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Research Article

PRELIMINARY PHYTOCHEMICAL SCREENING OF *LIMONIA ACIDISSIMA* LINN.

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ABSTRACT

Objective: To analyze the preliminary phytochemical characters of an important medicinal plant *Limonia acidissima* Linn. Methods: Studies were carried out in terms of sequential extraction in petroleum ether, methanol and water, total extractive values, powder behavior analysis of aerial parts and qualitative and quantitative estimation of phytochemicals. Results: The preliminary phytochemical analysis showed the presence of carbohydrates, proteins, lipids, alkaloids, flavonoids, terpenoids and tannins. Quantitative estimation of primary metabolites showed maximum amount of sugar, starch and phenol in stem, lipid and protein in leaf. Maximum yield were found in methanol extract (12.2%). Conclusions: These results are suggestive of primary bioactive compounds of commercially importance and may result in great interest in plants pharmaceuticals. These primary metabolites further can be used for biosynthesis of secondary metabolites or bioactive compounds.

Keywords: *Limonia acidissima,* Phytochemical, Solvents.

INTRODUCTION

Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies. Ayurveda, one of the oldest medicinal systems in the world, provides leads for a vast number of therapeutically useful compounds. Pharmacological studies have acknowledged the value of medicinal plants as potential source of bioactive compounds. Primary metabolites are of prime importance and essentially required for growth of plants like proteins, phenols, sugars, starch and lipids and are useful in flavoring, fragrances, insecticides, sweeteners and natural dyes [1]. The medicinal plants are rich in secondary metabolites which includes alkaloids, glycosides, amines, insecticides, steroids, flavonoids etc which have been used in drug and pharmaceutical industry [2].

Limonia acidissima Linn, syn. *Feronia limonia* (Rutaceae) is an aromatic, slow growing deciduous tree up to 9m tall grows all over India. Often polygamomonoecious tree with rough, spiny bark. The leaves are deciduous, alternate, dark-green, leathery, 3 to 5 inch long. The fruit is berry round to oval, globose, large, 2 to 5 inch wide, with a hard, woody rind, which is grayish-white, scurfy rind. The pulp is sticky brown, aromatic odorous, resinous, astringent, acid or sweetish, white seeds scattered through it. The fruits are used as a substitute for bael in diarrheoa and dysentery [3].

Different parts of plant are useful in treatment of several diseases such as fruits are stomachic, stimulant, astringent, diuretic, cardiotonic, tonic to liver and lungs, cures cough, hiccup, and dysentery, good for asthma, consumption, and tumors. Leaves are hepatoprotective, antioxidant, anti malarial, wound healing, analgesic and anti diabetic [4].

MATERIALS AND METHODS

Plant Material

Healthy plants of *Limonia acidissima* were collected from tonk district, Rajasthan and authenticated as *Limonia acidissima (RUBL 211890)* by the Herbarium, University of Rajasthan, Jaipur, Rajasthan, India.

Preparation of Extracts

The stem, leaf and fruit of *L. acidissima* were cut into small pieces, shade dried and powdered. The resultant was then subjected for successive extraction with petroleum ether, methanol, and water with soxhlet apparatus. The extracts were then concentrated in vacuum under reduced pressure using rotary flash evaporator, dried in desiccators and stored in refrigerator at 4°C till further use [5].

The extractive values were deduced using following formula:

Yield (%) = Dry weight of extract/Dry weight of plant powder×*100*

Preliminary Phytochemical Screening

Phytochemical screening of active plant extracts was done by following the standard method [6] for the qualitative analysis of various phytochemicals such as alkaloids, flavonoids, terpenoids and tannins.

Powder Behavior Analysis

Treatment of powder with different chemical reagents- acids like 1N HCl, H_2SO_4, HNO_3, Acetic acid and alkaline solutions like 1N NaOH and iodine was studied to detect the presence of phytoconstituents with colour changes under daylight.

Quantification of Primary Metabolites

Stem, leaf and fruit of *Limonia acidissima* were evaluated quantitatively to estimate the total levels of soluble sugars, starch, proteins, lipids and phenol following the established methods for the sugars, starch [7], lipid [8], protein [9] and phenol [10].

All experiments were repeated in triplicate and data were calculated as mean (±S.E.M).

RESULTS

The shade dried plant material subjected to sequential extraction in petroleum ether, methanol and water. Maximum yield were found in methanol extract (12.2%). Total extractive values are shown in Table 1.

Preliminary phytochemical investigation revealed that petroleum ether extract contains proteins, tannins, terpenoids and flavonoids, methanolic extract contains alkaloids, flavonoids, terpenoids, carbohydrates and proteins, aqueous extract contains flavonoids, alkaloids, terpenoids, carbohydrates and proteins (Table 2).

The powdered material of *Limonia acidissima* treated with different acids, bases and other chemicals. After treatment powder observed and fluorescence were tabulated in Table 3.

Leaf, stem and fruit of *Limonia acidissima* was evaluated quantitatively for the analysis of total soluble sugars, protein, starch, lipid and phenol (Figure 1).

