# Evaluation of Antibacterial and Antioxidant Activities of Essential Oil of Lime (*Citrus aurantifolia*) Pomace Powder

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**ABSTRACT:** *This study aimed to determine the chemical compounds, antioxidant and antimicrobial activities of Essential Oil (EO) derived from lime pomace. Gas chromatography/mass spectrometry (GC-MS) was used to determine the major components of the obtained EO. The antioxidant activities of this EO were determined by radical scavenging activity (DPPH) and the Ferric Reducing Antioxidant Power (FRAP) test. For antimicrobial activity, the disk diffusion method was used and the Minimum Inhibitory Concentrations (MIC) and the Minimum Bactericidal Concentration (MBC) were studied against common foodborne pathogens including; Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes, Escherichia coli, Salmonella typhimurium, and Pseudomonas aeruginosa. The result showed that the Gram-positive bacteria were more susceptible than gram-negative bacteria. The result shows that lime pomace powder with $IC_{50}$ 83.061 mg/mL has a high antioxidant capacity and also, the chemical analysis of the EO showed that the EO contained a complex mixture of several components and the main constituents were D-limonene (28.86%), α- terpinene (15.65%) and γ-terpinene (12.72%) respectively.*

**KEYWORDS:** *Antibacterial; Antioxidant activity; Chemical compounds; Lime pomace.*

## INTRODUCTION

Synthetic and natural antioxidants have been commonly used in the food industry to control oxidation and maintain quality. However, the use of synthetic antioxidants such as butylated hydroxyanisole (BHA), butylatedhydroxytolune (BHT), propyl gallate (PG), and tertbutylhydroquinone (TBHQ) has been declining in the food industry. Consumers are concerned about potential carcinogenic and health hazard from artificial additives and the FDA has also established limits on the number of synthetic antioxidants that can be added to food products [1, 2]. In recent years, the consumer desire for natural ingredients and chemical preservative-free foods has increased the popularity of natural antimicrobial agents [3]. For this purpose, aromatic plants and their extracts have been evaluated

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for their effectiveness for food safety and preservation applications. Most of their preservative properties are due to their Essential Oils (EOs) and other secondary plant metabolite components [4]. EOs have well-recognized properties such as antimicrobial as well as antioxidant properties. Due to their relatively safe status, EOs have some widespread applications in medicine, pharmaceutical and cosmetic industries and food industry [5]. The by-product produced during processing can be rich in protein, carbohydrate, oil and bioactive compound. Researchers are increasingly exploring the utilization of by-products [6, 7, 8]. Regarding the production of juice, jam and marmalade through processing of Citrus fruits, nearly half of the raw processed fruit goes to waste, namely its peels, seeds and pulps. Wastage of this potentially valuable resource, as well as serious disposal problems, has attracted the Citrus industry's attention to utilization of the Citrus pomace as a possible source of other nutrients [6]. Lime is an important medicinal plant that uses for therapeutic goal and around 80% of the population in this world uses herbal medicines to treat disease especially for infectious diseases. According to WHO medicinal plants are used in order to the therapeutic purpose and be used as a pioneering the synthesis, semisynthetic chemical drugs [7]. Lime (*Citrus lime*) is the third most important species of citrus in the world, behind orange and mandarin. Production of lemon and lime in 2016 in FAO countries was 15,981,800 million tones [9]. Lime has rich source of nutrients such as calcium, potassium, magnesium, phosphor and vitamins including A, E, C, $B_{12}$, and B6. Moreover, because of flavonoid compounds, antioxidant capacity of lime is high [10]. So that the major portion of vitamin C located in lime peel and pulp that acts effectively against obesity, diabetes, hyperlipidemia, cardiovascular diseases and cancer [11]. Citrus fruit pomaces exhibiting antimicrobial activity are rich with flavonoid glycosides, coumarins, β and ϫ- sitosterols and volatile compounds [12]. Flavonoids can function as direct antioxidants and free radical scavengers (DPPH) and have the capacity to modulate enzymatic activities and inhibit cell proliferation [13]. The aim of this study was to determine the chemical compounds, antioxidant, and antimicrobial activities of EO of lime pomace.

## EXPERIMENTAL SECTION

### *Preparation of lime pomace powder*

Ripe Mexican lime (*Citrus aurantifolia*) pomace were collected on 10/09/2017 from a local lime juice factory in Mashhad, Iran, then the limes (*C. lime*) pomace was dried under the shade in room temperature. After that, the dried pomaces were powdered using a mortar as well as electric blender and the powder was put on suitable containers and stored at 4 $^0$C [14].

### *Preparation of extract stock solution*

The stock solution of lime (*Citrus lime*) pomace extract was dissolving in a suitable volume of Dimethyl sulfoxide (DMSO) to get a concentration of 100 mg/ml. The concentration of extract can be calculated used the formula:

$$\text{concentration (\%)} = \frac{\text{Volume of extract}}{\text{volum of solvent}} \times 100$$

DMSO (stock: 1 mg/ml DMSO) [15, 16, 17].

