Egypt. J. Chem. Vol. 67, SI: M. R. Mahran, pp. **1645 - 1659** (2024)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Antimicrobial and Antioxidant Activity of Essential Oils Treated by Gamma Irradiation Extracted from Citrus Peels.

Amr K. Ali[1]*, Kamal M. El-sahy[2], Toliba A. O.[2], Hany A. Shawky[1], M. A. Abdelaleem[1]* and Khalid. R. A. Elbassiony[1]

[1] *Plant Research Department, Nuclear Research Center (NRC), Egyptian Atomic Energy Authority (EAEA), Cairo, Egypt.*
[2] *Food Science Department, Faculty of Agriculture, Zagazig University, Cairo, P.O. Box 13759, Egypt..*

Abstract

Background: A common problem among senior people, malnutrition greatly affects their quality of life, functional level, and health results. Orange and lemon peels was used as a source for essential oil (EO). Gamma radiation at does (0, 2, 4, 6, 8 and 10 kGy) were submitted to essential oils then essential oils were analyzed for their physicochemical properties and for chemical composition using GC/MS. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and Ferric-reducing antioxidant power (FRAP) were measured to examine the antioxidant activity of essential oils. Agar-well diffusion method was used to estimate antimicrobial activity. Chromatographic examination revealed that the primary constituent of orange and lemon peels EO was d-limonene. Antioxidant activity was significantly increased when essential oils were exposed to increasing levels of gamma irradiation up to 4 kGy. DPPH and FRAP values at 4 kGy were (51.16 and 76.81%) and (246.28 and 661.54 µM TE/mg) of orange and lemon peels EO, respectively. The maximum inhibition zone was shown with essential oils irradiated at a dose 4 kGy. Our study showed that lemon essential oils had the best antioxidant and antimicrobial activities compared to orange essential oils.

Keywords

Citrus peels EOs, Gamma radiation, GC/MS, Antimicrobial, Antioxidant activities.

1. Introduction

In recent years, many consumers have required natural preservatives in foods instead of synthetic and harmful chemicals. Therefore, interest in natural and non-synthetic antimicrobials has increased as possible replacements for traditional antimicrobials to spread shelf life and control foodborne pathogens. Generally, numerous essential oils have been strongly encouraged for their possible antimicrobial properties [1]. Because of the fact that citrus oils naturally add flavor to citrus juices, the Food and Drug Administration (FDA) considers these substances as generally recognized as safe (GRAS) [2].

Citrus plants (*Rutaceae family*) characterize one of the greatest vital fruit crops grown worldwide [3, 4], mainly in the Mediterranean area [5]. Orange, lemon, clementine, grapefruit, mandarin, and bergamot are the citrus varieties that are typically used in food or manufacturing purposes uses (consumption of fresh fruit, drinks, beauty products, candies, and pharmaceuticals) [5, 6].

Citrus EOs and Flavonoids are widely recognized for their beneficial effects in possessing many biological activities, such as antioxidant, antimicrobial and cytotoxic properties, also using in food additives and in the cosmetic industry [7]

Citrus plants establish one of the most important and valuable sources of essential oils worldwide [4, 8, 9]. Essential oils of citrus, generally extracted from the peels [5, 6, 8, 9] similarly from leaves, flowers, young shoots, buds, seeds, and roots [3, 9, 10], are aromatic volatile liquids, simply extracted by cold pressing and steam distillation, and approximately ranged between 0.5 and 5.0% (w/v) [5, 9]. Essential oils are highly complex mixes of organic components, the main parts of which are aliphatic aldehydes, alcohols, esters, and monoterpenes and sesquiterpenes together with their oxygenated derivatives. These mixtures are utilized for a multitude of biological applications. [3, 6, 8, 11]. After obtaining EOs from citrus peels, large by-products/wastes are discarded and cause environmental problems. Furthermore, byproducts of citrus fruit, such as pulp residue, albedo, peels, and seeds, contain bioactive compounds that could be used in animal feed, flavoring, manufactured meals, and medical applications [12].

Chromatographic analysis (GC/MS) determined that, the primary constituent of citrus Eos is limonene. The amount of oil in it can range from 30% to 99%, depending on the type: 30–40% in sweet orange, 40–75% in lemon, and 68–98% in bergamot [13]. About a hundred different chemicals constitute orange essential oil (EO), which can be broadly classified into three categories: nonvolatile, oxygenated compounds, and terpene hydrocarbons. The terpene portion can establish from 50 to more than 90% of the oil. Citrus

*Corresponding author e-mail: mdabdelrazek.md@gmail.com.; (Mohamed A Abdelaleem).
Receive Date: 20 May 2024, Revise Date: 29 July 2024, Accept Date: 11 August 2024
DOI: 10.21608/ejchem.2024.291091.9741

fruits' famous scent is attributed to limonene [14]. Lemon Eo are complex mixtures of chemical substances such as limonene, citral, linalool, γ-terpinene and β-caryophyllene among others, which are represented by the three main classes terpenes, oxygenates, and sesquiterpenes [15-17]. The most important flavour mixture is citral, whereas linalool possesses extremely unique organoleptic properties. Additionally, the high fragrance flavor of lemon oil can be attributed to limonene, octanol, myrcene, and γ-terpene, among other components [15]. Lemon fruits are famous for their vital nutrients, medicinal, and cosmetic characteristics. Citrus lemons' main source of raw materials is fruit, specifically the juice and essential oils extracted from it [5, 6, 18].

One promising technological solution for addressing food safety and quality concerns is food irradiation. The world is irradiating over 60 products with this technique, which has been approved in over 50 nations, including Egypt [19, 20]. As per the Codex Alimentarius General Standard [21] dried aromatic herbs and spices can be sanitized by gamma irradiation up to a dose of 10 kGy. Nevertheless, the US Food and Drug Administration (FDA) and certain other nations have raised this limit to 30 kGy for these products [21-23]. The dose of 10 kGy is sufficient to assurance manufactured goods disinfestations and microbial sanitization [24, 25]. Radiation treatment may improve the concentration of specific phytochemicals and enhance the antioxidant and biological properties of a variety of materials [26, 27].

According to Hasan et al. [28] Citrus fruits can be used in food and nutraceuticals as antimicrobial and antioxidant components. Researchers have also focused a lot of focus on citrus essential oils' antioxidant properties. [29, 30].

Furthermore, because essential oils are natural extracts that are safe at low concentrations for food production, research on their benefits against a wide range of microorganisms, including pathogenic and food spoilage bacteria, has recently increased [31] and their usage as antioxidant, anticancer and antiviral was also investigated [32]. Essential oils exhibition antibacterial, antifungal, and insecticidal characteristics [33-37]. Burt [33] mentioned that, Essential oils are utilized in the food and pharmacological industries due to their antibacterial and Antioxidant characteristics. Protection of these activities is highly significant to plan and confirm the sanitization procedure of herbs.

The essential oils (EOs) and fatty acids (F.As) are the origins of various phytochemical substances and are commercially beneficial in food and they are several biological activities including antiviral, antibacterial, anti-parasitic, and anti-inflammatory [38].

Thus, the purpose of this investigation is to benefit from citrus peel waste, which causes an environmental pollution problem. Evaluate the effects of several doses of gamma irradiation on the physicochemical characteristics, chemical structure and on antioxidant and antimicrobial properties of EOs extracted from orange and lemon peels.

2. Material and Methods

2.1 Materials:

Orange (*Citrus sinensis*) and Lemon (*Citrus lemon*) fruits were obtained from local markets. The orange and lemon fruits were cleaned by hand; The endocarp was removed, and the peels were then cut using a sharp knife into very small pieces, divided into suitable samples and packaged in polyethylene pouches.

