J. Food and Dairy Sci., Mansoura Univ., Vol. 8 (2): 111- 116, 2017

Essential Oils of Citrus Fruit Peels Antioxidant, Antibacterial and Additive Value as Food Preservative

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ABSTRACT

Essential oils are major source of antibacterial natural compounds that could be used in storing fish. The essential oils were extracted from the fruit peels of *Citrus aurantifolia* (Christm.) Swingle (Key lime), *C. limon* (L.) Burm.f. (Lemon) and *C. paradisi* Macfad (Grapefruit) during January 2017 and analyzed using gas chromatography coupled with mass spectrometry (GC/MS). The essential oils antioxidant activities were determined using 2,2′-diphenypicrylhydrazyl (DPPH) and the *β*-carotene-linoleic acid assays. The *in vitro*animicrobal activities of the essential oils were determined using the microdilution methods against Gram positive and Gram negative bacteria. The food additive value of the essential oils was examined in sardine preserving for 2, 4 and 6 days against *Staphylococcus aureus*. Essential oil yields were 0.5, 0.53, and 0.6% for *C. aurantifolia*, *C. limon* and *C. paradisi*, respectively. Essential main constitutes were limonene (40.16%), β-Pinene (19.55%) and*α*-Citral (8.13%) for *Citrus aurantifolia*; limonene (57.20%), β-Pinene (8.91%) and γ-Terpinene (6.52%) for *Citrus limon*; limonene (73.5%), linalool (4.71%) and linalool oxide (4.16%) in *Citrus paradisi*. The highest antioxidant activities were found in the essential oil of *C. paradisi* with a percentage of 84.92 ± 0.5 and 92.45 ± 0.6 in the DPPH and β-Carotene-linoleic acid assays, respectively. The highest antibacterial activities were found in *C. paradisi* showing the lowest Minimum inhibitory concentrations (MIC) values against *B. cereus* (0.14 mg/mL), *L. monocytogenes* (0.21 mg/mL), *M. flavus* (0.12 mg/mL), *P. aeruginosa* (0.14 mg/mL), and *S. aureus* (0.16 mg/mL). Essential oils of *C. paradisi* showed the highest inhibitory activities against *S. aureus* in sardine during 2, 4 and 6day storage at 4±1°C. The essential oil of *C. paradisi* showed the most promising results as antioxidant, antimicrobial and might be used for sardine cold storage for short periods compared to other species.

Keywords: *Citrus*; *essential oils; antioxidants; antibacterial; food preserving; sardine*

INTRODUCTION

Food spoilage microorganisms and oxidation are among the most common causes of food deterioration. Particularly, peroxides and other components produced during the manufacturing process and food storage are the major cause of food quality deterioration, leading to rancidity and changes in the taste, smell, and color, and eventually the reduction of food quality (Mau *et al*., 2004; Dai *et al*., 2013). The existence and growth of bacteria in food can also lead to spoilage, toxin formation, and quality deterioration of food products (Celiktas *et al*., 2007). In addition, the consumption of spoiled food can cause a wide spectrum of human diseases. Therefore, there is a growing public health awareness worldwide concerning the food quality.

Synthetic preservatives have been widely used, although some of these compounds caused side effects in living organisms (Misra & Pavlostathis, 1997; and Tripathi *et al*., 2007). In addition, the resistance development of microorganisms to synthetic preservatives is another threat facing the continuous use of these chemicals. The growing interest in the replacement of synthetic food preservatives has promoted research on the screening of new antioxidants and antimicrobial preservatives from natural sources (Bajpai *et al*., 2008). In recent years, interest in the effective use of essential oils from plants in food preservation has been growing. The use of the essential oils may reduce the development of microbial resistance and food contamination.