### *Essential of lime pomace*

Dried lime pomace powder (100 g) was transferred to a two-L flask; then one L distilled water was added. The EO was extracted using a Clevenger-type apparatus (Germany) by steam distillation over a 3 h period. The obtained oils were filtered through a sterile syringe filter with a 0.45 μm pore size. The EO was dried over anhydrous sodium sulfate (Na2SO4) and stored in a sealed dark glass vial at 4 ˚C [18].

### *Chemical analysis*

Analyzed proximate composition of lime pomace powder were determined according to the Method of AOAC (2016). Crude protein content was determined by Kjeldahl method using an Auto Kjeldahl System (Kjeltec™ 2300, Foss, Sweden). The amount of total nitrogen in the raw materials were multiplied with both the traditional conversion factor of 6.25 [19] Moisture content by a dry measurement of protein percentage, ash percentage, calcium, phosphorus and dry matter content were measured by AOAC (2016) method [20].

### *Antioxidant Assessment*

Free Radical Scavenging Activity: 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals absorb at 517 nm, but upon reduction by an antioxidant compound, absorption decreases. Briefly, 50 μL of processed Solid Phase Extraction (SPE) MeOH extract or pure compound prepared at different concentrations was added to 2 mL of fresh 0.1 Mm solution of DPPH in methanol and allowed to react at 37 °C in the dark. After thirty minutes the

absorbance was measured at 517 nm [21]. The DPPH scavenging ability as percentage was calculated as:

$$\text{Radical scavenging capacity} = \left(\frac{A_{blank} - A_{sample}}{A_{blank}}\right) \times 100$$

$A_{sample}$ = Absorbance of blank

$A_{blank}$ = Absorbance of sample

Also, IC50 values of samples and standard compounds were determined by measuring the concentration of a sample to scavenge 50 % of radicals [22, 23].

Ferric Reducing Antioxidant Power: The determination of ferric reducing antioxidant power or ferric reducing ability (FRAP assay) of the extracts was performed as described by [24] with some modifications. The stock solutions prepared were 300 mM acetate buffer pH 3.6, 10 mM TPTZ (2, 4, 6-tri (2-pyridyl)-s-triazine) solution in 40 mMHCl, and 20 mM FeCl3·6H2O solution. Plant extracts or standard methanolic Trolox solutions (150 µL) were incubated at 37 °C with 2 mL of the FRAP solution (prepared by mixing 25 mL acetate buffer, 5 mL TPTZ solution, and 10 mL FeCl3·6H2O solution) for 30 min in the dark. Absorbance of the blue ferrous tripyridyltriazine complex formed was then read at 593 nm.

***GC-MS analysis***

The analysis of the EO was carried out using gas chromatography-mass spectrometry (Agilent technologies, USA). The chromatograph was equipped with a HP-5MS capillary column (30 m×0.25 mm ID×0.25 µm film thickness). Column temperature was programmed at 70 ˚C as an initial temperature and kept at 70 ˚C for 3 min, then gradually increased to 280 ˚C at a rate of 5 ˚C per min, holding at the mentioned temperature for 2-10 min. The temperature of the injector was 265 ˚C and helium was used as the carrier gas. The mass spectrometer was operated in electron ionization mode at 70 eV and ionization source temperature was considered at 250 ˚C. The constituents of the EO were identified and confirmed by comparison the gas chromatography retention indices to n-alkanes (C8–C24) and mass spectra with those of Iran National Institute of Standards and Technology commercial library, as well as with literature data [25].

***Bacterial strain***

*E. coli O157:H7* is an enterohemorrhagic, Gram-negative, facultative anaerobic bacterium that produces verotoxin. The route of infection is mainly through meat, dairy products, fruit, and water [26]. *Salmonella Typhimurium* is Gram-negative, facultative bacterium that is found in humans and warm-blooded animal hosts. It is sometimes transferred to food by contamination, often via stool [27]. It causes a foodborne disease called salmonellosis that is widespread globally [27]. *Pseudomonas aeruginosa* is an opportunistic pathogen that is able to survive in moist environments. *P. aeruginosa* is one of the causative agents of hospital-acquired infections, especially in burn patients, which is not only due to its high prevalence and severity but also because of its innate and acquired resistance to antibacterial drug [28]. *Listeria monocytogenes* is a Gram-positive bacterium that is widespread in the environment [29]. It can grow under refrigerated conditions and causes the foodborne illness listeriosis [30]. The reported frequency of listeriosis is low, but the mortality rate is higher than that of other bacteria causing foodborne illnesses [31]. *Bacillus cereus* is known as a facultative anaerobic, spore-forming bacterium that is widespread in nature [32]. It causes a foodborne illness and produces diarrheal and emetic toxins [32]. *Staphylococcus aureus* is an opportunistic pathogen that can cause food poisoning, as well as a variety of infections [33].

