IC-019
Pharmacognostical and Physiochemical study of the peels of *citrus maxima* with the physical, chemical, antioxidant and antimicrobial evaluation of peel's oil

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ABSTRACT

Background: Fruits of citrus family are known for its antioxidant and antimicrobial activity. The study aims to perform pharmacognostical and physiochemical 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 physiochemical 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 was determined for the *C.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* by means of the Agar well diffusion technique and by determining the MIC (Minimum Inhibitory Concentration)

Result: Pharmacognostical standards for the peel physical standards for the oil were developed and limonene were 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 mg/g rutin equivalent. Oil showed 58.30% inhibition of DPPH. The antibacterial activity of oil was much effective against *E. coli.*

Keywords: Pharmacognostical, physiochemical parameters, *C. maxima* oil, *Escherichia coli, Staphylococcus aureus*, *Streptococcus agalactiae*

1. Introduction:

1.1. Introduction to herbal medicine and *Citrus maxima*

Herbal medicines are in high demand right now, and more people are coming to accept them every day. Because there is a great number of readily accessible raw material, World Health Organization or WHO encourages and advocates the application of traditional herbs or cures in the healthcare industry. In nature, plants are extremely intricate. The effectiveness of the plant medicine depends on their Species, location, and harvesting techniques. Standardization of herbal drugs is crucial to detect improper herb authentication, microbial adulterations, pesticide residue and other parameters that will affect the effectiveness of the crude drugs. According to the WHO (World Health Organization), identifying the identity and degree of purity of such materials requires first describing a medicinal plant's macroscopic and microscopic characteristics.[1-2]

Citrus maxima belongs to the family of Rutaceae and this family has approximately 160 genera and Citrus one the important genus of all is cultivated on all continents. An estimated eighty five million tons of various varieties of citrus are cultivated annually around the world. One of the world's most significant perennial fruit crop is citrus plant. After China and Brazil, India produces more citrus than any other country. More than 150 countries around the world grow citrus, 53 of which do so commercially. India produces about 7–8% of the citrus crop worldwide. Citrus plants are the third most cultivated crop in the country, behind banana and mango, with 10.86 lakh hectares under cultivation and 14.262 Million tonnes produced annually. The Citrus fruits are known for their unique flavor and fragrance. They are also a great source of dietary fiber, 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]

1.2. Taxonomy of Pomelo fruit (*Citrus maxima*)[2]

Biological name: *Citrus maxima* (J. Burm.) Merr.

1.2.1. Taxonomical Classification

Plantae	: Kingdom
Tracheophyta	: Phylum
Magnoliophyta	: Division
Magnoliopsida	: Class
Sapindales	: Order
Rutaceae	: Family
Aurantioideae	: Sub family
Citrus	: Genus
Maxima	: Species

1.2.2. Habit / Habitat: A tree that is 16–50 feet (5–15 meters) tall with a 4–12 inch crooked trunk. Northeastern area in Assam and Tirupura up to 1,500 meters. It is native to India's east.

1.2.3. Plant Description: *Citrus maxima* is a perennial shrub that grows all over India and is also referred to as papanus.

1.2.4. Morphology [6-8]

Leaves : Large, 10.5–20 cm (4–8 in) long, elliptic to oblong-shaped, evergreen leaves. Often marginalized, pubescent below. acute tip, Complete margin, uneven base, distinct smell

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*

Fig 1: Unripe *C. maxima* fruit on the tree

Fig 2: Fresh ripe fruits of *C. maxima*

1.2.5. Purposes

Previous studies on *C. maxima* oil demonstrates the antibacterial activity against the broad spectrum and numerous pathogenic bacteria and was majorly active against *Bacillus licheniformis* bacteria.[9] Studies also explains the antioxidant activity with some positive and some negative results on 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 anti-oxidant and anti-bacterial activity on the given strains

The study aims to perform pharmacognosy, phytochemistry and physiochemical analysis for 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, anti-oxidant activity and anti-microbial activity of the extracted oil. According to the folklore claim *C. maxima* plant are highly medicinal and the peels were traditionally used for its anti-microbial, brain tonic, sedative and anti asthmatic activity but there is 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 done for the screening of *C. maxima* peel oil for its antioxidant potential and antimicrobial efficacy agents against a panel of microbes linked to skin diseases.