Essential oils are a complex mixture of compounds, mainly monoterpenes, sesquiterpenes, and their oxygenated derivatives (alcohols, aldehydes, esters, ethers, ketones, phenols and oxides). Other volatile compounds present in ssential oils include phenylpropenes and specific sulphur- or nitrogen-containing substances. Generally, essential oil composition is a balance of various compounds, although in many species one constituent may prevail over all others (Cowan, 1999; and Spadaro, 2012). Plant essential oils, in general, have been recognized as an important natural source of biologically active compounds. Antioxidant activities of essential oils obtained from plants have been investigated in different systems (Elansary *et al*., 2016; and Si Said *et al*., 2016). It has been reported that the antioxidant effect of essential oils is related to their chemical composition. The essential oils with high contents of phenolic compounds, such as thymol and carvacrol showed strong antioxidant activity (Shahidi *et al*., 1992; Radonic & Milos, 2003; and Kulisic *et al*., 2004). On the other hand, many studies have been published on the antimicrobial activities of essential oils against different types of microorganisms in raw and processed food (Chouliara *et al*., 2007; Oral *et al*., 2009; and Djenane, 2015).

To investigate natural antimicrobials and antioxidants from Egyptian local plants, the chemical composition, antimicrobial and antioxidant activities of essential oils isolated from *Citrus aurantifolia*, *C. limon* and *C. paradisi* growing in Alexandria, Egypt.

MATERIALS AND METHODS

Materials

Gallic acid, methanol, ethanol, chloroform, Tween 20, BHT, *β*-carotene, linoleic acid , 2,2`-diphenypicrylhydrazyl (DPPH), Triptic Soy broth (TSB), Dimethyl Sulfoxide (DMSO) were purchased from Sigma Aldrich Egypt. The fruit peels of *Citrus aurantifolia* (Christm.) Swingle (Key lime),*C. limon* (L.) Burm.f. (Lemon) and *C. paradisi*Macfad (Grapefruit) were collected during January 2017 and vouchered in Department of Pomology, Faculty of Agriculture, Alexandria University.

Methods

Preparation and analysis of essential oils

Plant parts were subjected to hydro-distillation in a Clevenger-type apparatus for 1 h. Water was removed using anhydrous sodium sulfate following essential oils extraction. Gas chromatography (Hewlett Packard 5890)/mass spectrometry (Hewlett Packard 5989B) (GC-MS) apparatus was used to detect the composition of the essential oil following dilution in diethyl ether. Gas chromatography column (30 m, 0.25 mm i.d., film thickness 0.25 µm) HP-5MS (5% diphenyl) dimethylpolysiloxane capillary column. Injector temperature was maintained at 240°C; column temperature, isothermal at 70°C and held for 2 min, then programmed to 280°C at 6°C/min and held at this temperature for 2 min. The carrier gas (Helium) at the rate of 1 mL/min. The effluent of the GC column was introduced directly into the ion source of the MS. Spectra were obtained in the EI mode with 70 eV ionization energy. Oil composition was identified by comparing their retention indices and mass spectra with the NIST Mass Spectral Library (Adams, 2007).

Antioxidant activity assay

The antioxidant capacity of the essential oils were determined using the methods of 2, 2′-diphenypicrylhydrazyl (DPPH) and the *β*-carotene-linoleic acid assay (Elansary and Mahmoud, 2015). The experiments were repeated twice in triplicates. The absorbance was measured at 517 nm and 470 nm in the DPPH and *β*-carotene-linoleic acid assays, respectively. BHT was used as positive control and a blank was prepared in the same manner and the antioxidant capacities of each sample were compared to the BHT and the blank. Antioxidant activity was expressed as the percentage in both methods of DPPHradical or *β*-carotene-linoleic acid using the following equation: (% Antioxidant activity=$Abs_{control}$-Abs_{sample}/$Abs_{control}$ x 100), Abs = absorbance at 517 nm and 470 nm in the DPPH and *β*-carotene-linoleic acid assays, respectively.

