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Research Article

Bio-Reactivity of Orange Essential Oil Extracted from Peel Waste

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Abstract

This study aimed to characterize the essential oil of oranges extracted from Valencia orange peel waste from two different sources (Dakahlia Governorate (Sample A) and from Alexandria Governorate (Sample B), Egypt) and to determine the levels of pesticide residues in the oil and their compliance with standard specifications. The study also aimed to evaluate its biological effects by investigating its antioxidant and antimicrobial activity, as well as its cytotoxicity against certain types of cancer cells. The essential oil was obtained using freeze-thaw assisted hydrodistillation of the aqueous waste from orange peeling, a step in the orange juice production line. Chemical composition analysis and pesticide residue detection were performed using GC-MS. Nine components were identified in both samples (A and B) through mass spectra and linear retention indices. Limonene was found to be the predominant compound in the essential oil, comprising 90.22% and 93.15%, respectively. When comparing the chemical composition of the two oils, a significant difference in the percentage of components was observed, but three major compounds were identified: limonene, linalool, and β-myrcene. Sample (A) showed significant pesticide contamination at levels exceeding the Egyptian Standard Specification, while sample (B) was within safe limits. The antioxidant activity of the oils was tested using a free radical scavenging method with 2,2-diphenyl-1-picrylhydrazyl (DPPH). Both oils exhibited relatively strong antioxidant activity, with the oil from sample B being the most potent, registering 35.8 µg/mL. Furthermore, the antimicrobial evaluation using the disc diffusion method demonstrated the oil's ability to inhibit microbial growth, particularly Gram-positive bacteria. The oil also showed a strong anticancer effect, especially at IC50, 10, 16, and 8.5 µg/mL. This study concludes by confirming the efficiency of the freeze-thaw extraction method and assisting hydro distillation in extracting essential oil from orange peel residue solutions. It characterizes the oil to elucidate its potent antioxidant, antimicrobial, and anticancer properties.

Keywords: Orange Essential Oil; Extraction; Chemical Composition; Pesticide Residues; Antioxidant Activity; Antimicrobial Activity; Anti-Cancer Activity

Introduction

Oranges (Citrus sinensis) rank among the world's most cultivated fruits, with global production typically spanning 75–80 million metric tons annually across major producers like Brazil, China, India, the US, and the EU [33]. Egypt has solidified its position as a significant player in the global orange market. In the 2022/23 season, the country's orange production reached a record 3.16 million metric tons. Global orange juice production is experiencing notable shifts due to climatic challenges and disease pressures, while Egypt is emerging as a significant player in the processing sector. Processing oranges produces a large amount of waste, including all residues remaining after the juice extraction process, such as peels, seeds, and pulp, constituting between 50 and 65% of the total fruits' weight [23]. Every year 110–120 million tons of citrus waste are generated worldwide from citrus processing industries creating huge challenges regarding pollution of land, soil, underground water table, and overall wet/semi-solid waste management [24]. Through innovative utilization strategies, this waste can be transformed from an environmental burden into a valuable resource that contributes to sustainability and economic development. Citrus peels contain BCs, such as polyphenols, pigments (carotenoids), vitamins, sugars, dietary fiber (pectin, cellulose, hemicellulose, and lignin), and essential oils (EOs) [45]. The food industry is trying to isolate the bioactive compounds (BCs) obtained from waste to produce functional ingredients and nutritional supplements with therapeutic potential for nutrition and health [39]. These days, scientists and botanists alike are interested in the extraction of chemicals from its natural sources [21]. Fruit peels, blossoms, and leaves all contain secondary metabolites called citrus essential oils (CEOs), which are primarily made up of aromatic chemicals. [1] The peels contain more essential oil than the leaves. CEOs consist of 85–99% volatile and nonvolatile chemicals, with species-specific percentages ranging from 1–15% [20]. The volatile terpene and terpenoid molecules, such as monoterpenes, monoterpene hydrocarbons, monoterpene alcohols, monoterpene aldehydes, sesquiterpenes, and sesquiterpene alcohols, are responsible for the biological action of CEOs [9]. They can treat metabolic diseases and have antibacterial, anticancer, and anti-inflammatory properties [31]. Along with a few additional compounds including alpha-pinene, beta pinene, sabinene, limonene, β-ocimene, and linalool, D-limomen makes up more than 85% of its essential oil [7]. The biological properties of orange peel essential oil, such as its antioxidant, anti-inflammatory, antiaging, antibacterial, antifungal, and anti-aflatoxigenic properties, have been identified in recent years [6]. These properties are closely linked to the hydrocarbons, alcohols, esters, and aldehydes that make up these volatile oils [18]. The emergence of serious adverse effects from the use of synthetic antioxidants such butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) explains the increasing interest in finding natural antioxidants [32]. Continuous use of synthetic preservatives might lead to residual toxicity and the emergence of resistance in certain bacteria. Because they have demonstrated little to no harm to human health and have antimicrobial and antioxidant properties, natural and safe products like essential oils or compounds derived from plants have recently attracted scientific attention and been accepted by the food industry [35]. Numerous studies have demonstrated the positive effects of citrus-based dietary products on degenerative diseases, including cancer, hypertension, cardiovascular disease, and blood glucose decrease [34]. Therefore, investigating the possibility of turning this waste into commercially viable items is imperative.

