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Original Research Article

Pharmacognostical, Phytochemical and Pharmacological Evaluation on Leaves and Volatile Oil of *Citruslimon*. (Linn.)

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Abstract

Bijora, also known as *Citrus medica Linn*., is a significant plant in Ayurveda, although little is known about its pharmacological properties.

Objectives: The current study assessed pharmacognostic and physicochemical standards.

Methods: Methods used included macroscopy, microscopy, phytochemical screening, and GC MS analysis.

Results: Macroscopy reveals the fruit's organoleptic characteristics, while microscopic analysis reveals the existence of oil glands and capillaries in the rind that produce essential oils. When water-soluble ash is higher than acid-insoluble ash, it means there are fewer acid-insoluble siliceous materials. GC MS analysis of methanolic extract. Initial phytochemical analysis reveals the presence of carbohydrates, amino acids, flavonoids, tannins, phenolic compounds, and steroids.

Conclusion: Citrus medica fruit's pharmacognostic and phytochemical criteria are distinctenough to establish its validity.

Keywords: Bijora, Citrus medica, Pharmacognosy, Phytochemical Analysis

1. INTRODUCTION:

Citrus fruits are a crucial component of the human diet, providing essential nutrients such as vitamin C, folic acid, potassium, flavonoids, pectin, and dietary fibers. They also contain highly oxygenated triterpenoid compounds (limonoids), particularly in underutilized by-products of citrus juice production[1-3]. Citrus peels are rich in nutrients and contain many phyto-chemicals, making them efficient for drug or food supplement use. As antibiotic resistance increases, there is a search for alternative drugs that are considered safe. Methods of drug discovery systems using higher plants have been used, with a focus on ethnomedical approaches. Random selection followed by chemical screening or phytochemical screening methods have been used in the past and are currently followed in developing countries. However, these tests can be false-positive and false-negative, making it difficult to assess the biological effects of

different phytochemicals. The Central Drug Research Institute (CDRI) in India has evaluated over 35,000 species of plants for various biological activities, including antibacterials, antidiabetic, antifertility, antifungal, antitumor, cardiovascular, central nervous system depressant, cytotoxicity, and dirutic. There are no biologically active drugs for human use, but a large number of known and novel bioactive compounds have been isolated from active plants[3-7].

Ethnomedical information has been documented and valuable for initiating drug discovery systems. The WHO Traditional Medicine Programme provided useful evidence of ethnomedical studies for drug discovery systems several years ago. WHO-TRM centers worldwide ask their help to identify all plant-derived pure compounds used as drugs in their respective countries and survey pharmacopoeias of developed and developing countries to identify useful drugs[5,7-11].The latest trend to added value of natural sources is the improvement in separation techniques to isolate and purity of natural products, such as counter-current chromatography and analytical techniques to determine compound structures. Screening of natural product mixtures is now highly compatible with the expected timescale of high-throughput screening campaigns. Singh and Barrett point out that pure bioactive compounds can be isolated from fermentation broths in less than 2 weeks, and the structures of new compounds can be elucidated within 2 weeks. With advances in NMR techniques, complex structures can be solved with very little more than 1 mg of compound. It is recently demonstrated that it is possible to prepare a screening library of highly diverse compounds from plants, with compounds being pre-selected from an analysis of the dictionary of natural products to be drug-like in their physicochemical properties[9-11].

As alternative techniques are explored, the speed and efficiency of natural products can be increased for drug discovery. Volatile oils play a crucial role in various natural substances, including pharmaceuticals, food industries, perfumery, cosmetics, spices, herbal theraphy, and aromatherapy. They are complex mixtures containing many single compounds, contributing to their therapeutic or adverse effects. Understanding the composition of volatile oils is necessary for better and specially directed application. The modern pharmaceutical industry faces challenges such as a stagnant pipeline of new drug discovery systems, a deviating disease economy, and IPR regulations in developing countries. A Bangalore Declaration, funded by the Common Fund for Commodities (CFC) Amsterdam in collaboration with Biocentre and Food and Agriculture Organisation, Rome, held in Bangaluru, India, focused on providing sustainable livelihood opportunities for farmers and poor in the region through organic cultivation systems and managed collection[9]. The workshop emphasized the importance of providing affordable healthcare options in the form of quality traditional medicines and building regional brands in the global market. *Citrus limon (L)* is a widely cultivated plant belonging to the Rutaceae family, known for its medicinal properties. It has been used for treating stomach aches, vomiting, carminatives, refrigerant drinks, culinary uses, acute rheumatism, rheumatic gout, some forms of acute tropical dysentery, and diarrhea. Lemon juice and gunpowder have been successfully employed in acute rheumatism, rheumatic gout, acute tropical dysentery, and diarrhea. It also serves as an antidote to some acro-narcotic poisons. In India, lemon is used in day-to-day life for various purposes, including Siddha Medicin and Ayurveda, and is a main ingredient in many Indian cuisines. Lemon pickle or mango pickle is part of everyday lunch meals in southern India, and in Hindu Pooja, it takes a very important place.