Antibacterial activity assay

Four Gram-positivebacteria of *Bacillus cereus* (ATCC 14579), *Micrococcus flavus* (ATCC 10240), *Listeria monocytogenes* (clinical isolate), and *Staphylococcus aureus* (ATCC 6538) as well as Gram-negative bacteria of *Escherichia coli* (ATCC 35210) and *Pseudomonas aeruginosa* (ATCC 27853) were used as test organisms to determine antimicrobial activity. The bacterial strains were obtained from the Departments of Plant Pathology and Floriculture, Ornamental Horticulture, Faculty of Agriculture, Alexandria, Egypt.

The antibacterial activities were expressed as minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations using the micro-dilution method (Espinel-Ingroff, 2001). The concentration of the bacteria was adjusted to 1.0×10^4 CFU/mL using sterile saline and then stored at 4±1°C up to 6 days. Essential oils (2 µL) were added to 100 µL Triptic Soy broth (TSB) containing a bacteria inoculum (1.0×10^4 CFU per well) in a 96-well microtitre plates followed by incubation at 37°C for 24 h in a rotary shaker to determine the MICs and MBCs. The MIC was defined as the lowest concentrations without visible growth (at the binocular microscope). To determine the MBC, serial sub-cultivation of 2 µL into microtitre plates containing 100 µL of TSB for each well followed by incubation for 24 h was performed. MBC was defined as the lowest concentration showing no visible growth and indicated killing off 99.5% of the original inoculum. A microplate manager at 655 nm was used to determine the optical density. Experiments were performed twice in triplicates. Negative control (5% DMSO) and positive control (streptomycin) were used.

Essential oils as food preservative in sardine

To examine the inhibitory effects of the essential oils of citrus fruit peelsagainst common sardine foodborne pathogens, Gram-negative bacterial strain of *Staphylococcus aureus* [ATCC (American type culture collection) 6538] was used. Fresh sardine was obtained from local coasts then transported in insulated and sterile box under refrigeration within 20 minutes to the laboratory where it was rinsed using sterile deionized water, headed and gutted. Finally, it was rinsed carefully and water was drained then the fish was refrigerated at 4±1°C.The fish was examined for bacterial contamination. The inoculation was performed by placing sardine samples in plastic sterile bags inoculated with *S. aureus* culture adjusted at bacterial viable count of 3.5 $\log_{10}$ colony forming units (CFU) g^{-1}. Immediately, serial concentrations (MIC, 1.5x MIC, and 2x MIC)of the essential oils (dissolved in DMSO 0.5%) were added to the bags as well as negative control then the bags were homogenizing and stored at 4±1°C aerobic conditions in the dark for one week to simulate local market cold storage. Bacterial count was measured at 2 days' intervals for 3 times using 20 g of treated fish. The fish was minced in sterile mortar, transferred to sterile plastic bags that contain 200 ml of 0.1% sterile peptone water then homogenized and the slurry was serially diluted in peptone water and spread plated on Baird-Parker agar (Oxoid; CM275, UK), then stored at 37ºC for 2 days and the bacterial counts were expressed as $\log_{10}$ CFU g^{-1}, the minimum inhibitory concentrations (MIC) from the previous experiments were used and experiments were repeated twice in triplicates (Dejnane, , 2015).

Essential oils antioxidant effect on Sardine lipids

For *in vitro*oxidative study, three different concentrations of fruit peels essential oils were added (MIC, 1.5x MIC, and 2x MIC) to 100g of sardine. Fish samples were placed into polystyrene trays and divided into four groups. The first group (control) was sprayed with sterile distilled water. The other 3 groups were similarly sprayed respectively with 1, 1.5, 2x MIC values. Samples from all treatments were wrapped and stored under aerobic conditions at 4 ± 1 °C for 6 days. On days 2, 4, and 6 of storage, two packs containing each sample from each group were opened for lipid oxidation analysis.

