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PRELIMINARY STUDIES ON PHYTOCHEMICAL AND ANTIBACTERIAL ACTIVITY OF *Limonia acidissima* L. PLANT PARTS

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ABSTRACT

The preliminary phytochemical analysis of *Limonia acidissima* plant parts such as bark, leaf, rind, pulp and seed showed the presence of alkaloids, flavonoids, steroids, saponins, glycosides, phenols, gum and mucilage, fixed oils and fats, resins and tannins. Among the five plant parts, pulp possessed high amount of protein and the carbohydrate content was more in seeds and rind is rich was amino acid. The methanolic extracts of *L. acidissima* plant parts were tested against *Escherichia coli* and *Staphylococcus aureus* using disc diffusion method. The extracts from different parts showed varying degrees of antimicrobial activity.

Key words: *Limonia acidissima*, stomachic, astringent, diuretic, cardiotonic, carminative, bowel infections, antidote.

INTRODUCTION

Existence of human beings on earth is possible because of the vital role played by plant kingdom. Many traditional societies all over the world value a large number of plant species for a wide variety of reasons viz., food, fibre, shelter, medicine etc. Worshipping and giving respect to sacred groves and sacred trees are ancient traditions in India.

Limonia acidissima L. (wood apple) is a member of the family Rutaceae and is a religious tree planted in temples and gardens. It is an important indigenoustree of India known for its medicinal and processing properties.

The fruits are edible and considered to be a stomachic, astringent, diuretic, cardiotonic and tonic for the liver and lungs. The leaves are aromatic and carminative and are used for the treatment of indigestion and minor bowel infections of children. The roots are sometimes given as antidote to snakebites.

The present study was undertaken to evaluate the phytochemical constituents and the antibacterial efficacy of methanolic extracts of L. acidissima plant parts.

MATERIALS AND METHODS

The present study on Limonia acidissima (L.) was carried out at the

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COLLECTION OF PLANT MATERIALS

The fruits were collected from about 20 years old tree from the Institute of Forest Genetics and Tree Breeding, Coimbatore. After harvest, the fruits are kept in the sun for 2 weeks to fully ripen. The fruits were cracked with a hammer and the rind, pulp and seeds were separated. Bark and leaves were also collected from the same tree. The samples were shade dried and powdered.

BIOCHEMICAL ANALYSIS

About 250 mg of fresh bark, leaf, rind, pulp and seeds were taken separately and they were used for the biochemical analysis. Protein content was estimated by following the method of [1]Lowry *et. al.*, 1951 and seed protein was estimated by the method of [2]Basha and Cherry, 1976. Estimation of carbohydrates and amino acids was done by using Anthrone reagent and ninhydrin ([3]Moore and Stein, 1948) method respectively.

PHYTOCHEMICAL STUDIES

The powdered materials (bark, leaves, fruit rind, fruit pulp and seeds) were subjected to extraction with 95% methanol for 12 hours using a soxhlet unit. The extract was then evaporated to dryness on water bath.

The extracts obtained as above were then subjected to qualitative tests for the identification of various plant constituents like alkaloids, flavonoids, steroids, saponins, glycosides, phenols, gum and mucilages, fixed oil and fats, resins and tannins.

ANTIBACTERIAL ACTIVITY

The antibacterial activity was carried out against *E.coli* and *Staphylococcus aureus*.

The disc diffusion method was followed for antibacterial assay. Nutrient agar plates were prepared and inoculated by spread plate method. The extracts (0.1ml) were applied to sterile Whatman No. 1 filter paper disc (10 mm) and after drying them they were placed on the agar plates. Controls were carried out with respective solvent used for extraction. After 24 hours of ± 28ÚC inoculation, the inhibition zone surrounding the discs by the diffusion of compounds was measured in mm diameter and recorded.

RESULTS AND DISCUSSION

It was observed that the amount of protein was higher in pulp when compared to other parts of the plant (Table 1). Considerably less amount of protein was noted in the bark. The carbohydrate content was higher in seeds followed by pulp, leaves, rind and bark. The total amino acid content was comparatively higher in rind and lowest in the seeds. The leaf and pulp showed nearly same amount of amino acid (Table 1). As the fruit parts (pulp and seeds) possessed high amount of carbohydrate and protein, it was traditionally a “poor man’s food” in India.

Almost all the parts of *L. acidissima* are rich in secondary metabolites (Table 2). Among the identified constituents, alkaloid was higher in bark and rind compared to other parts. Saponins, steroids and glycosides were present in all plant parts. Flavonoids are present only in pulp and seeds. Phenols were found only in bark and leaves. Gum and mucilage is present in all the plant parts

tested except leaf. Leaf, pulp and seed possessed fixed oils and fats. Resins are present only in pulp. Bark, leaf and seeds showed the presence of tannins (Table 2). The presence of alkaloids, saponins, glycosides, steroids, fixed oils and fats, phenolic compounds, tannins and gum and mucilages, is in accordance with the findings by [4]Sheeja *et al.*,(2005) in their study on comparative phytochemical studies on the leaves of *Aegle marmelos* and *Feronia elephantum*.

The developed as well as developing countries nowadays use the medicinal plants because antibiotics have proved to be a blessing in disguise due to their higher cost, drug resistant strains and their harmful side effects. Generally extacts of all plant parts effectively controlled the growth ofboth gram negative and gram positive bacteria.

Among the 5 components of *L. acidissima* studied, the pulp extract showed greater inhibitory effect on both microorganisms, followed by seeds, leaf, bark and rind (Table 3). Similar effect of fruit pulp against microorganisms was found in *Caesalpinia digyna* ([5]Elizabeth, 2003). Similarly the stem bark of *Feronia elephantum* showed the antibacterial activity against gram positive and gram negative microorganisms as reported by [6]Rahman and Gray (2002).

The present study supports the contention that the tree can be exploited as a source of "Poor man's food" and it is important to plant these trees along roadsides or in plantations to conserve as ancient treasure and also it may be a valuable resource in the discovery of natural pharmace auticals products.

Table 1. Biochemical composition of L. acidissima

Plant Parts	Protein (mg/g)	Carbohydrate (mg/g)	Amino acid (mg/g)
Bark	10.53 ± 0.09	3.23 ± 0.09	9.10 ±0.29
Leaf	14.80 ± 0.16	6.40 ±0.08	10.86 ± 0.09
Rind	13.26 ± 0.24	5.33 ± 0.04	11.17 ± 0.08
Pulp	19.33 ± 0.12	9.38 ± 0.21	10.44 ± 0.08
Seed	16.50 ±0.83	12.72 ± 0.47	5.85 ± 0.24

Table 2. Phytochemical composition (Secondary metabolites) of *L. acidissima*

Sl. No	Active Principle	Bark	Leaf	Rind	Pulp	Seed
1	Alkaloids	+++	++	+++	+	+
2	Flavonoids	---	---	---	++	+
3	Saponins	+++	+++	++	+++	++
4	Phenols	+++	+++	---	---	---
5	Gum and muciulage	++	---	+	++	++
6	Fixed oil	---	+++	---	+++	+++

+++ high, ++ medium ,+ low and — absence.

Table 3. Anitbacterial activity of *L. acidissima*

Sample	Inhibition Zone (mm)		
	Escherichia coli	***Staphylococcus aureus***	***Control***
Bark	19	22	0
Leaf	21	28	0
Rind	17	24	0
Pulp	40	43	0
Seed	35	38	0

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