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EXTRACTION, PHYSICO-CHEMICAL ANALYSIS AND ANTIMICROBIAL POTENTIALS OF ESSENTIAL OIL FROM GRAPEFRUIT PEEL

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Abstract

Citrus oil governs the class essential oil from peels. This essential oil is present in the rind of the walls of a citrus fruit.. In the present investigation, grapefruit (*citrus paradisi*) peels are used for the extraction of citrus oil. Juices from citrus fruits are the most widely consumed beverage today in the world. Within the increased production of processed fruit, waste generated increased enormously. Peel of citrus fruit has numerous glands that contain oil that is typically recovered as a major byproduct. The essential oil is present in the fruit peel in great quantities. This study focuses mainly on steam distillation, where grapefruit peels dried or preheated before distillation. The citrus essential oil is a mixture of volatile compounds and mainly consists of monoterpene hydrocarbons. The grapefruit essential oil composed of 94% limonene, which is used as food flavoring agents to cosmetics. Twenty-four components were detected with total composition of 99.68% of terpenes and oxygenated terpenes. Composition data for grapefruit oil were characterized by FT-IR spectroscopy. The synthesized oil was tested for antimicrobial potential on *Escherichia Coli* and *Staphylococcus aureus*. The essential oil shows the inhibition of growth of those microbes. The waste obtained after study are used for vermicomposting which increase NPK value of the soil. Thus the disposal problem of grapefruit or any other citrus peels after essential oil extraction can be overcome by vermicomposting.

Key words: limonene, terpenes, grapefruit peel, *Escherichia Coli*, *Staphylococcus aureus.*

Introduction:

Humankind has used plants for many thousands of years and it's from this folklore that the use of aromatic plant compounds as medicine began. Oils were used in the embalming process, in medicines and in purification rituals [1] with an extremely broad range of biochemical effects, there are about 300 essential oils in general use today by proficient practitioners. Essential oils are a great benefit to help guard our bodies and homes from this onslaught of pathogens [2,3]. .Juices from citrus fruits are the most widely consumed beverages today in the world. Approximately 50 to 60% of processed fruits are transformed into citrus peel which is composed of peel, seeds and membrane residues. Within the increased production of processed fruit, waste generated increased enormously. Large amounts of waste pose the problem of disposal without causing environmental pollution [4,5]. Each citrus fruit has its own typical set of compounds that encompass the oil and that are responsible for its aroma and flavor [6,7]to products such as effervescent drinks, ice cream, cakes, air fresheners and perfumes. Recently developed extraction methods like supercritical fluid extraction, microwave assisted extraction and sox let method[8] have been used for oil extraction, nowadays they also used solvent extraction, water distillation and steam distillation methods.

The first modern day distillation of essential oil was performed by the Persian philosopher Avicenna (980-1037AD) who extracted the essence of rose petals through the "enfleurage" process [9]. Essential oils are broadly used as pharmaceutical components, in nutrition supplements and for cosmetic industry and aromatherapy [10]. Because of the vast amount of raw products used to make wholly natural essential oils, a lot of products on the market have been polluted with lower quality, commercial grade oils or contain other chemical substances to reduce the cost or increase the profit margin-a fact not usually revealed on the label. That is why it is important to study the chemical composition of the volatile fraction once the essential oil is extracted. [11,12]. Most commonly, the essence is extracted from the plant using a technique called distillation. One type of distillation places the plants or flowers on a screen. Steam is passed through the area and becomes "charged" with the essence. The steam then passes through an area where it cools and condenses. This mixture of water and essential oil is separated and bottled. Since plants contain such a small amount of this precious oil, several 100 pounds may need to produce a single ounce. The advantage of steam distillation is that it is a relatively cheap process to operate at a basic level, and the properties of oils produced by this method are well known.

Materials and methods

Preparation of Grapefruit Peel Sample

The grapefruit peel samples are collected from the nearby places and markets in Neyyattinkara, Kerala. The collected sample of grapefruit peels is cleaned and pith is manually separated from the outer

colored part of the peels. That is because of the reason that the majority of the oil in oil sac present in them. The peels were dried and powdered.