Table 1: Successive solvent extractive values of *Limonia acidissima*

S. No.	Solvent	Extractive Value (mg/gdw)	Color of Extractive
1.	Petroleum Ether	7.44	Greenish Yellow Powder
2.	Methanol	12.2	Brownish Sticky
3.	Aqueous	6.22	Brownish Powder

Table 2: Preliminary Phytochemical Test for Different Extracts of *Limonia acidissima* (Obtained by Successive Solvent Extraction)

Solvents	**Petroleum Ether**			**Methanol**			**Aqueous**		
Metabolites	**L**	**S**	**F**	**L**	**S**	**F**	**L**	**S**	**F**
Protein	+	++	+++	++	+	+++	+	++	+++
Carbohydrate	-	-	-	++	+	+++	++	+	+
Tannin	+++	+++	++	-	-	-	-	-	-
Flavonoid	+++	++	+	+++	++	++	+++	++	+
Alkaloid	-	-	-	+++	++	+	++	++	+
Terpenoid	++	+	++	+++	++	++	++	++	+

+, ++, +++ Relative intensities - No reaction L-leaf, S-stem, F-fruit

Table 3: Behavior of aerial parts of (Powdered) *Limonia acidissima* on the treatment with different chemical reagents

S. No.	Treatment	Color after treatment in day light
1.	Powder+ HCl	YW-BN
2.	Powder+ Acetic Acid	GN-YW
3.	Powder+10% $NaOH+CuSO_4$	DA-GN
4.	Powder + conc. H_2SO_4	BN-BL
5.	Powder+ Acetic Acid +H_2SO_4	DA-BN
6.	Powder+ conc. HNO_3	YW- RD
7.	Powder+ conc. HNO_3+excess NH_3	YW-BN
8.	Powder+50% $FeCl_3$	DA-GN
9.	Powder+ Acetic Acid +$FeCl_3$+H_2SO_4	BL-BN
10.	Powder+5% Iodine solution	GY-BN

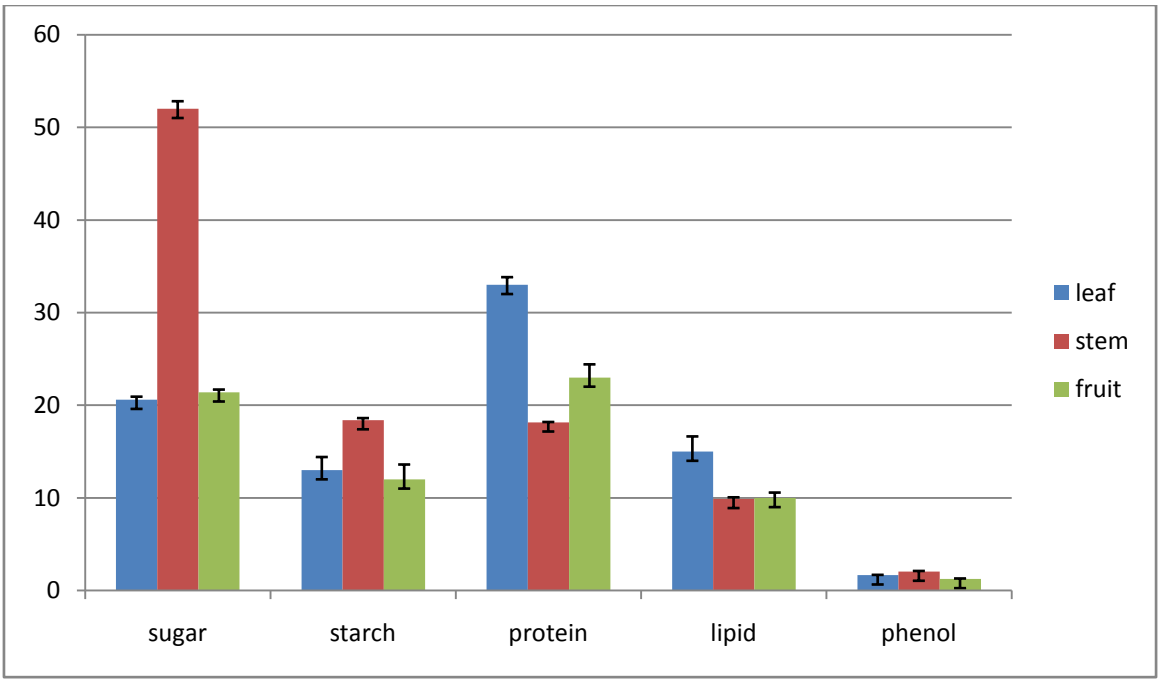

Fig. 1: Concentration of primary metabolites in *Limonia acidissima Linn*

DISCUSSION AND CONCLUSION

A primary metabolite is directly involved in the normal growth, development, and reproduction. Many primary metabolites lie in their impact as precursors or pharmacologically active metabolites in pharmaceutical compounds such as antipsychotic drugs [10].

Carbohydrates are probably the most abundant and widespread organic substances in nature, and they are essential constituents of all living things. Carbohydrates serve organisms as energy sources and as essential structural components. Highest amount of sugars was observed in stem (52±0.81mg/gdw).

Starch plays a vital role in the biochemistry of both plants and animals and has important commercial uses. It is one of the chief forms in which plants store food. Quantitative estimation of starch indicates that the content was comparatively higher in stem (18.4±0.21 mg/gdw).

Proteins are the beginners and builders of biochemical reactions. Essential body processes such as water balancing, nutrient transport, and muscle contractions require protein to function. Total levels of protein were found to be higher in leaf (33±0.82 mg/gdw).

The main biological functions of lipids include energy storage, signaling, and acting as structural components of cell membranes. The total levels of lipid were found to be higher in leaf (15±1.63 mg/gdw).

Phenolic compounds are widely distributed in the plant kingdom. External stimuli such as microbial infections, ultraviolet radiation, and chemical stressors induce their synthesis. Ecologic functions include defense against microbial pathogens and herbivorous animals [11]. Total level of phenol was found to be higher in stem (2.05±0.3mg/gdw).

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