



Research Article

Chemical composition and antibacterial activity of the peel essential oils extracted from citrus fruits

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Abstract

Citrus peel is an important source of essential oils (EOs). However, these EOs are not invested, although the annual production of citrus is high in Syria. The current study aimed to investigate chemical composition and antibacterial activity of some citrus peel EOs, namely: lemon (*Citrus limon*), orange (*C. sinensis*), grapefruit (*C. paradisi*), mandarin (*C. reticulata*) and bitter orange (*C. aurantium*). Gas chromatography-mass spectrometry (GC/MS) (gas chromatograph type: Agilent 7890A, auto sampler type: Agilent 7683B coupled to mass spectrometer, type Agilent 5975C, using DB-1 capillary column. EOs. concentration 1: 10 v/v in chloroform, injection volume 1 μ l, split ratio 1: 80), was used to identify the chemical composition of the EOs, which were extracted by hydrodistillation technique, and chemical composition was expressed as Mean \pm SD of three replications using SPSS V17 software. Minimal bactericidal concentration (MBC) was used to determine the antibacterial activity against five Gram-positive bacteria (*Bacillus cereus*, *B. licheniformis*, *Staphylococcus haemolyticus*, *S. lugdunensis*, *Enterococcus faecalis*) and five Gram negative bacteria (*Klebsiella oxytoca*, *Citrobacter koseri*, *Serratia liquefaciens*, *Pseudomonas fluorescens* and *P. luteola*). Limonene formed the vast majority of EOs (between 62.16 and 95.26% in lemon and orange EOs, respectively), but there were other active components, such as α -Pinene and β -Pinene. Lemon EO was the most effective one, with MBC values ranged between 4 μ l.ml⁻¹ (against *Bacillus cereus*) and 50 μ l.ml⁻¹ (against *Serratia liquefaciens*). *Pseudomonas luteola* (a Gram-negative bacterium) was the most sensitive species to citrus EOs (MBC values ranged between 4 and 50 μ l.ml⁻¹ for lemon and orange EOs, respectively); while *S. liquefaciens* (a Gram-negative bacterium) was the most resistant bacterium (MBC values were 50 and 150 μ l.ml⁻¹ for lemon and mandarin EOs, respectively) among all species studied in the current research.

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Introduction

Essential oils (EOs) are natural substances extracted from medicinal and aromatic plants, commonly by distillation processes. These compounds have an important role in food preservation contributing to safety and shelf-life extension of food products (Shehata et al., 2020). Interest in the application of essential oils in various fields is increasing continuously, and with their significant pharmacological activities, these aromatic medicinal plants are being implemented in pharmaceutical industries as antibacterial, anti-inflammatory, antiviral and antifungal agents (Aziz et al., 2018).

Citrus peel represents about 45% of the total fruit weight, which is considered as a processing byproduct, that creates environmental problems (Farhat et al., 2011). The major classical use of citrus EOs from citrus peels is as natural flavouring materials, which have great commercial importance (Mustafa, 2015). Over 400 volatiles of different chemical nature have been exclusively described in some citrus species (González-Mas et al., 2019). It has demonstrated that citrus EOs have a wide spectrum of bioactive properties such as antioxidant, antiviral, anticancer (Mancuso et al., 2019), antimicrobial (Jafari et al., 2011), anti-inflammatory, antiaging, antibacterial, antifungal, and anti-aflatoxigenic activities (Barreca et al., 2017; Celano et al., 2019; Hasiija et al., 2015; Kamal et al., 2013). Citrus EOs are largely employed as aromatizers in the food and pharmaceutical industries. In particular, lemon and bergamot EOs are used in the cosmetic industry, for the production of perfumes, detergents and body-care products (Osman, 2019). Essential oils are important materials because they can be used in any foods and are considered generally recognized as safe (GRAS) (Boudries et al., 2017).

