.0 mm, respectively at concentration 0.5-1.25 µl/ml of LGEO (Figure 2). In addition, it could be observed antimicrobial activity of LGEO on bacteria like *B. subtilis* and *Staph. aureus* as gram positive bacteria that values 10.5-13.0 and 10.0-13.5 mm, respectively (Figure 2). In context, it was observed inhibition of fungi and yeast by LGEO especially *A. flavus, A. niger*, and *S. cervisiae* recorded 6.5-9.5 mm, 6.0-9.8 mm, and 5.5-8.5 mm at concentration 0.5-1.25 µl/ml of CCEO, respectively (Figure 2). Where, LGEO concentrations increased due to increase of diameter inhibition zone in all tests microorganisms (Table 2). These finding are harmony with, Onawunmi (1989), who analyzed the action of essential oil at 0.05% to *Staphylococcus aureus, Bacillus subtilis* and *Escherichia coli* by agar diffusion method. Also, who reported the most effective essential oils were those that performed best against *S. aureus, B. cereus, L. monoctyogenes, E. coli*, and *S. Typhi*, key pathogen strains and markers of food quality. The inhibition areas are similar to those observed by Tyagi and Malik (2012) showed that comparing the disc diffusion assay's zone of inhibition (i.e., 13.5mm for the same amount of oil) to the zone of inhibition caused by the vapor phase antimicrobial efficacy evaluation. According to the current data, LGEO is quite effective in vapor phase against *E. coli*. In context, de Oliveira *et al*., (2013) discussed that the concentration of 1.56% was chosen as the Minimum Inhibitory Concentration (MIC), which caused inhibition zones with an average diameter of 5.33 mm to appear for *C. citraus*. Most researchers consider the lowest inhibitory concentration as the standard for evaluating the efficacy of antibacterial essential oils as stated (Burt, 2004).

Table (2) observed that the efficacy of CCEO on gram positive bacteria stronger than gram negative bacteria, that revealed in increased of diameter inhibition zone in gram positive bacteria. While, Gupta *et al*., (2016) they reported that all microorganisms with the exception of E. coli were very susceptible to lemongrass oils' potent antibacterial effects. Where, the typical inhibitory zone against bacteria measured 27 mm in diameter. But *B. subtilis* was the most sensitive bacterium to essential oil. Also they reported that Essential oils' antimicrobial properties are associated with their chemical makeup, and more specifically with the dominant constituent(s). Similar results were reported by Premathilake *et al*. (2018) who investigated such pathogenic bacterial strains as *E. coli, B. cereus*, and *S. aureus*. In comparison to the Gram-negative strain E. coli, gram-positive bacteria were more susceptible to the essential oil of *C. citratus* at all doses. In context, Abdel-Gwada *et al*. (2022) found that inhibition zones of Lemongrass essential oil against *E.coli, S. Typhi*, and *S.aureus*.

In context, it was observed inhibition of *A. flavus, A. niger*, and *S. cervisiae* about 6.5-9.5 mm, 6.0-9.8 mm, and 5.5-8.5 mm at concentration 0.5-1.25 µl/ml of CCEO, respectively (Figure 2). Where, with increased of concentrations of LGEO due to increase of diameter inhibition zones. Thus, use 1.25 µl of LGEO has higher effect on all fungi and yeast. Whereas, the diameter inhibition zones of fungi and yeast are smaller than their bacteria strains in our study. These findings are harmony with Gupta *et al*., (2016) who reported that with mean inhibition zone diameters of 20 mm and 27 mm, respectively, lemongrass essential oil demonstrated potent antifungal activity against both *A. niger* and *C. albicans*. The antimicrobial activity of lemongrass essential oil is usually higher against fungi than bacteria according to Premathilake *et al*. (2018). In context, *A. niger* was the most sensitive strain against lemongrass EO as mentioned (Mahanta *et al*., 2007). The high antifungal activity of lemongrass essential oil is attributed to the presence of two isomers of citral as reported (Leite *et al*., 2014). According to Harris (2002), Citral appears to interact mostly with the fungi's cell wall. Such interaction influences its synthesis, suppressing it, and ultimately leading to cell death. In context, Zhou *et al*. (2014) revealed that Citral significantly suppressed the growth of mycelium, and its antifungal effects were linked to the rupture of cell membranes and subsequent release of cellular components. The inhibiting activity of lemongrass essential oil may also stem from the synergistic effect of individual minor or major compounds according to Nguefack *et al*. (2012).