The food, beverage, cosmetic, and pharmaceutical industries all make extensive use of orange essential oils. Because of their effects on the environment and public health, regulations pertaining to residue levels of chemicals used for treatment are becoming more stringent. For this reason it is necessary to guarantee genuineness and the quality of essential oils and to determine all organic contaminants, even if a maximum limit of residues has not been established by legislation for this product type [10]. Orange oil, extracted through processes such as cold-pressing of orange peels, can exhibit significantly higher concentrations of pesticide residues than the original fruit due to the removal of water and concentration of lipophilic compounds.

The aim of the current study was to extract orange oil from the waste of Valencia orange peels, to identify the diverse and valuable bioactive compounds in the resulting essential oil and its functional properties, as well as to determine the quality and safety of the extracted oil in order to study the potential uses of a safe and bioactive essential oil in the food industry and to present a new approach to waste management and utilization.

Materials and Methods

Materials

Orange peel emulsion waste (20 liters) was collected from two juice factory, one in Dakahlia Governorate (Sample A) and the other in Alexandria Governorate (Sample B), Egypt.

All of the analytical-grade chemicals utilized in this study were acquired from Sigma-Aldrich Chemicals and Al Gomhouria Company.

Methods

Preparation of waste samples

The samples of Orange peel emulsion waste (A and B) were filtered separately using a 500-micron filter to remove large impurities, stems, and leaves that may be present in the waste. The samples were then cooled and transported to the laboratory of chemistry for extraction and characterization of oil.

Orange essential oil extraction

Orange essential oil was extracted on two stages as follows Stage one: Freezing and thawing

The method used in this study was according to Xu., *et al*. [46] with some modification. Orange peel emulsion waste (OPEW) was frozen at -18˚C for 24 hours, then thawed at 20-25˚C, repeated three times (60-75% of the water is removed).

As a result of this treatment, the OPEW forms three layers from top to bottom: a clear layer of oil, an emulsion (oil + water + solids), and frozen water at the bottom. The oil is drawn off before thawing using a pipette or pouring then thawed and the water separated from the emulsion using a separating funnel.

Stage two: Extraction the remaining essential oil by hydro-distillation method

The emulsion is distilled (to extract the remaining oil that was not removed in the first stage) and the water is separated separately (to ensure the water is free of oil).

The layer below the oil layer obtained after extraction by Freezing and Thawing and containing (essential oil and solids) was subjected to Clevenger apparatus through hydro-distillation for 3 hours to obtain orange essential oil. Using a Clevenger extractor is recommended at the Lab scale for essential oil extraction according to Guenther, (1972) [19]. After distillation, orange essential oil was dried by a glass separator, filtered two times, preserved in dark closed bottles for preventing light and oxygen exposure, and kept in the fridge at 4˚ ± 1C.

The yield of the oil extracted using the method of extraction was calculated using equation.