In this study, the chemical composition of peel EOs of common citrus species cultivated in Syria (lemon, orange, grapefruit, mandarin and bitter orange) was investigated. In addition, antibacterial activities of these EOs against some Gram-positive bacteria (*Bacillus cereus*, *B. licheniformis*, *Staphylococcus haemolyticus*, *Staphylococcus lugdunensis*, *Enterococcus faecalis*), and some Gram-negative bacteria (*Klebsiella oxytoca*, *Citrobacter koseri*, *Serratia liquefaciens*, *Pseudomonas fluorescens* and

Pseudomonas luteola), expressed as MBC value, using broth macrodilution method, were evaluated.

Materials and methods

Plant materials

The current study was performed in the laboratories of the National Commission for Biotechnology (NCBT), Damascus-Syria. Citrus fruit samples [lemon (*Citrus limon*), orange (*C. sinensis*), grapefruit (*C. paradisi*), mandarin (*C. reticulata*) and bitter orange (*C. aurantium*)] were purchased from local market in Damascus, Syria. The fruits were washed with distilled water and wiped with clean cloth. The flavedo layer were manually grated (with a household grater) into small pieces, which were immediately used for essential oils extraction.

Extraction of EOs

The grated fresh peels (flavedo layer) were submitted to hydrodistillation, in which the plant materials are immersed directly into water inside the vessel and the whole mixture is boiled, the device includes a heating source, vessel, a condenser to convert vapor from vessel onto liquid and a decanter to collect the condensate and separate essential oils from water (Aziz et al., 2018); using Clevenger type apparatus. The upper oily layer was separated from the lower aqueous layer by centrifuging at 6000 rpm for 20 min. The upper oily layer was dried over anhydrous sodium sulphate and stored in glass vial with Teflon sealed caps at -20°C in the absence of light until analyzed (Kırbaşlar et al., 2009; Boudries et al., 2017).

Gas chromatography-mass spectrometry analysis

The EOs were analyzed by gas chromatograph (Agilent 7890A, autosampler Agilent 7683B) coupled to mass spectrometer (Agilent 5975C), using capillary column (DB-1, 30 m × 0.25 mm × 0.20 µm). GC/MS spectra were obtained according to the following conditions: Helium carrier gas; flow rate 1 ml min⁻¹; split rate 1: 80; injection volume 1 µl; injection temperature 250°C; the oven temperature program: 60°C for 4 min, 60→64°C at 1°C min⁻¹,

64→155°C at 2.5°C min⁻¹, 155→250°C at 5°C min⁻¹.

The ionization mode used was electronic impact at 70 electron volt. The ion source temperature and the quadrupole temperature were 230°C and 150°C, respectively, and the scanning range m/z was 30–450. The relative percentage of the components was calculated from GC/MS peak areas. Identification was confirmed by comparison of the component mass spectral fragmentation patterns with those stored in the MS database (National Institute of Standards and Technology and Wiley libraries). The identification was farther confirmed by comparison of holding time of sample component with some available standards (Limonene, α -Pinene, β -Pinene, α -Terpineol, β -Linalool, β -Myrcene, *O*-Cymene).

Bacterial strains source, subculture and maintenance

Bacterial strains (*Bacillus cereus*, *B. licheniformis*, *Staphylococcus haemolyticus*, *S. lugdunensis*, *Enterococcus faecalis*, *Klebsiella oxytoca*, *Citrobacter koseri*, *Serratia liquefaciens*, *Pseudomonas fluorescens* and *P. luteola*) were isolated from food samples; and identified by morphological, physiological and biochemical tests, as well as Polymerase Chain Reaction to ensure the identification (data is not showed here). The strains were preserved in broth medium (Luria-Birtani (LB) broth + 20% (v/v) glycerol) and stored at -80°C. Ten μ L of stored culture, were subcultured by streaking on LB agar plates and incubation at 37°C for 24 h. Good isolated colonies were chosen to prepare bacterial suspension for antibacterial activity assay.