One of the primary alcohols included in lemongrass essential oil, geraniol, apparently does not interact with ergosterol or prevent the formation of fungus cell walls as part of its mode of action as mentioned (Leite *et al*., 2015). Whereas, Pereira *et al*. (2015) suggested that, two monoterpene alcohols, geraniol and citronellol, have antifungal effects on *Trichophyton rubrum* by inhibiting the formation of ergosterol. In general, the high lipophilic nature and low molecular weight of the essential components of lemongrass oil, terpenes and terpenoids, determine the oil's high antifungal activity, which likely involves rupturing the cell membrane, resulting in cell death, or impeding sporulation and germination of fungi. Citral, citral diethylacetal, and limonene are thought to be the major biologically active compounds present in these oils that are responsible for much of their antifungal activity; however, oils generally contain many more minor compounds which may contribute to antifungal activity as well according to Sellamuthu *et al*. (2012).

Table 2. Antimicrobial activity of LGEO

	Gram Negative Bacteria		Gram Positive Bacteria		Fungi		Yeast
Inhibition zone (mm) concentration	***E.coli***	***S.typhi***	***B.subtilis***	***Stap. aureus***	***A.flavus***	***A.niger***	***S.cervisiae***
0.5 µl/ml	6.5	7.5	10.5	10.0	6.5	6.0	5.5
0.75 µl/ml	7.5	8.5	11.5	11.5	7.0	7.0	5.5
1 µl/ml	8.5	9.0	12.0	13.0	8.5	8.6	7.5
1.25 µl/ml	11.5	12.0	13.0	13.5	9.5	9.8	8.5

