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PHYTOCHEMICAL INVESTIGATION AND IN VITRO ANTI-OXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF LIMONIA ACIDISSIMA

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ABSTRACT

This study explores the phytochemical profile and biological activities of *Limonia acidissima* (Wood Apple) root extract. Ethanolic extraction revealed the presence of alkaloids, flavonoids, saponins, phenols, and glycosides. Quantitative assays confirmed high phenolic and flavonoid content, correlating with strong antioxidant potential. The extract showed dose-dependent and significant anti-inflammatory effects via protein denaturation inhibition. Physicochemical analysis supported extract purity and pharmacological relevance. Findings validate traditional medicinal claims, highlighting the root extract as a promising natural therapeutic candidate. Future work should isolate active compounds and assess efficacy through in vivo and clinical studies.

KEYWORDS: Limonia acidissima, phytochemical screening, Anti-oxidant activity, Anti- inflammatory activity.

INTRODUCTION

Limonia acidissima L. (Rutaceae), commonly known as Wood Apple, is a tropical fruit tree native to India, Sri Lanka, and Southeast Asia. Widely used in Ayurveda and Siddha medicine, it is valued for its antioxidant, antimicrobial, and anti-inflammatory properties. Historical records mention its use in ancient Ayurvedic texts, and it holds cultural significance in religious rituals. Belonging to the Rutaceae family, Wood Apple is applied in

treating digestive disorders, respiratory issues, and infections. Its pulp is rich in vitamins, minerals, and fibre, supporting gastrointestinal health, while leaves, bark, and roots show anti-diabetic, hepatoprotective, and immunomodulatory effects. Beyond medicine, the fruit is processed into juices, jams, candies, and functional foods, offering both nutritional and commercial potential.

PLANT PROFILE:- Limonia acidissima Linn is a medicinal traditional plant belong to Rutaceae family and it is commonly known as wood apple.

GEOGRAPHICAL SOURCE: This plant is native to India, Sri Lanka, and parts of Southeast Asia. It grows widely in dry tropical regions and is cultivated as well as found in the wild.

MORPHOLOGY ODOUR: Aromatic TASTE: Sour and Astringent SHAPE AND SIZE: The fruit is round to oval, 5-12 cm in diameter EPICARP: Hard, woody, rough-textured outer shell MESOCARP AND ENDOCARP: The pulp is brownish, sticky, and aromatic, containing numerous seeds embedded in mucilage.

PLANT DESCRIPTION:-Habit: A slow-growing, deciduous tree Leaves: Pinnate, alternate, and compound with ovate leaflets Bark: Rough, brownish with fissures Flower: Small, greenish-white, occurring in clusters Fruit: Spherical with a thick woody rind that does not split upon ripening Root: Tap root system with well-developed lateral roots.

Table 1: Taxonomical classification of *Limonia acidissima.*

Kingdom	Plantae
Order	Sapindales
Family	Rutaceae
Sub Family	Aurantioideae
Genus	Limonia
Species	L. acidissima

AIM AND OBJECTIVE

AIM: To investigate the phytochemicals constituents of Limonia acidissima and evaluate their role in in-vitro anti-oxidants and anti-inflammatory activities.

OBJECTIVES: 1. Collection, authentication and extraction of Limonia acidissima.

2. Perform phytochemical screening

3. Evaluate the in vitro antioxidant activity (DPPH Assay) and Anti-inflammatory activity.

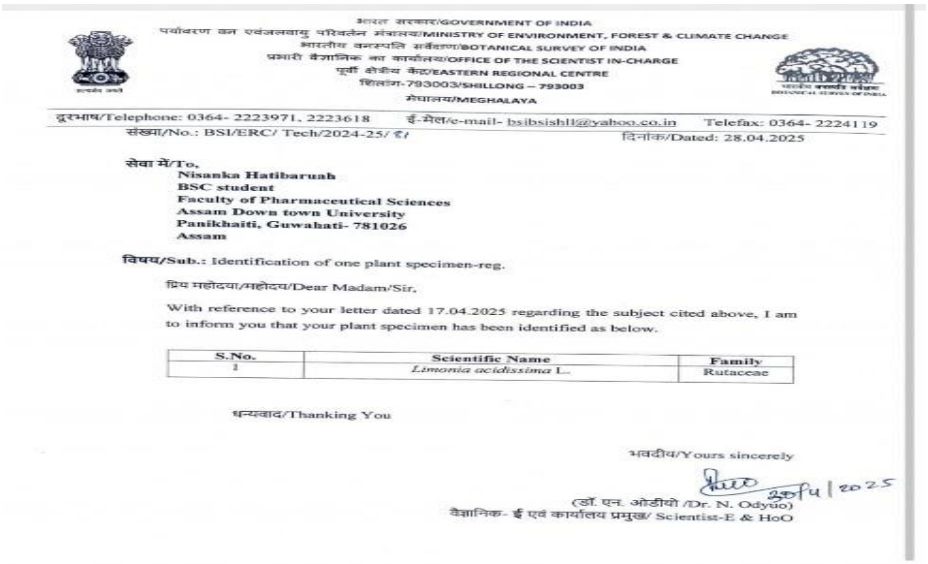
MATERIAL AND METHODS

CHEMICALS: - Folin-Ciocalteu reagent, Sodium carbonate (Na2CO3) solution (7.5% w/v), Standard Gallic acid, Aluminium chloride (AlCl3) solution (10% w/v), Sodium nitrite (NaNo2) solution (5% w/v), Sodium hydroxide (NaOH) solution (1M), Quercetin (Standard), Distilled Water, Sample Extract.

