



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

Amr K. Ali<sup>1\*</sup>, Kamal M. Alsahy<sup>2</sup>, Toliba A. O.<sup>2</sup>, Hany A. Shawky<sup>1</sup>, M. A. Abdelaleem<sup>1\*</sup> and Khalid. R. A. Elbassiony<sup>1</sup>

<sup>1</sup> Plant Research Department, Nuclear Research Center (NRC), Egyptian Atomic Energy Authority (EAEA), Cairo, Egypt.

<sup>2</sup> 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 doses (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  $\mu\text{M TE/mg}$ ) 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](mailto:mdabdelrazek.md@gmail.com); (Mohamed A Abdelaleem).

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fruits' famous scent is attributed to limonene [14]. Lemon Eo are complex mixtures of chemical substances such as limonene, citral, linalool,  $\gamma$ -terpinene and  $\beta$ -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  $\gamma$ -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 disinfections 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 brasiliensis* (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  $^{60}\text{Co}$  gamma chamber was used in the Cyclotron Project at the Nuclear Research Center of

the Egypt Atomic Energy Authority (with a dosage rate of 395.1 Gy/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-diphenylpicrylhydrazyl (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<sup>-5</sup> mol/l of DPPH solution (2.4 mg of DPPH in 100 ml of methanol), (0.1 mL) of essential oil was added to 3.9 ml of a 6 × 10<sup>-5</sup> 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.

### Antioxidant activity (Inhibition)%

$$= \left[ \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \right] \times 100$$

**Where:**  $A_{\text{control}}$  is the absorbance of the control response (absorbance of DPPH solution) and  $A_{\text{sample}}$  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 MFeCl<sub>3</sub>, 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.

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

## 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 physicochemical 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 physicochemical properties of citrus peel essential oils at varied applied levels.

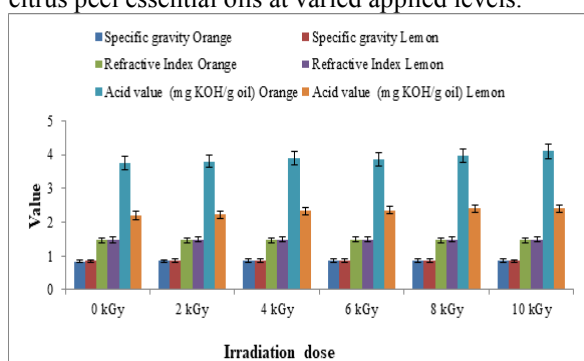


Figure 1. Physicochemical properties of orange and lemon peels essential oils

### 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,  $\beta$ -myrcene,  $\alpha$ -pinene, terpinolene,  $\beta$ -pinene,  $\gamma$ -terpinene, linalool and 3-carene. The effect of gamma irradiation is varied from one-to-one compound, where  $\alpha$ -pinene, terpinolene,  $\beta$ -myrcene,  $\gamma$ -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,  $\alpha$ -terpineol, geranyl acetate, linalool, 4-terpenol, neryl acetate and  $\alpha$ -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	$\alpha$ -pinene	19.259	-	-	0.48	1.29	1.18	1.26
2	$\beta$ -Thujene	20.325	0.66	0.54	0.17	0.56	0.52	0.55
3	$\beta$ -Terpinene	21.915	0.37	0.2	-	-	-	-
4	$\beta$ -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	$\alpha$ -Phellandrene	23.311	-	-	-	0.1	0.08	0.1
7	$\gamma$ -Terpinene	23.426	0.28	-	0.14	0.38	0.35	0.38
8	(+)- $\delta$ -Carene	23.449	-	0.17	-	-	-	-
9	D-Limonene	24.29	94.67	97.04	97.36	92.19	92.87	92.4
10	$\beta$ -Phellandrene	25.221	-	-	-	0.59	0.53	0.53
11	Isoterpinolene	26.516	-	0.02	0.01	-	0.06	0.06
12	$\beta$ -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	$\beta$ -Copaene	34.562	-	-	-	0.06	0.05	0.06
26	Valencene	35.119	0.18	-	-	0.23	0.18	0.21
27	$\alpha$ -Muuroleone	36.85	0.13	-	-	-	-	-
28	Calarene	40.718	0.04	-	-	-	-	-
29	cis-Muurolo-4(15),5-diene	40.758	-	0.01	0.01	0.06	-	-
30	$\beta$ -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	$\alpha$ -Thujene	19.902	0.13	0.14	0.14	0.31	0.29	0.33	
2	$\alpha$ -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	$\beta$ -Pinene	21.729	-	-	-	-	2.77	-	
6	$\beta$ -Thujene	21.921	0.14	0.16	0.16	-	-	-	
7	$\beta$ -Myrcene	22.177	1.5	1.67	1.61	3.11	-	3.1	
8	$\alpha$ -Phellandrene	22.288	-	-	-	0.33	-	-	
9	3-carene	23.455	1.96	2.31	1.97	12.36	11.78	12.48	
10	$\alpha$ -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	$\gamma$ -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	$\alpha$ -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	$\alpha$ -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 EOs 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