2. Research Methodology

Plant material - Collection and identification

Pomelo fruits were obtained from *Citrus maxima* trees from Jharkhand, India and authenticated by the 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 was then coarsely powdered and kept at room temperature in an airtight container.

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 safranin: 0.1% (w/v) solution.[12]

Powder microscopical analysis

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

Quantitative microscopy-linear measurements

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

Physiochemical analysis

Physical and chemical characteristics like total ash, acid insoluble ash, and water-soluble ash value, foaming index, loss on drying and swelling index was assessed in accordance with Indian Pharmacopeia specifications. The official methods were followed when calculating the percentage of ash value, a measure of the crude drug's purity, and presence of polar and non-polar compounds were 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 swelling index assess the amount mucilage present in the sample. Foaming index were performed to assess the presence of saponin in the sample. All the parameter were performed as per the Indian Pharmacopoeia 1996, WHO 1998, 2011 and Kokate 2017.[16-19]

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 utilizing 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.[20]

The powdered medication was tested using a limit test for heavy metals and arsenic to ascertain the toxicity of heavy metals in accordance with the Indian Pharmacopoeia 1996 standard procedure.[21]

Extraction of Essential oil[22]

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 over a period of 3 hrs. The obtained oils were separated and stored in a dark glass vial, sealed and kept at 4°C.

1.1. 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 along of the peel's oil was determined with the equations below.

$$\text{Density of oil} = \frac{\text{Weight of sample}}{\text{Volume of sample}} \times 100$$

$$\text{Specific gravity of oil} = \frac{\text{Weight of oil extract}}{\text{weight of water}} \times 100$$

1.2. Characterization of Pomelo oil[23]

The percentage (v/w) is used to express the content of volatile oil. A Shimadzu GC (gas chromatography) fitted with a column made of stainless steel (2 m x 2 mm) filled of SE-30 10% Chromosorb-W was used to perform the GC-MS analysis. Oven program: 240 °C (5 ms), 260 °C (10 ms); nitrogen as the carrier gas; 40 ml/min flow rate; 240 °C for the injector and 240 °C for the detector. To identify individual components, the values of the spectra were compared with literature from libraries like NIST and WILEY using a mass spectra database. When a compound is compared to a homologous series of n-alkanes, its retention time on a gas chromatographic column is measured to determine its LRI. The linear retention index (LRI) can be obtained from the retention time with the formula given below.

$$\text{LRI (Linear Retention Index)} = 100 \times \left(\frac{(t-tn)}{(tn-1-tn)} + n\right)$$

were, "t" represents the time of retention of the component

"n" represents the number of carbon in present in the previous n-alkane
"n+1" represents the number of carbon in succeeding n-alkane

1.3. Total phenol content[24]

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 were 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, three ml of sodium carbonate solution (2%; Na2CO3) were 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 calibration curve was plotted. The total phenolic content was represented as mg of gallic acid equivalent per gram of Pomelo oil.

1.4. Total Flavonoids content

The flavonoid content was determined using the colorimetric technique with aluminum 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 aluminum chloride solution were mixed together. A Schrodinger (UV-Vis spectrophotometer) was used to analyze 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 amount of flavonoids was calculated and was represented as milligram of rutin equivalent/gram of the Pomelo oil.

1.5. 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 following equation, DPPH radical scavenging activity (%) was calculated.

$$\text{Percentage radical scavenging activity} = \frac{A_{control} - (A_{sample} - A_{blank})}{A_{control}} \times 100$$

were, $A_{control:}$ Absorbance of control (DPPH) solution
A_{sample}: Absorbance of sample (Pomelo oil) solution
A_{blank}: Absorbance of blank solution

2.10 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 Gentamycin were used as standard for the study.

