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Chemical composition and active compounds in Bitter orange (*Citrus aurantium*) peels

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ABSTRACT

Background: Citrus fruits are significant to functional food science for two main reasons: the diversity of their chemical and nutritional content and their organic residues, which increase their economic value. The present study was conducted on the aqueous extract of dried bitter orange (*Citrus aurantium*) peels. It aimed to determine the overall chemical composition of the extract, and identify any bioactive compounds that may hold nutritional and industrial value.

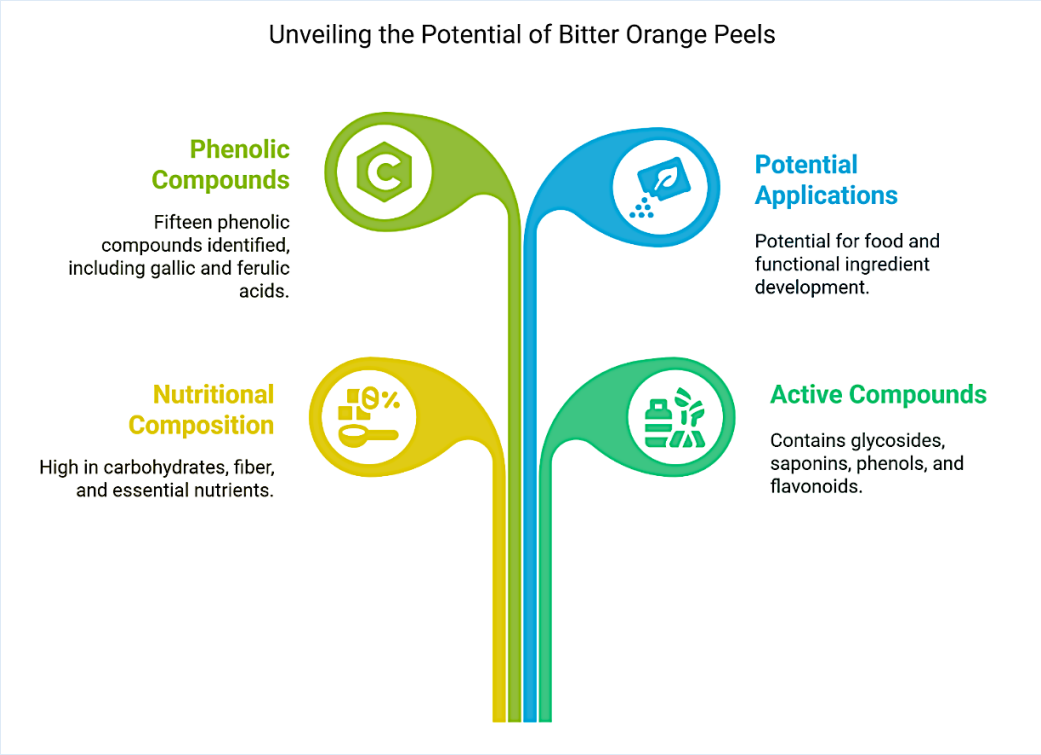
Objective: This study aimed to determine the overall composition of locally grown bitter orange peels and to determine the active ingredients that can be extracted from them both qualitatively and quantitatively.

Results: The results indicated that the percentages of moisture, carbohydrates, protein, fat, fiber, and ash reached 5.34%, 70.25%, 1.95%, 3.50%, 15.38% and 3.58%, respectively. The pH value for the aqueous peels extract was 4.30. Qualitative results indicated the presence of glycosides, saponins, phenols, flavones, coumarins alkaloids in the extract of bitter orange peels. Quantitative results, obtained via HPLC analysis, included the concentrations of active compounds in the prepared extract, which varied according to the type of extract. The aqueous extract of bitter orange peels contained the active compounds gallic acid (0.019) µg/mL, vanillic acid (1.149) µg/mL , caffeic acid (0.077) µg/mL, 4-hydroxybenzoic acid (3.390) µg/mL, coumaric acid (0.033) µg/mL, catechol (0.002) µg/mL, naringenin (0.330) µg/mL, ferulic (0.320) µg/mL, chlorogenic (0.262) µg/mL of flavonoids, and vitamin C (448 µg/mL).