2.2 Chemicals and reagents:

Sodium phosphate buffer, potassium ferric cyanide, (BHT) The synthetic antioxidant butylated hydroxytoluene (purity 99.9%) and tri-chloroacetic acid (TCA) were bought from local chemical producers. The analytical quality chemicals and solvents used were El-Gomhoria Company for Chemicals and Drugs, located in Cairo, Egypt. The chemical 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) is manufactured by Sigma Company, located in St. Louis, MO, USA.

2.3 Microbial strains:

Two negative strains of *Salmonella typhimurium* (ATCC 98031) and *Escherichia coli* (ATCC 35218), as well as two Gram-positive strains of *Staphylococcus aureus* (ATCC 20231) and *Bacillus subtilis* (ATCC 9372). They were bought from the Egyptian Microbial Culture Collection (EMCC) at the Faculty of Agriculture, Ain Shams University, Cairo, Egypt. And a pathogenic fungus *Aspergillus brasilinesis* (ATCC 16404) and *Candida albicans* (ATCC 10231) were purchased from the Regional Center for Mycology and Biotechnology, Faculty of Science, El-Azhar University, Cairo, Egypt.

2.4 Extraction of essential oils:

According to the method of Shakir and Salih [39], Lemon and orange fruit was washed and clean it of any foreign materials, then peeled by using sharp knife to obtained outer layer. Then use (300 grams) of peels for extraction of essential oils from each lemon or orange peels were extracted by Hydrodistillation method for 3-4 hr. by a clevenger apparatus unit. The volatile oil was trapped in small volume and removed time to time from the side arm. After the distillation, the oil was dried over anhydrous sodium sulphate and kept in deep freezer until analysis.

2.5 Irradiation treatment of essential oils:

Gamma radiation was applied to orange and lemon peels (EO) at doses of 0, 2, 4, 6, 8, and 10 kGy. A Russian 60Co gamma chamber was used in the Cyclotron Project at the Nuclear Research Center of

the Egypt Atomic Energy Authority (with a dosage rate of 395.1Gy/h) for all radiation treatments.

2.6 Physicochemical properties:

Specific gravity and acid value were measured with minor modifications by [40]. Orange peel essential oil's refractive index was calculated and released by Dănilă et al. [41]. An Abbe's Refractometer of the EO was used to calculate the refractive indices of the EOs.

2.7 Chemical composition of citrus essential oils by gas chromatography (GC–MS):

The structure of citrus EOs was examined by gas chromatography (Agilent 8890) using a series capillary column DB-5MS, 60 m x 250 µm id x 0.25 µm film thicknesses, and a mass spectrometer detector (Agilent 5977B). The carrier gas, helium gas, was utilized at a fixed pressure of 65 kPa. A 1 µL amount of EO was administered with a 1:50 split ratio and a 4-minute solvent delay. The oven was programmed to rise from 50 to 240°C in increments of 5°C per minute, or until the target temperature was achieved. Volatile constituents were recognized by matching the obtained mass spectra and retention indices by those of accurate standards and the National Institute of Standards and Technology's (NIST) MS Library [42, 43].

2.8 Antioxidant capacity of citrus peels essential oil

2.8.1 DPPH radical scavenging activity:

The principle of the DPPH assay is based on the color change of the DPPH solution from purple to yellow as the radical is quenched by the antioxidant .A modified form of the Brand-Williams et al. [44] was used to test the antioxidant activity. Utilizing a method that makes use of the free radical 2, 2-diphenyl-picrylhydrazyl (DPPH), antioxidants are permitted to react with the stable radical in a methanol solution. The DPPH radicals' staining was seen together with the reaction's decline in absorbance at a certain wavelength. At 515 nm, DPPH absorbs in its radical form; however, absorption is eliminated when an antioxidant species reduces the compound. A 6×10^{-5} moll/l of DPPH solution (2.4 mg of DPPH in 100 ml of methanol), (0.1mL) of essential oil was added to 3.9 ml of a 6×10^{-5} mol/L methanol DPPH e solution. After giving the mixture a good shake, it was left at room temperature for half an hour and kept out of the light according to Kheira et al. [45].. Absorbance (A) was measured at 515 nm using a spectrophotometer. And the blank was methanol.

$$\textbf{Antioxidant activity (Inhibition)}\% = \left[\frac{(\text{Acontrol} - \text{Asample})}{\text{Acontrol}}\right] \times 100$$

Where: $\mathbf{A_{control}}$ is the absorbance of the control response (absorbance of DPPH solution) and $\mathbf{A_{sample}}$ is the absorbance (of the essential oil with DPPH solution). Furthermore, as a positive control, butylated hydroxyl toluene (BHT), a synthetic antioxidant, was employed.

2.8.2 Ferric reducing antioxidant power (FRAP) assay:

The principle of the FRAP assay based on the method described measures the ferric reducing ability (FRAP). At low pH, when a ferric-Trotripyridyltriazine (FeIII-TPTZ) complex is reduced to ferrous (FeII) form, an intense blue color with an absorption maximum at 593 nm develops. The examination was conducted by the methodology of Benzie and Strain [46] with slight adjustments to be made in micro-plates with a recently prepared TPTZ reagent (300mM Acetate buffer (PH ≈ 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM MFeCl3, in a ratio of 10:1:1 v/v/v, correspondingly). 190 uL of the recently prepared TPTZ reagent were varied with 10 uL of the sample in a 96-well plate (n = 3). The reaction was let to incubate for thirty minutes in the dark at room temperature. The blue color that resulted from the incubation period was detected at 593 nm. By the linear reversion equation taken as of the linear dose-response curve of Trolox, The ferric-reducing capacity of the samples is given as µM TE/ mg sample.

$$y = 0.0019x + 0.0874 \qquad R^2 = 0.9985$$

2.9 Antimicrobial activity of citrus peels essential oil

2.9.1 Diffusion assay for screening the inhibitory effect of essential oils:

The antibacterial characteristics of EOs extracted from orange and lemon peels were studied in vitro. by the agar well diffusion method with some modifications [3, 47]. Mueller-Hinton sterile agar for an antibacterial test and sterilized potato dextrose agar (PDA) for an antifungal test. A cork borer (8 mm) was burned and used to bore one central well in each plate, which contained (50 µl) for an antibacterial test and (100 µl) for an antifungal test. The plates were saved at 7°C for 3 h to let the oil diffusion and then incubated at 37°C for 24 h (for bacterial growth) and 28°C for 72 h (for fungal growth) [48]. Subsequent the incubation time, the inhibitory zone was measured in millimeters. Three duplicates of each experiment were conducted.

2.10 Statistical analysis:

Data were examined by SPSS [49] analytical software version 18.0 (SPSS Inc., Illinois, and USA). Three replicates of each analysis were performed, and the results were reported as mean ± standard deviation. A one-way analysis of variance (ANOVA) was performed on the data, and as a post-hoc test, a Duncan test was used to compare the means. Levels of significance were determined using a 95% confidence level ($p < 0.05$).

3. Results and discussion

3.1 Effect of gamma irradiation on the physiochemical properties of essential oil:

Specific gravity of both orange and lemon peels EO are ranged from about 0.84 to 0.86 as mentioned in Figure 1, and all data represented in our study

demonstrated that there were no noteworthy significant variations in control samples in comparison to irradiated ones. The results seem to agree with [50, 51], who stated that after being exposed to 10 kGy of radiation, the specific gravity of the irradiated peppermint and coriander EOs remained unchanged. Similar results were obtained by other studies [37, 52-54]. Regarding refractive index (RI), the average number of RI is about 1,471 to 1,474 in orange peel essential oil and is about 1.477 to 1,484 in lemon peels EO. So, our study found that there were no significant differences after irradiation of essential oil. Similar results attained by Parthasarathy et al. [55] and Abdelaleem [56] who noted that only the fourth decimal number affected the refractive index of irradiated rosemary EOs at dosages of 2, 4 and 6 kGy. In this sense, the previous data are in similar to results observed by other researches [40, 57-59].