% yield= Weight of oil extracted/ Weight of sample used x100

Chemical composition of orange peel essential oil:

Using a mass spectrometer (Agilent 5977B GC/MSD), gas chromatography (Agilent 8890 GC System), and an HP-5MS fused silica capillary column (30 m, 0.25 mm i.d., 0.25 mm film thickness), the Gas Chromatography–mass spectrometry (GC-MS) analysis of orange peel essential oil was carried out in accordance with the procedure outlined by Tran., *et al*. [43]. Initially set at 50 ˚C, the oven temperature was designed to rise from 50 to 200 ˚C at a rate of 5 ˚C/min and from 200 ˚C to 280 ˚C at a rate of 10 ˚C/min, with a 7-minute hold at 280

˚C. The carrier gas, helium, flowed at a rate of 1.1 mL/min. With a split ratio of 1:50, 1 µL of the essential oil solution was injected into the GC after it had been dissolved in diethyl ether (20 µL essential oil / mL diethyl ether). The injection temperature was 230 ˚C. At 70 eV, mass spectra in the electron impact mode (EI) were acquired, covering the m/z range of 39 to 500 amu. By comparing them with information from the National Institute of Standards and Technology's (NIST) mass spectra repository, isolated peaks were identified. The area of the peak that corresponds to each chemical was used to calculate the relative percentage of each compound. Gas Chromatography-Mass Spectrometer (GC Mass) unit, Central Laboratories Network Chromatography (CLN-CE) Lab, National Research Center, Dokki, Egypt, is where this analysis was carried out.

Detection of Pesticide Residues of orange peel essential oil:

The Pesticide analysis of orange peel essential oil was conducted as described by Tran., *et al*. [43] using EN 15662:2018 test. Gas chromatography (Agilent technology, GC), often coupled with mass spectrometry (GC-MS), and is used to detect the presence of pesticide residues. The oil is diluted in a suitable solvent such as hexane or pentane and injected into a gas chromatograph equipped with a fused silica capillary column (e.g., SLB-5ms, 10 m × 0.10 mm inner diameter, 0.10 µm film thickness). The oven temperature

program typically starts at 40˚C and increases rapidly (e.g., 50°C/min) to 320˚C. Hydrogen or helium is used as the carrier gas at a constant linear velocity (e.g., 81.5 cm/s). Identification is based on retention times and comparison of mass spectra with standards and libraries. Quantification is performed by peak area normalization or using internal standards). The Pesticide analysis was conducted at, Pesticide Residual lab, Chemical and Food Test Labs, General Organization For Export and Import Control, Damietta, Egypt.

Phytochemical characteristics of orange peel essential oil:

Total phenolic content

Using the Folin-Ciocalteu reagent and a calibration curve with gallic acid as a standard, the total phenolic compounds were extracted from the oil samples using the method outlined by Ebrahimzadeh., *et al*.[14] and quantified by spectrophotometry.

Total flavonoids content

Flavonoids were determined using the colorimetric aluminum chloride method in accordance with the procedures outlined by Calabro., *et al*.[5] Each plant extract's 0.5 ml solution was combined with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water. The mixture was then allowed to sit at room temperature for half an hour. A twin beam Perkin Elmer UV/Visible Spectrophotometer was used to detect the reaction mixture's absorbance at 415 nm. A calibration curve was created by producing quercetin solutions at concentrations ranging from 12.5 to 100 mg ml-1 in methanol in order to calculate the total flavonoid contents as quercetin.

Antioxidant Activity (IC50) by DPPH Assay

The antioxidant capacity of the essential oil samples is commonly evaluated by the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay. A stock solution of DPPH in methanol is prepared, and various concentrations of the essential oil diluted in methanol or ethanol are mixed with the DPPH solution. After incubation in the dark at room temperature for 30 minutes, the decrease in absorbance at 517 nm is measured using a UV-Vis spectrophotometer. The percentage of DPPH radical scavenging is calculated relative to a control without oil. The IC50 value, representing the concentration of oil required to reduce the initial DPPH concentration by 50%, is determined from a dose-response curve. This method is described by Diouf., *et al*. [11].

Antimicrobial assay of orange peel essential oil

Six species of pathogenic bacteria were tested for the orange peel essential oil. antimicrobial activity: three Gram-negative bacteria (Salmonella enterica, Escherichia coli 0157 H7 ATCC 51659, and Pseudomonas aeruginosa NRRL B-272), three Gram-positive bacteria (Bacillus cereus EMCC 1080, Staphylococcus aureus ATCC 13565, and Listeria monocytogenes, yeast and Candida albicans. Orange oil in two concentrations—100% and 50% v/v—was made and tested. The produced concentrations' antibacterial activity was assessed using the disc diffusion technique on nutrient agar. After being injected into Tryptic soy broth tubes, the tested pathogenic bacteria were cultured for four hours at 37˚C. 0.5 Mc-Farland was used to modify the turbidity of these cultures. Sterile cotton swabs were used to create a consistent bacterial lawn on the surface of solid nutrition agar plates. Following a half-hour drying period, the discs impregnated with the tested concentration were put onto the surface of the dried nutrient agar plates. As a positive control, ceftriaxone sodium salt (1.0 mg/mL) was employed. For a whole day, the plates were incubated at 37˚C. According to Mehaya., *et al*. [25], antibacterial activity was assessed by calculating the diameter of the zone of inhibition (mm).