Novelty: This study highlights the underutilized potential of bitter orange (*Citrus aurantium*) peels by demonstrating their rich content of bioactive compounds, including multiple phenolic acids and flavonoids. Through qualitative and chromatographic analysis, the research identifies nutritional and medicinal components, positioning bitter orange peels as a valuable resource for functional food development.

Conclusion: The bitter orange peel extract contained several types of alkaloids, the most important of which are: methaqualone, promethazine, epigallocatechin, tramadol, and berberine. Additionally, bitter orange peel contains both water-soluble and fat-soluble vitamins. For this reason, the vitamin C levels were also very high in the aqueous extract of bitter orange peel.

Keywords: bitter orange, peel extract, chemical compounds, phenolic compounds, vitamins.



Graphical Abstract: Chemical composition and active compounds in Bitter orange (*Citrus aurantium*) peels

INTRODUCTION

Citrus aurantium is taxonomically a member of the citrus family *Rutaceae*, which includes 150 genera and 1500-1600 species. This family belongs to the order *Sapindales*, a subdivision of the phylum *Angiosperm*, and the class *Dicotyledons (Eudicots)*. Locally, *C. aurantium* is called bitter orange or *Naranj* in Iraq [1]. Bitter orange is naturally widely cultivated in subtropical regions, including India, Africa, the Middle East, the Mediterranean basin, and South America. Its most important commercial sources come from Spain, Sicily, and the hot regions of South America.

The plant is described as an evergreen tree with compound leaves that contain clear venation between

the blade and the leaf stalk. The flowers are bisexual, radially symmetrical, and pentamerous. The orange fruit includes a large amount of volatile and fixed oils, in addition to flavonoids [2-5]. The fruit, flowers, and leaves are rich sources of biologically active compounds such as flavonoids, alkaloids, essential oils, coumarins, terpenoids, tannins, vitamins, and minerals [6-8].

Bitter orange peel was first described in a monograph published in the *European Pharmacopoeia*, paragraph 10.1 [9]. It was defined as the dried outer and middle peel of the ripe fruit of *Citrus aurantium*, partially freed from the white spongy tissue of the middle and inner peel.

The documents of the European Food Safety Authority (EFSA) address the safety of ingestion of dried hydroalcoholic extracts obtained from unripe, dried fruit and the dried peels of ripe or unripe citrus fruits, which were used as food supplements, based on real-life case studies [10]. The scientific opinion on the Qualified Presumption of Safety (QPS) approach was utilized for the safety assessment of plants and botanical preparations [11]. The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) Panel and the EFSA Panel on Food Contact Materials, Enzymes, and Flavors have evaluated some individual components of bitter orange extract and several related flavonoids that define flavorings for chemical use in foods [12-14]. These components have demonstrated a wide range of health-promoting properties such as antioxidants, anti-inflammatory, antidiabetic, anti-anxiety, antimicrobial, and anti-cancer effects [15].

The peels of the bitter orange plant are used as a natural stomach and carminative remedy [16]. Bitter orange is also used as an antispasmodic, antiseptic, hypotonic hypnotic, and to promote skin cell health [20-21]. In Chinese medicine, bitter orange fruits are used for weight loss, as they are thought to increase calorie expenditure via heat generation in the muscles without stimulating the central nervous system. It is also thought that bitter orange fruit works to release fatty acids, enhancing an individual's ability to exercise by optimizing energy metabolism. The bioactive compounds behind these curative properties are alkaloids, which can be found at a concentration of 0.8% in bitter orange peels.

Food waste is a global issue, so research regarding methods of reduction is of great importance. One such method is finding uses for secondary waste, like peels, seeds, and pomace, from fruits and vegetables. For example, citrus peels can be dried and converted into a powder rich in fiber, biologically active compounds, technological properties, and numerous health benefits. Citrus waste is an ideal candidate to reduce food waste, as it constitutes 25-30% of food residues during the food manufacturing chain. Further, citrus waste is a rich source of phenolic compounds, as citrus peels contain a higher number of polyphenols than the flesh.

This study aimed to determine the overall composition of locally grown bitter orange peels and to identify the active ingredients that can be extracted from them qualitatively and quantitatively.

MATERIALS AND METHODS

Bitter orange peels: The fruits were cleaned to remove dust and dirt. Then, they were peeled, so only the flesh remained. The peels were taken and cut into small squares, and placed in trays for drying. They were dried in an oven at 40°C until the weight was stable. Afterwards, the dried peel samples were ground and placed into tightly sealed plastic containers, isolated from light and air. The containers were stored in the refrigerator at 4°C.