COLLECTION OF PLANTS Limonia acidissimalinn was collected from kollegal, Chamarajanagar district of Karnataka was used as sample. The freshly separated root part of the plant were cut into small pieces, washed and dried at room temperature for a week and then crushed into powder with electrical grinder and stored in an air tight container for further uses.

AUTHENTICATION

The fresh Roots of Limonia acidissima linn was collected from Chamarajanagar district of Karnataka, India and it was identified and authenticated by Dr. N. Odyuo (Botanist) and the Reference No. BSI /ERC/ Tech/ 2024-25/ 81 from the herbarium of the Botanical Survey of India (BSI), Meghalaya, Shillong, 793003.



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संख्या/No.: BSI/ERC/ Tech/2024-25/ 81 दिनांक/Dated: 28.04.2025

सेवा में/To,
Nisanka Hatibaruah
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Faculty of Pharmaceutical Sciences
Assam Down town University
Panikhaiti, Guwahati- 781026
Assam

विषय/Sub.: Identification of one plant specimen-reg.

प्रिय महोदया/महोदय/Dear Madam/Sir,

With reference to your letter dated 17.04.2025 regarding the subject cited above, I am to inform you that your plant specimen has been identified as below.

S.No.	Scientific Name	Family
1	*Limonia acidissima* L.	Rutaceae

धन्यवाद/Thanking You

भवदीय/Yours sincerely

(डॉ. एन. ओडीयो /Dr. N. Odyuo)
वैज्ञानिक- ई एवं कार्यालय प्रमुख/ Scientist-E & HoO

PREPARATION OF PLANT MATERIALS

The collected plant material was first thoroughly washed under running tap water to remove soil, dust, and other surface impurities. After initial cleaning, the roots were rinsed with distilled water to ensure removal of any residual contaminants. The clean roots were then cut

into small pieces using a clean, sharp stainless steel knife to facilitate uniform drying. These cut segments were spread on clean trays and dried under controlled conditions at room temperature (approximately 25–30°C) for 7 days, avoiding direct sunlight to prevent degradation of sensitive phytochemicals. The dried root material was then coarsely powdered using an electric grinder and stored in a clean, dry, airtight amber-colored glass container to protect it from light and moisture.

PLANT EXTRACTIONS

200 grams of dried powder was extracted using hot 90%, ethanol in a Soxhlet apparatus at 55-65°C for 72 hours. After that, the ethanol was removed using a rotary vaccum evaporator under low pressure and controlled pressure and stored extract for further uses.

Table 2: Preliminary Phytochemical Screening Of *Limonia Acidissima.*

Sl. no	Plant constituents	Test/ reagents	Petroleum Ether extract	Chloroform extract	Ethyl acetate extract	Ethanol extract	Water extract
1	alkaloids	Dragondroff reagents	present	Present	present	present	present
2	Flavonoids	Naoh test	present	Present	present	present	present
3	phenols	Ferric chloride sol.	present	Present	present	present	present
4	Glycoside	Kellar-kiliani test	Absent	Absent	Absent	present	present
5	Carbohydrate	Fehling test	present	Present	present	Absent	Absent
6	Saponin	Froth formation test	present	Present	present	present	present
7	Protein	Xanthoprotic test	Absent	Absent	Absent	Absent	Absent
8	streoids	Libermann-Burchads test	Absent	Absent	Present	present	Present
9	Tannins	Gelatin test	Absent	Absent	Present	present	present
10	Triterpene	Salkowski test	Absent	Absent	Present	present	present
11	Diterpene	Copper acetate test	Absent	Absent	present	present	present
12	Anthraquinone	Borntrager test	Absent	Absent	Absent	present	present

***IN-VITRO* ANTIOXIDANT ACTIVITY (DPPH)**

The radical scavenging activity was determined using DPPH free radical assay.[32]

- Initially, 1 mg/ml stock solution of *Limonia acidissima* and was prepared
- 25, 50, 100, 200 and 400 µg/ml concentration of *Limonia acidissima* in methanol were prepared.
- 5 ml DPPH (100 µm) solution was added varying concentration of plant extracts.
- Then the mixtures were incubated at 37±2ºc in an incubator for 30 minutes.
- Their absorbance was measured at 540 nm by using methanol as blank
- Here ascorbic acid and gallic acid were used as reference standard and triplicate readings

were taken in the study.

- Inhibitory activity percentage was calculated according to the following equation:

$\%Inhibition = A_o - A1 / A_o \times 100$

Where,

A_o = absorbance of the control, A1 = represent the absorbance of the sample

Table 3:- In-Vitro Anti-Oxidant Activity Of Limonia Acidisiima Root Extract.

