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Pharmacognostic Studies and Biological Activities of *Limonia acidissima*

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ABSTRACT

Plants are an important source of medicines and play a vital role in achieving the WHO's goal of universal well-being. People are becoming aware of the medicinal value of natural needs. Among, many medicinal plants, *Limonia acidissima L*. which belong to Rutaceae family, emerged out to be one of the most valuable plants owing multiple medicinal properties. Wood apple or *Limonia acidissima L*. contains 15% citric acid, potassium, calcium and iron salt. The seed and pulp contain fats and protein. The present work was undertaken to lay down the physio-chemical evaluation, phytochemicals and biological parameters. Later the pulp powder of wood apple was extracted with and antidiabetic activity,where later two activities were performed by bioautography ethanol by Soxhlet method. The preliminary phytochemicals analysis revealed presence of all tested compounds like Alkaloids, Tannins, Phenolic compounds, Saponins, Steroids and flavonoids. When HPTLC was performed, plates were observed under UV Light and it gave characteristic banding pattern which is unique to *Limonia acidissima L*. The ethanolic extract of *Limonia acidissima L*. showed a good antibacterial, antioxidant method.

ARTICLE DETAILS

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1. INTRODUCTION

Plants are one of the major source traditional medicines which are in uses since ages. Natural plant-based products have already gained popularity because of its low side effects andits popularity is rising with widening range of applications from pharmaceuticals to cosmetics.The plant based bioactive compounds have been an important source of drug discovery and development. Plant-derived drugs continue to be an important resource for combating serious diseases, particularly in developing countries [1]. Traditional medicines are still used to treat 60-80% of the world's population for common illnesses. Medicinal plants have a long history in many parts of World, and they continue to be useful and applicable tools for treating ailments [2]. Many research groups are now engaged in medicinal plant research because natural products of higher plants may provide a new source of antimicrobial agents as well as anticancer agents.

Wood apple also known as elephant apple or monkey fruit, which belongs to family Rutaceae.The scientific name of this is *Limonia acidissima LINN* or *Feronia elephantum.* Some of the other Indian names include Kapitha, belada hannu, kaith,Etc [2].

2. MATERIALS AND METHODOLOGY

2.1 Collection of sample

Limonia acidissima L. fruits were collected from the local market of Bangalore. The fruit was stored in a room temperature. Later the shell of wood apple is cracked and the pulp in it was collected and dried in oven for 2-3 days and grinded it using to prepare the powder.

2.2 Organoleptic evaluation

Organoleptic evaluation is a qualitative method where we use sense organs to study characteristic feature of plant sample. It involves the study on both physical and sensory characteristics like colour, smell, taste, sight and touch etc. [4].

2.2.1 Moisture

2gms of powdered sample was measured and taken in a petri dish, where the weight of both empty petri dish and the petri dish with powdered sample is noted and kept it for drying in ovenfor about one hour and allow it to cool down in desiccator

and weight of it was measured taken.

$$\text{Moisture (\%)} = \frac{(w_2 - w_3)}{(w_2 - w_1)} \times 100$$

Where,

W_1 = weight of empty petri dish W_2 = weight of sample + petri dishW_3 = weight of sample after drying

2.2.2 Determination of total ash

Two grams of powdered sample was previously ignited, dried and tared silica crucible. spreadthe materials in an even thin layer and then place it in the electric burner under low flame andignite slowly to obtain carbon residue. Keep the crucible in the furnace and adjust the maximumtemperature heat to 500-600^o c until the drug is free from carbon and then cooled. This kept in

a desiccator for 15-20 min and weighed using electronic balance and noted down the readings[3].

$$\text{Total Ash (\%)} = \frac{(B-C)}{(A)} \times 100$$

Where,

A- Sample weight in grams B-Weight of dish+ contents after ignition

C-Weight of the empty dish in grams.