Lipid oxidation was measured by the 2-thiobarbituric acid (TBA) method of Djenane *et al*. (2015). Fish samples of 10 g were taken and mixed with 20 mL trichloroacetic acid (10%), using an Ultra-Turrax T25 macevator (Janke & Kunkel, Staufen, Germany).

Samples were centrifuged at 2300 rpm for 30 min at 5 °C; supernatants were filtered through quantitative paper (MN 640 W, Machinery-Nagel GmbH & Co. KG, Düren, Germany). 2 mL of the filtrate were taken and mixed with 2 mL of thiobarbituric acid (20 mM); tube contents were homogenized and incubated at 97 °C for 20 min in boiling water. Absorbance was measured at 532 nm. The concentration of the samples was calculated using a calibration curve. TBA-RS values were expressed as mg malondialdehyde/kg sample.

Statistical analyses

The data was presented as means± standard deviations (SD) and subjected to Least significant differences (LSD) test in the ANOVA implemented in SPSS (PASW Ver. 21) at a level of significance of $P \leq 0.05$.

RESULTS AND DISCUSSION

Essential oil constitutes

Essential oil ratios were 0.50, 0.53, and 0.60% for *C. aurantifolia*, *C. limon* and *C. paradisi*, respectively.Fruit peels essential oils varied in their chemical composition as shown in Table 1. *Citrus aurantifolia* main oils constitutes were limonene (40.16), β-Pinene (19.55), α-Citral (8.13), γ-Terpinene (6.33), α-Terpineol (3.61), and terpinen-4-ol (2.68). *Citrus limon* main oils constitutes werelimonene (57.20), β-Pinene (8.91), γ-Terpinene (6.52), α-Citral (4.86), β-Citral (3.73), and α-Terpineol (3.48). While, *Citrus paradisi* main oil constitutes were limonene (73.5), linalool (4.71), linalool oxide (4.16), β-Citral (2.76), β-Fenchyl alcohol (1.89), andnootkatone (1.68). The essential oils compositions of *Citrus aurantifolia*, *C. limon* and *C. paradisi* are in agreement with previous investigations on these crops (Bourgou *et al.*, 2012; Okunowo *et al*., 2013; Sarrou*et al*., 2013). Sarrou *et al*. (2013) reported that *C. aurantifolia* fruit peels from Greece were mainly composed of limonene (90%) and in our study the percentage of limonene was 40.16%. Also, Sanei-Dehkordi *et al*. (2016) reported that the peels were composed of 90% limonene. However, Abderrezak *et al*. (2014) reported that the major fruit peel from Algeria essential oils constitutes were linalool, cis-linalool oxide, trans-carveol, endo-fenchyl acetate and carvone.Bourgou *et al* (2012) reported that *Citrus limon* fruit peels main oil constitutes were limonene (37.63–69.71%), β-pinene (0.63–31.49%) and γ-terpinene (0.04–9.96%) which is in agreement with this study. Okunowo *et al.* (2013) reported that the fruit peels essential oil of *Citrus paradisi* composition by GC-MS analyses was D-Limonene (75.05%) and β-myrene (7.25%) which is matching the result of this study (limonene,73.5%). The variability in the essential oil composition is strongly related to environmental and genetic factors (Duarte *et al*., 2010).

Table 1. Major constituents of essential oils extracted from plant species

Plant name	Essential oil ratio (Fresh weight)	Major components	%
Citrus aurantifolia	0.50	Limonene	40.16
		β-Pinene	19.55
		α-Citral	8.13
		γ-Terpinene	6.33
		α-Terpineol	3.61
		Terpinen-4-ol	2.68
Citrus limon	0.53	Limonene	57.20
		β-Pinene	8.91
		γ-Terpinene	6.52
		α-Citral	4.86
		β-Citral	3.73
		α-Terpineol	3.48
Citrusparadisi	0.60	Limonene	73.50
		Linalool	4.71
		Linalool oxide	4.16
		β-Citral	2.76
		β-Fenchyl alcohol	1.89
		Nootkatone	1.68