Antibacterial activity assay

Antibacterial activity was determined by MBC broth macrodilution method (Sheeladevi & Ramanathan, 2012) with some modifications, as follows: Mueller Hinton broth (MHB) was prepared and poured into sterile screw cap-test tubes. Cell suspension (10⁶ cfu/ml) of each bacterial strain was prepared. The essential oils were prepared at different concentrations (final concentrations 4, 10, 20, 40, 50, 100 and 150 μ L.mL⁻¹) and finally tween 80 was added to the mixture in concentration of 0.5%, the overall volume was 1 ml in each test tube. Ten μ L of prepared bacterial suspension (10⁶ cfu/ml) was inoculated into each test tube containing the mixture of EO, tween 80 and MHB, and the test tubes were incubated at 37°C for 24 h. Mueller Hinton broth containing 0.5% tween 80 (without addition of EOs), was used as control. After the incubation period was passed, 10 μ L of each tube were pipetted and inoculated into a Petri dish containing Luria-Birtani agar. The inoculated Petri dishes were incubated for 24 h. at 37°C and the presence (or absence) of growth was recorded. The lowest concentration of EO at which every bacterium did not demonstrate any visible growth on the Petri dish after incubation was determined as MBC.

Results and discussion

Chemical composition of citrus peel EOs

The GC/MS analysis of citrus EOs demonstrated overall 45 different compounds in the five citrus species. Qualitative and quantitative analyses of the EO profiles are listed in Table 1, which includes the area percentage of the identified compounds, as well as the sum of oxygenated compounds, aldehydes, alcohols and esters.

Table 1. Chemical composition of citrus peel essential oils

compound	Retention Time (Min.)	Lemon%	Orange%	Grapefruit%	Mandarin%	Bitter Orange%
α -Phellandrene(2)	4.846	0.38±0.02	0.00±0.00	0.00±0.00	0.24±0.01	0.00±0.00
α -Pinene(1)	5.037	1.43±0.07	0.56±0.03	0.62±0.03	1.07±0.05	0.59±0.03
Camphene(1)	5.487	0.04±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

... continued Table 1

compound	Retention Time (Min.)	Lemon%	Orange%	Grapefruit%	Mandarin%	Bitter Orange%
β -Phellandrene(1)-(2)	6.365	1.08 \pm 0.05	0.60 \pm 0.03	0.63 \pm 0.03	0.11 \pm 0.01	0.16 \pm 0.01
β -Pinene(1)	6.461	6.96 \pm 0.35	0.07 \pm 0.00	0.06 \pm 0.00	0.50 \pm 0.02	0.3 \pm 0.02
β -Myrcene(1)	7.113	1.53 \pm 0.08	1.85 \pm 0.09	2.11 \pm 0.11	1.99 \pm 0.10	2.08 \pm 0.10
Octanal(1)-(3)-(4)	7.639	0.13 \pm 0.01	0.30 \pm 0.01	0.30 \pm 0.02	0.11 \pm 0.01	0.11 \pm 0.01
Isoterpinolene(1)	8.210	0.33 \pm 0.02	0.00 \pm 0.00	0.00 \pm 0.00	0.18 \pm 0.01	0.00 \pm 0.00
<i>O</i> -Cymene(1)	8.608	0.42 \pm 0.02	0.00 \pm 0.00	0.00 \pm 0.00	0.23 \pm 0.01	0.00 \pm 0.00
Limonene(1)	8.889	62.16 \pm 0.56	95.26 \pm 0.83	93.41 \pm 0.71	86.76 \pm 0.67	95.21 \pm 0.80
1R- α -Pinene(1)	9.397	0.09 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
β -Ocimene(1)	9.910	0.13 \pm 0.01	0.00 \pm 0.00	0.37 \pm 0.02	0.16 \pm 0.01	0.33 \pm 0.02
γ -Terpinene(1)	10.375	12.96 \pm 0.65	0.11 \pm 0.01	0.06 \pm 0.00	7.82 \pm 0.39	0.00 \pm 0.00
cis-Linalooloxide(1)	11.114	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.08 \pm 0.00
Terpinolene(1)	11.910	0.65 \pm 0.03	0.00 \pm 0.00	0.00 \pm 0.00	0.41 \pm 0.02	0.00 \pm 0.00
β -Linalool(3)-(5)	12.687	0.21 \pm 0.01	0.34 \pm 0.02	0.13 \pm 0.01	0.13 \pm 0.01	0.24 \pm 0.01
Nonanal(3)-(4)	12.957	0.11 \pm 0.01	0.00 \pm 0.00	0.08 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
Isopulegol(3)-(5)	15.691	0.18 \pm 0.01	0.00 \pm 0.00	0.13 \pm 0.01	0.00 \pm 0.00	0.00 \pm 0.00
Berbenol(3)-(5)	16.366	0.05 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
Terpinene-4-ol(3)-(5)	16.799	0.26 \pm 0.01	0.00 \pm 0.00	0.06 \pm 0.00	0.08 \pm 0.00	0.00 \pm 0.00
2-Pinene-4-ol(3)-(5)	17.403	0.06 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
α -Terpinol(3)-(5)	17.605	0.37 \pm 0.02	0.00 \pm 0.00	0.00 \pm 0.00	0.03 \pm 0.00	0.07 \pm 0.00
Decanal(3)-(4)	18.689	0.08 \pm 0.00	0.35 \pm 0.02	0.62 \pm 0.03	0.12 \pm 0.01	0.24 \pm 0.01
β -Citral(3)-(4)	20.551	2.02 \pm 0.10	0.11 \pm 0.01	0.05 \pm 0.00	0.00 \pm 0.00	0.06 \pm 0.00
Pergamiol(3)-(5)	21.545	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.12 \pm 0.01