Preparation of orange peel extract: The aqueous extract of the bitter orange peel sample was prepared according to the method of [22]. 10g of sample and 300ml of distilled water were combined at 70°C and left for 30 minutes on a magnetic mixer. The mixed sample was filtered using a Buechner funnel and vacuum

through filter paper (Whatman No. 1). After filtering, the sample was concentrated in a rotary evaporator at 60 °C until the volume of the extract reached 20ml. Then, the concentrated extract was poured into a Petri dish and placed in an electric oven to dry at 40°C for 24 h. The dried powder was skimmed and collected in clean, dry bottles and stored in the refrigerator until use.

Estimation of gross composition: Chemical analysis of orange peels included the determination of moisture, protein, fat, ash, carbohydrates, and fiber according to the standard methods described in [23].

Detection of active compounds in the extracts: Standard methods were used to determine various phytochemical components of the *C. aurantium* aqueous extract, such as phenols, glycosides, and saponins [24], flavonoids [25], alkaloids [26], and tannins [27-28], (Table 1).

Table 1. Preliminary phytochemical tests for Bitter Orange *(Citrus aurantium)*.

Phytoconstituents	Test	Observation
Flavonoid	2 g extract +5 ml of 20% ammonia solution+ 2 ml H_2SO_4 concentrated	Yellow
Glycosides	5 ml extract +1 ml H2SO4+ 2ml glacial CH3COOH +1 drop FeCl3	Brown ring
Tannins	2ml extract + 2 ml HCl 1% +heat	Red precipitate
Phenol	2 ml extract +2 ml FeCl3 (5%)	Blue
Saponins	0. 5 ml extract + 0.5 ml H2O + heat	Foam
Alkaloids	Mayer reagent	White precipitate
	Wagner reagent	Brown precipitate
	Dragendorff	Orange precipitate
	Picric acid	Yellow precipitate

Determination of active compounds using HPLC: The active and phenolic compounds of the dry (aqueous) bitter orange peel extract were determined using high-performance liquid chromatography (HPLC). The HPLC was performed on a SYKMA machine made in Germany, according to the method [29] in the Food Safety Laboratories of the Environment and Water Department/Ministry of Science and Technology. The dried sample was prepared by adding 3mL of methanol for homogenization, then injecting 100µL using an autoinjector under the following separation conditions:

C18-ODS column, dimensions (25cm × 4.6mm × 5µm). Mobile phase A was used to separate compounds, A = 0.1% acetic acid : (50 ACN : 50 MEOH)

. UV detector with a wavelength of 285nm and a temperature of 25°C, flow rate of 0.1 ml/min.

Determination of vitamins using HPLC: Water-soluble and fat-soluble vitamins were measured according to the method described by [30].

Study of the inhibitory activity of the aqueous extract of bitter orange peels on experimental microorganisms: Mueller-Hinton agar media was poured into petri dishes. Four holes were made in each dish using a cork piercer. Different concentrations of bitter orange peel extract (µg/ml) were added and incubated at 37°C for 24 h to measure the inhibition zones of bacterial growth for each concentration [31].

RESULTS AND DISCUSSION

Approximate analysis: Figure 1 shows the percentages of chemical content of three replicates of dried bitter orange peel waste using the electric dryer method. The moisture, ash, carbohydrate, fat, fiber, and protein

contents were 5.34%, 3.58%, 70.25%, 3.50%, 15.38%, and 1.95%, respectively. It was noted that the results were close to those found in [32-33], at 5.49%, 3.48%, 70.78%, 3.2%, 15.2%, and 1.85%.

However, they differed slightly from what was found in [34], where moisture content was 3.86%, ash content was 5.72%, carbohydrate content was 67.98%, fat content was 1.79%, fiber content was 15.74%, and protein made up the remaining 8.76%. This difference could be due to several reasons, including the difference in the cultivated bitter orange varieties, environmental conditions, climatic factors like soil type, exposure to sunlight, and rainfall, and agricultural factors (organic farming, maturity status, growth area, fertilization, and irrigation). In addition to the differences in the methods of estimating components or the analytical methods that were used, the relative concentrations of the chemical components from the bitter orange peel extract were important in evaluating the quality with regard to color, appearance, nutritional value, and aroma [35-36].