Minimal Inhibitory Concentrations (MICs) of orange peel essential oil

The MIC values of orange peel essential oil were determined by the tube dilution method Drobniewski., *et al*. [12]. In short, the carefully produced dilution series of the tested ingredient was added to molten agar tubes that had been allowed to cool in a water bath at 50˚C. The tubes were then vortexed well and transferred into sterile, previously labeled petri dishes. Once the agar surface had completely dried at room temperature, 1µl of a microbial suspension containing 107 CFU/ml was detected. Plates were incubated as advised, and the lowest concentration at which the growth vanished was noted.

Determination of potential cytotoxicity of orange peel essential oil on human cancer cell line Principle: The Sulphorhodamine-B (SRB) assay was used to measure cytotoxicity in accordance with the protocol described by Srour., *et al*. [40]. The sulforhodamine B colorimetric assay is employed to screen for cytotoxicity. Nat. Protoc. 2006:1, 1112-1116. SRB is an aminoxanthrene dye that has two sulphonic groups and is brilliant

pink. It is a protein stain that provides a sensitive indicator of the amount of protein in cells by attaching itself to the amino groups of intracellular proteins in slightly acidic environments.

Procedure:

- Using a 200 µl fresh medium, cells were seeded in 96-well microtiter plates at an initial concentration of $4x10^3$ cells/ well and allowed to adhere to the plates for a full day.
- orange peel essential oil was added at various quantities (1.56, 3.25, 12.5, and 25 µg/ml).
- Three wells were utilized for every medication concentration. For 48 hours, the plates were incubated.
- The cells were fixed for an hour at 4 ˚C using 10 µl of cold trichloroacetic acid at a final concentration of 10%.
- 50 µl of 0.2% SRB diluted in 1% acetic acid was used to stain the plates for 30 minutes at room temperature in the dark after they had been cleaned with distilled water using an automatic washer (Tecan, Germany). The plates were washed with 1 % acetic acid and air-dried.
- Using an ELISA microplate reader (Sunrise Tecan reader, Germany), the optical density (O.D.) of each well was measured spectrophotometrically at 570 nm after the dye was dissolved in 200 µl/well of 10M tris base (pH 10.5). The mean values of each oil concentration were computed by automatically subtracting the mean background absorbance.

Calculation

The percentage of cell survival was calculated as follows:

Surviving fraction = O.D. (treated cells)/ O.D. (control cells).

The IC50 values (the concentrations of drug required to produce 50% inhibition of cell growth) were also calculated using prism version 5.

Statistical analysis

Minitab 18 was used to calculate the MIC value and statistical significance of the antibacterial activity [26]. The analysis of variance test (ANOVA, One Way analysis) was used to find the means ($p<0.05$). Significant changes across treatments were compared using Fisher's LSD (Least Significant Difference) Method ($\alpha = 0.05$) [17].

Results and Discussion

Extraction of orange peel essential oil by Freeze-thaw assisted Hydro-distillation

The obtained results showed that, the highest quantity of essential oil was obtained during the first stage, reaching 172.1 and 176 ml for samples A and B, respectively. However, the quantities obtained decreased the second stage, as shown in Table (1). The percentage of OPEO extracted from the solid layer by Hydro distillation reached 26.63 and 17.19 ml for samples A and B, respectively. Finally, the total amount of orange essential oil obtained at the end of the extraction process was 198.7 and 193.2 ml, with extraction rate from the residue estimated at 0.99 and 0.966% for samples A and B, respectively.

To get good results, hydro-distillation is done under ideal circumstances. The yield of essential oils is influenced by a number of elements, such as harvest time, growing stage, various portions, and environmental circumstances [28]. This explains why varied essential oil yields can be obtained using the same extraction technique.