Antioxidant activity of leaves essential oils

The total antioxidant activities of essential oils were determined using the DPPH and linoleic acid assays (Table 2). The highest antioxidant activities were found in the essential oil of *C. paradisi* with a percentage of 84.92 ± 0.5 and 92.45 ± 0.6 in the DPPH and β-Carotene-linoleic acid assays respectively compared to lower values in *C. aurantifolia* and *C. lemon*. C. *paradisi*was followed by *C. aurantifolia* then *C. lemon*. Sarrou *et al*. (2013) reported that *C. aurantifolia* fruit peels showed good antioxidant activities using DPPH assay. Castro-Vazquez *et al*. (2016) studied the antioxidant activities of the essential oils of fruit peels of *C. paradisi* and reported that the essential oils extracted from peels has outstanding antioxidant activities. Karoui & Marzouk (2013) reported that *Citrus aurantium* peel and juice showed antioxidant activities lower than standard antioxidants. The antioxidant activities of the essential oils is mainly attributed to major oil constitutes (Elansary & Ashmawy, 2013; and Elansary & Mahmoud, 2015) such as limonene which is commonly reported *Citrus* sp. (Sarrou *et al*., 2013; and Sanei-Dehkordi *et al*., 2016).

Table 2. Antioxidant activity of essential oils using the corresponding concentrations measured by DPPH and *β*-carotene-linoleic acid methods[a]

Essential oil	DPPH radical scavenging %	*β*-Carotene-linoleic acid %
Citrus aurantifolia	68.25 ± 0.3	75.74 ± 0.5
Citrus limon	76.93 ± 0.5	83.63 ± 0.2
Citrus paradisi	84.92 ± 0.5	92.45 ± 0.6
Butylated hydroxytoluene (BHT)	91.61 ± 0.1	98.12 ± 0.5

[a]**Values are expressed as meansof triplicate±SD.**

Antibacterial activities of the essential oils

All essential oils showed antibacterial activities against studied bacteria (Table 3). The highest antibacterial activities were found in *C. paradisi* showing the lowest MIC values against *B. cereus* (0.14mg/mL), *L. monocytogenes* (0.21 mg/mL), *M. flavus* (0.12 mg/mL), *P. aeruginosa* (0.14 mg/mL), and *S. aureus* (0.16 mg/mL). Okunowo *et al* (2013) reported that the fruit peels essential oil of *Citrus paradisi* from Nigeria showed strong antibacterial activities against *B.*

cereus, *Enterococcus faecalis*, *E. coli*, *Klebsellia pneumonia*, *Pseudococcus* sp., *Salmonella typhmurium*, *Shigella flexneri* and *S. aureus* using inhibition zone method. Uysal *et al*. (2011) reported that *Citrus paradisi* fruit peel essential oil was mainly composed of limonene (90%) and showed strong antibacterial activities against *S. aureus, Staphylococcus epidermidis, E. coli, Enterococcus faecalis, Salmonella typhimurium* andothers and their inhibition zones were from 11 to 53 mm. One report indicated that the essential oils of *Citrus limon* showed moderate antibacterial activities against *S. aureus, E. coli, and Pseudomonas aeruginosa* (Bourgou *et al*., 2012).

