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Pharmacognostic, phytochemical, physicochemical property and antimicrobial activity studies of lemon peel oil

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ABSTRACT

The Study was mainly aimed to establish the local ethno-medicinal claim of Assam that lemon peel oil has Antimicrobial property. Again when searching for the Microscopic Characters of lemon leaf for identification, data's were not available easily and then the aim of the study was broadened to include evaluation of pharmacognostic, phytochemical, physicochemical property and to make the data's available easily for further use. The Lemon Peel Oil as every other volatile oil has shown antimicrobial activity, but was active against both Gm + ve and $Gm _ve$ organism. When compared with Standard drug Amoxicillin the activities were less potent but can be useful if explored either alone or in combination with other antimicrobial agents.

Key Words: Lemon Peel Oil, Microscopy, Physicochemical property, Antimicrobial activity, Paper disc diffusion method.

INTRODUCTION

Citrus fruits are recognized as an important component of the human diet, providing a variety of constituents important to human nutrition, including vitamin C, folic acid, potassium, flavonoids, pectin, and dietary fibres. Citrus also contains significant amounts of highly oxygenated triterpenoid compounds (limonoids), particularly in underutilized by-products of citrus juice production.

Citrus fruits are mainly used by juice processing industries while the peels are generally wasted. The citrus peels are rich in nutrients and contain many phyto-chemicals, they can be efficiently used as drugs or as food supplements too. Since there is an increase in the number of antibiotic resistance pathogens, there is always a search of an alternative drug that is regarded as safe. Citrus peels if proved to have antibacterial activity; they can also be used in same food industry which generates large peel wastes as a food preservative.

The peel of *Citrus* fruit is a rich source of flavanones and many polymethoxylated flavones, which are very rare in other plants [Ahmad *et al.* (2006)]. The antimicrobial abilities of essential oils, among which citrus oils, are also shown to be a particularly interesting field for applications within the food and cosmetic industries [Caccioni *et al.*, (1998)]. It has also been used as an anti-diabetic (Hamendra and Anand (2007)]), antimicrobial [Caccioni *et al.* (1998)], antifungal [Stange Jr *et al.* 1993], hypotensive agent

[Kumamoto *et al.* (1986)], antioxidant [Proteggente *et al.* (2003)]; [Kanaze *et al.* (2008)], carminative, insect repellent, antibacterial, larvicidal, antiviral, uricosuric, anti-yeast, antihepatotoxic and antimutagenic agent [Han (1998)].

MATERIALS AND METHODS

Plant Material-The plants used in this study was *Citrus limon* (common name: Lemon), family Rutaceae. The peels were collected from the local market.

Extraction Procedure- Extraction of volatile oil from lemon peel by using Clevenger's apparatus.

Microscopy Study:[3]

Qualitative microscopic evaluation was carried out by taking transverse sections of fresh Leaf of lemon. The thinnest section was selected and cleared by boiling with chloral hydrate solution for 20 minutes and then observed under microscope.

Physicochemical parameters:[4]

The determination of various physicochemical parameters such as total ash, water soluble ash, pH were calculated as per Indian Pharmacopoeia.

Preliminary phytochemical screening of powder of lemon leaf: [3]

For preliminary phytochemical screening powder was subjected to various qualitative chemical tests to determine the presence of various phytoconstituents like glycosides, tannins, phytosterols, fixed oils and fats, proteins and amino acids, flavonoids, saponins, gums and mucilage's etc.

Antimicrobial activity:[5,6]

Media: Nutrient Agar solid media.

Bacterial strains: Both Gm +ve (*S. aureus*) and Gm –ve strains (*E. coli*) were collected from were collected from Dept. of Microbiology and were incubated at $37^{\circ} \pm 0.1 \text{ }^{\circ}\text{C}$ for 24 hrs by inoculation into nutrient broth.

Method: Disk Diffusion method.

Nutrient Agar media was prepared and Sterilized in a flask and cooled to 45-50 °C and was distributed by pipette (25ml) in each pre sterilized petridishes, previously inoculated with 0.01ml of the nutrient broth cultures and swirled to distribute the medium homogenously. Disks injected with various concentrations of Volatile Oil (250 μ l/ml, 500 μ l/ml & 1000 μ l/ml) were placed on the solid agar medium by pressing slightly. The treated petridishes were placed at 4°C for one hour and then incubated at 37°±0.1°C for 24 hrs. Same has been done for Standard drug Amoxicillin and Control (Tween80). Lastly, the zones of Inhibitions formed on the media were measured with a transparent ruler in millimeters.