Method Sample	Freeze-Thaw (stage one)	Hydro-distillation (stage two)			
		Solid layer	Thawed water	Total (ML\20L)	Yield (%)
A	172.1	26.63	Not Significant	198.7	0.99
B	176	17.19	Not Significant	193.2	0.966

Table 1: Extraction of orange peel essential oil by Freeze-thaw assisted Hydro-distil

A= Orange peel emulsion waste from Dakahlia Governorate. B=Orange peel emulsion waste from Alexandria Governorate.

Chemical composition and chromatographic profile of orange peel essential oil

The chemical composition and relative abundance of the various kinds of chemicals found in orange peel essential oil samples are displayed in Table (2).

Nine components were found in the orange peel essential oils used in this investigation. Limonene (90.22 and 93.15%), β-myrcene (4.37 and 2.36%), linalool (0.83 and 1.15%), αpinene (1.92 and 1.01%), sabinene (1.00 and 0.57%), decanal (0.54 and 0.65%), and valencene (0.47 and 0.62%) were the primary constituents of the orange essential oils for samples A and B, respectively. The results are in line with the findings of Velázquez-Nuñez., *et al*. [44], who stated that limonene makes up the majority of the orange essential oil (94.88 to 96.62%), with a few other components identified as αpinene, β-myrcene, linalool, and decanal components.

Furthermore, Yang., *et al*. [47] noted that extrinsic (geographical origin, environment, and isolation techniques) and intrinsic (genetics, subspecies, and plant age) elements may have a significant impact on orange essential oils. For example, limonene (71.80%), β-myrcene (4.55%), sabinene (1.39%), linalool (3.89%), and α-pinene (1.17%) were the main volatile compounds of orange essential oils in the study by Duman., *et al*. [13]. These amounts were significantly different from the current study.

Sample Component	**A**		**B**	
	AS (%)	RT	AS (%)	RT
α-Pinene	1.92	6.328	1.01	11.883
Sabinene	1.00	7.293	0.57	7.293
β-Myrcene	4.37	7.697	2.36	7.714
γ-Terpinene	0.65	8.245	----	----
D-Limonene	90.22	8.783	93.15	8.956
Linalool	0.83	10.671	1.15	10.677
Decanal	0.54	13.605	0.65	13.605
Valencene	0.47	21.240	0.62	21.240
Trans-Limonene oxide	----	----	0.49	11.763

Table 2: The main compounds found in of orange peel essential oil for sample (A) and (B).

As% = Area Sum% RT = Retention time

A= Orange oil extracted from the waste obtained from Dakahlia Governorate. B= Orange oil extracted from the waste obtained from Alexandria Governorate.

Pesticide residues in orange peel essential oil samples:

Result in Table (3) show the concentration of pesticide residues in the orange peel essential oils. Sample A showed extensive contamination: fourteen of fifteen monitored pesticides were detected above LOQ, with several exceeding ESS limits; notably, pyrimethanil reached 1497.19 mg/kg versus a legal limit of 0.05 mg/kg. In contrast, orange oil B displayed low residue burdens, with all quantified pesticides remaining below national ESS values. This strong between-sample variability agrees with previous multi-residue surveys of citrus essential oils, which report that peel-derived oils can accumulate lipophilic pesticides and occasionally surpass regulatory maximum residue levels, while other batches remain compliant [30].

Elshabrawy., *et al*. [15] stated that orange oils found that certain fungicides and insecticides in commercial oils were well above EU MRLs, whereas other batches were compliant, showing strong variability between sources similar to your contrast between A and B.

Sample Residues	A (mg/kg)	B (mg/kg)	LOQ	ESS
Buprofezin	<LOQ	<LOQ	0.005	0.01
Fludioxonil	0.006	<LOQ	0.005	0.01
Piperonnyl butoxide	0.065	0.01	0.005	0.02
Mepronil	0.025	0.007	0.005	0.02
Iprovalicarb	0.044	0.005	0.005	0.02
Pyrimthanil	1497.19	<LOQ	0.005	0.05
Pyriproxifen	0.058	<LOQ	0.005	0.01
Thiabendazole	0.123	0.009	0.005	0.02
Disulfoton sulfone	0.035	<LOQ	0.005	0.01
Malathion	0.075	<LOQ	0.005	0.01
Fenpropathrin	0.128	<LOQ	0.005	0.01
Chlorpyrifos	0.148	0.007	0.005	0.02
2-phenylphenol	0.087	0.009	0.005	0.02
Cyhalothrin	0.163	<LOQ	0.01	0.02
Tebuconazole	0.039	0.006	0.005	0.02

Table 3: Levels of pesticide residues in orange peel essential oil samples.