80g grapefruit peels sample is taken in a distillation flask and add 500ml of distilled water to it. Heat is supplied to the distillation unit by a temperature controlled basket heater. At the initial stage, experiment is carried out at a temperature of 88^0cfor 60 min. time period. The mixtures of hot vapors were allowed to pass through a condenser and the distillate is collected in a beaker. This distillate has two layers, one dense layer and other less dense layer. The top layer is citrus oil and the bottom layer was water, which was separated using a separating funnel. Separated oil layer contains some amount of water, that was removed by adding diethyl ether.

Physico-chemical analysis of essential oil

Colour of Grapefruit peel oil

The colour of the oil was recorded by visual observations.

Determination of pH of Grapefruit peel oil

In beaker 25 ml of oil sample was taken and pH of sample was determined by using labtronics auto digital pH meter model LT-11.

Determination of acid value

Dissolved 1-2 ml oil in 50 ml of neutral solvent in 250 ml conical flask. Add few drop of phenolphthalein. Titrate the content against 0.1 N KOH Shake constantly until pink colour which persist for 15 sec is obtained

Determination of saponification value

Accurately weigh out 1-2 g of oil into 250 ml conical flask, add 25 ml alcoholic KOH and dissolve the oil completely. Connect air condenser to the flask and boil for about 30 min on a boiling water bath. Cool to room temperature; add 2-3 drop of phenolphthalein indicator and mix. Titrate against 0.5N HCl until the pink colour disappear Treat the blank simultaneously in the absence of oil.

Oil yield determination (%)

The oil content after the extraction was transferred into a measuring cylinder which was placed over water bath for 30 min at 70°C to ensure complete evaporation of solvent and volume of the oil was recorded and expressed as oil content (Abubakar.*et. al*. 2014).

Analysis of Essential Oil

Determination of chemical constituents

The oil samples were analyzed using the GC-MS A capillary column was used for separation of the component. Helium gas was used as the carrier gas with a flow rate of 1.1 mL/min. Temperature program for the oven was from50 to 250°C at a rate of 5 min-1 with holding time of 1 to 10 min. Injector temperature was 250°C, and the injection volume was 1.0 µL. The identification of components of the essential oil was based on comparison of Kovats retention indices and mass spectra that corresponded with data (Adam, 1989) and mass spectra libraries The quality for the perfumery of oil was evaluated on the basis of the addition of the phytoconstituents: citral, β-myrcene, neral, citronellal, citronellol and geraniol.

Phytochemical Studies (Qualitative analysis)

The extracts were subjected to preliminary phytochemical screening following the methodologies;

Test for alkaloids:2 ml filtrate was mixed with 1% HCl and about 6 drops of Mayor‘s reagents. A creamish or pale yellow precipitate indicated the presence of respective alkaloids.

Test for amino acids and proteins: 1 ml of the extract was treated with few drops of Ninhydrin reagent. Appearance of purple color shows the presence of amino acids.

Test for tannins: 1 ml of the extract was treated with few drops of 0.1% ferric chloride and observed for brownish green or a blue-black coloration.

Test for anthraquinones (Borntrager's test): 1 ml of the extract solution was hydrolyzed with diluted Conc.H_2SO_4 extracted with benzene. 1 ml of dilute ammonia was added to it. Rose pink coloration suggested the positive response for anthraquinones.

Test for saponins: Froth test for saponins was used. 1g of the sample was weighed into a conical flask in which 10ml of sterile distilled water was added and boiled for 5 min. The mixture was filtered and 2.5ml of the filtrate was added to 10ml of sterile distilled water in a test tube. The test tube was stopped for about 30 second. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins.

Test for protein: 3 ml sample of extract was treated with 4% Sodium Hydroxide and few drops of 1% Copper Sulphate was added. Violet or pink colour appears the presence of protein.

Test for terpenoids (Salkowski test): 5 ml of extract was mixed in 2 ml of chloroform, and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface was formed to show positive results for the presence of terpenoids.

Determination of IR spectra

The infrared spectrum of the oil was obtained using shimadzu FT-IR spectrophotometer and structure determined with the help of correlation charts. The samples were examined neat by placing them in between potassium bromide pellets. The solvent spectrum was also obtained to aid in analytical identification. .