... continued Table 1

compound	Retention Time (Min.)	Lemon%	Orange%	rapefruit%	Mandarin%	Bitter Orange%
Thymol(3)-(5)	22.143	0.00±0.00	0.00±0.00	0.00±0.00	0.05±0.00	0.00±0.00
Citral(3)-(4)	22.245	2.74±0.14	0.13±0.01	0.05±0.00	0.00±0.00	0.00±0.00
(±)-Lavadulol acetate(3)-(6)	27.421	1.43±0.07	0.00±0.00	0.13±0.01	0.00±0.00	0.04±0.00
β-Cu-bebene(2)- (3)	28.377	0.00±0.00	0.00±0.00	0.07±0.00	0.00±0.00	0.20±0.01
cis-Geraniol(2)- (3)-(5)	28.441	0.93±0.05	0.00±0.00	0.11±0.01	0.00±0.00	0.00±0.00
Caryophyllene(2)	29.712	0.44±0.02	0.00±0.00	0.62±0.03	0.00±0.00	0.11±0.01
α-Bergamotene(2)	30.778	0.89±0.04	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
α-Caryophyllene(2)	31.401	0.00±0.00	0.00±0.00	0.07±0.00	0.00±0.00	0.00±0.00
β-Farnesene(2)	32.045	0.09±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Germacrene D(2)	32.818	0.00±0.00	0.00±0.00	0.09±0.00	0.00±0.00	0.06±0.00
Acoradien(2)	32.996	0.03±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
(Z)-β-Farnesene(2)	33.162	0.04±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Eremophilene(2)	33.450	0.17±0.01	0.32±0.02	0.00±0.00	0.00±0.00	0.00±0.00
α-Cubebene(2)	33.593	0.00±0.00	0.00±0.00	0.05±0.00	0.00±0.00	0.00±0.00
γ-Elementene(2)	33.608	0.09±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
cis-α-Bisabolene(2)	34.207	0.09±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
β-Bisabolene(2)	34.448	1.35±0.07	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
(+)-δ-Cadinene(2)	35.027	0.00±0.00	0.00±0.00	0.18±0.01	0.00±0.00	0.00±0.00
α-Santalol(3)-(5)	41.099	0.07±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
α-Bisabolol(3)-(5)	42.578	0.05±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
(1) Monoterpenes		86.69±0.43	97.86±0.49	96.63±0.48	99.12±0.50	98.59±0.49
(2) Sesquiterpenes		4.63±0.09	0.92±0.02	1.71±0.03	0.34±0.01	0.52±0.01
(3) oxygenated compounds		8.68±0.17	1.23±0.02	1.66±0.03	0.51±0.01	0.88±0.02
(4) Aldehydes		1.43±0.03	0.00±0.00	0.13±0.00	0.00±0.00	0.04±0.00
(5) Alcohols		5.08±0.10	0.89±0.02	1.10±0.02	0.22±0.00	0.41±0.01
(6) Esters		2.17±0.04	0.34±0.01	0.43±0.01	0.29±0.01	0.43±0.01