2.2.3 Determination of acid insoluble ash

Add 25 ml of Hydrochloric acid to the crucible or silica dish containing the total ash that was obtained, cover with a watch glass, and bring the mixture to a gentle boil for five minutes on ahot plate or burner. Filter the insoluble matter onto the ash-free filter paper, then rinse the filterpaper several times with hot water until the filtrate is neutral or free of acid. The insoluble matter should be transferred from the filter paper to a dry crucible, ignited to a constant weightin a muffle furnace at 450–500° C, and allowed to cool for 30 minutes before being weighed immediately. Now, determine the proportion of acid-insoluble ash [4].

$$\text{Acid insoluble ash (\%)} = \frac{(B-C)}{(A)} \times 100$$

Where,

A = Sample weight in grams B = Weight of dish + content after ignition in grams

C = Weight of empty dish in grams

2.2.4 Determination of Extractive values:

2 grams of powdered plant material of both plants understudy were extracted with ethanol and water. Thus obtained extracts were allowed to dry in room temperature. After complete evaporation, weigh nature and color of the extracts were recorded [4].

$$\text{Alcohol soluble extractive value (\%)} = \frac{(B-C)}{(A)} 4 \times 100$$

Where,

A – Weight of the plant material;B – Weight of the dish + residue;

C – Weight of the empty dish.

2.3 Fluorescence studies

The fluorescent examination evaluating the behavior of the powder samples with various chemical regents such as, methanol, ethanol, water, concentrated HCl, H_2SO_4 and HNO_3 were observed in daylight as well as under UV radiation. Fluorescent analyses of all the plant powders were carried out according to the methods of Chase and Pratt [3, 4]

2.4 Powder microscopy studies

The powdered plant material was soaked in 10% Nitric acid overnight. The sample is washed with distilled water the following day. Slides are prepared by staining the soaked plant material with saffranine and observed under microscope and the images were captured [5, 4].

2.5 Preliminary phytochemical evaluation

Plants contain phytochemicals or secondary metabolites, which are biologically active compounds like steroids, flavonoids, alkaloids, phenols, tannins, saponins, and others. It is vitalto evaluate these phytochemicals as these are liable for its remedial viability. These phytochemicals are found in leaves, flowers, bark, roots, and other plant parts. To identify thechemical components in the extract, a variety of chemical tests were performed [6].

2.6 HPTLC Studies

Place sufficient quantity of suitable mobile phase in to the chromatographic/glass chamber. Close the chamber and allow it to stand for at least 30 mins at constant room temperature for complete saturation. And the silica plate was placed on the spraying chamber of the HPTLC the amount of concentration of the sample to be sprayed on the plate is given as input throughthe software ISHAAN after the process of spraying the plate is air dried at room temperature, then placed in the already saturated solvent chamber i.e., toulene:ethylacetate (9:1) and then the solvent is made to run up to the specified distance on the silica plate. After that the plate isair dried and visualized under the visible light, UV light at 254nm and at long UV at 366nm. Then the derivatization was carried with the anisaldehyde- Sulphuric acid solution. Then the graphical presentation of the rf values is designed through the software named JUST TLC [7].

2.7 Bioautography Antioxidant activity and Anti-Diabetic activity

A solvent system of toluene, ethyl acetate and formic acid (5:4:1) was taken to develop the chromatogram. Air-dry the chromatogram for the complete removal of solvents. Later spray the chromatogram with a solution of 0.2% DPPH in Methanol/Ethanol observe antioxidant activity and with iodine solution for anti-diabetic activity. Chromatograms were examined in visible light [8, 9, and 10].

2.8 Anti-Bacterial activity

The in-vitro antibacterial activity test was conducted against

three strains of bacteria i.e., *Escherichia coli, Bacillus tequilensis, Pseudomonas aeruginosa*. Where the *E.coli* anti-bacterial activity was determined by well diffusion method and *Bacillus tequilensis, Pseudomonas aeruginosa* activity was determined by disc method. Where 10 µl of gentamycinis used as a positive control and 10 µl of DMSO as standard is used and the samples are addedat different concentrations such as 10, 20 and30 µl [11, 12, and 9].

