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RESEARCH ARTICLE

A Systematic Insight into the Pharmacognostical, Phytochemical, Chemo-fingerprinting and Pharmacological Assessment of *Citrus maxima*

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Abstract

Background: Fruits of the citrus family are known for their antioxidant and antimicrobial activity. **Aim:** The study aims to perform pharmacogenetic and physicochemical analysis of the peel along with the determination of the physical standards, phytochemical analysis, phytochemical identification, antioxidant, and antimicrobial properties of Pomelo peel oil. **Methods:** Pharmacognostical and physicochemical parameters of the pomelo peel were analysed using the standard procedure recommended by WHO. The essential oil was extracted from the pomelo peel using Clevenger's apparatus, and physical properties, total phenolic content, total flavonoid content, and chemical analysis with GC-MS were determined for the *Citrus maxima* oil, utilizing accepted procedures. Using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, the antioxidant activity of the *C. maxima* oil was also assessed. Antibacterial assay of the Pomelo oil was evaluated against *Escherichia coli, Staphylococcus aureus,* and *Streptococcus agalactiae* using the Agar well diffusion technique and by determining the MIC (Minimum Inhibitory Concentration). **Results:** Pharmacognostical standards for the peel and physical standards for the oil were developed, and limonene was observed as the major constituent in the oil, and Total phenol content was 62.42 ± 4.03 mg of equivalent gallic acid/g of *C. maxima* oil. The total amount of flavonoids in the pomelo oil is 16.48 ± 0.92. Oil of *C. maxima* showed potential antioxidant and antimicrobial activity. **Conclusion:** The study has successfully established pharmacognostical standards and physical properties for Pomelo peel oil, identifying limonene as a major constituent. The oil demonstrated significant antioxidant and antimicrobial activity, highlighting its potential for therapeutic applications.

Major Findings: Pomelo peel essential oil was found to contain limonene as the major component and demonstrated significant antioxidant and antimicrobial activity against *E. coli*, *S. aureus*, and *S. agalactiae*. In silico docking supported its potential antibacterial efficacy, highlighting its therapeutic value.

Keywords: Antioxidant, Antimicrobial, Pharmacognostical, Physicochemical Parameters

1. Introduction

In today's era, there is a high demand for Herbal medicines, and their popularity is on an increasing trend. Due to easily accessible raw materials, the World Health Organisation (WHO) recommends traditional herbs as effective remedies in the healthcare industry. Plants are extremely intricate. The effectiveness of the plant medicine depends on its species, location, and harvesting techniques. Standardisation of herbal drugs is crucial to detect improper herb authentication, microbial adulterations, pesticide residues, and other parameters that may affect the effectiveness of the crude drugs. According to the WHO (World Health Organisation), identifying the identity and degree of purity of such materials requires describing

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a medicinal plant's macroscopic and microscopic characteristics.[1,2].

Citrus maxima belongs to the family of Rutaceae, which has approximately 160 genera. Citrus fruit is cultivated in more than 150 countries; 53 countries grow it commercially. As per the estimation, the annual global production of citrus fruits stands at 85 million tons. After China and Brazil, India is the largest producing country. India produces about 7–8 % of the citrus crop worldwide. Citrus plants are India's third most cultivated crop, behind banana and mango, with 10.86 lakh hectares under cultivation, and 14.8 million tons were produced in 2022, with an annual growth rate of 5.37 %. Citrus fruits are known for their unique flavour and fragrance. They are also a great source of dietary fibre, phytochemicals, and vitamin C, all of which are vital for human health because of their antioxidant qualities and ability to fend off a variety of chronic illnesses. Antioxidants such as ascorbic acid, phenolic compounds, and flavonoids are prevalent in citrus fruits and juices[3–5].

Taxonomy of Pomelo fruit (*Citrus maxima*)[2]
Biological name: *C. maxima* (J. Burm.) Merr.
Taxonomical Classification
Kingdom: *Plantae*
Phylum: *Tracheophyta*
Division: *Magnoliophyta*
Class: *Magnoliopsida*
Order: *Sapindales*
Family: *Rutaceae*
Subfamily: *Aurantioideae*
Genus: *Citrus*
Species: *maxima*

A tree that is 16–50 feet (5–15 meters) tall with a 4–12 inch crooked trunk. In Northeastern states such as Assam and Tripura, it is grown at an altitude of 1,500 meters. *Citrus maxima* is a perennial shrub that grows all over India and is also referred to as Papuans. Leaves: Large, 10.5–20 cm (4–8 in) long, elliptical to oblong-shaped, evergreen leaves. Often marginalized, pubescent below the acute tip, complete margin, uneven base, distinct smell[6–8].