Table 1 shows that the total number of identified compounds was 37, 12, 23, 17 and 17 compounds in lemon, orange, grapefruit, mandarin and bitter orange, respectively. The monoterpene hydrocarbon namely Limonene was the predominant compound representing 62.16, 95.26, 93.41, 86.76 and 95.21% in lemon, orange, grapefruit, mandarin and bitter orange, respectively.

Antibacterial activity of citrus EOs

In vitro antibacterial activity of five EOs (lemon, orange, grapefruit, mandarin and bitter

orange) was investigated against 10 bacterial species (Gram-positive bacteria: *Bacillus cereus*, *B. licheniformis*, *Staphylococcus haemolyticus*, *S. lugdunensis* and *Enterococcus faecalis*; and Gram-negative bacteria: *Klebsiella oxytoca*, *Citrobacter koseri*, *Serratia liquefaciens*, *Pseudomonas fluorescens* and *Pseudomonas luteola*), using MBC method. Eight concentrations of each EO (4, 10, 20, 30, 40, 50, 100 and 150 $\mu\text{L.mL}^{-1}$) were prepared by adding the suitable amounts of each EO to MHB and using tween 80 (0.5% V/V) as emulsifier. The MBC values are shown in Table 2.

Table 2. Minimum bactericidal concentration ($\mu\text{L.mL}^{-1}$) of citrus peel EOs against Gram-positive and Gram-negative bacteria

Bacteria	Minimum Bactericidal Concentration ($\mu\text{L.mL}^{-1}$)				
	Lemon	Orange	Grapefruit	Mandarin	Bitter orange
<i>Bacillus cereus</i>	4	40	50	20	50
<i>Bacillus licheniformis</i>	20	100	50	40	50
<i>Citrobacter koseri</i>	10	100	20	100	50
<i>Enterococcus faecalis</i>	20	100	50	40	40
<i>Klebsiella oxytoca</i>	40	150	100	100	100
<i>Pseudomonas fluorescens</i>	40	50	50	50	40
<i>Pseudomonas luteola</i>	4	50	40	40	10
<i>Serratia liquefaciens</i>	50	100	100	150	100
<i>Staphylococcus haemolyticus</i>	40	150	50	100	50
<i>Staphylococcus lugdunensis</i>	40	50	50	40	50

Table 2 revealed that most Gram-negative bacteria were more resistant to citrus EOs than Gram-positive bacteria, and this result was consistent with that of Nazzaro et al. (2013), who found that Gram-negative bacteria are more resistant compared to Gram-positive bacteria to the plant-origin antimicrobials, because lipopolysaccharide outer membrane forms a selective barrier to protect the bacteria from several antibiotics, detergents and dyes that would otherwise damage the inner membrane (Yap et al., 2021; Stefanello et al., 2008).

Lemon had the most effective EO with MBC values ranged from 4 to 40 $\mu\text{L.mL}^{-1}$, while orange EO was the least effective one with MBC values ranged between 40 and 150 $\mu\text{L.mL}^{-1}$ (Table 2). The MBC values of *K. oxytoca* were 40, 150, 100, 100, 100 $\mu\text{L.mL}^{-1}$ for lemon, orange, grapefruit, mandarin and bitter orange, respectively. This result reflects their contents of α -Pinene,

which is generally known as an antimicrobial agent (Hosni et al., 2010).

Discussion

The content of Limonene in lemon EO in the current study (62.16%) was very close to that recorded in Turkey by Kirbaşlar et al. (2009) (61.8%); while it was higher than that found by Ben Hsouna et al. (2017) (39.74%) in Tunisia.