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

Calculated mean is for triplicate evaluations  $\pm$  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 brasiliensis*). 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,  $\gamma$ -terpinene,  $\alpha$ -pinene, linalool,  $\beta$ -pinene and  $\beta$ -myrcene. Our findings correlate with other studies [89-91] who reported that orange oil had an impact on *Bacillus subtilis*, *St. aureus*, *Salmonella typhimurium*, *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  $\leq 12$  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 brasiliensis* 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,  $\gamma$ -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.

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.

Irradiation dose	Inhibition Zone (mm)											
	Gram (+) bacteria				Gram (-) bacteria							
	<i>Staphylococcus aureus</i>		<i>Bacillus subtilis</i>		<i>Escherichia coli</i>		<i>Salmonella typhimurium</i>		<i>Candida albicans</i>		<i>Aspergillus brasiliensis</i>	
	Orange	Lemon	Orange	Lemon	Orange	Lemon	orange	Lemon	Orange	lemon	orange	Lemo
0 kGy	16.0 ±0.2 00 <sup>BC</sup>	26.3±1 .527 <sup>BC</sup> D	18.3± 0.577 B	25.3± 0.577 D	19.3±1 .154 <sup>AB</sup> C	23.6± 0.577 D	19.0± 1.000 C	23.6± 0.577 C	14.0 ±1.0 0 <sup>F</sup>	35.0 ±1.0 0 <sup>E</sup>	15.3±1.527 B	19.0± 0.100 B
2 kGy	19.0 ±1.0 0 <sup>A</sup>	28.3±1 .154 <sup>AB</sup> A	21.6± 0.577 A	40.3± 1.527 A	16.6±0 .577 <sup>C</sup> C	30.3± 1.527 BC	23.0± 1.732 A	28.6± 1.154 A	24.0 ±1.0 0 <sup>B</sup>	40.3 ±.57 7 <sup>D</sup>	18.3±1.527 A	40.0± 0.200 A
4 kGy	20.0 ±1.0 0 <sup>A</sup>	31.6±2 .081 <sup>A</sup> A	20.6± 1.154 A	41.6± 1.527 A	21.6±1 .527 <sup>A</sup> A	37.3±3 .055 <sup>A</sup> A	24.0± 1.000 A	29.3± 2.000 AB	24.0± 0.577 C	26.0 ±.57 7 <sup>A</sup>	16.0±2.000 BC	45.0± 0.300 C
6 kGy	19.0 ±1.0 0 <sup>A</sup>	29.6±1 .527 <sup>AB</sup> C	16.6± 0.577 C	34.3± 2.081 B	20.0±2 .000 <sup>AB</sup> B	31.6± 2.886 B	22.0± 2.000 AB	24.6± 0.577 C	17.0 ±1.0 0 <sup>E</sup>	45.0 ±1.0 0 <sup>B</sup>	14.6±1.527 B	33.6± 0.300 B
8 kGy	18.3 ±1.5 2 <sup>AB</sup>	26.6±3 .785 <sup>BC</sup> B	18.6± 0.577 B	29.0± 1.000 C	21.6±1 .527 <sup>A</sup> A	29.3±1 .154 <sup>BC</sup> BC	19.6± 2.081 BC	27.3± 2.516 AB	20.6 ±.57 7 <sup>C</sup>	42.6 ±2.0 8 <sup>C</sup>	15.0± 1.000 B	37.0±0.200 BC
10 kGy	15.0 ±0.1 52 <sup>C</sup>	24.3±0 .577 <sup>CD</sup> E	18.6± 0.577 B	25.3± 0.577 D	18.3±1 .527 <sup>BC</sup> BC	27.6± 0.577 C	17.3± 0.577 C	25.3± 1.154 BC	19.0 ±1.0 0 <sup>D</sup>	39.0 ±1.0 0 <sup>D</sup>	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  $\beta$ - Myrcene,  $\beta$ -Thujene,  $\gamma$ -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.

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.

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