2.10.1. Agar Well Diffusion Method

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 milliliter; 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 the concentration of 5µl on the filter paper disc were used as standard. The inhibition zones on the agar plates were then measured in millimeters after being incubated for 24 hours at 37 °C. Each experiment was conducted three times for accuracy.

2.10.2. Minimum Inhibitory Concentration

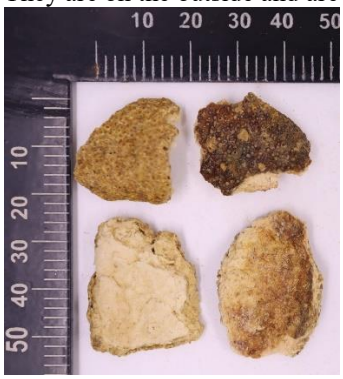
The MIC is the lowest drug concentration, measured in mg/µL, that inhibits microbial growth. It was carried out on microliter plates with 96 wells. The technique of broth dilution was employed to establish the lowest inhibitory concentration. 500 mL of distilled water was combined with 14 g of nutrient 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 completely filled with broth media, while the last well is partially filled with bacteria and broth. Pomelo oil mixture, broth, along with stains of bacteria were added in 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 were

repeated with gentamycin and readings were taken after a 24-hour interval, the second reading was taken after the first one.

2. Results And Discussion

2.1. 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 in 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.

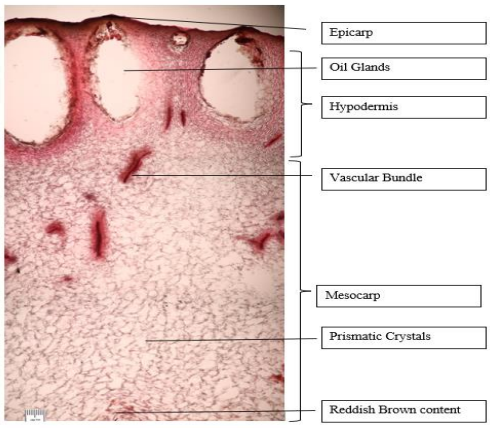


Macroscopic Image of ***Citrus maxima*-Fruit Rind**

S.No.	Organoleptic Characteristics	Observation
		Fruit Rind
1	Size and Shape	Dried flat cut pieces of fruit peel, thickness up to 0.5 cm.
2	Colour	Outer surface: Yellow to yellowish brown Inner surface: Creamish colour to yellowish white.
3	Surface characteristics, texture	Rough texture, numerous elevations observed due to oil glands in the surface.
4	Odour	Aromatic.
5	Taste	Bitter and astringent.

Microscopical analysis

C. 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 (Fig.3 & 4)



S.No.	Tissue Characteristics	Observation
		Fruit Rind
1	Pericarp	Comprises of epicarp and hypodermis.
2	Epicarp	A layer made of polygonal cells, filled with reddish / yellowish brown contents and thick cuticle.

3	Hypodermis	Layers of parenchymatous cells embedded with oil glands.
4	Mesocarp	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.