A= Orange oil extracted from the waste obtained from Dakahlia Governorate. B= Orange oil extracted from the waste obtained from Alexandria Governorate**.**

<LOQ = Less than limit of Quantification. ESS= Egyptian Standard Specification.

Total polyphenols, total flavonoids and antioxidant activity for orange peel essential oil

Result in Table (4) show the total polyphenols, total flavonoids and antioxidant activity for orange peel essential oil samples. From the obtained results, it could be notated that, both of orange peel essential oil samples were content trace amounts of total polyphenols and total flavonoids. This could be due to its high moisture content (In the water waste used in extraction), which promotes enzymatic reaction and that may result in the loss of antioxidant compounds [8]. Citrus polyphenols are found mainly in juice and peel tissues, staying in solid or water-soluble forms not in the volatile EO fraction [16]**.**

In this study, antioxidant activity (AA) values of orange peel essential oils were recorded 37.5 and 35.8 µg/Ml for samples A and B, respectively. Although the IC_{50} values show moderate antioxidant activity, this is mainly attributed to the major volatile components such as D-limonene, γ-terpinene, and others, rather than polyphenols or flavonoids [22]. The results obtained are consistent with those of Sarrou., *et al*. [38], who found that the oils exhibited strong antioxidant activity. A scavenging activity for C. aurantifolia plant showed an antioxidant activity of 19.29%.

Sample Parameters	A	B
Total Polyphenols	Negligible	Negligible
Total flavonoids	Trace amount	Trace amount
Antioxidant Activity(IC50 by DPPH)	37.5 µg/Ml	35.8 µg/Ml

Table 4: Phytochemical analysis for orange peel essential oil samples.

A= Orange oil extracted from the waste obtained from Dakahlia Governorate. B= Orange oil extracted from the waste obtained from Alexandria Governorate.

Antimicrobial activity of orange peel essential oil:

Table (5) displays the antimicrobial activity data for the orange peel essential oil and bacterium standard (ceftriaxone), as determined by the disc diffusion method. Orange peel essential oil demonstrated moderate antibacterial efficacy across tested pathogens, with disc diffusion zones at 100% concentration ranging from 7.0–15.5 mm (mean ± SD), declining significantly at 50% (6.0–11.0 mm; $p < 0.05$), indicative of concentration-dependent membrane disruption primarily mediated by limonene and monoterpenes. Notably, Gram-positive bacteria exhibited heightened susceptibility: *Bacillus cereus* yielded the largest zone (15.50 ± 0.71 mm), statistically comparable to ceftriaxone (12.00 ± 1.41 mm; superscript A/B equivalence), while *Staphylococcus aureus* (11.50 ± 0.71 mm) and *Listeria monocytogenes* (10.00 ± 0.00 mm) were inferior to the antibiotic benchmark (16.00–31.50 mm). Gram-negative strains (*Salmonella enterica, Escherichia coli, Pseudomonas aeruginosa*) registered weaker inhibition (7.5, 8.0 and 7.0 mm vs. 29.5, 21.0 and 31.0 mm ceftriaxone), reflecting lipopolysaccharide barrier resistance, with no activity in the negative control (solvent alone). Antifungal effects were selective: modest against Candida albicans (6.50 ± 0.29 mm, akin to ceftriaxone's 7.00 ± 0.58 mm) but absent for *Penicillium verrucosum* and at 50% dilution.

The findings are consistent with the majority of research looking into how essential oils work against foodborne pathogens and organisms that cause food spoiling. According to these investigations, essential oils are often a little more effective against Gram-positive bacteria than Gram-negative ones [4].