Petioles: Broad and winged; Flowers: White, Large; Stamens: 17-25.

Fruit: Generous, pyriform or globose, pale yellow, with a thick rind and a yellow-to-crimson pulp. Figure 1 below shows the Unripe *C. maxima* fruit on the tree, and Figure 2 represents the fresh ripe fruits of *C. maxima.*

Previous studies on *C. maxima* oil demonstrate the antibacterial activity against a broad spectrum of and numerous pathogenic bacteria and were majorly active against Bacillus licheniformis bacteria[9]. Studies also explain the antioxidant activity with some positive and some negative results on the antimicrobial effect of the extract from *Citrus maxima* peel, and the phytochemical analysis showed the presence of alkaloids, terpenoids, and flavonoids. There is no clear study of the *C. maxima* peel oil as an antioxidant and antibacterial activity on the given strains.

The study aims to perform pharmacognosy, phytochemistry, and physicochemical analysis of the peels of *Citrus maxima*. Study is further continued with the extraction of oil, chemical analysis through GC-MS, total phenolic and total flavonoid content, antioxidant activity, and anti-microbial activity of the extracted oil. According to the folklore claim, *C. maxima* plants are highly medicinal, and the peels were traditionally used for their anti-microbial, brain tonic, sedative, and anti-asthmatic activity, but there is a lack of scientific evidence for the same[2]. The plant is also reportedly a source of vitamin C, and the pulp that remains after the juice is extracted is said to be used to treat wrinkles and pimples as well as to soften facial skin. The oil from the plant is also used in a variety of preparations to nourish the skin and reduce skin itching. Thus, this study is

Figure 1. Unripe *C. maxima* fruit on the tree.

Figure 2. Fresh ripe fruits of *C. maxima.*

done for the screening of *C. maxima* peel oil for its antioxidant potential and antimicrobial efficacy against a panel of microbes linked to skin diseases.

2. Materials and Methods

2.1 Collection and Identification of the Plant Material

Pomelo fruits were obtained from *Citrus maxima* trees from Jharkhand, India, and authenticated by Scientist E of Central National Herbarium, Botanical Survey of India, Howrah, Mr. K. Karthigeyan, with an authentication number of CNH/Tech.II/2023/21. We thoroughly rinsed the ripe fruits of pomelo under running water. Fruit peels were divided and then allowed to dry in the shade. The dehydrated peels were then coarsely powdered and kept at room temperature in an airtight container.

2.2 Morphological Analysis

Pomelo fruit peels' macroscopic characteristics, including colour, odour, taste, shape, and size, were investigated and reported using standard methods given by Youngken 1948[10] and Ferguson 1956[11].

2.3 Microscopical Analysis

Transverse sections of pomelo peel were stained with Safranin 0.1 % (w/v) solution. The required quantity of safranin powder was dissolved in 0.025 M borax solution to create a safranin: 0.1 % (w/v) solution[12].

2.4 Powder Microscopical Analysis

Powder of the peels of *Citrus maxima* was performed using Wallis 1965[12,13].

2.5 Quantitative Microscopy-Linear Measurements

The fibre, stone cell, and starch grain lengths and widths were measured using the methodology described by Divakar (2002)[14] and Kokate (2016)[15].

2.6 Physicochemical Analysis

Physical and chemical characteristics like total ash, acid insoluble ash, and water-soluble ash value, foaming index, loss on drying, and swelling index were assessed by the Indian Pharmacopoeia specifications. The official methods were followed when calculating the percentage of ash value, a measure of the crude drug's purity, and the presence of polar and non-polar compounds was identified using the extractive values. By using the WHO-recommended cold maceration method, the extractive values of substances that are soluble in water and alcohol were estimated. Loss on drying represents the presence of moisture, and the swelling index assesses the amount of mucilage present in the sample. Foaming index was performed to assess the presence of saponin in the sample. All the parameters were performed as per the Indian Pharmacopoeia 1996, WHO 1998, 2011, and Kokate 2017[16–19].

2.7 Extraction of Essential Oil[20]

Dried pomelo peel powder of 200 g was transferred for extraction in a round-bottom flask, and essential oil was extracted using a Clevenger-type apparatus for 3 hrs. The obtained oils were separated and stored in a dark glass vial, sealed, and kept at 4°C.