Figure 1 shows the pH value (acidity) results from the aqueous extract of bitter orange peel,4.30, which is consistent with what was found by [34]. The variation in pH values that were shown in the results may be due to the differences in the quality and concentration of active ingredients found in different varieties. Additionally, the presence of tannins in orange peels may be the reason for low pH values [32]. From the table above, we can conclude that citrus peels are characterized by a very high-water content (≥ 75%), which makes this by-product highly perishable and requires immediate use or stabilization to prevent fermentation and mold growth. The dry extract was mainly composed of soluble sugars (6.52–47.81 g/100 g), proteins (1.79–9.06 g/100 g), and minerals (2.52–10.03 g/100 g). The dried citrus extract was characterized by a low-fat content (0.48–4.00 g/100 g), as confirmed by [37].

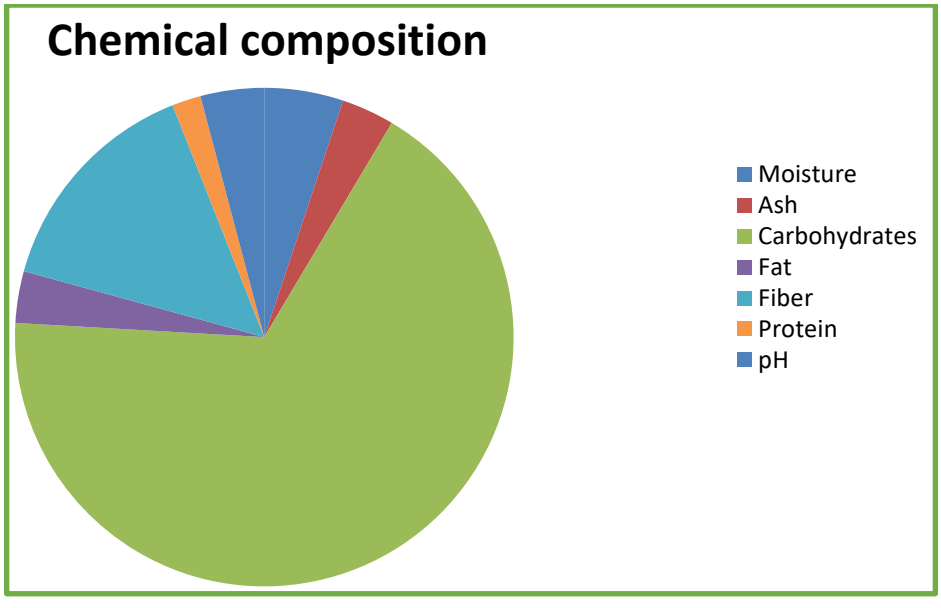


Figure 1. Chemical content of bitter orange peels

Qualitative detection of active ingredients in plant extracts: The preliminary detection of chemical compound and active ingredient content of the aqueous extract of bitter orange peels is shown in Table 1. The results indicate the presence of flavonoids, glycosides, tannins, phenols, saponins, and alkaloids in the bitter orange peel extract.

The results regarding the active ingredient content of the orange peel extract were consistent with those of [15], who demonstrated that citrus peels were rich with nutrients and contain numerous phytochemicals for use in dietary supplements. Our results were consistent with other studies that have examined a range of phytochemicals, including alkaloids, tannins, flavonoids, saponins, phenols, and glycosides, in bitter orange peel extracts, seeds, and leaves [38]. Lu and Yip [39] reported that phytochemical analysis of *Citrus maxima* revealed the presence of important classes of plant components, such as alkaloids and saponins. These compounds act as a natural antibiotic, which helps the body fight infections. This was similar to the results that were obtained from the current study.

The presence of these bioactive compounds may be due to the genetic makeup of the bitter orange plant. Although the concentrations of these compounds may be affected depending on several factors, including the type of soil and the environmental conditions related to plant cultivation, the genetic makeup of bitter oranges could make these plants very significant to functional food science [32].

Table 1. Chemical detection of active compounds in bitter orange peels.