Tested Microorganism		Inhibition Zones (mm) mean ± SD			
	Orange oil concentration		Ceftriaxone (1 mg/ml)	Negative Control	
	100%	50%			
Gram positive(+)	*Bacillus cereus*	15.50 ± 0.71^{A}	11.00 ± 1.41^{B}	12.00 ± 1.41^{B}	ND
	Staphylococcus aureus	11.50 ± 0.71^{B}	9.00 ± 0.00^{C}	16.00 ± 1.41^{A}	ND
	Listeria monocytogenes	10.00 ± 0.00^{B}	8.50 ± 0.71^{B}	31.50 ± 2.12^{A}	ND
Gram Negative(-)	*Salmonella enterica*	7.50 ± 0.71^{B}	6.00 ± 0.00^{C}	29.50 ± 0.71^{A}	ND
	Escherichia coli	8.00 ± 0.00^{B}	6.00 ± 0.00^{C}	31.00 ± 1.41^{A}	ND
	Pseudomonas aeruginosa	7.00 ± 0.00^{B}	6.00 ± 0.00^{B}	21.00 ± 1.41^{A}	ND
Fungi	*Penicillium verrocosum*	ND	ND	10 ± 1.00 ^{C}	ND
	Candida albicans	6.5 ± 0.29^{A}	ND	7 ± 0.58^{C}	ND

Mean ± Std. deviation; sharing a letter within the same column means no significance ND = not detected.

Table 5: Antimicrobial activity of orange peel essential oil (inhibition zones in mm mean ± SD).

Gram-positive bacteria exhibit greater sensitivity to orange peel essential oil primarily due to fundamental differences in cell envelope architecture that govern hydrophobic compound penetration. lacks an outer membrane barrier, allowing direct diffusion of lipophilic monoterpenes like D-limonene (90%) into the lipid bilayer [27].

Furthermore, according to Yu., *et al*. [48], orange essential oil's main constituent was D-limonene. Additionally, they stated that D-limonene has proven effective against foodborne bacterial and fungal infections, including *Listeria monocytogenes*, *Aspergillus niger, Colletotrichum falcatum, and Staphylococcus aureus.*

Minimal inhibitory concentration (MIC) for orange peel essential oil:

The MIC values show the minimum amount of orange oil needed to prevent bacteria from growing visibly [41].

Table (6) reports the minimal inhibitory concentrations (MICs) of orange peel essential oil against representative Gram-positive, Gram-negative, and fungal strains, expressed in mg/mL. Gram-positive bacteria (*Bacillus cereus and Staphylococcus aureus*) showed relatively low MIC values of 0.5 ± 0.00 mg/mL, indicating higher susceptibility, whereas Listeria monocytogenes required a markedly higher concentration (6.0 ± 0.00 mg/mL), suggesting a more tolerant phenotype among the Gram-positive group. In contrast, Gram-negative bacteria (*Salmonella enterica and Escherichia coli*) displayed the highest MICs, 20.0 ± 0.00 and 10.0 ± 0.00 mg/mL respectively, confirming that substantially larger doses of OEO are needed to inhibit their growth; this is consistent with the protective effect of the outer membrane in Gram-negative species described for citrus and other essential oils [29].This was consistent with earlier findings Geraci., *et al*. [18], which can be explained by differences in their cell structure. Gram-negative bacteria's outer peptidoglycan layer is an inefficient permeability barrier since the prions they contain would restrict solute access and lessen their susceptibility to antibacterial agents Interestingly, Pseudomonas aeruginosa exhibited a lower MIC (0.25 ± 0.00 mg/mL) than other Gram-negatives, comparable to the yeast Candida albicans (0.25 ± 0.00 mg/mL). The mold Penicillium verrucosum showed the greatest sensitivity, with the lowest MIC recorded (0.125 ± 0.00 mg/mL), indicating a strong antifungal effect at relatively low oil concentrations. Tao and Zhou. [42] stated that Essential oils alter the shape of fungal hyphae by causing cytoplasmic loss and mycelial distortion. These oils also led to significant changes in extracellular conductivity, release of cellular components, and total lipid content.

Tested organisms		**MIC value (mg/ml)**
Gram positive (+)	*Bacillus cereus*	0.5 ± 0.00 ^{D}
	Staphylococcus aureus	0.5 ± 0.00 ^{D}
	Listeria monocytogenes	6 ± 0.00 ^{C}
Gram Negative (-)	*Salmonella enterica*	20 ± 0.00 ^{A}
	Escherichia coli	10 ± 0.00 ^{B}
	Pseudomonas aeruginosa	0.25 ± 0.00 ^{E}
Fungi	*Penicillium verrocosum*	0.125 ± 0.00 ^{F}
	Candida albicans	0.25 ± 0.00 ^{E}

Table 6: Minimal Inhibitory Concentration (MIC value (mg/ ml)) for orange peel essential oil.

Mean ± Std. deviation; sharing a letter within the same column means no significance.