Extract (LA)	Concentration	Absorbances (540nM)	%Inhibition	IC50(ug/ml) ±SD
Control		0.72		253.53±0.18
Ethanol	25	0.69	4.1666667	
	50	0.57	20.833333	
	100	0.46	36.111111	
	200	0.32	55.5555555	
	400	0.22	69.444444	

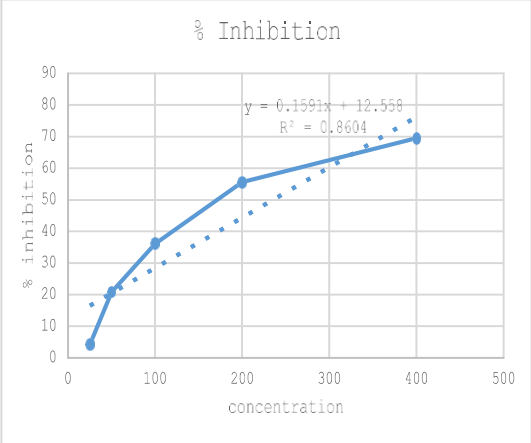

Figure 1: Percentage Inhibition Of Dpph Free Redicals By Ethanolic Extract Of Limonia Acidissima.

IN-VITRO ANTI-INFLAMATORY ACTIVITY

The reaction mixture comprised 2.8 mL of phosphate-buffered saline (PBS, pH 6.4), 0.2 mL of egg albumin (from fresh hen's egg), and 2 mL of ethanol extract of *Limonia acidissima* Linn at various concentrations (100, 200, 300, and 500 µg/mL). Double-distilled water was used to make up the volume to 5 mL. The mixtures were incubated at 37 ± 1°C in a BOD incubator for 15 minutes and then heated at 70 ± 2°C for 5 minutes in a water bath. After cooling, the absorbance was measured at 540 nm using a UV-visible spectrophotometer (Shimadzu UV-1800).

A solution without the extract served as the control, and diclofenac sodium (at a final concentration of 100 µg/mL) was used as the standard for comparison. The experiment was performed in triplicate.

The percentage inhibition of protein denaturation was calculated using the formula.
%inhibition = 100 (Vt / Vc - 1)

Where, Vt = absorbance of test sample, Vc = absorbance of control.

The extract/drug concentration for 50% inhibition was determined by plotting percentage inhibition with respect to control against treatment concentration.

Table 4: In-Vitro Anti-Inflammatory Activity Of Limonia Acidissima Root Extract.

Extracts (LA)	Concentration (µg/ml)	Absorbance (540nm)	% Inhibition	IC50 (µg/ml) ± SD
Control		0.52		
Ethanol extract of *Limonia acidissima* Linn	100	0.432	16.92	369.69 ± 0.09
	200	0.312	40	
	300	0.278	46.53	
	500	0.211	59.42	

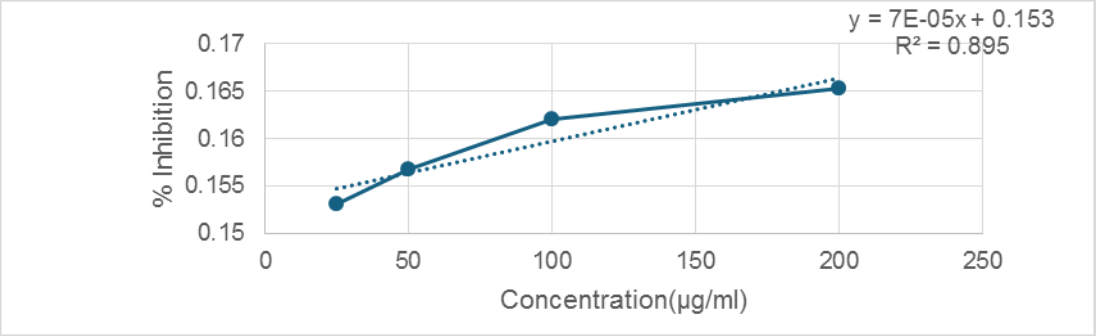

Figure 2: Percentage Inhibitiom Of Protein Denaturation By Ethanolic Root Extract Of Limonia Acidissima.

DISCUSSION AND CONCLUSION

The present study demonstrated that the ethanolic root extract of Limonia acidissima possesses promising antioxidant and anti-inflammatory activities, which may be attributed to the presence of phytochemicals such as alkaloids, flavonoids, phenols, tannins, saponins, and triterpenes. The extract exhibited appreciable total phenolic and flavonoid contents and showed concentration-dependent antioxidant activity in the DPPH assay (IC_{50} = 235.53 ± 0.18 µg/mL) along with moderate anti-inflammatory activity in the protein denaturation assay

(IC_{50} = 369.69 ± 0.09 µg/mL). These findings support the traditional medicinal use of Limonia acidissima and indicate that the plant could serve as a potential natural source of antioxidant and anti-inflammatory agents. However, further studies involving isolation of active constituents, mechanistic investigations, in vivo pharmacological evaluation, and clinical studies are required to confirm its therapeutic efficacy and safety.

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