2.8 Physical Properties of Pomelo Oil

The physical properties like colour, odour, pH, density, solubility, viscosity, specific gravity, and refractive index of *C. maxima* oil obtained from the peels were estimated. Specific gravity and Density of the peel's oil were determined with the equations below.

Density of oil = (Weight of sample /Volume of sample) X 100

Specific gravity of oil (Weight of oil extract/ weight of water) X 100

2.9 Determination of Heavy Metal Contamination

Even in trace amounts, heavy metals can have toxic effects that lead to intoxication and pose a health risk. In addition to their therapeutic benefits, medicinal plants can be toxic due to the presence of heavy metals and other impurities. This is something to keep in mind when utilising them to treat different diseases. This is done to keep contaminants out of the raw materials used to make medicinal plants. Therefore, a heavy metal estimation was done[21].

The powdered medication was tested using a limit test for heavy metals and arsenic to ascertain the toxicity of heavy metals by the Indian Pharmacopoeia 1996 standard procedure.[17].

2.10 GC-MS Sample Preparation

The sample for GC–MS was prepared by dissolving 5 ml of extracted oil in methanol. The solution was

centrifuged at 3000 rpm for 15 min, and an aliquot of 1 µl was injected for the–MS experiment.

2.10.1 GC-MS Analysis[20,22]

The content of the volatile oil is expressed as a percentage v/w. GC-MS was carried out using a Shimadzu gas chromatograph with a SE-30 10 % Chromosorb-W packed stainless steel column (2 m x 2 mm).

Oven programme: 60°C (5 min), 60°-260°C (5°C/min), 260°C (10 min); carrier gas – nitrogen, flow rate 40 ml/min; injector temperature 240°C; detector temperature 240°C. Individual components were identified by a database of mass spectra matching with literature available in libraries like NIST and WILEY by comparison of their mass spectrum values. The LRI of a compound is an expression of its retention time on a gas chromatographic column relative to a homologous series of n-alkanes. The following equation is used to calculate the Linear Retention Index (LRI) from the retention time.

$$LRI = 100\left(\frac{t - tn}{tn - 1 - tn} + n\right)$$

t = retention time of component
n = carbon number of preceding n-alkane
n+1 = carbon number of the subsequent n-alkane

2.11 Total Phenol Content[23]

The overall phenolic content of the Pomelo oil was calculated using gallic acid and a Folin-Ciocalteu reagent (Sigma-Aldrich Chemie) as a reference compound. 46 ml of distilled water was added to 0.1 ml of the solution containing the pomelo oil. The mixture was then shaken vigorously before 1 ml of Folin-Ciocalteu reagent was added. Three minutes later, 3 ml of sodium carbonate solution (2 % Na2co3 solution) was added, and the mixture was gently shaken for two hours. The solution's absorbance was estimated at 760 nm. Gallic acid was treated as the standard in the same procedures, and a calibration curve was plotted. The total phenolic content was represented as mg of gallic acid equivalent per gram of pomelo oil.

2.12 Total Flavonoids Content[24]

The flavonoid content was determined using the colourimetric technique with aluminium chloride. The calibration curve was obtained using Rutin. Several concentrations of pomelo oil were made for this test. 500 µl of diluted pomelo oil and 500 µl of a 2 % methanolic aluminium chloride solution were mixed. A Schrodinger (UV-Vis spectrophotometer) was used to analyse the absorbance of the reaction mixture at 430 nm after each formulated mixture had been incubated at room temperature for 15 minutes. The calibration curve for rutin (in the range of 5 to 60 mg/ml) was obtained using the same method. The amounts of flavonoids were calculated and were represented as milligrams of rutin equivalent/gram of Pomelo oil.

2.13 Determination of DPPH (2,2-Diphenyl-1-Picrylhydrazyl) Radical Scavenging Activity[25]

Pomelo Oil's antioxidant activity was measured using Sigma-Aldrich (DPPH radical scavenging assay). The process is adding 5 µl of DPPH solution (0.004 % in methanol) to 50 µl of oil in aliquots of different concentrations. After giving the mixture a good shake, it was allowed to sit for half an hour at room temperature in the dark. The absorbance of the solution was then calculated at 517 nm. DPPH solution was used as a negative control, and Ascorbic acid as a positive control. Less absorbance of the reaction mixture implied greater free radical scavenging activity. Using the following equation, DPPH radical scavenging activity (%) was calculated.