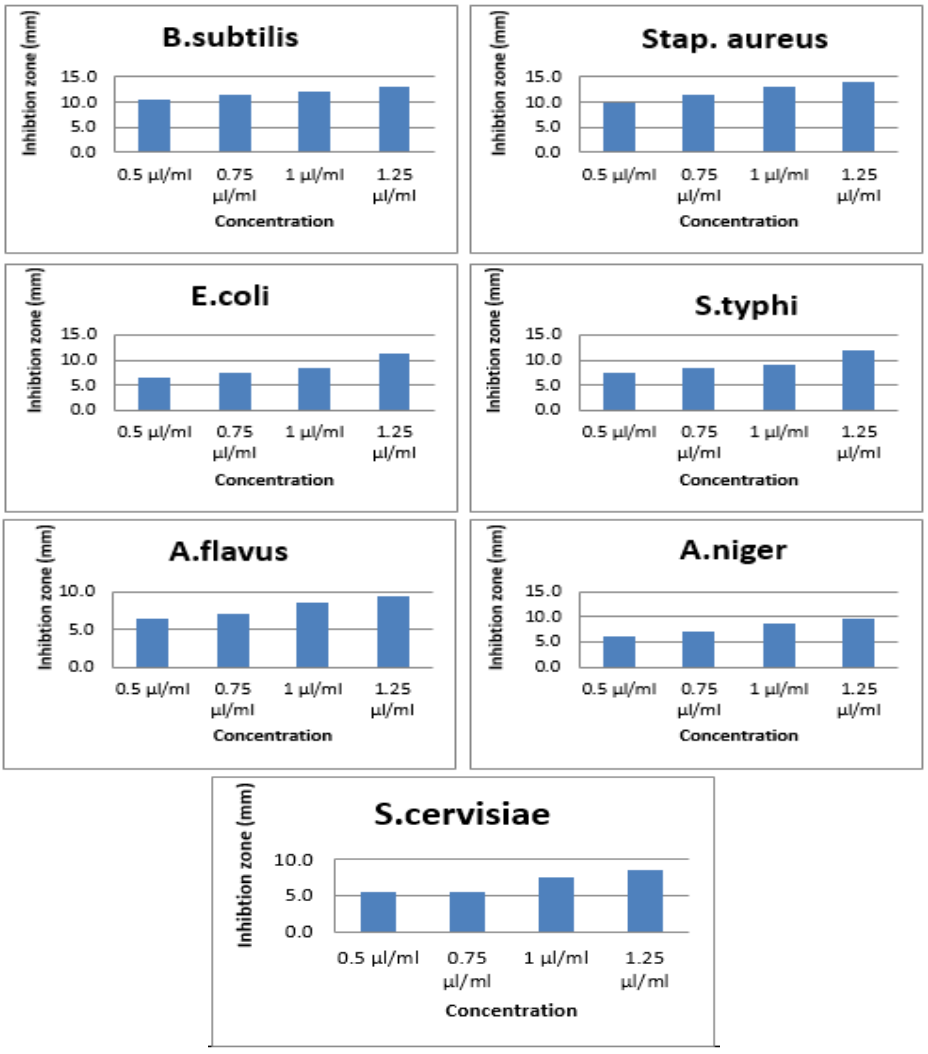

Figure 2. antimicrobial activity of lemongrass essential oil

Antioxidant activity of CCEO:

Our study elicited that CCEO had antioxidant capacity which was DPPH scavenging activity (%) of essential oils (39.26-75.8%) at concentration 0.5-1.25 µl/ml of CCEO (Table 3). Hence, 39.26% registered for 0.5 µl/ml concentration as lower concentration and on higher concentrations (75.8%) was registered, which was the highest compared with BHT 200ppm (70.36 %) (Figure 3). This finding is harmony with Kumar *et al*. (2017) who reported that there was an incremental trend of radical scavenging with increasing concentration of oil. The presence of active principles, such as citral in lemongrass, may be the cause of these oils increased capacity to scavenge free radicals. Also is going to, Soares *et al.*, (2013) who reported that the *C. citratus* EO was found to have similar to that exhibited by the synthetic antioxidant BHT. However, the oil has a significant benefit over BHT in this regard: because they are derived from plants, plant-based antioxidants do not cause the adverse effects, which caused by synthetic antioxidants like BHT.

On the other hand Table (3) showed that antioxidant activity of essential oils (ferric reducing assay), hence, there was an incremental trend of ferric reducing assay with

increasing concentration of oil. When, concentration 0.5 µl/ml, ferric reducing assay was 0.507, and concentrations 0.75, 1, and 1.25 µl/ml, ferric reducing recorded 0.613, 0.715, and 0.827, respectively. In literature, Lemongrass essential oil's antioxidant qualities are thought to be a result of the oil's complex chemical makeup, as even minute components can affect and control the activity of the entire oil. Incorporating *C. citratus* essential oil into the composition of nutraceuticals or/and functional foods may be worthwhile given its antioxidant qualities. In food items, antioxidants can scavenge free radicals and delay lipid oxidation, which is the primary reason why food quality degrades. In many countries, the application of essential oils is not regulated whatsoever. Inappropriate and sporadic usage of lemongrass essential oil may also cause health issues brought on by mutations, carcinogenic effects, and genetic harm as stated (Sousa *et al*., 2010).

Table 3. Antioxidant activity of LGEO:

concentrations	DPPH scavenging activity (%)	Antioxidant activity (ferric reducing assay).
0.5 µl/ml	39.26	0.507
0.75 µl/ml	49.25	0.613
1 µl/ml	59.05	0.715
1.25 µl/ml	75.8	0.827
BHT (200 ppm)	70.36	0.801