***Antimicrobial activity***

Six food borne pathogens including three gram positive *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 10876), *Listeria monocytogenes* (ATCC7644) and three gram negative *Escherichia coli* (NTCC 12900), *Pseudomonas aeroginosa* (ATCC 1430) *Salmonella typhimurium* (ATCC 14028),) bacterial species used in the present study were obtained from Persian Type Culture Collection. The antibacterial potential of EO were measured by the disk diffusion method. Briefly, Muller Hinton Agar plates were culture with a standardized inoculums ($1.5 \times 10^8$ CFU/ml equal to 0.5 McFarland) of each bacterial strains. Then the disks contain specific amount of EO were carefully placed at the labeled seeded plate. The plates were incubated aerobically at 37º C for 24 hours. Inhibition zone is index of antibacterial properties that determined in mm and inhibition zone ≥ 12mm was considered as good inhibitory effect of extract [34].

***Determination of MIC and MBC***

A broth microdilution assay was used to determine the MIC in which visible growth of the bacterium was inhibited.

Briefly, EO dilutions of 1.25, 2.5, 5, 10, 20, 40, 80, 160 mg/mL were prepared using a 96 well microtiter plate [35]. Twenty µl EO (stock) with specific concentration and 160 µL nutrient broth (Merck, Darmstadt, Germany) were transferred to each well. Then, 20 µl inoculum was added to each well [36]. The final concentration of bacteria in each microwell was $10^5$ CFU/ml (estimated using the surface plate counting method).As a positive control, 20 µl inoculum was added to 180 µl nutrient broth lacking EO (0%). A well containing only 200 µl broth was prepared as a negative control. To evaluate the possible contamination of EO, 20 µl EO and 180 µl broth were also transferred to a well. The microplate was covered with a sterile plate sealer and the contents were mixed for 2 min using a plate shaker [37]. The plates were incubated at 37 ˚C for 24 h. Bacterial growth was visually determined by turbidity in the wells and the growth in each well was compared with that of the growth control EO free well [36,37]. The minimum concentration of EO that reduced 99.9% of the bacterial population after incubation at 35-37 ˚C for 24 h was considered the MBC [38]. The wells with no visible growth in the MIC determination assay were used for this test. A sterile swab was applied to the contents of the well and spread on the surface of nutrient agar plates. Then, the plates were incubated at 37 ˚C for 24-48 h. The concentration of EO in those wells that yielded plates with no visible colonies was determined to be the MBC [39].

## STATISTICAL ANALYSIS

The data (means ± SD) were analyzed by using one way analysis of variance (ANOVA) followed by Duncan's post hoc test to compare the means between treatments and differences were considered significant when $p < 0.05$.

## RESULTS AND DISCUSSION

Analyzed proximate composition of lime pomace powder is shown in Table 1.

The results of DPPH assay of three different concentrations of lime pomace powder is shown in Table 2. The result shows that lime pomace powder with $IC_{50}$ 83.061 mg/mL has high antioxidant capacity.

The chemical analysis of the EO showed that the EO contained a complex mixture of several components (Table 3). The main constituents were D-limonene (28.86%), α- terpinene (15.65%) and γ-terpinene (12.72%).

***Table 1: Composition and proximate analysis of lime pomace powder.***

| Lime pomace powder analyzed | (%) |
|---|---|
| Crude protein | 8.4 |
| Moisture | 5.9 |
| Ash | 6.7 |
| Dry matter | 94.1 |

*****Dry matter is all compound except moisture*

***Table 2: Free Radical Scavenging Activity (DPPH).***

| Concentration | Percentage of inhibition (%) |
|---|---|
| 0.05 | 62.291 |
| 0.1 | 67.213 |
| 0.15 | 70.491 |

***Table 3: Chemical analysis of the major components of lime pomace essential oil.***

| component | Retention time(min) | Area sum (%) |
|---|---|---|
| 3-methylheptane | 4.16 | 0.01 |
| 1,3-dioxolane | 4.32 | 0.72 |
| Cis-1-ethyl-3-methylcyclopentane | 4.51 | 1.42 |
| n-octane | 4.75 | 8.26 |
| P-cymenene | 17.32 | 4.13 |
| linalool | 20.9 | 1.12 |
| fenchol | 8.19 | 1.29 |
| α-pinene | 13.81 | 0.61 |
| camphene | 8.96 | 0.38 |
| decane | 10.71 | 2.08 |
| m- cymenene | 11.58 | 2.65 |
| D-limonene | 11.77 | 28.86 |
| isoborneol | 12.20 | 1.52 |
| ϒ-terpineol | 12.82 | 2.18 |
| Terpinene-4-ol | 24.84 | 1.05 |
| α- terpinene | 16.89 | 15.65 |
| β-linalool | 14.28 | 2.22 |
| Fenchyl alcohol | 14.78 | 1.47 |
| Endo-borneol | 16.66 | 1.71 |
| γ-terpinene | 18.62 | 12.72 |
| α- bergamotene | 25.74 | 2.41 |
| β-bisabolene | 27.48 | 3.59 |