On the other hand, although there was no apparent change in the acid value, the irradiation treatment caused a slight increase in all samples, where the acid value of control orange EO was 3.76, increased to 4.10 (mg KOH/g oil) at dose 10 kGy, and was 2.20 in lemon essential oil, increased to 2.40 (mg KOH/g oil) at dose 10 kGy. This research agrees with [58]. A low acid value in essential oil means that it has a long shelf life [40, 60]. The statistical studies revealed that there is no significant difference ($p \leq 0.05$) in the effects of gamma irradiation on physiochemical properties of citrus peel essential oils at varied applied levels.

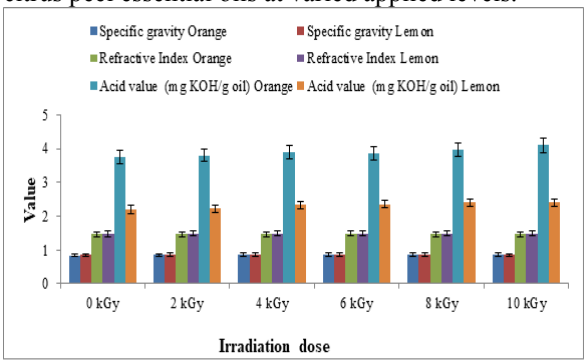

Figure 1. Physicochemical properties of orange and lemon peels essential oils

3.2 Chemical composition of citrus essential oils by GC–MS

Tables 1 and 2 show chemical category and GC data of the compounds recognized in EOs of the unirradiated and the irradiated orange and lemon peels at the dose levels of 0, 2, 4, 6, 8 and 10 kGy. Effect of gamma irradiation on chemical composition of orange and lemon peels essential oils has been carried out, initially, because the biological actions of plant extracts are mostly caused by their abundance in chemical combinations, and secondly, to comprehend the outcomes obtained in subsequent research on antioxidants and antimicrobials [41, 61, 62]. Gamma irradiation made slight variations in the quantitative structure of some components and the greatest vital changes were documented for the most prominent ingredients of the orange and lemon peels essential oils. Thus, D-Limonene (predominant compound in orange peel essential oil) amount increase for 2 and 4 irradiation doses, but decrease at doses 6, 8 and 10 kGy compared to unirradiated sample. Our data are similar to [63-67] who proved that the d-limonene quantity was greatly present (66–93%) in all citrus EOs.

As mentioned in Table 2, the main compounds of lemon peels Eos were limonene, β-myrcene, α-pinene, terpinolene, β-pinene, γ-terpinene, linalool and 3-carene. The effect of gamma irradiation is varied from one-to-one compound, where α-pinene, terpinolene, β-myrcene, γ-terpinene, and 3-carene were increased due to radiation dose increased. On the contrary, amount of limonene decreased with radiation dose increased. Linalool was totally diminished at doses 6, 8 and 10 kGy.

The chemical composition described in our study, is largely comparable to that reported in previous studies in citrus peels essential oils and agrees with other researches [11, 68-70].

This irregular change is similar to that described by Thongphasuk et al. [71] and Douar-Latreche et al. [72]**.** Variability was seen in the compounds' reactivity to radiation. Thus, gamma radiation effects on the constituents of essential oils are additionally influenced by a number of variables, including temperature, sample condition, radiation dose, and dose rate. Gamma radiation exposure can adjust the yield of essential oil extraction and the composition of its components [73]. The attendance of limonene, α-terpineol, geranyl acetate, linalool, 4-terpenol, neryl acetate and α-pinene in the structure of lemon peel essential oil provides antimicrobial activity in the oil [41, 58].

The precise mechanism through which radiation induces changes in the structure of volatile oils remains poorly comprehended. However, the recent study's findings regarding the variations in ingredient content following gamma irradiation may be attributed to the radiation sensitivity of the compounds at the dose utilized [74].

The dissimilar contents of terpenoids in Egyptian citrus peel essential oils can be impacted by the kind of soil, the site, and the weather in which the types are refined. These factors may be responsible for variations in the proportion of essential oils. Furthermore, the season of harvest, the fruit's maturity, and the extraction techniques can all have an effect on the terpenoid concentrations in citrus essential oils [75].

Table 1. Chemical composition (%) of orange peel essential oils at different irradiation doses

No	Compounds Name	RT (min)	Peak area (%) of identified compounds					
			0 kGy	2 kGy	4 kGy	6 kGy	8 kGy	10 kGy
1	α-pinene	19.259	-	-	0.48	1.29	1.18	1.26
2	β-Thujene	20.325	0.66	0.54	0.17	0.56	0.52	0.55
3	β-Terpinene	21.915	0.37	0.2	-	-	-	-
4	β-Myrcene	22.259	1.7	1.49	1.36	3.13	2.9	3.02
5	Octanal	22.848	0.13	0.09	0.07	0.2	0.19	0.2
6	α-Phellandrene	23.311	-	-	-	0.1	0.08	0.1
7	γ-Terpinene	23.426	0.28	-	0.14	0.38	0.35	0.38
8	(+)-δ3-Carene	23.449	-	0.17	-	-	-	-
9	D-Limonene	24.29	94.67	97.04	97.36	92.19	92.87	92.4
10	β-Phellandrene	25.221	-	-	-	0.59	0.53	0.53
11	Isoterpinolene	26.516	-	0.02	0.01	-	0.06	0.06
12	β-Ocimene	26.663	-	-	-	0.06	0.05	0.06
13	Linalool	26.779	0.56	0.21	-	0.45	0.4	0.44
14	Limonene oxide	26.794	-	-	-	0.06	0.05	0.06
15	Linalyl formate	26.802	-	-	0.19	-	-	-
16	(-)-Perillic alcohol	28.462	-	-	0.04	-	-	-
17	Isocarveol	28.582	-	0.03	-	-	-	-
18	(R)-(+)-Citronellal	28.851	-	0.01	-	0.09	0.09	0.09
19	Caryophyllene	28.971	0.78	-	-	-	-	-
20	Decanal	30.813	0.32	0.17	0.14	0.35	0.32	0.34
21	Neral	31.31	-	-	-	0.07	0.06	0.07
22	Citral	32.356	-	-	-	0.11	0.11	0.11
23	Carvone	32.639	-	0.02	0.02	-	-	-
24	Humulene	32.993	0.13	-	-	-	-	-
25	β-Copaene	34.562	-	-	-	0.06	0.05	0.06
26	Valencene	35.119	0.18	-	-	0.23	0.18	0.21
27	α-Muurolene	36.85	0.13	-	-	-	-	-
28	Calarene	40.718	0.04	-	-	-	-	-
29	cis-Muurola-4(15),5-diene	40.758	-	0.01	0.01	0.06	-	-
30	β-Farnesene	40.811	-	-	-	-	-	0.06
Total Hydrocarbons Compounds			98.94	99.49	99.55	98.65	98.77	98.69
Total Oxygenated Compounds			1.01	0.51	0.44	1.33	1.22	1.31
Grand Total			99.95	100	99.99	99.98	99.99	100

(-) = Not Detected

Table 2. Chemical composition (%) of lemon peel essential oils at different irradiation doses