Lemon EO contained high amounts of α -Pinene (1.43%), which was higher than that recorded by Ben Hsouna et al. (2017) (0.46%), while β -Pinene (1.08%) was lower than that recorded in the same research (25.44%); as well lemon EO contained high amounts of β -Phellandrene, γ -Terpinene, β -Citral, Citral, cis-Geraniol, α -Bergamotene and β -Bisabolene (1.43, 6.96, 12.96, 2.02, 2.74, 0.89, 1.35 and 0.93%, respectively); and traces of Camphene, Berbenol, 2-pinene-4-ol, Acoradien, (Z) β -

Farnesene, β -Farnesene, γ -Elemene, cis- α -pinene (<0.1%, each compound). (see Figure 1).

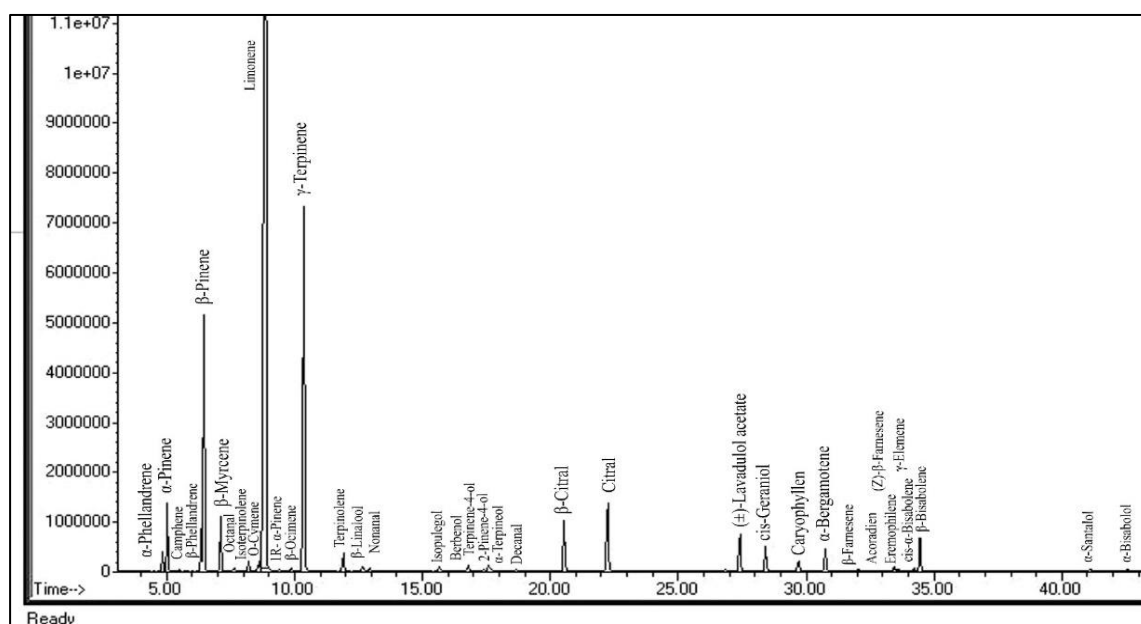


Figure 1. Chemical composition of lemon peel essential oil

Orange EO had the highest content of Limonene among the other citruse EOs in the current study (95.26%), which was higher than (71.54%) as recorded by Farahmandfar et al. (2020); while the contents of β -Myrcene, β -Linalool, α -Pinene and Decanal in the current study (1.85, 0.34, 0.56 and 0.35%, respectively) were lower than (7.20, 4.11, 1.85 and 1.21%, respectively) as recorded by Farahmandfar et al. (2020), but the content of β -Myrcene (1.85%), was close to (1.79%) as recorded by Alparslan et al. (2017) in Turkish orange. Orange EO in the current study contained moderate amounts of α -Pinene, β -Phellandrene, Octanal, γ -Terpinene and Decanal (0.56, 0.60, 0.30, 0.11 and 0.35%, respectively), and a trace of β -Pinene (0.07%). The content of β -Pinene in the current study was very close to that found in Turkish orange (0.05%) (Alparslan et al., 2017); while α -Pinene content (0.56%) was lower than that recoded in the same research (2.48%).