Percentage radical scavenging activity = [(Acontrol – (A sample – A blank))/ Acontrol] X 100, where Acontrol: Absorbance of control (DPPH) solution, A sample: Absorbance of sample (Pomelo oil) solution, A blank: Absorbance of blank solution.

2.14 Antibacterial assays[26]

Antibacterial activity of Pomelo oil was analysed against the strains of Escherichia coli, Staphylococcus aureus, and Streptococcus agalactiae. Agar nutrient medium was used to maintain the bacterial cultures, and gentamicin was used as a standard for the study.

2.14.1 Agar Well Diffusion Method[27]

The standard well diffusion method, as advised by the Clinical & Laboratory Standards Institute (CLSI), was used to test the efficacy of *C. maxima* oil against bacteria. First, the purity was examined after the bacterial species

were grown on nutrient agar medium for an entire night at 37 °C. After dissolving 14 g of nutrient agar in 500 ml of distilled water, the medium was autoclaved for 15 minutes at 121 °C. After adding 4 ml of the medium to each of three sterile petri plates, they were promptly sealed. Using a standard density of 0.5 McFarland (1108 cells per millilitre; Bio-Mérieux, Marcy I'Etoile, France), bacterial suspensions were prepared using 0.9 % sodium chloride solution. A sterile swab was used to apply these bacterial suspensions onto the agar plates. Wells were created on the agar plates using inoculating loops. A mixture of 360 microliters of Citrus oil and 640 microliters of DMSO was prepared and added to the wells using a pipette, with 30 microliters added to each well. Gentamycin loaded at a concentration of 5µl on the filter paper disc was used as a standard. The inhibition zones on the agar plates were then measured in millimetres after being incubated for 24 hours at 37°C. Each experiment was conducted three times for accuracy.

2.14.2 Minimum Inhibitory Concentration

The MIC is the lowest drug concentration, measured in mg/µL, that inhibits microbial growth. It was carried out on microliter plates with 96 wells. The technique of broth dilution was employed to establish the lowest inhibitory concentration. 500 ml of distilled water was combined with 14 g of nutrient broth to make broth media, which was then autoclaved at 121 °C for 15 minutes. Each well of the 96-well titration plate received a 100 µL of broth. In wells 2 to 12, 50µL of bacterial solutions were added to the plate. The first column wells of the plate were then filled with 50µL of the extracted oil. Next, a dilution of two-fold was created. The first well is filled with broth media, while the last well is partially filled with bacteria and broth. Pomelo oil mixture, both, along with strains of bacteria were added to all the other wells. Additionally, the serial dilution was carried out before adding the bacteria. At 37 °C, plates were incubated for 24 hours. The same above procedure for the extract was repeated with gentamycin, and readings were taken after a 24-hour interval; the second reading was taken after the first one.(28)

3. Results and Discussion

3.1 Morphological Analysis

The macroscopic characteristics of dried and fresh pomelo peel differ. Fresh peel has a bright green to yellow outer surface, which changes to brownish yellow when dried. Fresh peel's inner surface is white; after drying, it turns whitish brown. Fresh peels are highly aromatic, whereas dried peels are much less aromatic. The flavour is bitter and sour. Fruits have an ovoid or globular shape. Peels after drying can be shaped into a triangle or spiral. These thin strips have approximate measurements of 0.1 to 0.2 cm thick, 2 to 2.5 cm wide, and 1 to 2 cm long. They are on the outside and are covered in numerous tiny pits that are oil glands. The Macroscopic characters of *Citrus maxima* fruit Rind are depicted in Figure 3.

The dried peels were up to 0.5 cm thick, and the colour of the outer surface was yellow to yellowish brown, and the inner surface was creamish to yellowish white. Surface characteristics of the inner surface are creamish colour to yellowish white, and the outer

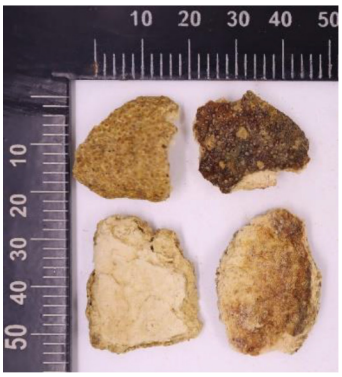

Figure 3. Macroscopic Image of *Citrus maxima*- fruit rind.