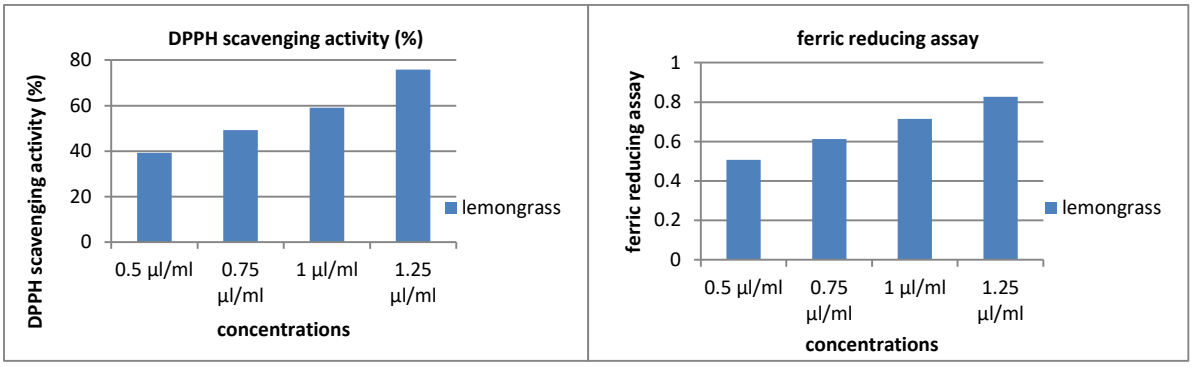

Figure 3. Antioxidant activity of lemongrass oil

Application of lemongrass essential oil for preserved fresh Valencia orange (*Citrus sinensis*) juice:

Organoleptic evaluation, which is relevant to all food states, is one of the most important elements that can be utilized as a conclusive indicator of a food's quality from the perspective of the customer. The tabulated findings showed that all orange juice samples with very good or above color scores did not have their appearance and color altered by lemongrass essential oils (table 4). Where, there is no significant difference in odor, taste, and overall palatability between S1 (orange juice without CCEO) and S2T1 (orange juice with 0.5 µl/ml). It was discovered that the evaluation of the juice to which lemongrass essential oil was added for both flavor and overall palatability decreased as the content of lemongrass essential oil increased. Both the S2T2 and S2T3 treatments had extremely good overall palatability ratings of 7.55 and 7.40, respectively. For sample S2T4, the treatments' lowest values for aftertaste and general palatability were 6.05 and 6.95. These results are approach with these of de Sousa *et al*., (2005).

Table 4. Organoleptic evaluation of orange juice treated with essential oils of lemongrass

Treatments	Appearance	Color	Odor	Taste	Aftertaste	Overall palatability
S1	8.45^{a}	8.70^{a}	8.55^{a}	8.55^{a}	8.65^{a}	8.58^{a}
S2T1	8.55^{a}	8.50^{a}	8.40^{a}	8.20^{ab}	8.10^{b}	8.25^{a}
S2T2	8.70^{a}	8.65^{a}	8.55^{a}	7.80^{b}	7.50^{c}	7.55^{b}
S2T3	8.44^{a}	8.75^{a}	7.10^{b}	7.60^{b}	7.50^{c}	7.40^{b}
S2T4	8.65^{a}	8.50^{a}	6.98^{b}	6.95^{b}	6.95^{d}	6.05^{c}
LSD	0.372	0.374	0.671	0.623	0.406	0.397

(S1= orange juice without CCEO. S2T1= orange juice with 0.5 µl/ml CCEO. S2T2= orange juice with 0.75 µl/ml CCEO. S2T3= orange juice with 1.0 µl/ml CCEO, S2T4= orange juice with 1.25µl/ml CCEO- ^{a,b,c} Values with different superscript on the same column are significantly different (p<0.05)).

Microbiology examinations of orange juice samples treated with CCEO:

Table (5) showed that, control sample S1 (without EOs) has the highest value of viable cell count of bacteria, after 8 days at 4 °C was 137 ± 1.09 CFU/ml, this limit is not consistent with the microbial limit standards as stated by NFSA Decree (2021). Samples of orange juice treatment with LGEO have lower counts compared to another treated samples at four storage periods (12 days). Samples with 0.5 µl/ml orange juice has highest total count of bacteria 38 ± 4.4 CFU/ml compared with samples with 0.75 µl/ml orange juice 36 ± 3.7 CFU/ml, and samples with 1.00 µl/ml orange juice 34 ± 2.9 CFU/ml. Throughout our findings CCEO using given the best results in total count test, in bacteria and fungi compared with control sample. General, increasing of EO concentrations lead to reduce of total counts of treated samples, during four storage periods at 4°C. In microbiological test, no significant differences in bacterial count were observed for all samples compared to control sample, while no yeast or fungus was detected in all samples at zero time.