No	Compounds Name	RT (min)	Peak area (%) of identified compounds					
			0 kGy	2 kGy	4 kGy	6 kGy	8 kGy	10 kGy
1	α-Thujene	19.902	0.13	0.14	0.14	0.31	0.29	0.33
2	α-Pinene	20.354	1	1.06	1.04	3.04	2.81	3.14
3	Camphene	21.149	0.08	0.11	0.11	0.41	0.39	0.44
4	Sabinene	21.343	-	-	-	-	0.3	0.34
5	β-Pinene	21.729	-	-	-	-	2.77	-
6	β-Thujene	21.921	0.14	0.16	0.16	-	-	-
7	β-Myrcene	22.177	1.5	1.67	1.61	3.11	-	3.1
8	α-Phellandrene	22.288	-	-	-	0.33	-	-
9	3-carene	23.455	1.96	2.31	1.97	12.36	11.78	12.48
10	α-Terpinene	23.516	-	1.45	1.16	1.07	0.93	1.07
11	1,4-Cineol	23.724	-	-	-	0.85	0.76	0.85
12	p-Cymene	23.735	6.21	3.44	2.68	8.09	7.24	8.2
13	Limonene	24.044	78.14	74.6	75.16	51.82	56.82	51.37
14	cis-Sabinene hydrate	24.29	0.52	-	-	1.99	-	-
15	γ-Terpinene	25.372	2.15	4.41	4.96	6.55	5.7	6.48

Continue table 2

16	p-Mentha-3,8-diene	25.893	0.09	0.08	0.09	-	1.65	2.02
17	Terpinolene	26.533	3.16	5.36	5.84	5.23	4.37	5.05
18	Linalool	26.791	1.81	1.7	1.75	-	-	-
19	Isopulegol	26.893	-	-	-	0.42	0.31	0.37
20	Fenchol	28.021	0.11	-	0.11	-	-	-
21	2-Carene	28.211	-	-	0.11	-	-	-
22	Myrcenol	28.467	-	0.09	-	-	-	-
23	cis-Verbenol	28.49	0.13	0.08	0.08	-	-	-
24	Isoborneol	30.184	0.09	0.14	0.14	-	-	-
25	p-Cymen-8-ol	30.43	0.1	0.11	0.09	-	-	-
26	α-Terpineol	30.831	0.25	0.37	0.33	-	-	-
27	Neral	32.131	0.88	1.07	1.02	1.63	1.31	1.38
28	Citral	33.102	1.11	1.3	1.26	1.75	1.47	1.5
29	Cinnamaldehyde	33.646	0.22	0.25	0.19	-	-	-
30	1-Chloro-5-methylhexane	33.858	0.09	-	-	-	-	-
31	Neral dimethyl acetal	34.392	-	-	-	0.42	0.45	0.75
32	Geranial dimethyl acetal	34.899	-	-	-	0.62	0.65	1.13
33	α-Farnesene	35.862	0.12	-	-	-	-	-
34	Methyl methanthranilate	38.012	-	0.08	-	-	-	-
Total Hydrocarbons Compounds			94.68	94.79	95.03	92.32	95.05	94.02
Total Oxygenated Compounds			5.31	5.19	4.97	7.68	4.95	5.98
Grand Total			99.99	99.98	100	100	100	100

(-) = Not Detected

3.3 Antioxidant activity of citrus peels essential oil

3.3.1 Free radical scavenging assay (DPPH) and Ferric reducing power assay (FRAP):

The antioxidant properties of the EOs of irradiated and unirradiated samples were achieved by the reducing capabilities and scavenging of radicals DPPH and FRAP.

Antioxidant activity can be assessed using a DPPH assay, which measures the capacity of the essential oil to scavenge the stable free radical DPPH by the donation of a hydrogen atom or an electron. Citrus EOs contain antioxidant properties that might delay or prevent cell damage induced by physiological oxidants [36, 76]. Antioxidants react with DPPH, converting it to 1,1- diphenyl-2-picryl hydrazine, due to its rapid hydrogen accepting ability, which intercepts the spread of the free radical oxidation chain, forming stable end products that do not cause further lipid oxidation [77]. Free radicals are widely known for causing cell death and tissue damage, which leads to chronic illnesses [78]. Many studies have demonstrated the role of EOs regarding their free radical removal capacity [79], which is due to their beneficial antioxidant properties, allowing them to counteract cellular damage caused by physiological oxidants.

As mentioned in Table 3, the orange and lemon peel essential oils possessed significant radical scavenging activity, ranging from DPPH 42.1 to 76.8%. Clearly, compared to orange essential oil, the EO from lemon exhibited the greatest radical-scavenging activity. Limonene, which characterizes the major compounds of orange and lemon EOis well-known for its antioxidant capacity as a result of the hydrogen donation [30, 67]. [30, 80-82] observed that the stable, purple-colored radical DPPH could be reduced to 54.67% by lemon peel EO to a yellow-colored radical, DPPH-H. Furthermore, free radical scavenging activity of essential oil might be attributed to the phenolic compounds extracted in essential oils, recognized by their antioxidant activity against reactive oxygen class [83]. All the essential oils of both lemon and orange peels, but for reducing power, were a smaller amount active than the standard BHT.

Regarding the irradiation treatment, irradiated samples have the highest antioxidant activity compared to non-irradiated samples at different doses. Data in Table 3 showed that, the best irradiation dose is 4 kGy in both essential oils. The improved antioxidant capacity might be due to the degradation of molecules to other phenolic molecules or for of the differences in the conformity of molecules that donate in antioxidant activity [84, 85]. As well as, Pérez et al. [86] have detected that irradiation increase antioxidant activity of ethanolic and methanolic extracts of rosemary (*Rosmarinus officinalis* L.). Additional research demonstrated that the application of radiation can enhance the concentration of certain phytochemicals, as well as the biological value and antioxidant activity of materials that are not the same [26, 27]. Such increased levels in phytochemicals and improvement in the antioxidant activity can communicate additional health benefits in citrus peels essential oils in addition to a likely enhancement in the oxidative stability.

Data in the present study are similar with Shalaby et al. [87] who reported that the antioxidant activity (DPPH%) for orange essential oil are (54.23%).

The oil's antioxidant activity, as shown by ferric reducing power, usually increased as the radiation dose increased, reaching a maximum at dose levels of 4 kGy (Table 3). Gamma radiation's effects on the constituents of essential oils are also dependent on a number of factors, including temperature, plant species, sample condition, radiation dose, and dose rate [73, 88].

Table 3. Antioxidant activity of irradiated and unirradiated essential oils extracted from orange and lemon peels at different doses

	DPPH %		FRAP (μM TE/mg)	
BHT (200 mg/L)	91.5±1.053^{A}		---	
Irradiation dose	Orange	Lemon	Orange	Lemon
0 kGy	43.69±1.190^{C}	54.36±3.563^{F}	194.17±1.694^{D}	501.72±1.095^{F}
2 kGy	48.92±1.735^{B}	65.15±1.516^{D}	214.52±1.897^{C}	631.54±1.324^{C}
4 kGy	51.16±1.375^{B}	76.81±3.255^{B}	246.28±2.127^{A}	661.54±1.688^{A}
6 kGy	44.22±0.795^{C}	71.19±0.871^{C}	221.72±1.692^{B}	635.22±2.127^{B}
8 kGy	42.16±2.640^{C}	62.86±0.469^{D}	197.16±2.785^{D}	613.29±1.995^{D}
10 kGy	44.08±0.865^{C}	58.63±1.420^{E}	174.70±1.609^{E}	570.14±2.126^{E}

Calculated mean is for triplicate evaluations ± SD; means with different superscripts in the same column are regarded statistically different ($p \leq 0.05$).