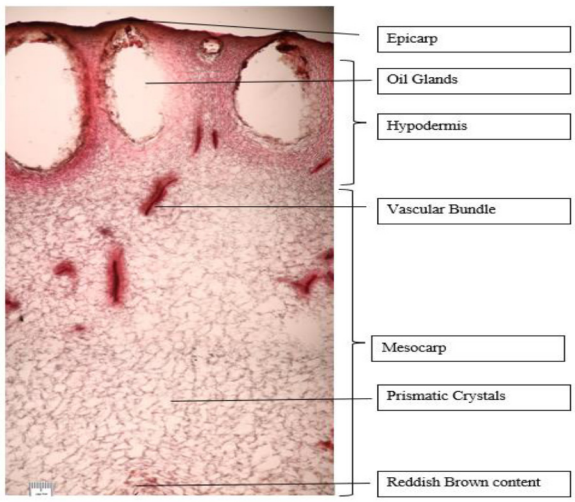

Figure 4. T.S. of the *C. maxima* peel showing characteristic features.

surface has a rough texture, and numerous elevations are observed due to oil glands on the surface. Peels had an aromatic odour and a bitter and astringent taste.

3.2 Microscopical Analysis

Citrus maxima Peel's T.S. reveals an epidermis layer, which is followed by two to three rows of Tiny cells of parenchyma in the hypodermal layer. There is a layer of large, porous mesocarp cells beneath the hypodermis. Large oil glands with an oval shape are embedded in the mesocarp cells and beneath the hypodermis. Fibres of varying lengths are also visible in T.S. (Figure 4).

The Pericarp comprises epicarp and hypodermis, Epicarp contains a layer made of polygonal cells, filled with reddish/yellowish brown contents and thick cuticles. Hypodermis layers of parenchymatous cells are embedded with oil glands. The mesocarp has layers of spongy parenchymatous cells consisting of vascular strands scattered all over the mesocarp. Prismatic crystals of calcium oxalate are found all over the mesocarp.

3.3 Powder Microscopical Analysis

The results of the powder microscopical analysis were performed, and the observations are given in Figure 5.

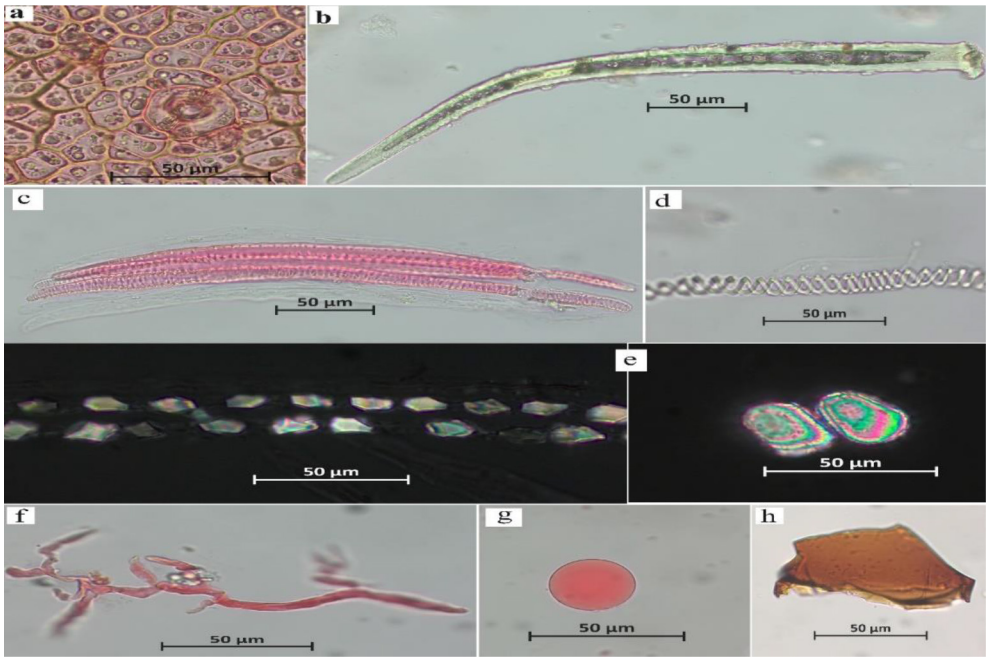

Figure 5. Powder microscopy of *Citrus maxima* fruit rind.