An investigation aiming to scientifically explore the crucial medicinal use of lemon, especially its antimicrobial activity in vivo, is inevitable [10-14]. By focusing on the bioactivity of volatile oils, we can address the unsettling situation faced by the modern pharmaceutical industry and provide affordable healthcare options for farmers and the poor in developing countries.

3. Materials and Methods

3.1 Plant Material

In the surrounding areas of Bundelkhand, Uttar Pradesh, fresh leaves of the Citrus limon Linn. plant were collected, and a voucher specimen was maintained for future reference. G.C.

Analysis The analytical GC was performed using a Varian 3300 gas chromatograph equipped with a capillary column made of silicon DB-1 (30 meters by 0.25m). The film had a thickness of 0.25 micrometers, and nitrogen was used as the primary carrier gas. The flow rate is 1.5 milliliters per minute. The temperature was programmed to range from 800C to 2500C at 40C per minute. The temperature of the injector was 2500 degrees Celsius, and the detector's (FID) temperature was 3000 degrees Celsius[9-13].

3.2. GC-MS Analysis

Analytical Evaluation At 2500 degrees Celsius and 70 electron volts, GC-MS analysis was performed using QP-2000 equipment. With a film thickness of 0.25 microns, the GC column Ulbon HR-1 is similar to the ov-1 fused capillary and measures 0.25 millimetres by 50 metres. The temperature was initially set at 1000 degrees Celsius for six minutes, then raised to 2500 degrees Celsius at a pace of 100 degrees Celsius per minute. There was a flow rate of 2 milliliters per minute, an FID detector, and helium as the carrier gas (9).

3.3 Isolation of oil

Hydro-distillation was performed on the plant material in accordance with the procedure that was suggested in the British Pharmacopoeia, 2003. Following drying on anhydrous sodium sulphate, the oil was kept at a temperature of forty degrees Celsius in the absence of light (10, 11).

4. Result and discussion:

4.1. Pharmacognosy of Citrus limon (Lemon)

The pharmacognosy of Citrus limon, also known as the lemon, involves studying its physical, chemical, and biological properties relevant to medicine and pharmacy. Here's an overview:

Macroscopic Features[9-13]:

- Tree: Small evergreen tree, 3-6 meters tall, with spiny branches and glossy green leaves.
- Leaf: Elliptical, aromatic, dark green on top, lighter green and smooth below, with noticeable veins.
- Flower: Solitary or clustered, white with five petals.
- Fruit: Yellow oval citrus fruit with rough skin and juicy, acidic pulp.

Chemical Constituents[9,11]:

- Primary metabolites: Carbohydrates (sugars, starch), proteins, amino acids, organic acids (citric acid), minerals.
- Secondary metabolites: Volatile oils (limonene, citral), flavonoids (hesperidin, eriocitrin), phenolic acids (ferulic acid, caffeic acid), coumarins, coumarins, triterpenes, vit amins (vitamin C).

Traditional Uses[9-12]:

- Antibacterial, antifungal, antiviral properties.
- Digestive aid, helps relieve nausea and vomiting.
- Immune system booster due to high vitamin C content.
- Wound healing and skin disinfectant.
- Febrifuge (reduces fever).
- Anticancer and antioxidant properties (research ongoing).

Pharmacological Activities[8-13]:

- Antibacterial and antifungal due to volatile oils and other constituents.
- Antioxidant activity protects cells from damage.
- Anti-inflammatory properties may help with various conditions.
- Cardiovascular health benefits suggested by some studies.
- Potential role in cancer prevention, but more research needed.

4.1.2 Morphological features of *C.limon* [9,11]

It is a tree with a yellowish-green, spreading habit.

Shape: Light green, oblong to elliptic ovate
Scarcely winged, lanceolate, sharp-pointed
Colour: Yellowish green
Margin : Subserrated margin
Petiole: Narrowly winged
Flowers: Axillary single or in small clusters
Fruits: Oblong ovoid berry (7.5 -12.5cm)

4.3. PHYSICOCHEMICAL EVALUATION OF ISOLATEDVOLATILEOIL

The results of physicochemical analysis, and GC-MS analysis were as follows.