3. RESULT AND DISCUSSION

3.1 Organoleptic Evaluation

Table 3.1.1 Organoleptic Evaluation of *Limonia acidissima L.* Pulp

Plant	Colour	Texture	Odour	Taste
Limonia acidissima L.	Cream to brown	Resinous	Pleasant	Sour or sweet and astringent

3.2 Physico-chemical evaluation of *Limonia acidissima L.*

Sample	Total ash (%)		Acid insoluble ash (%)		Water Extract value(%)		Alcohol Extract Value(%)		Moisture (%)	
	OV	LM (NMT)	OV	LM (NMT)	OV	LM (NLT)	O V	LM (NLT)	OV	
***Limonia* acidissima L (Dried Pulp)**	5.4	6	0.6	1	31.75	25	29.82	12	6.65	**NA**

OV- Obtained values; LM- Limit as prescribed by Ayurvedic Pharmacopeia of India
NMT- Not more than NLT- Not less Than

3.3 Fluorescence Studies

Table 3.3.1. Fluorescence studies of *Limonia acidissima* L. Pulp

Sl. No	Sample + Reagent	UV Light
1.	Powder as such	Cream colour
2.	Powder+ Water	Yellowish Cream
3	Powder +HCl	Fluorescent Grey
4.	Powder +Con.H_2SO_4	Greenish Black
5.	Powder+HNO_3	Dark Brown
6.	Powder + Acetic acid	Yellowish Brown
7.	Powder + Ethanol	Cream
8.	Powder + Methanol	Cream

3.4 Powder Microscopy

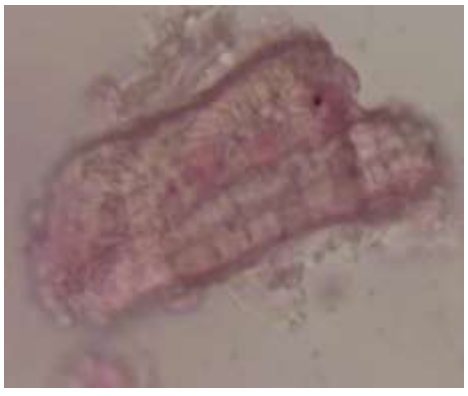


Fig 3.4.1. (a) Inner epidermis showing Glandular trichomes

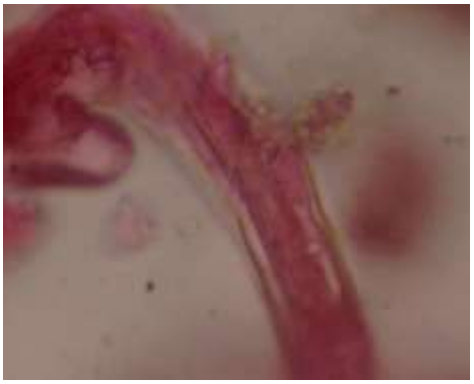


Fig 3.4.2. (b) Covering Trichomes

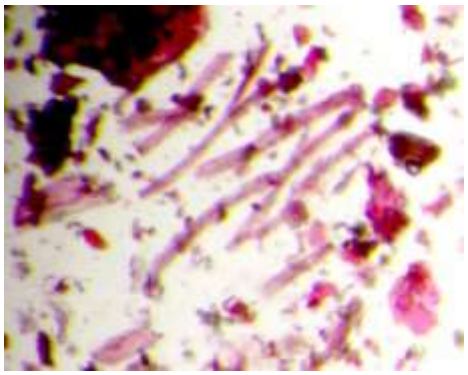


Fig 3.4.3.(c) Fibers

Fig 3.4.4. (d) Fibro vascular tissue

3.5 Preliminary Phytochemical analysis

Table 3.5.1. Preliminary phytochemicals analysis of *Limonia acidissima L.*

Phytochemicals	Results
Alkaloids	Present
Saponins	Present
Tannins	Present
Phenolic Compounds	Present
Steroids	Present
Flavonoids	Present