Active compound	Result	color
Alkaloids	+	White precipitate Brown precipitate Orange precipitate Yellow precipitate
Saponins	+	foam
Tannins	+	red precipitate
Glycosides	+	brown ring
Phenolic compounds	+	blue
flavonoids	+	Yellow

(+) means the presence of the compound in the plant, (-) means the absence of the compound in the plant

Identification and quantification of phenolic compounds using HPLC: Table 2 includes the results of the separation and identification of the polyphenols present in the aqueous extract of bitter orange peel using high-performance liquid chromatography (HPLC) technology. The polyphenols identified included gallic acid (0.019 µg/mL), vanillic acid (1.149 µg/mL), caffeic acid (0.077 µg/mL), 4-hydroxybenzoic acid (3.390 µg/mL), coumaric acid (0.033 µg/mL), catechol (0.002 µg/mL), naringenin (0.330 µg/mL), ferulic acid (0.320 µg/mL), and chlorogenic acid (0.262 µg/mL). The polyphenol identification was completed via comparison with their respective phenolic or flavonoid standards. During this comparison, it was noted that the retention time (RT) is close to the RT of the standard compounds used in the analysis. The results were similar to those that confirmed that phenolic acids (caffeic, coumaric, and ferulic) are characteristic of citrus fruits These results are also similar to those found by [40], who found that the peel is the richest part of citrus fruits in flavonoids. The absence of some compounds depends on the type and polarity of the solvents. This is attributed to the polar and nonpolar nature of the compounds present in the bitter orange peel extract, the polarity of the solvent--acetone and methanol were used to extract the bitter orange peel in this study--and the extraction conditions. [41] indicated that one of the important points in the extraction process is the use of solvents and water, due to their ability to separate the desired compounds. Different concentrations of the

mixture of compounds depend on the affinity with the solvent. Limonene is non-polar and does not dissolve in water. It dissolves in non-polar solvents, including hexane, chloroform, and ether. On the other hand, naringin dissolves in polar solvents, such as ethanol and methanol.

All flavonoids in citrus fruits can be classified into the following groups: flavanones, flavones, and flavanols. Citrus flavonoids have health benefits, including anticancer, antiviral, and anti-inflammatory activities. They also reduce capillary fragility and inhibit platelet aggregation in humans. Studies have shown that phenols exhibited a wide range of biological effects, including antimicrobial and antiviral effects, anti-inflammatory, anti-allergic, and anticoagulant effects. Phenolic carboxylic acids, such as caffeic and gallic acid, have beneficial effects on human health by preventing degenerative diseases, such as cardiovascular disease and cancer. Coumaric acid can act as a direct scavenger of reactive oxygen species, preventing lipid peroxidation. It lowers blood cholesterol levels and enhances the resistance of low-density lipoprotein (LDL) to oxidation [38, 42-43].

Table 2. Detection of flavonoids in bitter orange peels

	Flavonoid compounds	Concentration of the compound in the aqueous extract of bitter orange peels µg/mL
1	Gallic acid	0.019
2	Vanillic acid	1.149
3	Caffeic acid	0.077
4	hydroxy-4Benzoic acid	3.390
5	Coumaric	0.033
6	Catechol	0.002
7	Ferulic acid	0.320
8	Naringenin	0.330
9	Chlorogenic acid	0.262

Vitamins in orange peels: Table 3 shows the vitamin content of bitter orange peels: it contains the concentrations of vitamins C, B2, and B1 obtained from HPLC analysis after comparison with the standard compounds of a sample of aqueous orange peel extract. The table indicates a high content of vitamin C in the peels, a characteristic of citrus fruits. Again, it was noted that the RT was close to the retention time of standard compounds used in the analysis. The RT of this compound was close to the standard RT of 4,680 minutes at a concentration of 448 µg/mL. Vitamin C has high nutritional importance, as it is a powerful antioxidant. It helps strengthen the immune system, protect cells from damage caused by free radicals, and enhance iron absorption. Vitamin C also enhances collagen production, which contributes to the health of the skin, blood vessels, and bones [44]. The results were consistent with those found by [45] in their study of aqueous extracts of citrus peels, with concentrations ranging from 400-500 µg/mL. The peels are also rich in vitamin A, as long as they are fresh and naturally dried. The RT was observed to be similar to that of the standard compounds used in the analysis, vitamin B2, or riboflavin. The RT of this compound was observed to be similar to the standard RT of 5.257 minutes at a concentration of 0.697 µg/mL. Riboflavin aids in energy production and is sensitive to light. These results were similar to those found by Shahidi et al. (2020) in their study on agricultural waste as a source of bioactive compounds in the food industry and fermentation, with