Table 1. Linear measurements of the fibres

Linear measurements of the fibres						
Parameters	**Length (µm)**			**Width (µm)**		
	Minimum	**Average**	**Maximum**	**Minimum**	**Average**	**Maximum**
Fibers	270	505.5	750	15	12	7.5

Table 2. Linear measurements of the calcium oxalate crystals

Linear measurements of the calcium oxalate crystals.						
Parameters	**Length (µm)**			**Width (µm)**		
	Minimum	**Average**	**Maximum**	**Minimum**	**Average**	**Maximum**
Calcium oxalate crystals	13.75	24	55	13.75	19.8	27.5

a. Layer of epicarp cells in surface view with paracytic stomata, b. Unicellular trichomes, c. Tracheids stained by safranin reagent, d. Spiral vessels, e. Fibre crystal made up of prismatic crystals of calcium oxalate viewed under polariser, f. Fragments of oil gland cells, g. Oil globules stained with Sudan red stain and h. Brownish orange content.

Powder characteristics of *Citrus maxima* include a layer of epicarp cells in surface view with paracytic stomata, Unicellular trichomes are rarely observed, pitted tracheids with borders were observed, and spiral vessels. Fragments of oil gland cells from the hypodermis were present. Crystal fibres were made up of prismatic crystals of calcium oxalate. Oil globules were present and stained red with Sudan Red III. Brownish-orange content was observed throughout.

3.4 Quantitative Microscopy-linear Measurements

The diameter of the starch grains, the length and width of the fibres, and the stone cell composition of the powdered peels of *Citrus maxima* fruits were all measured linearly. Tables 1,2, and 3 present the findings. It guarantees this plant's quality.

3.5 Physicochemical Analysis

The physicochemical parameters are primarily used to evaluate the drug's quality and purity. A drug's ash values provide information about its inorganic or earthy composition. More value is found in water-soluble ash than in acid-insoluble ash. Extractive values help to identify expired or tampered drugs as well as provide information about the chemical components of the drug. The findings imply that the powdered drug has a high extractive value in water. The swelling index is high, representing the presence of high mucilage, and there is no mucilage, which represents the absence of saponin, and loss on drying is also higher, which represents the presence of high moisture content in the fresh peels. The total ash was observed to be 3.3 %, acid-insoluble ash was about 0.98 %, and water-soluble ash was about 1.8%. The alcohol soluble and water-soluble extractive values are about 8.4 % & 29.4 %, respectively. Loss on drying of the fresh peel was 21.01 %, and no foaming Index was found, and the swelling index was high, about 10.1.

Table 3. Linear measurements of the starch grains

Linear measurements of the starch grains.			
Parameters	**Diameter (µm)**		
	Minimum	**Average**	**Maximum**
Starch Grains	25	34.38	50

3.6 Essential Oil Extraction

The oil extracted from the dried peel of pomelo fruits is pale yellow, highly aromatic, and the yield of the oil was calculated as 3 %.

3.7 Physical Properties of Pomelo Oil

Oil extracted by Clevenger's method of extraction produced pale yellow oil and was highly aromatic, with a pH of 5.7. The density of a substance about water is measured by its specific gravity. Pomelo oil's specific gravity was 0.8511, and the density of the pomelo oil was 0.8509 g/ml. Due to its lower density than water, the oil was completely insoluble in water, and it floated on top of the water layer. The viscosity of the oil was determined using an Ostwald viscometer to find the internal resistance of the fluid to motion. The viscosity of the pomelo oil was 0.86442 centipoises. Refractive index of the oil was measured using an Abbe refractometer, and it was found to be 1.36 at 300°C.

3.8 Heavy Metal Analysis

The experimental results showed that all the heavy metals were below the limit of quantification at a specific wavelength. Lead (Pb) was observed at the wavelength of 220.353, and the amount of lead was below the limit of quantification. Cadmium (Cd) was observed at the wavelength of 226.502, and the amount of lead was below the limit of quantification. Mercury (Hg) was observed at the wavelength of 184.887, and the amount of lead was below the limit of quantification. Arsenic (As) was observed in the wavelength of 193.696 and the amount of lead was below the limit of quantification. Limit of quantification: Pb (1.0mg/kg), Cd (0.1mg/Kg), Hg (0.5mg/kg), As (2.0mg/kg)

3.9 Characterisation of Pomelo Oil

Essential oil with a 3 % yield and a pale yellow colour was produced by distilling dried pomelo peel. The essential oil was chemically analysed using GC-MS, and the results revealed that it was made up of a

complex mixture of various ingredients. The primary components were, in order, DL-limonene (69.98 %), Beta-Myrcene (6.23 %), 2H-1-Benzopyran-2-one, 7-[(3,7-dimethyl-2,6-octadienyl) oxy] (2.78 %), Alpha-Pinene (2.04 %), and Silane, dimethyl (1.88 %), Bicyclo [3.1.0] hexane, 4-methylene-1-(1-methylethyl) (1.84 %), Decanal (1.54 %), Caryophyllene (1.53 %), Octanal (1.37 %), 7-Oxabicyclo [4.1.0] heptane, 1-methyl-4-(1-methylethenyl)(0.85 %), Linalool(0.56 %) (Table 4).