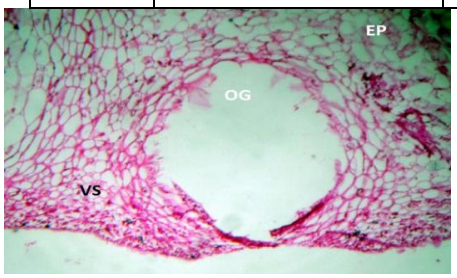


Fig 3: TS of *Citrus maxima* peel 10 X

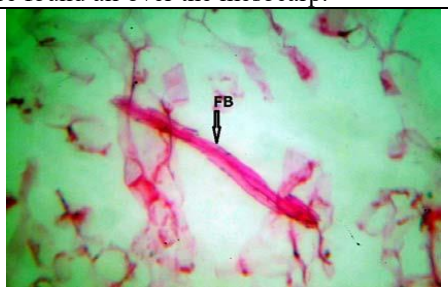
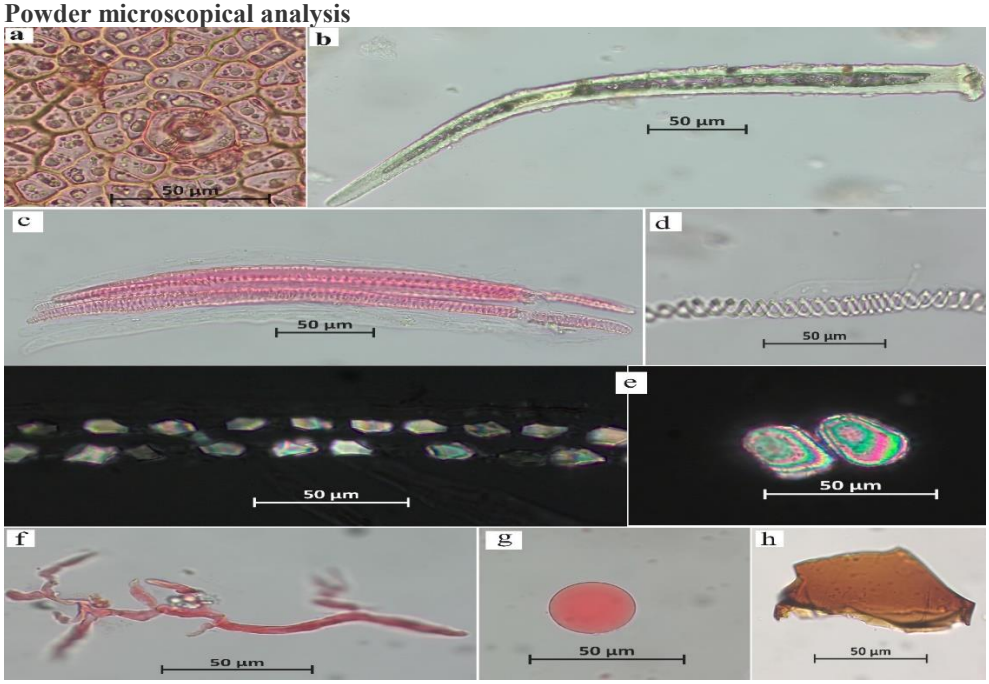


Fig 4: TS of *Citrus maxima* peel 100 X showing fibre

VS- Vascular bundle, OG- Oil gland, EP – Epidermal cell

Powder microscopical analysis



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

S.No.	**Powder Characteristics**	**Observation**
		Fruit Rind
1.	Epicarp	Layer of epicarp cells in surface view with paracytic stomata.
2.	Trichome	Unicellular trichomes (rarely observed).
3.	Tracheids	Border pitted tracheid's.
4.	Vessels	Spiral vessels.
5.	Gland cells	Fragments of oil gland cells from hypodermis.
6.	Crystals	Crystal fibres made up of prismatic crystals of calcium oxalate.
7.	Oil	Oil globules stained by Sudan red stain.
8.	Content	Brownish orange content

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. Table 1,2 and 3 present the findings. It guarantees this plant's quality.

Parameters	Length (µm)			Width (µm)		
	Minimum	Average	Maximum	Minimum	Average	Maximum
Fibres	270	505.5	750	15	12	7.5

Table No 1: Linear measurements of the fibres

Parameters	Length (µm)			Width (µm)		
	Minimum	Average	Maximum	Minimum	Average	Maximum
Calcium oxalate crystals	13.75	24	55	13.75	19.8	27.5

Table No 2: Linear measurements of the calcium oxalate crystals.

Parameters	Diameter (µm)		
	Minimum	Average	Maximum
Starch Grains	25	34.38	50

Table No 3: Linear measurements of the starch grains.