3.4 Antimicrobial activity of citrus peel essential oils:

The study included six distinct strains of pathogenic microorganisms, including two gram positive bacteria (*Staphylococcus aureus and Bacillus subtilis*), two gram negative bacteria (*Escherichia coli and Salmonella typhimurium*) and two fungal strains (*Candida albicans* and *Aspergillus brasilinesis*)**.** The antibacterial activity of the citrus peel essential oils was assessed using the Agar well diffusion assay.

The radiation dose of 4 kGy was the best dose, giving the greatest inhibition zone for all used microbial strains. Moreover, as shown in Table 4, lemon peel EO had greater antibacterial activity against human pathogenic bacteria than orange peel EO. Greater antimicrobial properties are displayed by the synergistic interaction of bioactive substances, i.e., limonene, γ-terpinene, α-pinene, linalool, β-pinene and β-myrcene. Our findings correlate with other studies [89-91] who reported that orange oil had an impact on *Bacillus subtilis, St. aureus, Salmonella typhiomurium*, *Aspergillus flavus* and *Candida lypolitica,* however, the inhibition zone of orange oil was smaller than that of lemon oil, despite the greater antibacterial activity seen for lemon at the various concentrations tested.

Therefore, this results arrangement with Aruna et al. [53] who discovered that the orange and lemon peel oils inhibition zone against *S. aureus* measured was 18 – 22 mm, whereas the inhibition zone around *E. coli* measured 11 – 16 mm. As well, our data contract with Hamdan et al. and Moosavt et al. [30, 92] who reported that the volatile oils mainly EOs of *Citrus spp.* have exposed bactericidal and fungicidal properties.

Strong activity occurred in the inhibition zone, which was > 20 mm; moderate activity occurred in the inhibition zone, which was < 20 to 12 mm; and no inhibition zone, which was ≤ 12 mm [93]. Thus, the irradiated and unirradiated essential oil extracted from lemon peels classified as the strongest antimicrobial agent compared to orange peels essential oils, where, it had a moderate to strong antimicrobial activity.

Several researchers have proposed that the antibacterial activity of essential oils may be attributed to their capacity to enter cells through bacterial membranes, have inhibitory effects on the functions of the cell, and have lipophilic characteristics [94-96].

An significant characteristic of essential oils and their constituents is hydrophobicity, allowing the essential oils to distinct the lipids of the bacterial cell membrane and mitochondria cause the bacterial cell to become more permeable [33, 97]. Some Gram positive and Gram negative bacteria have been shown to be inhibited by essential oils when they come into contact with microbial cell membranes. [16, 98].

Essential oils can be used to prevent the growth of bacteria by affecting the bacterial outer membrane, changing the fatty acid content, causing potassium ions and protons to leak out, interfering with the intake of glucose, and inhibiting enzyme activity or cell lysis [99].

On the other hand, it was noticed that the lemon peel EO accessible additional antifungal activity against (*Aspergillus brasilinesis* and *Candida albicans*) than orange EO. Table 5 showed that, the inhibition zone of irradiated EO has significant differences compared with non-irradiated essential oils. The radiation dose of 4 kGy followed by 2 kGy was the best dose that gave the greatest inhibition zone for fungi and yeast. As previously mentioned in Table 2&3, The constituents limonene, γ-terpinene, linalool and terpinen-4-ol may be responsible for *Citrus sinensis* L. peel Essential oils' antifungal action [100, 101].

D-Limonene was the main constituent in the planned essential oils, with variable fractions. Furthermore, according to the literature, D-Limonene has demonstrated effectiveness against foodborne bacterial and fungal pathogens, for example *Colletotrichum falcatum, Listeria monocytogenes, Aspergillus niger* and *Staphylococcus aureus* [102].

According to Jing et al. [103], there is a linking among the chemical structures of the most common compounds in EOs and their antimicrobial activity. According to Viriato [104], EO is abundant in terpenes like limonene, which are thought to serve as a natural antifungal because of their polar chemical configurations, which are both hydrophobic and lipophilic and allow them to interact with the components of the fungal cell membrane. According to recent research, essential oils changed the fungal mycelium's membrane permeability and integrity, which led to the leakage of cellular constituents [96, 105].

Table 4. Zones of inhibition (mm) for both irradiated and unirradiated orange and lemon peels essential oils against a selection of Gram-positive, Gram-negative bacteria and pathogenic fungal strains; well diameter 8.0 mm.

	Inhibition Zone (mm)											
	Gram (+) bacteria				**Gram (-) bacteria**							
	Staphylococcus aureus		***Bacillus subtilis***		***Escherichia coli***		***Salmonella typhimurium***		***Candida albicans***		***Aspergillus brasiliensis***	**n**
Irradiation dose	**Orange**	**Lemon**	**Orange**	**Lemon**	**Orange**	**Lemon**	**orange**	**Lemon**	**Orange**	**lemon**	**orange**	**Lemon**
0 kGy	16.0±0.200^{BCD}	26.3±1.527^{BC}	18.3±0.577^{B}	25.3±0.577^{D}	19.3±1.154^{AB}	23.6±0.577^{D}	19.0±1.000^{C}	23.6±0.577^{C}	14.0±1.0$0^{F}$	35.0±1.0$0^{E}$	15.3±1.527^{B}	19.0±0.100^{B}
2 kGy	19.0±1.0$0^{A}$	28.3±1.154^{AB}	21.6±0.577^{A}	40.3±1.527^{A}	16.6±0.577^{C}	30.3±1.527^{BC}	23.0±1.732^{A}	28.6±1.154^{A}	24.0±1.0$0^{B}$	40.3±.57$7^{D}$	18.3±1.527^{A}	40.0±0.200^{A}
4 kGy	20.0±1.0$0^{A}$	31.6±2.081^{A}	20.6±1.154^{A}	41.6±1.527^{A}	21.6±1.527^{A}	37.3±3.055^{A}	24.0±1.000^{A}	29.3±1.154^{A}	26.0±1.0$0^{A}$	54.6±.57$7^{A}$	16.0±2.000^{BC}	45.0±0.300^{C}
6 kGy	19.0±1.0$0^{A}$	29.6±1.527^{AB}	16.6±0.577^{C}	34.3±2.081^{B}	20.0±2.000^{AB}	31.6±2.886^{B}	22.0±2.000^{AB}	24.6±0.577^{C}	17.0±1.0$0^{E}$	45.0±1.0$0^{B}$	14.6±1.527^{B}	33.6±0.300
8 kGy	18.3±1.52^{AB}	26.6±3.785^{BC}	18.6±0.577^{B}	29.0±1.000^{C}	21.6±1.527^{A}	29.3±1.154^{BC}	19.6±2.081^{BC}	27.3±2.516^{AB}	20.6±.57$7^{C}$	42.6±2.0$8^{C}$	15.0±1.000^{B}	37.0±0.200^{BC}
10 kGy	15.0±0.152^{C}	24.3±0.577^{CDE}	18.6±0.577^{B}	25.3±0.577^{D}	18.3±1.527^{BC}	27.6±0.577^{C}	17.3±0.577^{C}	25.3±1.154^{BC}	19.0±1.0$0^{D}$	39.0±1.0$0^{D}$	14.3±1.154^{B}	29.0±0.100^{D}

Calculated mean is for triplicate evaluations ± SD; means with different superscripts in the same column are regarded statistically different ($p \leq 0.05$).