3.10 Total Phenol Content

Phenolic compounds play significant roles in plant defence against pathogens and herbivore predators. They are successful in controlling human pathogenic infections. The main redox properties of phenolic compounds, which enable them to function as reducing agents, hydrogen donors, etc., are responsible for their antioxidant properties. The total phenol content of *C. maxima* oil was 62.42± 4.03 mg Gallic acid equivalent/g of *C. maxima* oil. The concentration and the absorbance of the control and various concentrations of the standard have been represented in Table 5 below.

3.11 Total Flavonoid Content

Flavonoids show interference in the production of reactive oxygen species and the quenching of free radicals. Tangeretin (4',5,6,7,8-Pentamethoxyflavone) is present in the oil of pomelo peel, which shows its capacity as an antioxidant. These antioxidant properties are dependent on the presence of easily oxidizable hydroxyl groups and how much polymerisation they have undergone. The total flavonoid content of the pomelo oil is 16.48± 0.92 mg/g rutin equivalent. The concentration and the absorbance of the control and various concentrations of the standard have been represented in Table 6 below.

3.11.1 2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

With ascorbic acid as standard, the antioxidant activity of pomelo oil was ascertained. Ascorbic acid, used

Table 4. Chemical analysis of the major components of *C. maxima* peel oil

S. No.	Compound	Retention time(min)	% content
1	DL-limonene	6.306	69.98
2	Beta-Myrcene	5.434	6.23
3	2H-1-Benzopyran-2-one, 7-[(3,7-dimethyl-2,6-octadienyl) oxy]	25.282	2.78
4	Alpha-Pinene	4.544	2.04
5	Silane, dimethyl	1.165	1.88
6	Bicyclo [3.1.0] hexane, 4-methylene-1-(1-methylethyl)	5.152	1.84
7	Decanal	8.665	1.54
8	Caryophyllene	11.606	1.53
9	Octanal	5.678	1.37
10	7-Oxabicyclo [4.1.0] heptane, 1-methyl-4-(1-methylethenyl)	7.614	0.85
11	Linalool	7.116	0.56

Table 5. Total phenol content

Concentration	Absorbance
20	0.06±0.01
40	0.18±0.02
60	0.25 ±0.01
80	0.50±0.02
100	0.49± 0.01
Pomelo oil	0.2944 ± 0.01

Table 6. Total flavonoid content

Concentration	Absorbance
10	0.05±0.02
20	0.09±0.01
30	0.13 ±0.02
40	0.18±0.01
60	0.02± 0.01
Pomelo oil	0.0816 ± 0.01

as a positive control, demonstrated 10.18 % activity, whereas pomelo oil demonstrated 62.64 % inhibition of DPPH, indicating a significant difference ($p<0.05$). The presence of limonene in high quantities helps in showing the potential antioxidant activity. The percentage inhibition of the standard at different concentrations and the IC50 of pomelo oil are represented in Table 7 below.

3.12 Antibacterial Properties of Pomelo Oil

3.12.1 Agar Well Diffusion Method

By measuring the zone of inhibition, the in vitro antibacterial properties of *C. maxima* peel oil counter to bacteria like *S. aureus, S. agalactia* and *E. coli* were evaluated. Data from the well diffusion method showed that Pomelo oils significantly inhibited the growth of the various bacterial strains that were put to the test. The zone of inhibition for Pomelo oil's antibacterial activity against E. coli was 15.24 ± 1.94 mm, making it the most effective. S. agalactiae and S. aureus each had a zone of inhibition measuring 11.33 ± 1.86 mm and 12.56 ± 2.03 mm, respectively. All bacterial strains' growth was significantly ($p< 0.05$) constrained by the application of Pomelo oil. According to the zone of inhibition value, the order of the bacterial strains was E. coli > S. agalactiae >S. aureus. As a result, S. aureus has the highest rate of growth and the lowest value of zone of inhibition, while E. coli has the highest value and the least growth. The zone of inhibition of *C. maxima* oil is comparable to the standard gentamycin and is more effective on *E. coli.* Antibacterial activity of *C. maxima* peel oil and gentamycin against bacterial strains is shown below in Table 8.