3.6 HPTLC Profiling

- Stationary phase: silica gel 60 F254 (E. Merck) precoated TLC Plates
- Mobile phase: toluene:ethyl acetate (9:1 v/v)
- Developing chamber: twin trough glass chamber
- Mode of application: band
- Saturation time: 30 mins
- Derivatization: Anisaldehyde-sulphuric acid

Application Table Parameter:

Track No	Vial	Volume (Ul)	Std/Sample
1	1	6	Sample
2	1	6	Sample

Development of chromatogram/ procedure

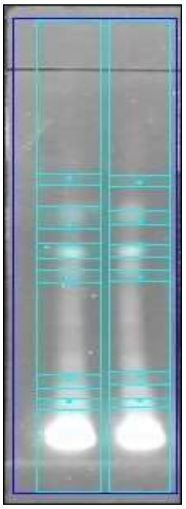
Sample solutions were applied to the Silica gel 60 F254 (E. Merck) precoated TLC plates as sharp bands by means of Aspire Spraylin sample applicator. The spots were dried in a current of air. Chromatography was carried out in a glass chamber (Aspire). The mobile phase was poured into a twin trough glass chamber whole assembly was left to equilibrate and for pre-saturation for 30 min. The platewas then developed until the solvent front had travelled at a distance of 80mm above the base of the plate, at 20ºC and 50% relative humidity. The plate was visualized for detection by observing it under UV 254,366nm and after derivatization. The densitometric scan was drawn using just TLC software attached to Aspire HPTLC