Conclusion

The recent study examined the efficacy of Egyptian citrus peel byproducts assessment through the manufacture of essential oils with antioxidant and antimicrobial activities. The values of physiochemical properties were not significantly affected compared to non-irradiated samples. D-Limonene and β- Myrcene, β-Thujene, γ-Terpinene, Neral, Citral, and linalool were the main constituents detected in the two essential oils. The study showed that the radiation dose that gave the highest antioxidant and antimicrobial values was 4 kGy. The antioxidant capacity of the citrus peel essential oils was proved by double dissimilar antioxidant activity examinations (DPPH and FRAP assay) where, lemon essential oil showed scavenging activities greater than orange essential oil. Therefore, citrus essential oils either irradiated or unirradiated characterized as natural and safe replacements to spread the shelf life of food products as well as, may be integrated in cosmetics and medicinal preparations due to greatly valued biological activities.

References

1. Calo, J.R., et al., *Essential oils as antimicrobials in food systems – A review.* Food Control, 2015. **54**: p. 111-119.
2. Cohen, S.M., et al., *FEMA GRAS assessment of natural flavor complexes: Citrus-derived flavoring ingredients.* Food and Chemical Toxicology, 2019. **124**: p. 192-218.
3. Ben Hsouna, A., et al., *Citrus limon essential oil: Chemical composition, antioxidant and antimicrobial activities with Its Preservative Effect against Listeria monocytogenes inoculated in minced beef meat.* Lipids in Health and Disease, 2017. **16**.
4. Agarwal, P., et al., *Citrus essential oils in aromatherapy: Therapeutic effects and mechanisms.* Antioxidants, 2022. **11**(12): p. 2374.
5. Palazzolo, E., V.A. Laudicina, and M.A. Germanà, *Current and Potential Use of Citrus*

Essential Oils. Current Organic Chemistry, 2013. **17**: p. 3042-3049.
6. Rosa, A., et al., *Chemical characterization, antioxidant and cytotoxic activity of hydroalcoholic extract from the albedo and flavedo of Citrus limon var. pompia Camarda.* Journal of Food Measurement Characterization, 2023. **17**(1): p. 627-635.
7. Ahmed, H.A., et al., *Nano-Formulations of Hesperidin and Essential Oil Extracted from Sweet Orange Peel: Chemical Properties and Biological Activities* Egyptian Journal of Chemistry, 2021. **64**(9): p. 5373-5385.
8. Li, Y., et al., *The chemical composition and antibacterial and antioxidant activities of five citrus essential oils.* Molecules, 2022. **27**(20): p. 7044.
9. Brah, A.S., et al., *Toxicity and therapeutic applications of citrus essential oils (CEOs): A review.* International Journal of Food Properties, 2023. **26**(1): p. 301-326.
10. Fancello, F., et al., *Chemical characterization, antioxidant capacity and antimicrobial activity against food related microorganisms of Citrus limon var. pompia leaf essential oil.* LWT-Food Science Technology, 2016. **69**: p. 579-585.
11. González-Mas, M.C., et al., *Volatile compounds in citrus essential oils: A comprehensive review.* Frontiers in Plant Science, 2019. **10**: p. 12.
12. Suri, S., A. Singh, and P.K. Nema, *Current applications of citrus fruit processing waste: A scientific outlook.* Applied Food Research, 2022. **2**(1): p. 100050.
13. Moufida, S.d. and B. Marzouk, *Biochemical characterization of blood orange, sweet orange, lemon, bergamot and bitter orange.* Phytochemistry, 2003. **62**(8): p. 1283-1289.
14. Ngele, K., V. Olugbue, and U. Okorie, *Phytochemical constituents and Antimicrobial effect of unripe epicarp of orange friuts (Citrus sinensis) against Escherichia coli and Staphylococcus aureus.* Int. J. of Sci. Nature, 2014. **5**(3): p. 418-422.
15. Benvenuti, F., F. Gironi, and L. Lamberti, *Supercritical deterpenation of lemon essential oil, experimental data and simulation of the semicontinuous extraction process.* The Journal of Supercritical Fluids, 2001. **20**(1): p. 29-44.
16. Ibrahim, F.M., et al., *Egyptian Citrus Essential Oils Recovered from Lemon, Orange, and Mandarin Peels: Phytochemical and Biological Value.* Horticulturae, 2024. **10**(2): p. 180.
17. Petretto, G.L., et al., *Waste Citrus limon leaves as source of essential oil rich in limonene and citral: Chemical characterization, antimicrobial and antioxidant properties, and effects on cancer cell viability.* Antioxidants, 2023. **12**(6): p. 1238.
18. Klimek-Szczykutowicz, M., A. Szopa, and H. Ekiert, *Citrus limon (Lemon) phenomenon—a review of the chemistry, pharmacological properties, applications in the modern pharmaceutical, food, and cosmetics industries, and biotechnological studies.* Plants (Basel), 2020. **9**(1): p. 119.
19. Ehlermann, D.A.E., *Wholesomeness of irradiated food.* Radiation Physics and Chemistry, 2016. **129**: p. 24-29.
20. Maherani, B., et al., *World Market Development and Consumer Acceptance of Irradiation Technology.* Foods, 2016. **5**(4).
21. Codex, *Codex general standard for irradiated foods.* 2003: p. 106-1983.
22. Pérez, M.B., S.A. Banek, and C.A. Croci, *Retention of antioxidant activity in gamma irradiated argentinian sage and oregano.* Food Chemistry, 2011. **126**: p. 121-126.
23. Rosiak, N., et al., *Antioxidant potential of resveratrol as the result of radiation exposition.* Antioxidants, 2022. **11**(11): p. 2097.
24. Molins, R.A., *Food irradiation: principles and applications*. 2001: John Wiley & Sons.
25. Rady, A.H., et al., *Impact of gamma radiation on antioxidant activity in faba bean (Vicia faba L.) and the potential of meatballs formulation with inclusion of the powder of irradiated beans.* Journal of Food Science Technology, 2020. **57**: p. 2975-2984.
26. Zevallos-Concha, A., et al., *Effect of gamma irradiation on phenol content, antioxidant activity and biological activity of black maca and red maca extracts (Lepidium meyenii walp).* Toxicology Mechanisms Methods, 2016. **26**: p. 67 - 73.
27. Pereira, E., et al., *Effects of gamma radiation on the bioactivity of medicinal and aromatic plants: Mentha × piperita L., Thymus vulgaris L. and Aloysia citrodora Paláu as case studies.* Food and Function, 2018. **9**(10): p. 5150-5161.
28. Hasan, M.M., et al., *Antimicrobial activity of peels and physicochemical properties of juice prepared from indigenous citrus fruits of Sylhet region, Bangladesh.* Heliyon, 2022. **8**(7): p. e09948.
29. Guo, J.-j., et al., *Comparative analysis of chemical composition, antimicrobial and antioxidant activity of citrus essential oils from the main cultivated varieties in China.* Lwt, 2018. **97**: p. 825-839.
30. Moosavy, M.-H., et al., *Antioxidant and antimicrobial activities of essential oil of lemon (Citrus limon) peel in vitro and in a food model.* Journal of Food Quality and Hazards Control, 2017. **4**: p. 42-48.