Results showed that all samples did not detected yeast and fungi at zero time, Table (5) and these findings are consisted with Adjou *et al*. (2017) who did not find any viable cell count of yeast and fungi in the same conditions. Even in 4 days of storage no detected yeast and fungi exception control sample Table (5). Whereas, bacteria, fungi, and yeast were the highest in S1, compared with, other samples that mean about 45 ± 3.6 CFU/ml, and 23 ± 4.6 CFU/ml, respectively. In addition, it did not detective *Salmonella*, *E.coli*, and *S.aureus* during our study. Results reported that limits of viable cell count of microorganisms in treated orange juice were in allowed limits for 8 days of storage at 4 °C, which were reported by NFSA Decree (2021). Similarly, was observed that the bacterial count increased 100 CFU/ml in control to 137 ± 4.6 CFU/ml, and that of fungi and yeasts to

50 CFU/ml to 62 ± 5.3 CFU/ml, thus excluding subsequent storage (8 days). In the subsequent storage period (12 days) microbial count for all samples exceeded the allowable safety limit, that reported by NFSA Decree (2021).

Table 5. Microbiology test of juice sample treated with lemongrass essential oil stored 4°C.

Samples	Storage periods at 4°C							
	0 Time		4 Days		8 days		12 days	
	Total count CFU/ml	Yeast, Mold CFU/ml	Total count CFU/ml	Yeasts, Molds CFU/ml	Total count CFU/ml	Yeast, Mold CFU/ml	Total count CFU/ml	Yeast, Mold CFU/ml
S1	33 ± 0.57	ND	45 ± 0.69	23 ± 0.69	137 ± 1.09	62 ± 1.06	-	-
S2 T1	33 ± 1.12	ND	38 ± 0.49	ND	45 ± 0.79	29 ± 0.99	102 ± 1.09	45 ± 1.01
S2 T2	32 ± 0.87	ND	36 ± 1.08	ND	43 ± 0.94	27 ± 0.98	100 ± 1.08	43 ± 1.02
S2 T3	32± 0.94	ND	34 ± 0.99	ND	41 ± 0.99	25 ± 0.93	99 ± 1.10	42 ± 1.04
LSD	3.202	-	3.697	-	3.323	2.655	3.121	3.263

(S1= orange juice without CCEO . S2T1= orange juice with 0.5 µl/ml CCEO. S2T2= orange juice with 0.75 µl/ml CCEO. S2T3= orange juice with 1.0 µl/ml CCEO. Mean ±SD . CFU/ml=Colony Forming Unit, ND=Not detected, LSD= Least Significant Difference)

Sensory attributes of studied orange juice samples after different periods of storage at 4°C:

Orange juice held at 4 °C was tested for its organoleptic qualities after being added Lemongrass essential oil (0.5-1.25 µl/ml). In Table (6), the statistical analysis of the received data ($p < 0.05$) is presented. The appearance and color of the items during storage varied significantly. The odor, aftertaste, and general palatability are unremarkable after 8 days of cold storage. The average of S1, S2T1, and S2T2 were 7.59, 7.85, and 7.62, respectively, and there is no difference in overall palatability between these averages. The samples (S2T1, S2T2, and S2T3) with the lowest overall palatability scores were 5.65, 6.50, and 6.75 correspondingly after 12 days of cold storage. These results were going with that reported by Abed *et al*. (2022).

Table 6. Sensory evaluation of orange juice with essential oils of lemongrass stored 4°C.