riboflavin concentrations ranging from 0.3 to 0.7 µg/mL in bitter orange extract. The RT was also similar to that of the standard compounds used for vitamin B1 (thiamine), 7.010 minutes at a concentration of 0.205 µg/mL. Thiamine is essential for nerve function and carbohydrate metabolism, as it converts carbohydrates into energy. It also helps maintain healthy skin and eyes, and is an antioxidant as it reactivates the antioxidant glutathione and contributes to enzymatic reactions and cell growth. Like riboflavin, it is sensitive to light. The concentration results for thiamine were similar to those found [46] in their study on agricultural waste, which included concentrations ranging from 0.1 to 0.3 µg/mL.

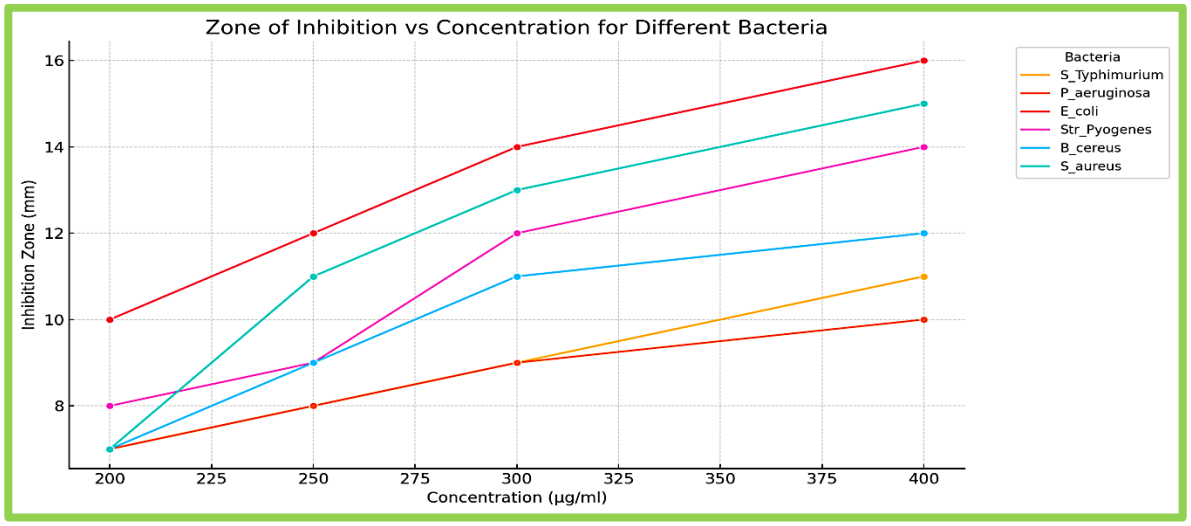
Table 3. Identification of vitamins in the aqueous extract of bitter orange peels using high-performance chromatography (HPLC).

	Vitamin compounds	Vitamin concentration in the aqueous extract of bitter orange peels µg/mL
1	C	448
2	B2	0.697
3	B1	0.205