Percentage of oil obtained :0.5 to 0.8%
Color: Paleto dark yellow or greenish yellow
Odor: Strong, Fragrant, Fragrant
Taste: Spicy, sour, aromatic taste

Characteristics Feel: Slightly sticky
Solubility: Solublepetroleumether, Toluene, chloroformand ethanol. It is mixed with water.
Refractiveindexat20ºC:1.4740–1.4755
Specific gravity20ºC :0,8560-08570
Opticalrotationat20ºC :+57-+65

4.3 PHARMACOLOGICAL STUDIES

***4.3.1IN VITRO* ANTIOXIDANT ACTIVITY**

4.3.1.1.DPPH Scavenging Activity

The percentage of DPPH free-radical and IC50 values obtained for volatile oil of citrus limon and ascorbic acid are tabulated in Table 1 and Figure 4. DPPH scavenging activity of volatile oil was comparable with that of standard ascorbic acid [9,10].

Table 1: Percentage Inhibition of Volatile Oil of *Citrus limon* andStandardAscorbic AcidagainstDPPHat517nm

S.No.	Conc.in µg/mL	Percentageinhibitionby standardAscorbicacid	Percentageinhibition byVOCL
1	6.25	48.21± 0.27	41.36± 0.43
2	12.5	51.18± 0.78	44.51± 0.69
3	25	59.97± 1.12	55.29± 0.83
4	50	74.65± 0.34	68.02± 0.82
5	100	79.84± 0.71	72.86± 0.63
6	200	92.82± 1.28	81.04± 0.76
	IC_{50}	30.09µg/mL	49.57µg/mL

*meanofthreereadings±SEM

Table 2: PercentageInhibitionof AscorbicAcidandVolatileOilof*C.limon*AgainstNitricOxideat 546nm

S.No.	Ascorbicacid		*C. limon*	
	Conc. inµg/mL	Percentageinhibition bystandardAscorbicacid	Conc.inµg/mL	Percentageinhibition byVolatileOil
1	22.22	47.19± 0.53	2.78	41.57± 0.92
2	44.44	54.31± 0.81	5.56	52.43± 2.01
3	88.89	60.67± 1.06	11.11	65.92± 2.61
4	177.78	65.92± 1.33	22.22	71.16± 2.91
5	222.22	84.64± 1.33	44.44	94.01± 2.14
	IC_{50}	84.56µg/mL		11.74µg/mL

*meanof threereadings±SEM

The IC_{50} value for volatile oil was foundto be 11.74µg/mLwhile forascorbic acid it was 84.56 µg/mLwhich indicates that the volatile oil had averypotentnitricoxidescavengingactivity.