***Table 4: Zone of inhibition (mm) of lime pomace essential oil on selected bacteria that cause food spoilage.***

| Organisms | 100% | 50% | 25% |
|---|---|---|---|
| *E.coli o157H:7* | 14 | 13 | 12 |
| *P.aeroginosa* | 15 | 14 | 12 |
| *S.thyphimorium* | 13 | 12 | 11 |
| *S.aureus* | 17 | 16 | 14 |
| *B.cereus* | 17 | 16 | 15 |
| *L.monocytogenes* | 15 | 13 | 12 |

***Table 5: The MIC and MBC values of lime EO (mg/mL) against different bacteria.***

| Bacterial species | MIC (mg/ml) | MBC (mg/ml) |
|---|---|---|
| *E.coli o157H:7* | 20 | 40 |
| *P.aeroginosa* | 20 | 40 |
| *S.thyphomorium* | 40 | 80 |
| *S.aureus* | 5 | 10 |
| *B.cereus* | 5 | 10 |
| *L.monocytogenes* | 10 | 20 |

The antibacterial activity of EO of lime pomace were assayed against six gram positive and negative bacteria by disk diffusion method and the results of inhibition zones have shown in Table 4. Result showed that the inhibition zone on 100% concentration greater than the other concentration. The most susceptible bacteria in agar well diffusion method were *S.aureus, B.cereus, L.monocytogenes P.aeroginosa*with diameter of inhibition zones 17,17,15,15 mm respectively, while the most resistant bacteria were *E.coli o157H:7* and *S.thyphimorium.* The largest zone of inhibition obtained against *S.aureus* and *B.cereus* (17mm).

Gram-positive bacteria were more susceptible than gram-negative bacteria to this plant extract. Susceptibility differences between gram-positive and gram negative bacteria may be due to cell wall structural differences between these classes of bacteria. The Gram-negative bacterial cell wall outer membrane appears to act as a barrier to many substances including antibiotics [40].

Table 5 depicts the MIC and MBC of the EO of lime pomace. It ranged from 5 to 40 mg/ml. *S.aureus* and *B.cereus* was found to be highly sensitive to the EO exhibiting lowest MIC of 5 mg/ml.

The antimicrobial and antioxidant activity on lime being associated with flavonoids and essential oil [41]. Flavonoids such as hesperidine and naringin are beneficial nutrients that enhance the activity of white blood cells and boost the body's defenses [41,42] Essential oil includes monoterpenes, sesquiterpenese, alcohols, aldehydes, ketones and esters. Monoterpenes had a higher antimicrobial activity than did hydrocarbons. Carvone and limonene were active against wide spectrum of antifungal and antimicrobial activity [42, 43]. Chemical analysis of lime pomace EO identified limonene and α- terpineneas the major compounds which are also in close agreement with the findings of previous studies. For instance, *kamal et al* [44] found that β-myrcene and limonene were the most abundant components in pomaces of three Citrus species, including C. *reticulate*, C. *sinensis*, and C. *paradisii*. Lota [45] and Desta [46] reported limonene as well as γ-terpinene as the major monoterpenes in pomace EO of C. *reticulata* (mandarin) and lime EO, respectively. Also, Duman *et al* [47] found that limonene and gamma-terpinene were the most abundant components in oil of lemon (C. limon).

The relation between total phenol content and antioxidant activity has been widely studied in different foodstuffs, such as fruit and vegetables [48, 49] indicating that the free radical scavenging activity of fruits and vegetables significantly increases with a high concentration of total polyphenol content [50]. Meanwhile, the phenolic compounds have the ability to scavenge free radicals by donating a hydrogen atom from their phenolic hydrogen groups [51]. As found in this work, lime pomace EO was able to reduce the stable, purple-colored radical DPPH into yellow-colored DPPH-H by 83.061 mg/mL and it showed a good antioxidant capacity. This result is in agreement with those obtained by [52], demonstrating the scavenging abilities ranging from 20 to 70% of *Citrus* spp, EOs. Inan et al [53] studied the Effect of location and Citrus species on total phenolic, antioxidant, and radical scavenging activities of some Citrus seed and oils. Their results showed that the radical scavenging of lemon seed oil was 3.24–2.83 DPPH and the parameter levels were found lower in Citrus seed oils then seeds. The significant antioxidant activity of the tested EO might be related to the presence of monoterpenes, particularly limonene and *γ*-terpinene, which are the main compounds of EO and have been reported to have a good antioxidant activity [54]. Use of phenolic compounds