Sensory attributes	Storage period 4°C (Days)	Treatments				
		S1	S2T1	S2T2	S2T3	LSD
Appearance	0	8.45± 0.493	8.55± 0.545	8.70± 0.545	8.40± 0.765	0.255
	4	8.00± 0.543	8.20± 0.453	8.15± 0.765	7.80± 0.543	0.221
	8	6.70± 0.554	7.20± 0.654	7.30± 0.745	7.30± 0.454	0.442
	12	-	5.8± 0.865	7.2± 0.543	7.15± 0.454	0.422
Color	0	8.70± 0.567	8.50± 0.986	8.65± 0.475	8.70± 0.756	0.238
	4	7.55± 0.562	8.00± 0.876	8.05± 0.865	7.90± 0.565	0.206
	8	5.95± 0.467	7.35± 0.765	7.60± 0.097	7.75± 0.455	0.412
	12	-	5.95± 0.987	7.4± 0.676	7.5± 0.654	0.503
Odor	0	8.55± 0.654	8.40± 0.765	8.55± 0.49	7.15± 0.538	0.280
	4	8.15± 0.654	8.20± 0.456	8.05± 0.765	7.60± 0.343	0.243
	8	6.40± 0.765	7.45± 0.546	7.35± 0.456	7.60± 0.356	0.301
	12	-	6.4± 0.654	7.15± 0.466	7.6± 0.667	0.382
Taste	0	8.55± 0.657	8.20± 0.896	7.80± 0.543	7.60± 0.765	0.316
	4	7.95± 0.643	8.25± 0.365	6.98± 0.875	7.80± 0.456	0.274
	8	6.50± 0.733	7.35± 0.576	7.45± 0.334	7.70± 0.456	0.547
	12	-	6.5± 0.587	6.55± 0.356	6.5± 0.665	0.591
Aftertaste	0	8.65± 0.632	8.10± 0.864	7.50± 0.876	7.50± 0.765	0.296
	4	8.20± 0.098	8.05± 0.485	7.85± 0.543	7.85± 0.876	0.259
	8	6.40± 0.065	7.40± 0.876	7.50± 0.865	7.75± 0.898	0.513
	12	-	5.65± 0.754	7.15± 0.643	6.55± 0.496	0.476
Overall palatability	0	8.58± 0.432	8.25± 0.744	7.55± 0.598	7.25± 0.678	0.305
	4	7.80± 0.454	8.00± 0.985	7.75± 0.457	7.55± 0.878	0.264
	8	6.40± 0.665	7.30± 0.568	7.55± 0.687	7.55± 0.656	0.529
	12	-	5.65± 0.643	6.5± 0.567	6.75± 0.856	0.582

(S1= orange juice without CCEO . S2T1= orange juice with 0.5 µl/ml CCEO. S2T2= orange juice with 0.75 µl/ml CCEO. S2T3= orange juice with 1.0 µl/ml CCEO. Mean±SD. LSD=Least Significant Difference)

The results of sensory test showed that the orange juices without essential oils S1 (control) and orange juice treated with 0.5, 0.75, and 1µl/ml of CCEO were more acceptable, especially the taste and aftertaste, during the third storage period (8 days). However, with subsequent cold storage, most of the properties of product are significantly reduced. This is consistent with the results of the microbiology testing recommendations that S1 recommended to exclude S1 for its microbial increase on the limits allowed from the (NFSA Decree 1, 2021). Thus it is necessary to link the sensory evaluation with microbiology test.

Effect of LGEO applied on physicochemical of fresh orange juice after storage periods at 4 °C:

Data in table (7) show the physicochemical characteristics, namely °Brix, pH, and titratable acidity (citric acid equivalent) in orange juices with or without CCEO were evaluated immediately after the EO addition and during storage periods. The total soluble solids content showed nearly percentages after 0, 4, and 8 days of storage period (12.1, 12.1 and 12.0%) for control Sample (S1). While orange juice with lemongrass oil showed nearly percentages after 0, 4, 8, and 12 days (12.2, 12.1, 12.1 and 12.0%), (12.3, 12.2,12.2 and 12.1%), and (12.3,12.2,12.1 and 12.0%), respectively, for S2T1, S2T2, and S2T3 samples, respectively.

Table (7) reported that the pH was increased while total acidity (% as citric acid) was decreased after 0, 4 and 8 days of storage period, where were recorded (4.25, 4.28 and 4.33), (0.21, 0.20, and 0.19%) for control Sample (S1). While, pH of S2T1 sample after 0, 4, 8 and 12 days were 4.29, 4.31, 4.33 and 4.33, respectively, also, acidity were 0.22, 0.21, 0.20 and 0.19% (as citric acid), respectively. S2T2 sample recorded pH values 4.12, 4.19, 4.18 and 4.18, respectively and total acidity values 0.21, 0.20, 0.19 and 0.18%, respectively. S2T3 sample had pH values 4.14, 4.20, 4.21 and 4.21, and total acidity values 0.22, 0.20, 0.19 and 0.18%, respectively. These results are similarly observed a pattern of alteration in physicochemical parameters of EOs-supplemented orange juices (Kapoor *et al.,* 2014), they reported the increase in pH during storage may be due to decrease in acidity and increase in total sugar content as stated by Baruah and Mohan (1985), also, the decline in acidity could be due to conversion of acid into sugar and salts as stated by Ruttner et al. (1975).