Antimicrobial activity

The filter paper disc method was applied to the synthesized compounds. Each of the investigated isolates of bacteria was seeded in the tubes with nutrient broth. The seeded NB ($1cm^3$) were homogenized in the tubes with $9cm^3$of melted (45^0C) nutrient agar .The homogenous suspension was poured into Petri dishes. The discs of filter paper were cool. After cooling on the formed solid medium, the investigated compounds were placed with micropipette. After incubation for 24 hours in a thermostat at 25-27^0C, inhibition (sterile) zone diameters (including disc) were measured and expressed in mm. Every test was done in three replications. The antimicrobial activities of Grapefruit oil were tested against Escherichia *coli* and *Staphylococcus aureus*

RESULTS & DISCUSSION

Physical analysis of essential oil

It was observed that 1.75 ml oil obtained from 80 g of grapefruit peel powder i.e. 2.190 per cent yield of oil. The physical characteristics of essential oil was colourless, pH 6 acid value 2.50 (mgKOH/g) and Saponification value194.30 (mgKOH/g)

Table. Physical analysis of essential oil

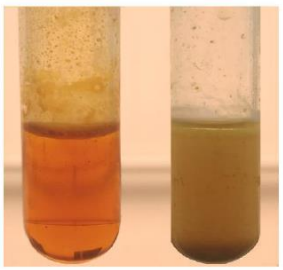
Sl.No	Parameters	Mean value
1.	Colour	Colourless
2	Taste	bitter
2.	pH	6
3.	Acid value (mgKOH/g)	2.50
4.	Saponification value	194.30

Chemical constituents present in grapefruit oil

Sl.No	Compounds	Composition (%)
1	α-Pinene	0.77
2	Camphene	0.01
3	Sabinene	0.49
4	β-Pinene	0.04
5	β-Myrcene	2.10
6	Octanal	0.34
7	Limonene	94.3
8	β-Ocimene	0.02
9	Linalool	0.11
10	Nonanal	0.05
11	trans-p-Mentha-2,8-dien-1-ol	0.14
12	cis-Limonene oxide	0.43
13	trans-Limonene oxide	0.30
14	Citronellal	0.04
15	α-Terpineol	0.13
16	Decanal	0.19
17	Carvone	0.41
18	α-Copaene	0.13
19	β-Cubebene	0.14
20	Caryophyllene	0.20
21	Germacrene D	0.01
22	δ-cadinene	0.04
23	Humulene	0.03
24	Caryophyllene oxide	0.04
	Monoterpene hydrocarbons	96.93

	Oxygenated monoterpenoids	1.62
	Sesquiterpene hydrocarbons	0.55
	Oxygenated sesquiterpenes	0.04
	others	0.60

Bromine test: A dilute Bromine -water solution is prepared and taken in a test tube. To that citrus oil extracted from grapefruit peels is added. If limonene is present in the oil extracted, the colour of the Bromine -water gets changes from red brown to pale yellow. This is because of the fact that the Bromine present in the Bromine – water solution occupies the space between the two double bonds present in limonene.



Confirmation test for limonene

Phytochemical Studies

Preliminary phytochemical analysis of peel extract of C. paradisi revealed presence of flavonoids, sterols, triterpenoids, coumarins, glycosides, reducing sugars and carbohydrates, but alkaloids, tannins, saponins, anthraquinones and lignin were not detected, and might be present in trace undetectable amounts by qualitative methods.

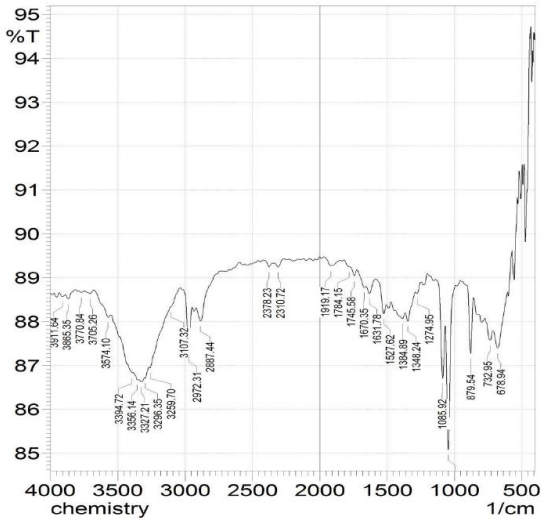
Table.4.3 Phytochemical examination of grapefruit peel by aqueous extraction

PHYTOCHEMICALS	TEST PERFORMED	INFERENCE
Alkaloids	Dragendroff‟s test	-
Carbohydrates	Fehlings test	+
Proteins/amino acids	Ninhydrin test	-
Terpenoids	Salkowski test	+
Tannins	Ferric chloride test	-
Flavonoids	Lead acetate test	+
Saponins	Foam test	-
Anthraquinones	Borntrager’s test	-