(a) Visible light

(b) Under UV at 254 nm

(c) Under UV at 365 nm

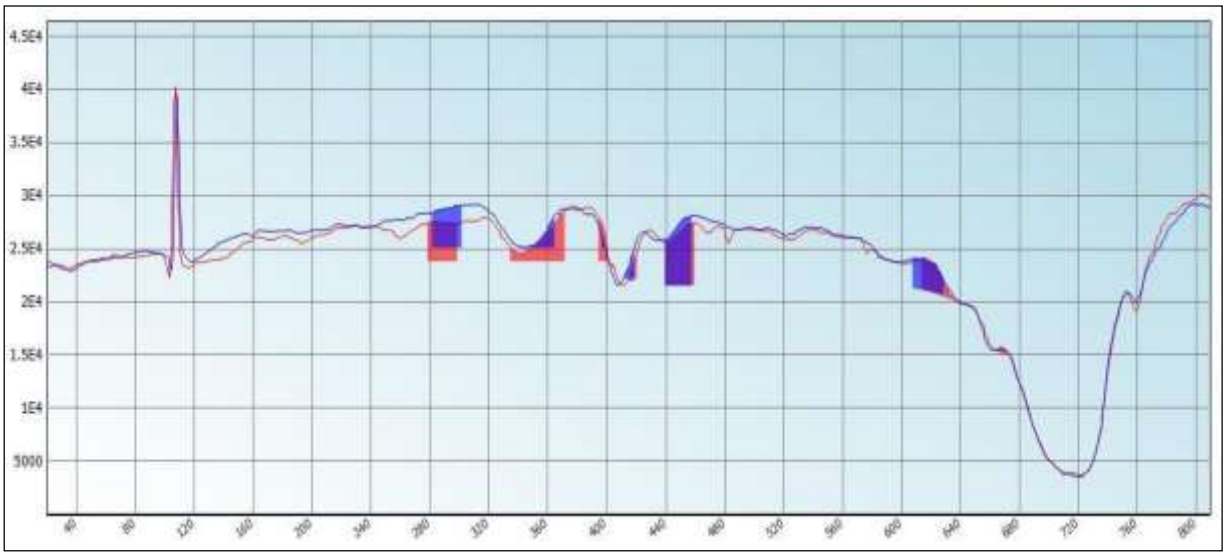
3.6.1. Plate



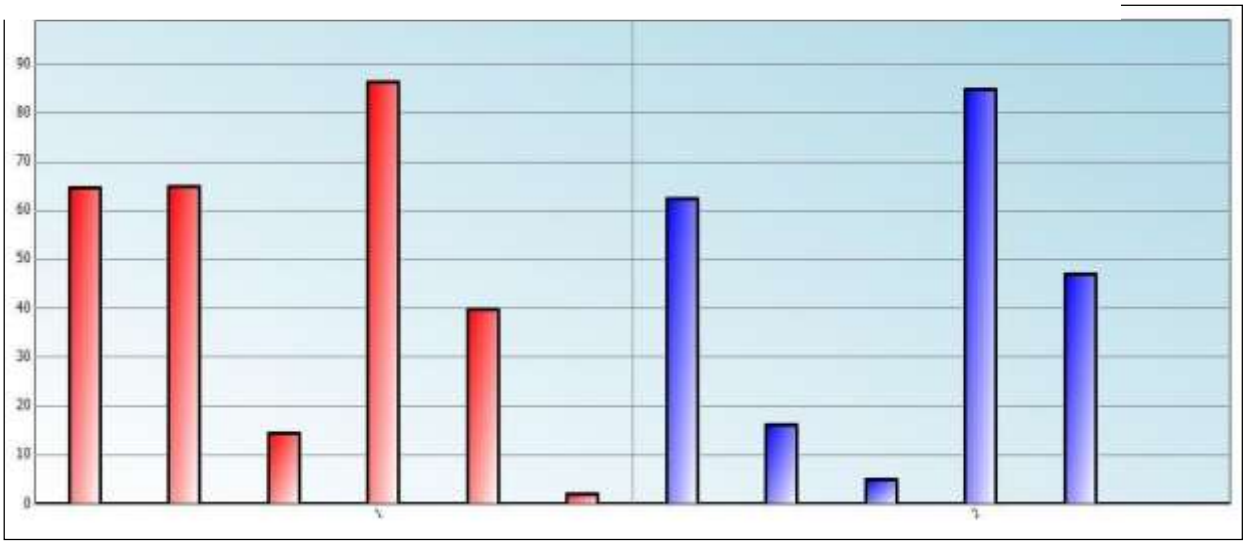
Silica coated TLC plate analysed by JustTLC software

3.6.2. Graph

VRIKSHA VIJNAN PRIVATE LIMITED BANGLORE



Graphical Analysis is performed using JUST TLC



Legend: Lane 1 Lane 2

Bar Graph analysis by JUSTTLC

Table 3.6.3Lanes

ID	Width	Bands	Volume
1	88	6	271.05
2	85	6	214.71

Table 3.6.4 Bands

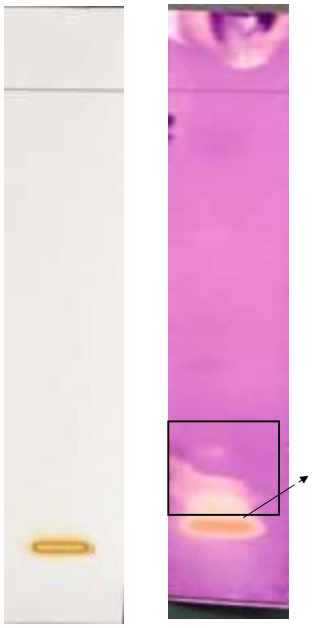
ID	Rf	Area	Volume
1_1	0.663	1760	64.43
1_2	0.565	3256	64.88
1_3	0.524	2288	14.19
1_4	0.452	1760	86.09
1_5	0.241	1936	39.57
1_6	0.195	1496	1.89
2_1	0.654	1700	62.25
2_2	0.571	2040	16.06
2_3	0.523	1870	4.93
2_4	0.454	1530	84.6
2_5	0.247	1785	46.87
2_6	0.197	1190	0

Result: The sample was plated with 6 µl concentration on TLC plate and was allowed to run in solvent system toulene:ethylacetate (9:1). Where 6 bands were observed at long UV at lightat 366nm with respective Rf values i.e., 0.663, 0.565, 0.524, 0.452, 0.241, and 0.195.