Table 7. Effect of refrigerated storage period on TSS, pH and acidity of orange juice preservation with LGEO:

Results	Storage period 4°C (Days)	Treatments			
		S1	S2T1	S2T2	S2T3
TSS (%)	0	12.1±0.11	12.2±0.20	12.3±0.08	12.3±0.09
	4	12.1±0.09	12.1±0.012	12.2±0.010	12.2±0.09
	8	12.0±0.08	12.1±0.12	12.2±0.13	12.1±0.12
	12	-	12.0±0.12	12.1±0.11	12.0±0.13
pH	0	4.25±0.06	4.29±0.08	4.12±0.04	4.14±0.10
	4	4.28±0.01	4.31±0.06	4.19±0.09	4.20±0.06
	8	4.33±0.11	4.33±0.11	4.18±0.09	4.21±0.10
	12	-	4.33±0.11	4.18±0.09	4.21±0.10
Total acidity (%)	0	0.21±0.11	0.22±0.07	0.21±0.08	0.22±0.11
	4	0.20±0.10	0.21±0.09	0.20±0.10	0.20±0.11
	8	0.19±0.11	0.20±0.09	0.19±0.01	0.19±0.11
	12	-	0.19±0.12	0.18±0.11	0.18±0.09

(Means± standard deviation , S1= orange juice without CCEO. S2T1= orange juice with 0.5 µl/ml CCEO. S2T2= orange juice with 0.75 µl/ml CCEO. S2T3= orange juice with 1.0 µl/ml CCEO)

CONCLUSION

The chemical preservatives used in the food and beverage industries had some benefits, but they also had some drawbacks that had a negative cumulative impact. As a result, recent advancements in food technology are focused on discovering safe and natural biocides as well as alternatives to chemical preservatives. Essential oils (EOs) are all-natural, aromatic oily liquids that can be made from any part of the plant using a variety of techniques. Lemongrass essential oil has antibacterial and antioxidant qualities in addition to being a natural taste ingredient, which helps extend the shelf life of foods. LGEO improvement of orange juice properties and extend of shelf life of juice that increased additive value of orange juice. The results of sensory test showed that the orange juices without essential oils S1 (control) and orange juice treated with 0.5, 0.75, and 1µl/ml of CCEO were more acceptable, especially the taste and aftertaste, during the third storage period (8 days).

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النشاط المضاد للميكروبات والمضادة للأكسدة لزيت حشيشة الليمون فى حفظ عصير البرتقال الطازج.

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الملخص

تناولت تلك الدراسة التأثير المضاد للميكروبات لزيت حشيشة الليمون مثل التأثير المضاد للبكتيريا الموجبة لجرام B.subtilis , Stap. aureus والبكتيريا السالبة لجرام E.Coli, S.Typhi ، وفطريات A.flavus, A.niger ، وخميرة S.cervisiae ، والتى أكدت نتائجها ان لزيت حشيشة الليمون تأثير قوى على تلك الكائنات الدقيقة. ومن خلال تلك الدراسه تم التأكيد على تمتع زيت حشيشة الليمون بنشاط مضاد للأكسدة قوى من خلال استخدام DPPH, FRAP . وهذا النشاط مرتبط بالتركيب الكيميائى لزيت حشيشة الليمون المحتوى على ٣٤ مركب ، واكثرهم تركيز هو α-citral و neral . فقد بينت النتائج أن زيادة تركيز زيت حشيشة الليمون حتى 1.25 µl/ml عززت من زيادة النشاط المضاد للميكروبات وايضا النشاط المضاد للاكسدة. وقد بينت النتائج التى تم تسجيلها عند تطبيق حفظ عصير البرتقال الطازج بالتبريد (4°C) بزيت حشيشة الليمون بالتركيزات المختلفة أن زيت حشيشة الليمون يعمل كمادة حافظة طبيعية لعصير البرتقال الطازج. ساهم بدرجة كبيرة فى تحسين الصفات الطبيعية للعصير وايضا الخواص الميكروبيولوجية والحسية للعصير. وتوصى الدراسة بان زيت حشيشة الليمون من البدائل الهامة والضرورية لحفظ المشروبات الطازجة والتى أعطت نتائج أكثر إيجابية وأمان لإستهلاك العصائر دون أى قلق أو خوف من وجود أى مواد مضافة للأغذية قد تكون ضارة بالغذاء وأيضا ضارة بالمستهلك.