RESULTS AND DISCUSSION

Microscopy : T.S. of Leaf : A transverse section of leaf showed the presence of following histological characters. [Figure-i]

- Upper epidermis
- Trichome
- Xylem vessel
- Lower epidermis
- Vascular bundle
- Spongy parenchyma.



Fig(i): T.S of lemon leaf

Powder Microscopic Observations:

During the powder microscopic Study of leaf of following observations were made like trichomes, xylem vessel, calcium oxalate and stomata.[Figure-ii]

MICROSCOPICAL CHARACTERISTICS OF POWDERED DRUG



Fig(ii): Powder Microscopy

Phytochemical Analysis of Leaf of lemon:[9,10]

The powder drug with different chemical reagents shows different colour when seen by naked eye. The different colours observed shows the presence of different types of phyto-constituents. Many drugs fluorescence when their powder is exposed to ultraviolet radiation. It is important to observe all materials on reaction with different chemical reagents under U.V. light. The fluorescence characteristics of powdered drug were studied under U.V. light after treating with different chemical reagents and is reported. The powder was subjected to different qualitative chemical tests.

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The presence of various phytoconstituents was observed during the test. The data obtained is specified in Table no i, ii & iii.

Reagent	Colour observed
Powder	Green
Powder + concentred HCL	Green
Powder + concentrated H2SO4	Black
Powder + 5% NaOH	Yellowish green
Powder + 5% FeCl3	Dark green

Table (i): Powder (Leaf) Analysis With Chemical Agents

Table (ii): Fluorescence Analysis Of Powdered Drug (Leaf)

Reagents	Fluorescence Observed
Powder + 1N NaOH in water	Yellowish green
Powder + 50% Hydrochloric acid	Light green
Powder + 50% Sulphuric acid	Light green
Powder + 50% Nitric acid	Yellowish red
Powder + Petroleum ether	Green
Powder + Chloroform	Yellowish green
Powder + Picric acid	Yellowish green
Powder + 5% Ferric chloride solution	Brownish green
Powder + 5% Iodine solution	Reddish brown

Table (iii): Phytochemical investigation (Leaf Powder)

TEST	OBSERVATION
Test for alkaloid	
1.Hager's test	+
2.Mayer's test	-
3.Dragendroff's test	+
4.Wagner's test	+
Test for Amino acid	
1.Ninhydrine test	-
Test for Carbohydrate (Molish test)	-
Test for volatile oil	+
Test for Fixed test (Saponification test)	-
Test for Glycoside	
1.Anthraquinone test	-
2.Saponin glycoside	+
Test for Tannin	-

Physicochemical parameters:[7,8]

The determination of physico-chemical parameter is important in determination of adulterants and improper handling of drugs. Table- iv shows the result of various physico-chemical parameters of powdered drug carried out using standard methods. Ash values are used to determine quality and purity of crude drugs. It indicates the presence of various impurities like carbonate, oxalate and silicate.

Table	(iv):	Physical	Evaluation	Parameters	(Leaf)
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Sl. no.	Parameter	value
1	Total Ash Value	10%
2	Water Soluble Ash	2%
3	P ^H of 1% Solution	4.2
4	P ^H of 10% Solution	3.5

Antimicrobial activity:[5,6,7,10]

The In-Vitro antimicrobial activities of volatile Oil Isolated from Lemon as well as that of Standard drug Amoxicillin and Control (Tween) are shown in Table v.

As seen from the table lemon oil have activity against both gram +ve and gram -ve bacteria as compared to the standard drug Amoxicillin and Tween80 as a control sample. Lemon shows maximum zone of inhibition for *S. aureus* 8mm and for *E. coli* 10mm in a dose dependent manner.

DRUG	CONCENTRATION	ZONE OF INHIBITION (mm)	
		S. aureus	E. coli
Lemon oil	250µl/ml	5	7
	500µl/ml	7	8
	1000µl/ml	8	10
Amoxycllin	250µg/ml	11	10
	500µg/ml	22	14
	1000µg/ml	28	20

Table (v): Antimicrobial activity of volatile oil of lemon peel & standard drug

Control Sample (Tween 80) has not shown any Zone of Inhibition.

CONCLUSION

From the phytochemical investigation it was found that in lemon leaf alkaloid, volatile oil and saponin glycosides are present. After performing the antimicrobial activity by using paper disk diffusion method, we found that lemon oil have activity against both gram +ve and gram -ve bacteria as compared to the standard drug Amoxicillin, and can be further studied for developing Herbal Antibiotic Preparation.

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