31. Kačániová, M., et al., *The antioxidant and antimicrobial activity of essential oils against Pseudomonas spp. isolated from fish.* Saudi Pharm J, 2017. **25**(8): p. 1108-1116.
32. Blowman, K., et al., *Anticancer properties of essential oils and other natural products.* Evidence-based complementary alternative medicine, 2018. **2018**.
33. Burt, S., *Essential oils: their antibacterial properties and potential applications in foods—a review.* International Journal of Food Microbiology, 2004. **94**(3): p. 223-253.
34. Thormar, H.E., *Front Matter. In Lipids and Essential Oils as Antimicrobial Agents.* 2011.
35. Zoubiri, S. and A. Baaliouamer, *Potentiality of plants as source of insecticide principles.* Journal of Saudi Chemical Society, 2014. **18**(6): p. 925-938.
36. Basavegowda, N. and K.-H. Baek, *Synergistic antioxidant and antibacterial advantages of essential oils for food packaging applications.* J Biomolecules 2021. **11**(9): p. 1267.
37. Seid, L., et al., *Valorization of citrus sinensis wastes for the production of orange essential oil: identification of chemical composition and antibacterial activity.* Journal of Fundamental Applied Sciences, 2022. **14**(3): p. 490-508.
38. Al-assaf, I.N., et al., *Essential oils and fatty acids of Thymus vulgaris seeds: chemical composition, antioxidant and antimicrobial activity* Egyptian Journal of Chemistry, 2023. **66**(3): p. 459-464.
39. Shakir, I.K. and S.J. Salih, *Extraction of Essential Oils from Citrus By-Products Using Microwave Steam Distillation.* Iraqi Journal of Chemical and Petroleum Engineering, 2015. **16**(3): p. 11-22.
40. Etta-francis, I., J. Akpa, and E. Ehirim, *Comparative Study on The Analysis and Utilization of Citrus Peels Essential Oil and Pectin.* ICONIC RESEARCH AND ENGINEERING JOURNALS (IRE). 2022. **volume 5** (Issue 7).
41. Dănilă, E., et al., *Chemical composition, antimicrobial and antibiofilm efficacy of C. limon and L. angustifolia EOs and of their mixtures against Staphylococcus epidermidis clinical strains.* Industrial Crops Products, 2018. **122**: p. 483-492.
42. Lan-Phi, N.T., et al., *Chemical and aroma profiles of yuzu (Citrus junos) peel oils of different cultivars.* Food Chemistry, 2009. **115**: p. 1042-1047.
43. Ren, J.-N., et al., *Characterisation of free and bound volatile compounds from six different varieties of citrus fruits.* Food Chemistry, 2015. **185**: p. 25-32.
44. Brand-Williams, W., M.-E. Cuvelier, and C. Berset, *Use of a free radical method to evaluate antioxidant activity.* LWT-Food science Technology, 1995. **28**(1): p. 25-30.
45. Kheira, M., et al., *Chemical Composition and Antioxidant and Antimicrobial Activities of Curcuma longa L. Essential Oils* Egyptian Journal of Chemistry, 2023. **66**(7): p. 283-295.
46. Benzie, I.F.F. and J.J. Strain, *The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay.* Analytical Biochemistry, 1996. **239**(1): p. 70-76.
47. Edogbanya, P., et al., *Comparative study on the antimicrobial effects of essential oils from peels of three citrus fruits.* MOJ Biology Medicines (Basel), 2019. **4**(2): p. 49-54.
48. Farouk, A., et al., *The Impact of Nanoencapsulation on Volatile Constituents of Citrus sinesis L. Essential Oil and their Antifungal Activity* Egyptian Journal of Chemistry, 2022. **65**(3): p. 527-538.
49. SPSS., *PASW STATISTICS (18) Command Syntax Reference. SPSS Inc., USA.* 2009.
50. Fazakerley, H., et al., *Olfactory and chemical changes in irradiated essential oils.* The International Journal of Applied Radiation and Isotopes, 1961. **11**(4): p. 174-183.
51. Abdelaleem, M., *Effect of gamma irradiation on the biochemical and microbiological properties of the volatile constituents of coriander fruits.* M.Sc. Thesis, Faculty of Agriculture, Cairo University, Egypt., 2008.
52. Kumar, A., *Physico-chemical and natural products investigations of essential oil from the rhizomes of Kaempferia galanga L.* Der Chemica Sinica, 2014. **5**(2): p. 91-94.
53. Aruna , T.H., G. Kumutha, K. Kanchana, S. Vellaikumar ,S., *Physicochemical, antioxidant and antimicrobial properties of citrus peel essential oils.* . Journal of Applied and Natural Science, 2022. **14**(2): p. 6.
54. Salamah, N., et al., *Authentication of citrus peel oils from different species and commercial products using FTIR Spectroscopy combined with chemometrics.* Pharmacia, 2024. **71**: p. 1-7.
55. Parthasarathy, V.A., B. Chempakam, and T.J. Zachariah, *Chemistry of spices*. 2008: Cabi.
56. Abdelaleem, M., *Studies on The Synergistic Effect of Some Irradiated Essential Oils in Some Food Products.* Ph.D. Thesis, Faculty of Agriculture, Ain Shams University, Cairo. Egypt., 2013.
57. Boughendjioua, H. and S. Djeddi, *Organoleptic and physicochemical properties of algerian lemon essential oil.* World Journal of Applied Chemistry, 2017. **2**(5): p. 96-100.

58. Benoudjit, F., L. Maameri, and K. Ouared, *Evaluation of the quality and composition of lemon (Citrus limon) peel essential oil from an Algerian fruit juice industry.* Algerian Journal of Environmental Science Technology, 2020. **6**(4): p. 1575-1581.
59. Annou, G., et al., *Physicochemical Indexes and Evaluation of Antioxidant, Antihemolytic and Antibacterial Activities of Citrus sinensis and Citrus limon Peel Essential Oils.* Al-Qadisiyah Journal For Agriculture Sciences, 2023. **13**(1).
60. Alam, M., *Uddin. MS Characterization of oil and lecithin from pioly (Aspidoparia morar) fish.* Journal of Innovations in Pharmaceutical Biological Sciences, 2017. **4**: p. 44-48.
61. Hosni, K., et al., *Composition of peel essential oils from four selected Tunisian Citrus species: Evidence for the genotypic influence.* Food chemistry, 2010. **123**(4): p. 1098-1104.
62. Al-Jabri, N.N. and M.A. Hossain, *Comparative chemical composition and antimicrobial activity study of essential oils from two imported lemon fruits samples against pathogenic bacteria.* Beni-Suef University Journal of Basic Applied Sciences, 2014. **3**(4): p. 247-253.
63. Vasek, O.M., et al., *Antibacterial activity of Citrus paradisi essential oil.* 2015.
64. Manaila, E., et al., *Comparation between chemical compositions of some essential oils obtained by hydrodistillation from citrus peels.* Revista de Chimie, 2016. **67**: p. 106-112.
65. Bozkurt, T., O. Gulnaz, and Y. Aka Kacar, *Chemical composition of the essential oils from some citrus species and evaluation of the antimicrobial activity.* IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT), 2017. **11**: p. 29-33.
66. Aydeniz, B., N.N. Demirel Zorba, and E. Yılmaz, *Antimicrobial activity of cold pressed citrus seeds oils, some citrus flavonoids and phenolic acids.* Rivista Italiana delle Sostanze Grasse, 2018. **95**: p. 119-131.
67. Bhandari, D.P., et al., *Volatile compounds and antioxidant and antimicrobial activities of selected citrus essential oils originated from Nepal.* Molecules, 2021. **26**(21): p. 6683.
68. Minh Tu, N.T., et al., *Characteristic odor components of Citrus sphaerocarpa Tanaka (Kabosu) cold-pressed peel oil.* Journal of Agricultural Food Chemistry, 2002. **50**(10): p. 2908-2913.
69. Sawamura, M., et al., *Volatile constituents of the peel oils of several sweet oranges in China.* Journal of essential oil research, 2005. **17**(1): p. 2-6.
70. Duarte, A.R., et al., *Genetic and environmental influence on essential oil composition of Eugenia dysenterica.* Journal of the Brazilian Chemical Society, 2010. **21**: p. 1459-1467.
71. Thongphasuk, P., et al., *Effects of gamma-irradiation on active components, free radicals and toxicity of Cassumunar ginger rhizomes.* International Journal of Pharmacy Pharmaceutical Sciences, 2014. **6**(7): p. 432-436.
72. Douar-Latreche, S., et al., *Effect of Gamma Irradiation on the Chemical Composition and Antioxidant Activity of Thymus algeriensis Extracts.* Journal of Essential Oil Bearing Plants, 2018. **21**(2): p. 449-461.
73. Yalcin, H., et al., *Effect of γ-irradiation on bioactivity, fatty acid compositions and volatile compounds of clary sage seed (Salvia sclarea L.).* Journal of Food Science, 2011. **76**(7): p. C1056-C1061.
74. Alloun, K., et al., *Effect of Gamma Ray Irradiation on Chemical Composition, Antioxidant, Antimicrobial, and Insecticidal Activities of Thymus pallescens Essential Oil.* Acta Chromatographica, 2017. **31**.
75. Matera, R., E. Lucchi, and L. Valgimigli, *Plant essential oils as healthy functional ingredients of nutraceuticals and diet supplements: A review.* Molecules, 2023. **28**(2): p. 901.
76. Al-Saman, M.A., et al., *Antimicrobial and antioxidant activities of different extracts of the peel of kumquat (Citrus japonica Thunb).* Journal of Food Measurement Characterization, 2019. **13**: p. 3221-3229.
77. Baliyan, S., et al., *Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of Ficus religiosa.* Molecules, 2022. **27**(4): p. 1326.
78. de Sousa, D.P., et al., *Essential oils: Chemistry and pharmacological activities.* Biomolecules, 2023. **13**(7): p. 1144.
79. Chen, X., et al., *Antioxidant activities of essential oils and their major components in scavenging free radicals, inhibiting lipid oxidation and reducing cellular oxidative stress.* Molecules, 2023. **28**(11): p. 4559.
80. Wei, A. and T. Shibamoto, *Antioxidant activities and volatile constituents of various essential oils.* Journal of agricultural food chemistry, 2007. **55**(5): p. 1737-1742.
81. Lin, L.Y., et al., *Lime (Citrus aurantifolia (Christm.) Swingle) Essential Oils: Volatile Compounds, Antioxidant Capacity, and Hypolipidemic Effect.* Foods, 2019. **8**(9): p. 398.
82. Teneva, D., et al., *Chemical composition, antioxidant activity and antimicrobial activity*