Table 3. Minimum inhibitory concentrations (MIC, mg/mL) of essential oils on bacterial strains

	B. cereus	***E. coli***	***L. monocytogenes***	***M. flavus***	***P. aeruginosa***	***S. aureus***
Citrus aurantifolia	0.21 ± 0.01	0.20 ± 0.01	0.33 ± 0.01	0.26 ± 0.03	0.20 ± 0.01	0.24 ± 0.01
Citrus limon	0.51 ± 0.01	0.26 ± 0.01	0.41 ± 0.01	0.82 ± 0.07	0.20 ± 0.01	0.43 ± 0.01
Citrus paradisi	0.14 ± 0.01	0.20 ± 0.01	0.21 ± 0.01	0.12 ± 0.03	0.14 ± 0.01	0.16 ± 0.01
Streptomycin	0.07 ± 0.01	0.09 ± 0.01	0.17 ± 0.01	0.9 ± 0.01	0.06 ± 0.01	0.20 ± 0.01

Values are expressed as means of triplicate ± SD

Essential oils inhibitory activities against *S. aureus* in sardine

Essential oils different concentrations (MIC, 1.5 MIC, and 2 MIC) showed variable inhibitory activities against *S. aureus* in sardine during 2, 4 and 6day storage at 4±1°C (Fig. 1). All essential oils showed inhibitory activities compared to untreated fish (control). Essential oils of *C. paradisi* showed the highest inhibitory activitiesagainst *S. aureus* in sardine during 2, 4 and 6day storage. *C. paradisi* essential oil was followed in the inhibitory activities by *C. aurantifolia* then *C. lemon*. Further, the use of essential oils with higher doses than the MIC values showed higher inhibitory activities and were close to the MBC values such as in *C. paradisi*. A clear gradual decrease in the bacterial growth was detected *in C. paradisi* treated fish during the storage period of 6 days. Djenane (2015) reported that the essential oil obtained from the fruit peel of *Citrus sinensis* L., *Citrus limonum* L. and *Citrus aurantium* L. from Algeria showed good antibacterial activities against *S. aureus* in sardine storage. Also,Chouliara *et al*. (2007) reported that the essential oil of oregano was prolonged the shelf life of fresh breast chicken meat during storage. In addition, Oral *et al.* (2009) reported that the essential oil of oregano extends the shelf life of chicken drumstick during storage.

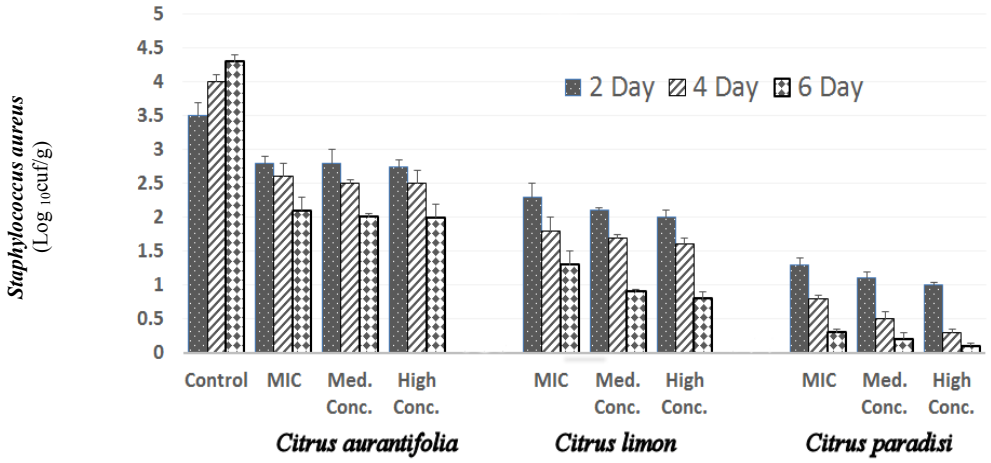

Figure 1. Variable inhibitory activities against of *Citrus* sp. essential oils against *S. aureus* in sardine during 2, 4 and 6-days of storage at 4±1°C. Three concentrations were used: MIC, medium concentration (1.5x MIC), and high concentration (2x MIC)