Coumarins	UV lamp	+
Sterols	Salkowski Liebermann	+
Lignins	Labat test	-
Glycosides	Glycosides test	+

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopy is one of the most widely employed techniques for functional groups identification The spectrum presents characteristic bands at 2972.31and 2914 cm^{-1} corresponding to CH_2 asymmetric and symmetric stretching vibrations. The signals which appeared between 2972.31 and 2887.44 cm^{-1} are caused by the asymmetrical and symmetrical stretching vibrations of C–H groups. Carbonyl compounds are often the strongest band in the spectrum that lies between the 1919.17 and 1527.62 cm^{-1}, its exact position being dependent on its immediate substituent. For a double-bonded functionality, conjugation plays an important role in the observed carbonyl frequency. This includes the connection to an aromatic ring or the conjugation to a C=C or another C=O The band which appeared at 1631.78 $cm^{-1}$was attributed to the stretching vibration of C=O The strong methylene/methyl band which appeared at 1527.62cm^{-1}, the weak methyl band which appeared at 1384.89 cm^{-1} and the methylene rocking vibration band which appeared at 732.95 cm-1are indicative of a long-chain linear aliphatic structure. Bands which appeared at 1274.95 and 1085.92 cm^{-1}were ascribed to the stretching vibrations of C-O-C and O-H, respectively. The absorption bands between 879.54-1274.95cm^{-1} indicate the methylene- cyclohexane ring vibrations (-CH_2-) and aromatic C-H in-plane bend. Finally, the absorption which occurred at about 879.54 cm^{-1} was evidenced for the limonene presence. The FTIR analysis of essential oils has made us to notice a similitude of absorption bands and only small differences between their intensities these citric essential oils is limonene and other terpenes and derivatives, such as: α-pinene, β-pinene, α-terpinene,1-terpinen-4-ol, terpinolene, o-cymol, 3-carene; 2-carene,α-terpineol, can be found in significant amounts. Limonene was found in a concentration over 94.3% in grapefruit. The results were confirmed by FTIR technique by which the vibrational modes of limonene were identified at 879.54 cm^{-1} (out of plane bending of terminal methylene group), 1435-1527.62 cm^{-1} (C-H asymmetric and symmetric bond) and 1631.78 cm^{-1} (C=O stretching vibration).



Fourier Transform Infrared Spectroscopy of grapefruit essential oil

Antimicrobial Activity

The oil was screened for their antimicrobial activities against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli Salmonella typhimurium Pseudomonas aeruginosa* .Based on the inhibition zone and MIC values, the order of sensitivity of the different bacteria was:

B. subtilis> E. coli > S. aureus> S. typhimurium> P. aeruginosa

Table. Antimicrobial activity of grapefruit essential oil

Bacterial Strain	**Zone of inhibition in grapefruit Oil (mm)**	**MIC (µL/mL)**
Bacillus subtilis	34	0.73
Staphylococcus aureus	23	6.05
Escherichia coli	25.6	6.05
Salmonella typhimurium	21.3	12.10
Pseudomonas aeruginosa	8.5	23

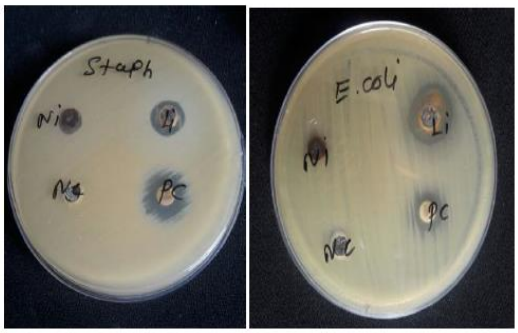


Fig: 4.3 Inhibition Zone of grapefruit Oil against *Staphylococcus aureus &Escherichia coli*

CONCLUSION

This study demonstrated support for the claimed uses of the plants in the traditional medicine probably due to the phytochemicals present. The peel of grapefruit is a very important part, as rich source of chemical constituents which is for prevention and cure of diseases. The peel (96% grapefruit) extract showed various degree of inhibitory activity against tested microorganism species of bacteria and fungi.. The results of the present study gave solid grounds that the *C. paradisi* peel extract possess a medicinal potential to develop new phytopharmaceutical drugs and cosmeceuticals.

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