According to earlier studies, Citrus peel generally has biological properties like antioxidant, anti-cancer, and antimicrobial activities.[27-28] According to the values, the antibacterial activity of the oil extracted from the peel of *C. maxima*, gram-negative bacteria are more resistant than gram-positive bacteria. This finding is consistent with earlier research; however, it differs from many other studies that found that gram-positive bacteria are more resistant to gram-negative bacteria since gram-negative bacteria's cell walls are more intricate than those of gram-positive bacteria.

Table 7. DPPH radical scavenging activity

Concentration	Absorbance	%inhibition	IC_{50}
Control	0.8		62.64
20	0.64±0.01	20.00	
40	0.58±0.02	34.38	
60	0.53±0.02	46.55	
80	0.48±0.02	60.38	
100	0.42±0.01	79.17	

3.12.2 Determination of MIC (Minimum Inhibitory Concentration) in Response to Bacterial Strains

MIC has the lowest concentration at which there is of 50 % growth of the observed absorbance. According to the data, there were differences in the antibacterial activity of the pomelo oil against the strains of bacteria that were studied. 7.82 mg/ml were the minimum MIC values that were noted for S. agalactiae. Escherichia coli and Staphylococcus aureus had MIC values of 15.05 mg/ml and 12.83 mg/ml, respectively. Pomelo oil was found to be more effective in the current study of gram-positive bacteria (7.82 mg/ml for S. agalactiae and 12.83 mg/ml for S. aureus) than gram-negative bacteria (15.05 mg/ml for E. coli). This is because gram-negative bacteria's walls are more complex and contain a higher phospholipid content than gram-positive bacteria. Whereas Gentamycin showed MICS of 6.2 ± 0.45 mg/ml against *E. coli* and 5.8 ± 0.65 mg/ml against *S. aureus,* and 3.2 ± 0.45 mg/ml against *S. agalactiae*. A lower Minimum Inhibitory Concentration (MIC) indicates a higher efficiency of oil against the bacteria. Minimum Inhibitory concentration (mg/ml) of *Citrus maxima* peel oil against bacterial strains is given below in Table 9.

Table 8. Antibacterial properties of *Citrus maxima* peel oil against various strains of bacteria

Antibacterial properties of *Citrus maxima* peel oil against various strains of bacteria		
Bacterial strains	**Zone of Inhibition (in mm) (*C. maxima* oil)**	**Zone of Inhibition (mm) (Gentamycin)**
Escherichia coli	15.24 ± 1.94	19.39 ± 0.61
Staphylococcus aureus	11.33 ± 1.86	20. 34 ± 0. 24
Streptococcus agalactiae	12.56 ± 2.03	15. 97 ± 0.45

Table 9. Minimum Inhibitory Concentration (MIC) (mg/ml) of *Citrus maxima* peel oil against the following bacterial strains

Minimum Inhibitory concentration (MIC) (mg/ml) of *Citrus maxima* peel oil against the following bacterial strains.		
Strains of bacteria	**MIC (mg/ml) (*Citrus maxima* peel oil)**	**MIC (mg/ml) (Gentamycin)**
Escherichia coli	15.05 ± 0.84	6.2 ± 0.45
Staphylococcus aureus	12.83 ± 0.49	5.8 ± 0.65
Streptococcus agalactiae	7.82 ± 1.32	3.2 ± 0.45

4. Conclusion

Standardisation helps in bringing up the sample identification, quality and purity of any drug in a cheaper way. In this study, the Pomelo peel was macroscopically, microscopically and physicochemically analysed to provide the identification standard for *C. maxima* fruit peel, which will help in the correct identification of raw material. Pomelo oil extracted from the peels of *C. maxima* fruits using Clevenger's apparatus was a pale yellow coloured oil with a tangy smell. Physical analysis was done to bring a standard for the analysis of the pomelo oil. GC-MS, Total phenol and flavonoid content showed the presence of limonene, phenol and flavonoid, respectively. The presence of these chemical constituents exhibited very decent antioxidant and antibacterial activity in Pomelo oil. Thus, this study opens a path for the preparation of various anti-oxidant and antibacterial formulations using pomelo oil as an active ingredient.

5. Author Contribution

Vijayalakshmi P: Writing – original draft, methodology, formal analysis, data curation, investigation; Malarkodi Velraj: Writing – review and editing, validation, data curation, supervision, project administration, investigation, conceptualization. Both the authors have read and agreed to the published version of the manuscript.

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