derived from various plants such as sage, rosemary, tea and grape seeds as natural antioxidants against oxidative processes of foods have received much interest in recent years. Our results showed antioxidant effect of lime in a dose-dependent manner. These findings confirm the work of Lau et al [55] for grape seed and of Coetzee and Hoffman [56] for αTC. As for green tea, a pro-oxidative dose effect was found seen, which is in contradicted with those of Tang [57], who found a clear antioxidative dose-response effect. At the same time other plant extracts, rosemary and sage extracts at 500 ppm [58], oregano and rosemary essential oils at 150 and 300 ppm [59] or at 100 and 200 ppm [60, 61] and a combination of marigold, purple coneflower, black currant and yellow bark at 1000 ppm [Botsoglou] have been shown to improve the oxidative stability of chicken meat. The antioxidant potential was also higher for lime pomace, radical savaging activity of pomace in the DPPH assay was 82 ± 1.23 µg/ with IC50 83.061 µg/mL and the FRAP assay was 92.25 ± 2.45 µMtrolox equivalents/g dry weight. The antioxidant properties and high content of flavonoids in the pomaces can make this waste material a good source of nutraceutical and healthy phenolic compounds, especially to be used as anti-ageing products, due to the high content of polyphenols. In this study, the EO of the lime pomace was found to have a significant inhibitory effect against *S. aureus* and *B. cereus*. The MIC and MBC values of 5 and 10% were obtained, respectively. This antimicrobial effect might be related to limonene and γ-terpinene in EO, which exert their toxic effects through the disruption of the bacterial membrane and the inhibition of respiration and ion transport processes [62]. In agreement with our study, Yosra et al [63] noted that the essential oils of *Ruta montana (R.M)* and *Ruta gravelons (R.G)* had the highest activity against *S. aureus*. Furthermore, *Taherkhani* [64] reported that the essential oil of *Artemisia ciniformis* have a significant inhibitory activity against *S. aureus*.

From these studies, it was observed that although lime pomace is effective against both groups of bacteria but its activity was high in Gram positive bacteria as compared to Gram-negative bacteria. These observations are in accordance with the earlier observations reported by and they also reported that Gram-negative organisms were less susceptible to the herbal extracts than Gram-positive isolates [46, 65]. It may possibly be due to the presence of high lipid content in the cell walls of Gram negative bacteria. Gram-positive bacteria such as *S. aureus*, and *B. cereus* contains teichoic acid in the peptidoglycan layer and is therefore inhibited by both lime pomace extracts and lime oil [66]. Furthermore, the outer membrane of Gram-negative bacteria is known to present barrier to penetration of numerous antibiotic molecules, and the periplasmic space contains enzymes, which are capable of breaking down foreign molecules introduced from outside thus providing greater resistance to them [67].

## CONCLUSIONS

This study indicated that the EO of *C. lime* pomace can be used as a potential natural antimicrobial as well as an antioxidant agent in the food industry. It would also be of interest to evaluate the antimicrobial activity of lime pomace EO against other foodborne pathogens. Moreover, Gram-negative organisms were less susceptible to the herbal extracts than Gram-positive isolates. It may possibly be due to the presence of high lipid content in the cell walls of Gram-negative bacteria. Further studies using different food models and storage conditions are suggested to improve the utilization of this EO as a natural alternative instead of synthetic preservatives.

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## REFERENCES

[1] Botterweck A., Verhagen H., Goldbohm R., Kleinjans J., Van Den B., Intake of Butylated Hydroxyanisole and Butylated Hydroxytoluene and Stomach Cancer Risk: Results from Analyses in the Netherlands Cohort Study, *Food and Chemical Toxicology*, **38(7)**: 599-605 (2000).

[2] Van den Brandt A., Goldbohm A., van't Veer P., Bode P., Dorant E., Hermus J., Sturmans F., Toenail Selenium Levels and the Risk of Breast Cancer, *American Journal of Epidemiology*, **140**: 20-26 (1994).

[3] Sadaka F., Nguimjeu C., Brachais H., Vroman I., Tighzert L., Couvercelle P., Review on Antimicrobial Packaging Containing Essential Oils and their Active Biomolecules, *Innovative Food Science and Emerging Technologies*, **20**: 350- 355 (2014).

[4] Fisher K., Phillips C., The Mechanism of Action of A Citrus Oil Blend Against *Enterococcus faecium* and Enterococcus Faecalis, *Journal of Applied Microbiology*, **106**: 1343-1349 (2009).

[5] Bansode D., Chavan M., Studies on Antimicrobial Activity and Phytochemical Analysis of Citrus Fruit Juices Against Selected Enteric Pathogens, *International Research Journal of Pharmacy*, **3(11)**: 122-6 (2012).

[6] Matthausa B., Özcan M., Chemical Evaluation of Citrus Seeds, on Agro-Industrial Waste, as a New Potential Source of Vegetable Oils, *Chemistry*, **63(3)**: 313-320 (2012).

[7] Behboud J., Amirreza E., Mohammad M., Antibacterial Effect of Salvia Officinalis Lam Extract, *Annals of Biological Research*, **2 (6):** 532-535 (2011).

[8] Acar R., Özcan M., Kanbur G., Dursun N. Some Physico-Chemical Properties of Edible and Forage Watermelon Seeds, *Iranian Journal of Chemistry and Chemical Engineering (IJCCE)*, **31(4)**: 41-47 (2012).

[9] FAO, Food and Agriculture Organization of the United Nations, (2016).