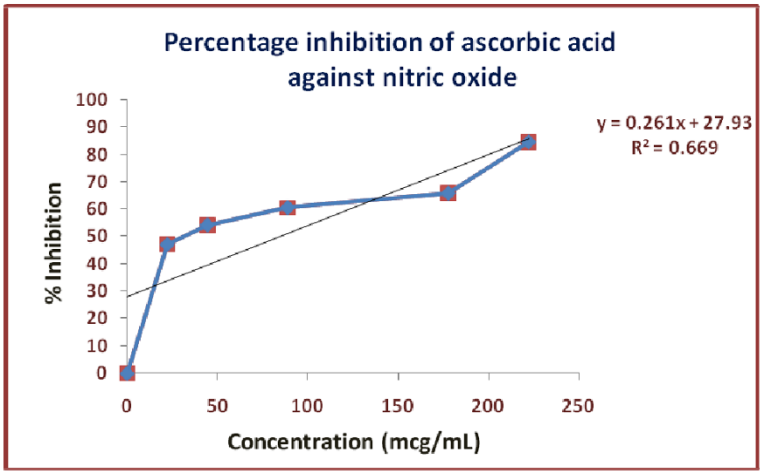

Fig.1: Nitric Oxide Radical Scavenging By Volatile Oil of *C.limon*

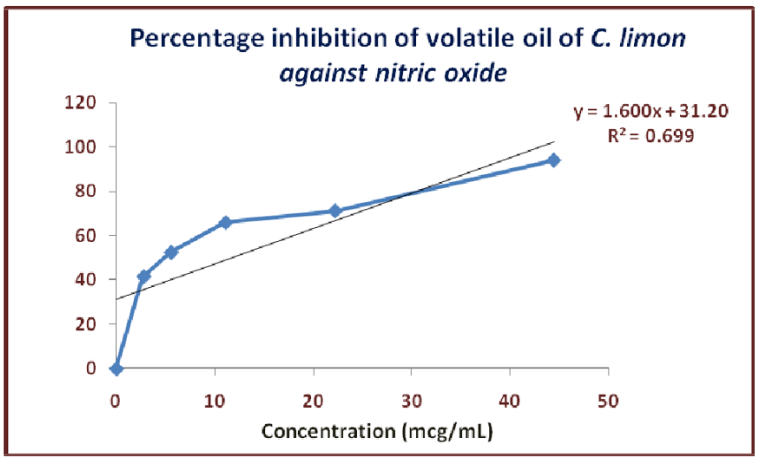

Fig.2: Percentage inhibition of Volatile Oil of *C.limon*

4.3.2 Ferric Reducing Power Antioxidant Assay

The absorbance for volatile oil was found to be 0.410±0.010 for a concentration of 400µg/mL, while for ascorbic acid it was 0.436±0.006 at a concentration of 50µg/mL. An increase in absorbance indicates increase in the reducing power of the volatile oil.

Table–3: Total Ferric Reducing Power Assay of Ascorbic Acid and VOCL

Ascorbicacid		VOCL	
Concentration (µg/mL)	Absorbance*	Concentration (µg/mL)	Absorbance*
10	0.243± 0.003	12.5	0.252± 0.003
20	0.278± 0.005	25	0.266± 0.002
30	0.362± 0.008	50	0.280± 0.003
40	0.413± 0.003	100	0.296± 0.003
50	0.436± 0.006	200	0.317± 0.004
		400	0.410± 0.010

*Meanof threereadings±SEM

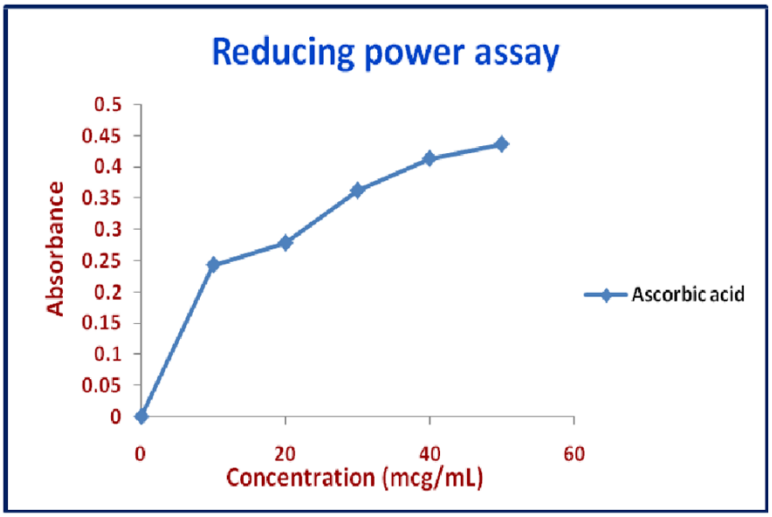

Fig. 3: Reducing Power Assay of Ascorbic Acid on PotassiumFerricyanide

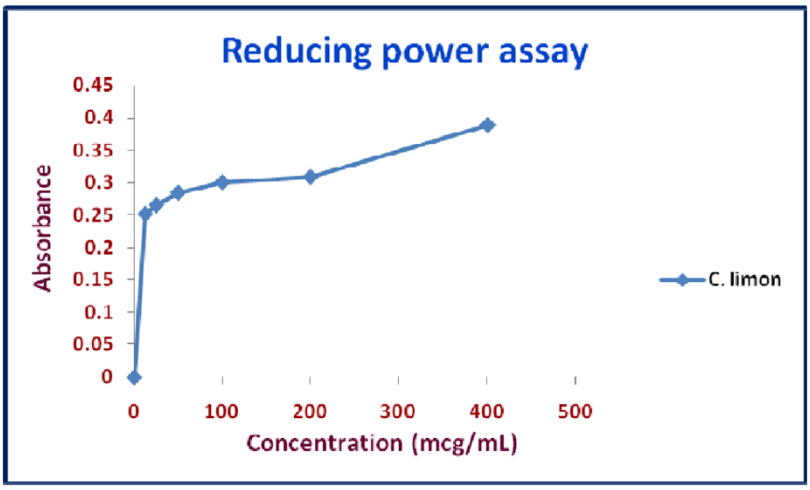

Fig. 4: Reducing Power Assay Volatile Oil of *C. limon* on Potassium Ferricyanide

The absorbance for volatile oil was found to be 0.410±0.010 for a concentration of 400µg/mL, while for ascorbic acid it was 0.436±0.006 at a concentration of 50µg/mL. Increase in absorbance indicates increase in reducing power of the volatile oil.