3.7 Bioautography

3.7.1. Antioxidant activity:

After spraying the TLC plate of sample with 0.2% DPPH (Diphenyl-p-picrylhydrazyl).



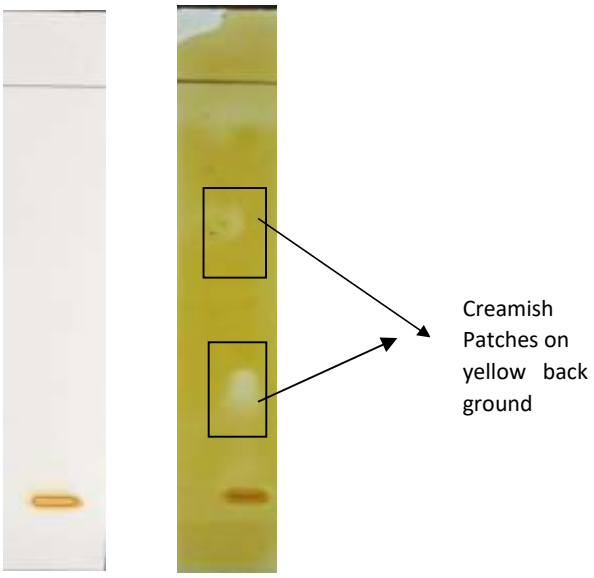
Cream patches onpurple background

After spraying DPPH

The above picture shows the antioxidant activity. The degree of antioxidant activity of the samples was determined qualitatively from observation yellowish white / cream colour bands on purple background.

3.7.2. Antidiabetic activity

After spraying the TLC plate of sample with iodine solution. The below picture shows theantidiabetic activity. The degree of anti-diabetic activity of the samples was determined qualitatively from observation of creamish colour bands on reddish background.



Visible Light **After spraying Iodine solution**

3.8 Anti-Bacterial activity

Antibacterial activity was conducted against three bacterial species i.e., *Escherichia coli, Bacillus tequilensis, Pseudomonas aruginosa* at different concentrations and the zone of inhibition.

Table 3.8.1. Antibacterial activity

	Zone of Inhibition in diameter (cms)			
Sl.No.	***Doses***	***E.coli***	***Bacillus tequilensis***	***Pseudomonas aruginosa***
1.	Standard (Gentamycin10 µl)	2.8	3	3.5
2.	Control (DMSO 10µl)	1	1.2	1.1
3.	Test samples			
a.	10 µl	0.5	0.7	1.1
b.	20 µl	0.7	0.9	1.2
c.	30 µl	0.8	1.2	1.2
d.	40 µl	1.0	-----	------

Where, *E.coli* activity is very less or almost negligible When all the doses are compared to standard Gentamycin. Where *B.tequilensis* and *P.aruginosa* showed a moderate activity as compared to standard gentamycin.

4. CONCLUSION

From the above studies, we can conclude that the traditional plants are the basis for the modernmedicines. The physico-chemical characters of powder of the fruit are evaluated which are within the range according to Ayurvedic Pharmacopeia of India. The ethanolic extract of the pulp is standardized by HPTLC fingerprinting where, 6 bands are observed at UV Light at 366nm with respective Rf values 0.66, 0.56, 0.52, 0.45, 0.24 and 0.19 and the screening of phytochemicals showed the presence of numerous potential phytochemicals such as Alkaloids,tannins, saponins, steroids, flavonoids and phenolic compounds. which are the main factors inplants to cure diseases.

The Extract of pulp shows varying antibacterial activity against the tested bacteria (i.e., both gram positive and gram-negative bacteria). The ethanolic extract of *Limonia* has shown the good antioxidant and antidiabetic activity which was revealed by bioautography method.

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