[10] Agu P., Olerumi O., Tuleun C., Nutritional Evaluation of Sweet Orange (citrus sinensis) Fruit Peel as Feed Resource in Broiler Production, *International Journal of Poultry Science*, **9**: 684-688 (2010).

[11] Gonzalez E., Domynguez R., Moreno A., Viguera C., Natural Bioactive Compounds of Citrus Lemon for Food and Health, *Journal of Pharmaceutical and Biomedical Analysis*, **51**: 327-345 (2010).

[12] Sultana B., Anwar F., Iqbal S., Effect of Different Cooking Methods on the Antioxidant Activity 311 of Some Vegetables from Pakistan, *Journal of. Food Science and Technology*, **43**: 290-312 (2010).

[13] Duthie G., Crozier A., Plant-Derived Phenolic Antioxidants, *Current Opinion in Lipidology*, **3(6)**: 447-51 (2000).

[14] Tumane M., Meshram G., Wasnik D., Comparative Study of Antibacterial Activity of Peel Extracts of Citrus Aurantium L. (Bitter Orange) and Citrus Medica L. (Lemon) Against Clinical Isolates from Wound Infection, *International Journal of Pharma and Bio Sciences*, **5**: 382-387 (2014).

[15] Gupta S., Gupta C, Prakash D., and Garg AP., Comparative Study of Antimicrobial Effects of Lemon Oil and Peel Extract against Food-Spoilage Microbes, *Journal Nutrition Health Food Science* **5(6)**:1-5 (2017).

[16] Alhoi H., Edy F., Nyoman L., Antimicrobial Activity of Lemon (Citrus limon) Peel Extract Against Escherichia coli, American Scientific Research Journal for Engineering, *Technology, and Sciences*, **39 (1)**: 268-273 (2018).

[17] Junab A., Bswajit D., Trideep S., Antimicrobial Activity of Lemon Peel (citrus lemon), *International Journal Current Pharmaceutical Research*, **9**:4 (2017).

[18] Ghasemi M., Mirjalili H., Hadian J., Chemical Profiles of the Essential Oil of Wild and In Vitro Regenerated Zataria Multiflora Boiss, (Lamiaceae), *Bulgarian Chemical Communications*, **46**: 362-367 (2014).

[19] Kjeldahl J., Neue Methode zur Bestimmung des Stickstoffs in Organischen Körpern, Fresenius, *Journal Analytic. Chemistry*, **22**: 366–382 (1883).

[20] AOAC, Official Methods of Analysis of AOAC, International 17th edition; Gaithersburg, MD, USA Association of Analytical Communities, (2016).

[21] Aras A., Doğru M., Bursal E., Determination of Antioxidant Potential of Nepeta Nuda Subsp. Lydiae, *Analytic Chemistry Letter*, **6(6)**: 758–765 (2017).

[22] Aras A., Bursal E., Alan Y., Türkan F., Alkan H., Kılıc O., Polyphenolic Content, Antioxidant Potential and Antimicrobial Activity of Satureja boissieri, *Iranian Journal of Chemistry and Chemical Engineering (IJCCE)*, **37(6)**: 209-219 (2018).

[23] Khairun Fadila S., Chun Hui A., Sook Mei K., Cheng Hock Ch., Chemical Constituents and Antioxidant Capacity of Ocimum basilicum and Ocimum sanctum, *Iranian Journal of Chemistry and Chemical Engineering (IJCCE)*, **38(2)**: 139-152 (219).

[24] Benzie I., Strain J., the Ferric Reducing Ability of Plasma (FRAP) as a Measure of Antioxidant Power: The FRAP Assay, *Analytic Biochemistry*, **239**:70-76 (1996).

[25] Adams P., "Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy", Allured Publishing Corporation, USA (2007).

[26] Henry M., McCann C., Humphry R., Morgan M., Willett A., Evans J., Gunn G., Tongue S., The British *E. coli O157* in Cattle Study (BECS): Factors Associated with the Occurrence of *E. coli O157* from Contemporaneous Cross-Sectional Surveys, *BMC Vet Research*, 5;**15(1)**:444 (2019).

[27] Eun-Jeong K., Ki-Hyun K., Se-Wook O., The Antimicrobial Effect of Thiamine Dilauryl Sulfate In Tofu Inoculated with *Escherichia coli O157:H7, Salmonella Typhimurium, Listeria monocytogenes* and *Bacillus cereus*, *Food Science Biotechnology*, **27(1)**: 283–289 (2018).

[28] Adachi J., Perego C., Graviss L., Dvorak T., Hachem R., Chemaly R., The Role of Interventional Molecular Epidemiology in Controlling Clonal Clusters of Multidrug-Resistant pseudomonas Aeruginosa i Critically Ill Cancer Patients, *American Journal Infection Control*, **37(6)**: 442 -6 (2009).

[29] Robert B., Listeriosis: A Primer, C*anadian Medical Association Journal*, **179(8)**: 795–797 (2008).

[30] Jalali M., Abedi D., Prevalence of *Listeria species* in Food Products in Isfahan. Iran, *International Journal of Food Microbiology*, **122**:336–340 (2008).