of essential oil from Citrus aurantium L zest against some pathogenic microorganisms. Zeitschrift für Naturforschung C, 2019. **74**(5-6): p. 105-111.
83. Ben Miri, Y., A. Arino, and D. Djenane, *Study of antifungal, anti-aflatoxigenic, antioxidant activity and phytotoxicity of Algerian Citrus limon var. Eureka and Citrus sinensis var. Valencia essential oils.* Journal of Essential Oil Bearing Plants, 2018. **21**(2): p. 345-361.
84. Adamo, M., et al., *Truffles decontamination treatment by ionizing radiation.* Radiation Physics Chemistry, 2004. **71**(1-2): p. 167-170.
85. Chen, C., et al., *Study on the Bacteriostasis of Lemon Essential Oil and the Application of Lemon Essential Oil Nanoemulsion on Fresh-cut Kiwifruit.* Frontiers in Sustainable Food Systems, 2024. **8**: p. 1394831.
86. Pérez, M.B., N.L. Calderon, and C.A. Croci, *Radiation-induced enhancement of antioxidant activity in extracts of rosemary (Rosmarinus officinalis L.).* Food chemistry, 2007. **104**(2): p. 585-592.
87. Shalaby, E.A., et al., *Biological Activities of Some Citrus Fruit Peels extracts.* Egyptian Journal of Chemistry, 2023. **66**(7): p. 51-61.
88. Koseki, P.M., et al., *Effects of irradiation in medicinal and eatable herbs.* Radiation Physics Chemistry, 2002. **63**: p. 681-684.
89. Mahmud, S., et al., *Volatile components, antioxidant and antimicrobial activity of Citrus acida var. sour lime peel oil.* Journal of Saudi Chemical Society, 2009. **13**(2): p. 195-198.
90. Mansour, A., et al., *Antibacterial effect and physicochemical properties of essential oil of Zataria multiflora Boiss.* Asian Pacific Journal of Tropical Medicine, 2010. **3**(6): p. 439-442.
91. Shehata, S.A., et al., *Effect of some citrus essential oils on post-harvest shelf life and physicochemical quality of strawberries during cold storage.* J Agronomy, 2020. **10**(10): p. 1466.
92. Hamdan, D.I., et al., *Chemical composition and biological activity of essential oils of Cleopatra mandarin (Citrus reshni) cultivated in Egypt.* Journal of Pharmacognosy Phytotherapy, 2013. **5**(5): p. 83-90.
93. Ait-Ouazzou, A., et al., *Chemical composition and antimicrobial activity of essential oils of Thymus algeriensis, Eucalyptus globulus and Rosmarinus officinalis from Morocco.* Journal of the Science of Food Agriculture, 2011. **91**(14): p. 2643-2651.
94. Guinoiseau, E., et al., *Cellular effects induced by Inula graveolens and Santolina corsica essential oils on Staphylococcus aureus.* European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology, 2010. **29**: p. 873-9.
95. Bajpai, V.K., K.-H. Baek, and S.C. Kang, *Control of Salmonella in foods by using essential oils: A review.* Food Research International, 2012. **45**(2): p. 722-734.
96. Simons, A., K. Alhanout, and R.E. Duval, *Bacteriocins, antimicrobial peptides from bacterial origin: Overview of their biology and their impact against multidrug-resistant bacteria.* Microorganisms, 2020. **8**(5): p. 639.
97. Friedly, E.C., et al., *In vitro Antilisterial Effects of Citrus Oil Fractions in Combination with Organic Acids.* Journal of food science 2009. **74**(2): p. M67-M72.
98. Calsamiglia, S., et al., *Invited Review: Essential Oils as Modifiers of Rumen Microbial Fermentation.* Journal of Dairy Science, 2007. **90**(6): p. 2580-2595.
99. Angane, M., et al., *Essential oils and their major components: an updated review on antimicrobial activities, mechanism of action and their potential application in the food industry.* Foods, 2022. **11**(3): p. 464.
100. Velázquez-Nuñez, M.J., et al., *Antifungal activity of orange (Citrus sinensis var. Valencia) peel essential oil applied by direct addition or vapor contact.* Food Control, 2013. **31**(1): p. 1-4.
101. Mancuso, M., et al., *Screening of antimicrobial activity of citrus essential oils against pathogenic bacteria and Candida strains.* 2019. **34**(3): p. 187-200.
102. Yu, H., et al., *Antifungal activity and mechanism of D-limonene against foodborne opportunistic pathogen Candida tropicalis.* Lwt, 2022. **159**: p. 113144.
103. Jing, L., et al., *Antifungal Activity of Citrus Essential Oils.* J Agric Food Chem, 2014. **62**(14): p. 3011-3033.
104. Viriato, A., *Terpenoids with antifungal activity for Candida Berkhout, causing nosocomial infections.* World Health, 2014. **38**(1): p. 40-50.
105. Magalhães, M.L., et al., *Biological activities of the essential oil from the Moro orange peel (Citrus sinensis (L.) Osbeck).* Flavour and fragrance journal, 2020. **35**(3): p. 294-301.

.