Inhibitory activity of bitter orange peel extract against some bacterial genera: The antibacterial activity of bitter orange peel extract was tested by measuring the inhibition zone against bacterial isolates from food sources, including Gram-negative bacteria (*Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Escherichia coli*), as well as Gram-positive bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes*, and *Bacillus cereus*). The sizes of the inhibition zones are shown in Figure 2. The inhibitory activity of the aqueous extract of bitter orange peels against some Gram-positive bacteria (*Staphylococcus aureus, Streptococcus pyogenes, Bacillus cereus*) and Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium*) was investigated by measuring the diameter of the inhibition halo. The results showed significant differences at the probability level ($P \leq 0.05$) in the inhibitory activity of the aqueous extract of bitter orange peels against bacterial growth. The aqueous extract of bitter orange peels at concentrations of 200, 250, 300, and 400 µg/mL showed effective antibacterial activity against the positive bacteria, *Staphylococcus aureus*, with an inhibition halo diameter of 7, 11, 13, and 15 mm, respectively, *Bacillus cereus* at concentrations of 200, 250, 300, and 400 µg/mL with inhibition corona diameters of 7, 9, 11, and 12 mm, and *Streptococcus pyogenes* at concentrations of 200, 250, 300, and 400 µg/mL with an inhibition corona of 8, 9, 12, and 14 mm. The aqueous extract of bitter orange peels at concentrations of 200, 250, 300, and 400 µg/mL also showed effective antibacterial activity against Gram-negative bacteria based on extract concentration. *E. coli* displayed an inhibition corona diameter of 10, 12, 14, and 16mm, respectively; *Pseudomonas aeruginosa* had inhibition corona diameters of 7, 8, 9, and 10mm; and *Salmonella typhimurium* had inhibition corona diameters of 7, 8, 9, and 11mm. The highest inhibitory corona diameter for Gram-positive bacteria was 15mm against *Staph. aureus* at a concentration of 400 mg/ml, followed by *Str. pyogenes* at 14mm and B. cereus at 12mm. The highest corona diameter of inhibition for negative bacteria was 16mm against *E. coli* at a concentration of 400 µg/mL, followed by *S. Typhimurium* at 11mm, and then *P. aeruginosa at* 10mm. The least effective inhibition for *P. aeruginosa* and *B. cereus* at a concentration of 200 µg/mL both gave a corona diameter of 7mm. This means that the aqueous extract of bitter orange peels decreases in effectiveness in tandem with concentration. The results corroborated what was found by researchers Alamholo and Shojaemehr (2020). The extract of bitter orange peels is affected by different solvents, including ethanol,

methanol, and hot water, towards the microorganisms *B. subtilis, B. cereus, Staph. aureus, S. typhi, P. aeruginosa*, and *E. coli*. The results showed that the aqueous extract was effective, but to a lesser extent compared to the alcoholic extracts, because the concentrations used were 100 and 200 µg/mL. The diameter of the inhibition halo obtained for the microorganisms *B. subtilis* (13mm), *B. cereus* (12mm), *S. aureus* (12mm), *S. typhi* (13mm), *P. aeruginosa* (6mm), *E. coli* (9mm), show that the aqueous extract has a greater effect on gram-positive bacteria than gram-negative bacteria. This is because the cellular structure of microorganisms plays a role in their susceptibility to the antibiotic properties of the extract. The antimicrobial activity of the aqueous extract of bitter orange peels is attributed to its content of phenolic compounds and flavonoids, which inhibit the synthesis of nucleic acids and vital enzymes, disrupt the cell wall, and incite apoptosis. Bacteria are among the common causes of many human diseases, and the tested bacteria are one of the contaminants that cause food spoilage [46].

Figure 1. The diameter of the inhibitory activity of bitter orange extract at different concentrations towards some bacterial genera.



Scientific innovation and practical implications: This study highlights the functional potential of bitter orange (*Citrus aurantium*) peels by identifying significant levels of bioactive compounds, including phenolic acids and flavonoids. These findings promoted the value of agricultural by-products, supporting sustainable food system initiatives. The identified bioactive compounds, such as gallic acid, ferulic acid, catechin, and naringin, suggest that bitter orange peel extract is a potent antioxidant with strong therapeutic potential. Practical applications include the development of functional foods, nutraceuticals, natural preservatives, and supplements aimed at reducing oxidative stress and promoting health. This research contributes to food innovation and waste reduction strategies by transforming waste into valuable functional ingredients. Future studies may be focused on clinical validation and industrial-scale extraction optimization.

CONCLUSION

Bitter orange peels are a by-product that is often neglected, albeit a source for many bioactive compounds. The current study showed that the extract of bitter orange peels obtained after drying and grinding them is rich in nutritional components and some active compounds with various applications. The results of qualitative and quantitative tests showed that bitter orange peels contained numerous types of flavonoids, alkaloids, tannins, saponins, and a wide range of

vitamins, which are considered highly effective antioxidants. These results indicated the possibility of exploiting the orange peels as a source of these compounds for inclusion in some food applications.

Abbreviations: mm: millimeter, gm: gram, mL: milliliter, °C: degrees Celsius, µm: micrometer, RT: retention time.

Authors' Contribution: Sara Thamer Hadi: Formal analysis, methodology, project administration, funding acquisition, validation, and writing the original draft. Ashraq Monir Mohamed: Data duration and formal analysis. Abdel Moneim elhadi sulieman: Methodology.

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