[31] Ross T., Dalgaard P., Tienungoon S., Predictive Modelling of the Growth and Survival of Listeria in Fishery Products, *International Journal of Food Microbiology*, **62**:231–245 (2000).

[32] Tarek F., Mansel W., "Bacillus cereus", Chapter 29, Foodborne Infections and Intoxications, 401-407, (2013).

[33] David M., Daum R., Community-Associated Methicillin-Resistant *Staphylococcus aureus*: Epidemiology and Clinical Consequences of an Emerging Epidemic, *Clinical. Microbiology*, **23**: 616–687 (2010).

[34] Nostro A., Ger P., Angelo D., Cannatelli A., Extraction Methods and Bioautography for Evaluation of Medicinal Plant Antimicrobial Activity, *Applied Microbiology*, **15**: 379-85 (2001).

[35] Saxena S., Uniyal V., Bhatt P., Inhibitory Effect of Essential Oils Against Trichosporonovoides Causing Piedra Hair Infection, *Brazilian Journal of Microbiology*, **43**: 1347-1354 (2012).

[36] Hashemi M., Ehsani A., Hassani A., Afshari A., Aminzare M., Sahranavard T., Azimzadeh Z., Phytochemical, Antibacterial, Antifungal and Antioxidant Properties of Agastachefoeniculum Essential Oil, *Journal of Chemical Health Risks*. **7**: 95-104 (2017).

[37] Moosavy H., Basti A., Misaghi A., Salehi I., Abbasifar R., Ebrahimzadeh A., Alipour M., Razavi E., Gandomi H., Noori N., Effect of Zataria multiflora Boiss. Essential Oil and Nisin on Salmonella Typhimurium and Staphylococcus Aureus in a Food Model System and on the Bacterial Cell Membranes, *Food Research International*, **41**: 1050-1057 (2008).

[38] Azizkhani M., Misaghi A., Basti A., Gandomi H., Hosseini H., Effects of Zataria Multiflora Boiss Essential Oil on Growth and Gene Expression of Enterotoxins A, C and E in Staphylococcus Aureus, *International Journal of Food Microbiology*, **163**: 159-165 (2013).

[39] Das P., Dutta S., Begum J., Anwar N., Antibacterial And Antifungal Activity Analysis of Essential Oil of Pogostemoncablin (Blanco) Benth, *Bangladesh Journal of Microbiology*, **30**: 7-10 (2016).

[40] Akthar M., Degaga B., Azam T., Antimicrobial Activity of Essential Oils Extracted from Medicinal Plants Against the Pathogenic Microorganisms: A Review, *Biological Sciences and Pharmaceutical Research*, **2(1)**:1-5 (2014).

[41] Adham A., Extract of Citrus Fruits Growing in Kurdistan/Iraq, *American Journal of Microbiology Research*, **3(5)**:155-159 (2015).

[42] Berk Z., Morphology and Chemical Composition in Citrus Fruit Processing, New York: Elsevier, 13-29 (2016).

[43] Brewer M., Prestat C., Consumer Attitudes Toward Food Safety Issues, *Journal of Food Safety*, **22(2)**: 67-83 (2002).

[44] Kamal M., Ashraf Y., Hussain I., Shahzadi L., Chughtai I., Antioxidant Potential of Peel Essential Oils of Three Pakistani Citrus Species: Citrus Reticulata, Citrus Sinensis and Citrus Paradisii, *Pakistan Journal of Botany*, **45**: 1449-1454 (2013).

[45] Lota L., Serra R., Tomi F., Casanova J., Chemical Variability of Peel and Leaf Essential Oils of 15 Species of Mandarins, *Biochemical Systematics and Ecology*, **29**: 77- 104 (2001).

[46] Desta B., Ethiopian Traditional Herbal Drugs, Part II, Antimicrobial Activity of 63 Medicinal Plants, *Journal Ethnopharmacol*, **39(2)**:129- 139 (1993).

[47] Duman E., Soltanbeigi A., Özcan M., Chemical composition of Essential Oil of Some Citrus spp. (sour, lemon, kamkuat, mandarin and orange) peels, *Journal of Medicinal and Spice Plants* **21(4)**: 153-159 (2016).

[48] Kiselova Y., Ivanova D., Chervenkov T., Gerova D., Galunska B., Yankova T., Correlation Between the *In Vitro* Antioxidant Activity and Polyphenol Content of Aqueous Extracts from Bulgarian Herbs, *Phytotherapy Research*, **20**: 961- 965 (2006).

[49] Klimczak I., Malecka M., Szlachta M., Gliszczynska-Swiglo A., Effect Of Storage on the Content of Polyphenols, Vitamin C and the Antioxidant Activity o Orange Juices, *Journal of Food Composition and Analysis*, **20**: 313-322 (2007).