Antioxidants activity on sardine lipids

Sardine fish might be oxidized quickly. TBA-RS values commonly determined as an index of lipid oxidation in fish which can effect on product sensory properties and consumers acceptance. For this purpose, TBA-RS test was conducted to test the antioxidant activity of fruit peels essential oilswhen added to Sardine. The TBA-RS test showed that the antioxidant activity of fruit peels essential oilssignificantly higherin treated sardine samples compared to the control, and they seem promising enough to inhibit lipid oxidation (Figure 2). There is inversecorrelation between oxidation levels and the concentration of fruit peels essential oils. Samples treated with C. *paradisi* essential oil showed higher antioxidant activity than *C. aurantifolia* and *C. lemon*. The malonaldehyde levels compared to the control samples for the treated samples with *C. paradisi* essential oil concentrations (MIC, 1.5xMIC, 2xMIC) for 6 days at 4± 1ºC were 1.91 and 1.64, 1.23 mg malonaldehyde/kg, corresponding to inhibition percentages of 34.48%,44.82 and 58.62%, respectively.The antioxidant activities of the essential oils is mainly attributed to major oil constitutes such as limonene which is commonly reported *Citrus* sp. (Sarrou *et al*., 2013; and Sanei-Dehkordi *et al*., 2016). Several previous studies suggested critical TBA-RS values for the association between the rancid odor detection and degree of lipid oxidation through sensory evaluation test. These results were in accordance with Viuda-Martos et al, (2010); and Djenane *et al*. (2015).

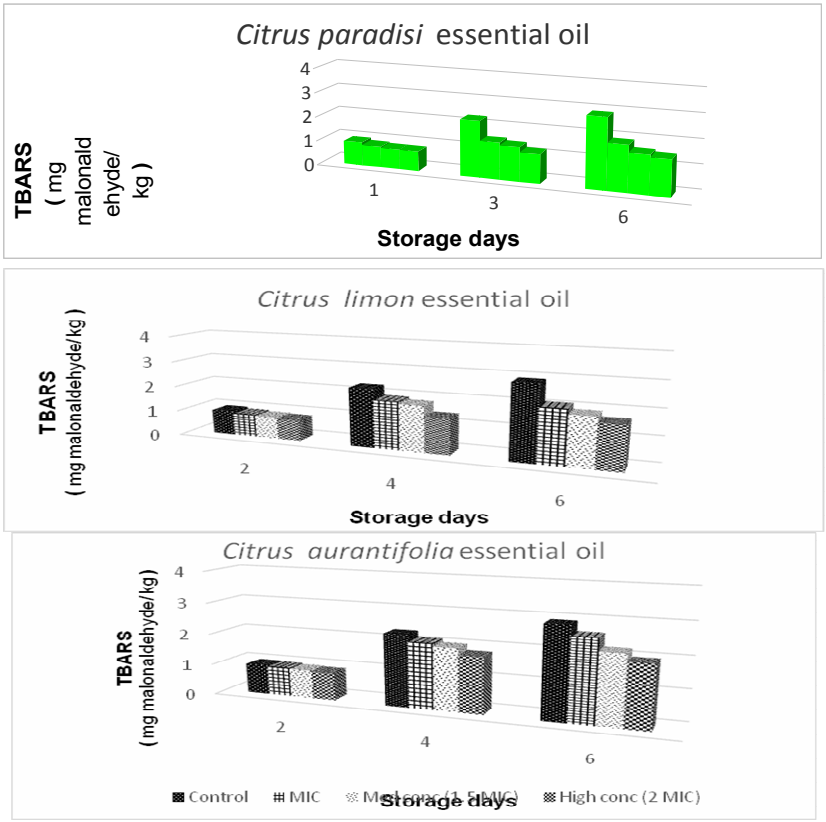

Figure 2. TBA-RS (mg malondialdehyde/Kg) in Sardine treated with fruit peels essential oils at (MIC, 1.5x MIC, and 2x MIC) at 4± 1˚C up to 6 days.