Grapefruit EO contained 92.5% Limonene, which was higher than that recorded in Turkish grapefruit (88.6%) (Uysal et al., 2011); as well it had high contents of β -Myrcene, β -Ocimene, Decanal and Caryophyllene (2.11, 0.37, 0.62

and 0.62%, respectively). β -Myrcene was higher than that recorded by Uysal et al. (2011), and so was decanal: 0.37% in current study and trace in the study of Uysal et al. (2011).

Grapefruit EO was unique to contain (+)- δ -Cadinene (0.18%) and traces of α -Caryophyllene, α -Cubebene (0.07 and 0.05%, respectively). The latter EO also contained moderate amounts of α -Pinene, β -Phellandrene, Octanal, β -Linalool, Isopulegol, (\pm)-Lavandulol acetate, cis-Geraniol (0.62, 0.63, 0.30, 0.13, 0.13, 0.13 and 0.11%, respectively), and traces of β -Pinene, γ -Terpinene, nonanol, Terpinene-4-ol, β -Cubebene, β -Citral, Citral and Germacrene D (<0.1%, each compound). The contents of α -Pinene and Germacrene D in the current study were compatible with that recorded by Uysal et al. (2011) (0.7% and trace, respectively); while β -Pinene and β -Cubebene in current study were less than that of Uysal et al. (2011) (1.2 and 0.2%, respectively).

The content of Limonene in mandarin EO (86.76%) was higher than that recorded by Badee et al. (2013) in the Egyptian cold pressed mandarin EO (65.57%). γ -Terpinene represented a high level (7.82%) in mandarin EO, but

it was lower than that recorded by [Badee et al. \(2013\)](#) (23.07%). Mandarin EO was characterized by high content of α -Phellandrene, β -Pinene, α -Pinene, β -Myrcene and *O*-Cymene (0.24, 0.50, 1.07, 1.99 and 0.23%, respectively), the first three compounds were lower than that recorded by [Badee et al. \(2013\)](#) (1.98, 2.07 and 1.76%, respectively). Mandarin EO was unique to contain a trace of Thymol (0.05%) and Isoterpinolene and Terpinolene (0.18 and 0.41%, respectively) together with lemon EO (0.33 and 0.65, respectively).

Bitter orange EO contained 95.21% Limonene, which was higher than that recorded by [Dugo et al. \(1993\)](#) (93.56% together with *O*-Cymene). The bitter orange EO was characterized by its higher contents of β -Myrcene and β -Cubebene than other citrus EOs (2.08 and 0.20%, respectively). The content of β -Myrcene was higher than that recorded by [Dugo et al. \(1993\)](#) (1.79%). Bitter orange EO was unique to contain *cis*-Linalooloxide and Pergamiol (0.08 and 0.12%, respectively), and it contained moderate amounts of α -Pinene, β -Phellandrene, β -Pinene, octanal, β -Ocimene and Caryophyllene (0.60, 0.16, 0.30, 0.107, 0.33 and 0.11%, respectively), and trace amounts of α -Terpineol, β -Citral, (\pm)-Lavandulol acetate and Germacrene D. The content of α -Pinene and α -Terpineol in the current study were higher than that recorded by [Dugo et al. \(1993\)](#) (0.56 and 0.042%, respectively); while the content of β -Pinene, Caryophyllene and Germacrene D was lower than that recorded by the same researchers (0.94, 0.052 and 0.108%, respectively).

The hydrophobicity of EOs, which enables them to partition with the lipids present in the cell membrane of bacteria and mitochondria, rendering them more permeable by disturbing the cell structures. This eventually results in the death of bacterial cell due to leakage of critical molecules and ions from the bacterial cell to a great extent. ([Chouhan et al., 2017](#)).