[50] Ghasemi K., Ghasemi Y., Ebrahimzadeh A., Antioxidant Activity, Phenol and Flavonoid Contents of 13 Citrus Species Peels and Tissues, *Pakistan Journal of Pharmaceutical Sciences*, **22**: 277-281 (2009).

[51] Thitilertdecha N., Teerawutgulrag A., Rakariyatham N., Antioxidant and Antibacterial Activities of *Nephelium lappaceum* L, Extracts, LWT, *Food Science and Technology*, **41**: 2029-2035 (2008).

[52] Frassinetti S., Caltavuturo L., Cini M., Della Croce C.M, Maserti E., Antibacterial and Antioxidant Activity of Essential Oils from Citrus spp, *Journal of Essential Oil Research*, **23**: 27-31 (2011).

[53] İnan O., Ozcan M., Aljuhaimi F., Effect of Location and Citrus Species on Total Phenolic, Antioxidant, and Radical Scavenging Activities of Some Citrus Seed and Oils, *Journal of Food Processing and Preservation*, **42(3)**:1-6 (2018).

[54] Conforti F., Statti G., Tundis R., Loizzo R., Menichini F., In Vitro Activities of Citrus Medica L. Cv. Diamante (Diamante Citron) Relevant to Treatment of Diabetes and Alzheimer's Disease, *Phytotherapy Research*, **21**: 427-433 (2007).

[55] Lau W., King J., Pre- and Post-Mortem Use of Grape Seed Extract in Dark Poultry Meat to Inhibit Development of Thiobarbituric Acid Reactive Substances, *Journal of Agriculture and Food Chemistry*, **51**:1602-1607 (2003).

[56] Coetzee M., Hoffman M., Effect Of Dietary Vitamin E on the Performance of Broilers and Quality of Broiler Meat During Refrigerated and Frozen Storage, *South African Journal Animal Science*, **31**:158–173 (2001).

[57] Tang Z., Kerry P., Sheehan D., Buckley J., Morrissey A., Dietary Tea Catechins and Iron-Induced Lipid Oxidation in Chicken Meat, Liver and Heart, *Meat Science*, **56**:285-290 (2000).

[58] Lopez-bote J., Gray I., Gomma A., Flegal J., Effect of Dietary Administration of Oil Extracts From Rosemary and Sage on Lipid Oxidation In Broiler Meat, *British Poultry Science*, **39**:235-240 (1998).

[59] Basmacioglu H., Tokusoglu O., Ergul M., The Effect of Oregano and Rosemary Essential Oils or Alpha-Tocopheryl Acetate on Performance and Lipid Oxidation of Meat Enriched with n-3 PUFA's in Broilers, *South African Journal of Animal Science*, **34**:197-210 (2004).

[60] Botsoglou A., Grigoropoulou H., Botsogl E., Govaris A., Papgeorgiol G., The Effects of Dietary Oregano Essential Oil and α-tocopheryl Acetate on Lipid Oxidation in Raw and Cooked Turkey During Refrigerated Storage, *Meat Science*, **65**:1193-1200 (2003).

[61] Papageorgiou G., Botsoglu N., Govaris A., Giannenas I., Iliadis S., Botsoglu E., Effect of Dietary Oregano Oil and α-tocopheryl Acetate Supplementation on Iron-Induced Lipid Oxidation of Turkey Breast, Thigh, Liver and Heart Tissues, *Journal of Animal Physic and Animal Nutrition*, **87**:324-335 (2003).

[62] Martins R., Salgueiro J., Goncalves R., Vila F., Tomi T., Adzet T., Casanova J., and Chemical Composition of the Bark Oil of CrotonStellulifer, an Endemic Species from S, Tomé e Príncipe, *Planta Medic*a, **66**: 647-650 (2000).

[63] Yosra B., Manef A., Sameh A. Biological Study from Ruta Plants Extracts Growing in Tunisia, *Iranian Journal of Chemistry and Chemical Engineering (IJCCE)*, **38(2)**: 85-89 (2019).

[64] Taherkhani M., Chemical Investigation and Protective Effects of Bioactive Phytochemicals from Artemisia Ciniformis, *Iranian Journal of Chemistry and Chemical Engineering (IJCCE)*, **35(2)**: 15-26 (2016).

[65] Escalona-Arranz J., Peres-Roses R., Urdaneta-Laffita I., Camacho-Pozo MI., Rodrigues J., Licea I., Antimicrobial Activity of Extracts from *Tamarindusindica* L. Leaves, *Pharmacogn Mag*, **6(23)**:242-247 (2010).

[66] Yokoyama K., Mizuguchi H., Araki Y., Kaya S., Ito E., Biosynthesis of Linkage Units for Teichoic Acids In Gram-Positive Bacteria: Distribution of Related Enzymes and Their Specificities For UDP-Sugars and Lipid-Linked Intermediates, *Journal of Bacteriology*, **171(2)**: 940–946 (1989).

[67] Duffy C., Power R., Antioxidant and Antimicrobial Properties of Some Chinese Plant Extracts. International, *Journal Antimicrobial Agents*, **17(6)**:527- 529 (2001).