4.4. ANTIBACTERIAL ACTIVITY

The results obtained for the susceptibility tests of the volatile oil against various Microorganisms are presented in Tables 4 to 6 and photographic documentation about this is presented in Figs.7 to 10. From Table 4, it can be seen that there was no growth against the tested microorganisms at a concentration of15µL/disc.

Table 4: Susceptibility testing of volatile oil C. limon to various microorganisms

S.No	Name of thedrug	Concentration (µL/disc)	1	2	3	4	5	6	7	8
1	Control(DMSO)	------	+	+	+	+	+	+	+	+
2	Standard (Amikacin)	--------	+	+	+	+	+	+	+	+
3	VolatileOilof *CitrusLimon*	5µL	_	_	_	_	+	+	+	_
		10µL	_	+	_	+	+	+	+	+
		15µL	+	+	_	+	+	+	+	+

NOTE:- (+) indicates growth; (-) indicates no growth1. *Chromobacteriumviolaceum*, 2.*Escherichiacoli*, 3.*Klebsiellapneumonia*, *Proteus Mirabilis*, 5.*Shigella Flexneri*, 6.*Streptocoocuspyogenes*, 7.*Staphylococcus aureus*, 8.*Pseudomonasaeruginosa*

4.5 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was defined as the lowest concentration of volatile oil that does not allow more than 20% growth of microorganisms after incubation in Nagar at 37ºC for 18–48 hours.

Table 5: MIC of VOCL against various microorganisms

S.No.	Nameofthe microorganism	Minimuminhibitoryconcentration (µL/disc)
1	*Chromobacteriumviolaceum*	15
2	*Escherichiacoli*	15
3	*Klebsiellapneumonia*	-
4	*ProteusMirabilis*	15
5	*ShigellaFlexneri*	15
6	*Streptococcuspyogenes*	15
7	*Staphylococcusaureus*	15
8	*Pseudomonasaeruginosa*	15

From theTable15,it can be observed that the MIC for *Chromobacterium violaceum*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus Mirabilis Shigella Flexneri*, *Streptocoocus pyogenes*, *Staphyolococcus aureus*and *Pseudomonasaeruginosa* MIC was15µL/disc.

Table 6: Antibiotic disc diffusion assay against various microorganisms

S.No.	Nameofthe microorganism	Zoneofinhibition(mm)*	
		Standard	VOCL
1	*Chromobacteriumviolaceum*	20±0.01	12±0.02
2	*Escherichiacoli*	22±0.05	26±0.06
3	*Klebsiellapneumonia*	24±0.04	--
4	*ProteusMirabilis*	26±0.02	26±0.04
5	*ShigellaFlexneri*	24±0.02	12±0.02
6	*Streptococcuspyogenes*	24±0.06	24±0.05
7	*Staphyolococcusaureus*	21±0.00	24±0.03
8	*Pseudomonasaeruginosa*	22±0.08	22±0.06

* mean 2 readings±SEM

The results obtained for the antibiotic disk diffusion technique are presented in Table 1 and Figures 1 to 14. From Table 6, it can be seen that the zones of inhibition of the volatile oil of Citrus limon for the organisms tested were lower than those produced by standard amikacin. From the above study, volatile oil of citrus limon inhibited growth. The above tested organism at a concentration of 15µL/disc, and also, the volatile oil of Citrus limon was more potent against the above micro-organisms and the standard drug amikacin.

Conclusion

This research is on Citrus Limon (Linon) Burm. Explores the pharmacognostic, phytochemical and pharmacological evaluation of A., a plant with a long history of ethnobotanical use in various diseases. Researchers, physicians, traders, and farmers often overlook the economic potential of the plant. Morphological evaluation revealed its normal character, while microscopic features revealed secretory cells, crystals and vascular bundles. Preliminary phytochemical investigation revealed the presence of carbohydrates, proteins, amino acids, flavonoids, saponins, terpenoids, tannins, phytosterols, mucilage and volatile oils. The volatile oil was isolated from fresh leaves and forty-three compounds were identified, including gamma dodecalactone, α citral, trans geraniol, capraldehyde, α terpineol, cis verbenol, (R)(+) citronellal, β linalool, β cis ocimene, Contains eucalyptol. , β limonene, and β pinene. The volatile oil showed good antioxidant properties, with a broad spectrum of activity against gram-positive and gram-negative organisms such as E.coli and streptococci. The study follows the ethical principle of 3Rs (reduction, refinement, replacement) to minimize harm to vertebrate animals used in medicinal screening activities.

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