CONCLUSION

Major essential oils constitutes such as limonene, β-Pinene andlinaloolmight be responsible for the high antioxidant activities for the three essential oils studies. All the three essential oils showed promising antibacterial activities against Gram-positivebacteria of *B. cereus*, *M. flavus*, *L. monocytogenes*, and *S. aureus* as well as Gram-negative bacteria of *E. coli* and *P. aeruginosa*. However, the highest antibacterial activities were found in *C. paradisi* showing the lowest Minimum inhibitory concentrations (MIC) values against *B. cereus* (0.14 mg/mL), *L. monocytogenes* (0.21 mg/mL), *M. flavus* (0.12 mg/mL), *P. aeruginosa* (0.14 mg/mL), and *S. aureus* (0.16 mg/mL). Essential oils of *C. paradisi* showed the highest inhibitory activities against *S. aureus* in sardine during 2, 4 and 6day storage at 4±1°C. The highest antioxidant, antimicrobial activities as well as the best essential oil for sardine preserving were found in the essential oil of *C. paradisi*which indicate that further investigations might be conducted using this oil for preserving other fish species in different food industries.

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الزيوت الطيارة لقشور ثمار الموالح كمضادات اكسدة ومضادات بكتريا و قيمتهاالمضافة في حفظ الأغذية

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*تعتبر*الزيوت الطيارة منالمصادر الرئيسية للمواد الطبيعية المضادة للبكتريا والتي يمكن استخدامها في حفظ الأغذية. تم استخلاص الزيوت الطيارة الموجودة في قشور ثمار الليمون الأخضر والليمون الأصفر والجريب فروت في يناير ٢٠١٧ وتحليلها باستخدام gas chromatography coupled with mass spectrometry (GC/MS) . تم تقدير النشاط المضاد للاكسدة في الزيت باستخدام طريقتي diphenypicrylhydrazyl (DPPH) -linoleic acid2,2′-و البيتا كاروتين –حمض لينوليك. كما تم تقدير النشاط المضاد للبكتريا الموجبة والسالبة لجرام للزيوت الطيارة بطريقة microdilution. تم تقدير القيمة المضافة للزيت الطيار المستخلص من الثمار كمادة حافظة لاسماك السردين لمدة ٢، ٤، ٦ أيام ضد نشاط بكتريا *Staphylococcus aureus*. اوضحت النتائج اننسب الزيت الطيار في قشورالثمار كانت٠,٥٣-٠,٦ % لليمون الأخضر والليمون الأصفر والجريب فروت تباعا. وكانت المكونات الأساسية للزيت الطيار هي الليمونين والبيتا بينين والسترال في الليمون الأخضر، الليمونين والبيتا بينين والجاما تربينين في الليمون الأصفر ، والليمونين واللينالول واللينالول أوكسيد في الجريب فروت. وجد ان اعلى نشاط مضاد للاكسدة في الجريب فروت بطريقتي DPPH والبيتا كاروتين –حمض لينوليك . كما ان اعلى نشاط مضاد للبكتريا وجد أيضا في الجريب فروت حيث كان له اقل تركيز مثبط للبكتريا ضد *B. cereus* (٠,١٤ملجم / مل) , *L. monocytogenes* (٠,٢١ملجم / مل)،*M. flavus* (٠,١٢ملجم / مل)،*P. aeruginosa* (٠,١٤ملجم / مل) و *S. aureus* (٠,١٦ملجم / مل). كما أظهرالزيت الطيار لقشور ثمارالجريب فروت اعلى نشاط مثبط*لنمو بكتريا الاستافيلوكوكس*اثناء التخزين المبرد لاسماك السردين لمدة٦أيام على٤±١درجة مئوية. كان الزيت الطيار لقشور ثمار الجريب فروت الأكثر فاعلية و الأعلى نشاط فى مضادات الاكسدة والبكتريا وحيث استخدم في التخزين المبرد لاسماك السردين لمدة زمنية تصل الى أسبوع على درجة حرارة ٤ °م.