Effect of orange peel essential oil on some types of cancer cells

According to results in table (7) and illustrated in figure (1) Orange peel essential oil showed clear, dose-dependent cytotoxicity against all three tested cancer cell lines, this is confirmed by the diagram with IC50 values of 8.5 µg/mL (MCF-7), 10 µg/mL (A549) and 16 µg/mL (HepG2), indicating a relatively strong ant proliferative effect, especially on breast and lung cancer cells. IC50 (half-maximal inhibitory concentration) for cancer cells is the concentration of a compound that reduces cell viability or proliferation to 50% of the untreated control, as estimated from a dose–response curve [37]. In cytotoxicity experiments on tumor cell lines, it is therefore used as an index of drug potency, with lower IC50 values indicating stronger anticancer effects [3].

The surviving-fraction curves in the figure demonstrate a steep initial decline in viability between 0 and 6.25–

12.5 µg/mL, followed by a plateau at higher concentrations, consistent with a typical sigmoidal dose–response pattern

measured by the SRB assay. At 25–50 µg/mL, all three cell lines retain only about 15–25% of control survival, confirming that orange oil exerts marked growth inhibition at relatively low micromolar-equivalent doses.

MCF-7 cells are the most sensitive, with the lowest IC50 (8.5 µg/mL), followed by A549 (10 µg/mL) and then HepG2 (16 µg/mL), suggesting some selectivity towards breast and lung cancer models. Saengha., *et al*. [36] indicated that the essential oil extracted from wild orange (*Citrus sinensis*) peel reduced the colonization capacity of the three cancer cells in a dose-dependent manner. At a concentration of 1.0% (v/v) of essential oils, the best results were observed at IC50 values of 0.15%, 0.30%, and 0.18% (v/v) At 48 hours for MCF-7 cells, 72 hours for HepG2, and 72 hours for HeLa, respectively. Amala., *et al*. [2] reported that monoterpene-rich citrus oils (limonene, γ-terpinene and oxygenated monoterpenes) can induce apoptosis and inhibit proliferation in breast and liver cancer models at low concentrations.

Oil conc: µg/mL	Surviving fraction (%)			Inhibition (%)		
	A549	HEPG2	MCF7	A549	HEPG2	MCF7
0.000	100	100	100	ZERO	ZERO	ZERO
6.250	75.6	67.2	69.8	24.4	32.8	30.2
12.500	35	58.9	20.2	65	41.1	79.8
25.000	22.8	24.2	18.2	77.2	75.8	81.8
50.000	16.5	18	13.6	83.5	82	86.4

Table 7: Effect of orange peel essential oil on some types of cancer cells.

A549: Lung Cancer HEPG2: Hepatocellular Carcinoma MCF7: breast cancer.

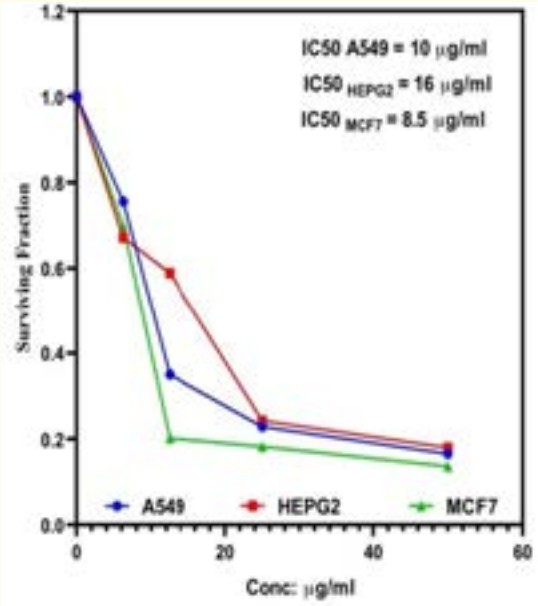

Figure 1: Effect of orange peel essential oil on some types of cancer cells.

Conclusions

In conclusion, citrus processing wastes (peels and wastewater) are of great importance to many industries, particularly the food and pharmaceutical sectors. To maximize their economic value, greater emphasis should be placed on managing the enormous quantities of citrus waste. This research presents a method for utilizing orange peel waste from food processing by extracting essential orange oil in an environmentally friendly manner. it emphasizes the potential of this byproduct as a source of bioactive compounds while investigating its antioxidant, antimicrobial, and anticancer properties.

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