2.2. Physiochemical analysis

The physiochemical 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 are helpful in identifying expired or tampered drugs as well as providing information about the chemical components of the drug. The findings imply that the powdered drug has a high extractive value in water. Sweeling 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 more which represents the presence of high moisture content in the fresh peels. (Table 1)

Table 4: Physicochemical parameters of dried peel powder of *Citrus maxima* Burm. Merr.	
Physicochemical Parameter (Values)	**Average**
Total ash	3.3%
Acid insoluble ash	0.98%
Water soluble ash	1.8%
Alcohol soluble extractive	8.4%
Water soluble extractive	29.4%
Loss on drying (fresh peel)	21.01%
Foaming Index	NIL
Swelling index	10.1

2.3. Heavy metal analysis

2.4. Essential oil Extraction

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

2.5. Physical properties of pomelo oil

The observation physical properties like colour, odour, pH, density, Viscosity, solubility, refractive index and specific gravity of *C. maxima* oil are displayed in Table 2. Oil extracted by Clevenger's method of extraction produced pale yellow oil and were highly aromatic with the pH of 5.7. The density of a substance in relation to water is measured by its specific gravity. Pomelo oil's specific gravity was 0.8511 and 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 water layer. Viscosity of the oil was determined using Ostwald viscometer to find the internal resistance of the fluid to motion. Viscosity of the pomelo oil was 0.86442 centipoises. Refractive index of the oil was measured using Abbe refractometer and it was found to be 1.36 at 30^0C.

Table 5: Physical properties of pomelo oil	
Parameter	Average Value
Colour	Pale yellow
Odour	Aromatic (citrus odour)
pH	5.7
Density	0.8409 g/ml
Specific gravity	0.8511
Viscosity	0.86442 centipoises
Solubility	Insoluble when dissolved in water, sparingly soluble when dissolved in alcohol and completely soluble in hexane
Refractive index	1.36 at 30^0C

2.6. Characterization of Pomelo oil

An 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 (Table 3). 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 6: Phytochemical identification of the chief components* of *C. maxima* peels oil.

Sl. No	Phytochemical Compound	Retention time(min)	% of total compound
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

* The minor components were excluded in the list.

2.7. 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 its antioxidant properties. The total phenol content of *C. maxima* oil was 62.42± 4.03 mg Gallic acid equivalent/g of *C. maxima* oil**.**

2.8. Total flavonoid content

Flavonoids interference in the production of reactive oxygen species and the quenching of free radicals. Tannins have astringent properties. Their capacity as antioxidants is dependent on the presence of easily oxidizable hydroxyl groups and how much polymerization they have undergone. The total flavonoid content of the pomelo oil is 16.48± 0.92 mg/g rutin equivalent.

2.9. **2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity**

With ascorbic acid standard, anti-oxidant activity of pomelo oil was ascertained. Ascorbic acid, used as a positive control, demonstrated 10.18% activity whereas pomelo oil demonstrated 58.30% inhibition of DPPH, indicating a significant difference ($p<0.05$).

2.10. **Antibacterial properties of Pomelo Oil**

By measuring the zone of inhibition, the *in vitro* antibacterial properties of *C. maxima* peel oil counter to bacteria's like *S. aureus, S. agalactia* and *E. coli*, was 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 (Table 4). 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 least growth. Zone of inhibition of *C.maxima* oil is comparable the standard gentamycin and was more effective on *E.coli*. Antibacterial activity of *Citrus maxima* peel oil and gentamycin against bacterial strains is show below in table 4.

Table 7: 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

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 to 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.

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

MIC is the lowest concentration at which there is reduction 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 against 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's. Where as Gentamycin showed MIC 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.agalacitae* 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 are given below.

Table No 8: Minimum Inhibitory concentration (MIC) (mg/mL) of *Citrus maxima* peel oil against 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

Conclusion:

Standardization 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 physiochemically analysed to provide the identification standard for *C. maxima* fruit peel which will help in correct identification of raw material. Pomelo oil extracted from the peels of *C. maxima* fruits using Clevenger's apparatus a pale yellow coloured oil with tangy smell was obtained. 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. Presence of these chemical constituents exhibited very decent antioxidant and anti-bacterial activity of the 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.

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