The recorded values of MBC against *C. koseri* were 10, 20, 50, 100 and 100 $\mu\text{l.ml}^{-1}$ for lemon, grapefruit, bitter orange, orange and mandarin, respectively; which can partially illustrated by the EOs content of (\pm)-Lavandulol acetate (1.43, 0.13, 0.04, 0, 0% in lemon, grapefruit, bitter orange, orange and mandarin, respectively). *Serratia liquefaciens* was the most

resistance species among all Gram-negative and Gram-positive bacteria with MBC values 50, 100, 100, 100 and 150 $\mu\text{l.ml}^{-1}$ for lemon, orange, grapefruit, bitter orange and mandarin, respectively; and these results are compatible with their contents of sesquiterpenes (4.63, 1.71, 0.92, 0.52 and 0.34% in lemon, grapefruit, orange, bitter orange and mandarin, respectively) and their contents of oxygenated components (8.68, 1.66, 1.23, 0.88, and 0.51 in lemon, grapefruit, orange, bitter orange and mandarin, respectively).

The EO extracted from lemon peels was the most effective against *B. cereus* (MBC 4 $\mu\text{l.ml}^{-1}$), followed by mandarin EO (MBC=20 $\mu\text{l.ml}^{-1}$), then orange EO (MBC 40 $\mu\text{l.ml}^{-1}$), and finally grapefruit and bitter orange (MBC 50 $\mu\text{l.ml}^{-1}$, each). The MBC value of lemon and orange EOs against *B. cereus* were lower than that found by [Fisher and Phillips \(2006\)](#) (1 and >4%, respectively).

The MBC values of the EOs against *B. licheniformis* were 20, 40, 50, 50 and 100 $\mu\text{l.ml}^{-1}$ for lemon, mandarin, grapefruit, bitter orange and orange, respectively. These results are almost compatible with the EOs content of α -Pinene (which is known to its antimicrobial activity, as we mentioned above).

[Leite et al. \(2007\)](#) recorded an antibacterial effect of α -Pinene on several strains of *Staphylococcus aureus*. In the current study, MBC values of *Staphylococcus lugdunensis* were 40, 40, 50, 50 and 50 for lemon, mandarin, orange, grapefruit and bitter lemon, respectively; which compatible with their contents of α -Pinene.

Conclusion

Citrus peel Eos are usually used for fragrance, cosmetic and flavor industries. Results showed that monoterpenes were the predominant compounds in all citrus Eos, which represented 99.12, 98.59, 97.86, 96.63 and 86.69% in mandarin, bitter orange, orange, grapefruit and lemon, respectively. The other main compounds were: oxygenated compounds (8.68, 1.66, 1.23, 0.88 and 0.51% in lemon, grapefruit, orange, bitter orange and mandarin, respectively), alcohols (5.08, 1.10, 0.89, 0.41 and 0.22% in lemon, grapefruit, orange, bitter orange and mandarin, respectively),

sesquiterpenes (4.63, 1.71, 0.92, 0.52 and 0.32% in lemon, grapefruit, orange, bitter orange and mandarin, respectively), esters (2.17, 0.43, 0.43, 0.34 and 0.29% in lemon, grapefruit, bitter orange, orange and mandarin, respectively) and aldehydes (1.43, 0.13 and 0.04% in lemon, grapefruit and bitter orange, respectively). Results revealed relatively high values of MBC, and Gram-positive bacteria were more sensitive than Gram-negative bacteria in general. The order of bacterial resistance towards citrus EOs, from most sensitive to most resistant was as follows: *Pseudomonas luteola*, *Bacillus cereus*, *Pseudomonas fluorescens*, *Staphylococcus lugdunensis*, *Enterococcus faecalis*, *Bacillus licheniformis*, *Citrobacter koseri*, *Staphylococcus haemolyticus*, *Klebsiella oxytoca* and finally *Serratia liquefaciens*. Lemon had the most effective EO among other citrus, followed by bitter orange, grapefruit, mandarin and finally orange. We suggest investigating the potential of citrus EOs to preserve food products as they possess antibacterial properties, good flavor and Generally Recognized As Safe (GRAS) to be used in food industry.

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Author's declaration and contribution

Authors declare that there is no conflict of interest. BA collected the citrus samples, extracted the EOs and planned the MBC method. RBA analyzed the chemical composition of citrus EOs, discussed the results, and wrote the manuscript. LA isolated and identified the bacterial strains, preserved and maintained the isolates, and conducted the MBC experiments. All authors read and approved the final version of the manuscript.

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