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Phytochemical Screening and Evaluation of the Bark and Leaves of *Albizia saman*

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S. Prema*[a], Dr. Venkatachary Jayanthy[b]

[a] *Research Scholar, Research and Development Center, Bharathiar University, Coimbatore, India*

[b] *Associate Professor, PSG College of Arts and Science, Coimbatore, India*

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ABSTRACT

Globally the research on traditional medicine is gaining momentum. Herbal drugs are rapidly becoming popular in recent years as an alternative and safe therapy and plants have been an exemplary source of medicine. The aim of the present study was to identify and investigate the qualitative and quantitative phytochemical constituents present in the bark and leaves of *Albizia saman* so as to use it for medicinal purpose. The crude powder extract of bark and leaves with methanol and 50% hydroethanol were used for phytochemical screening. Soxhlet extraction known for its high efficiency was used. The qualitative analysis revealed the presence of alkaloids, flavonoids, coumarin, saponin, tannins, terpenoids, phenolic compounds and cardiac glycosides in both bark and leaves extract of *Albizia saman*. Quantification revealed cardiac glycosides as the prominent phytoconstituent of bark. Comparing the extraction of bark and leaf, it was observed that the hydroethanolic extract of the bark was good and methanolic extraction was best suited for leaves for phytochemical extraction. Presence of cardiac glycosides, saponins, flavonoids and phenols in the bark and leaves can be exploited for the use of Albizia *saman* as a potent medicinal plant and can be beneficial in the pharmaceutical and alternative medicine industries.

INTRODUCTION

Medicinal plants are considered as natural chemical factories since they are the sources of organic chemical constituents and the richest resource of drugs of traditional medical systems. Phytochemicals are naturally occurring bioactive compounds having a defense mechanism to cure and protect from various diseases [1]. They are of two categories i.e., primary and secondary constituents. Primary constituents have chlorophyll, proteins, sugar and amino acids whereas secondary constituents contain terpenoids, alkaloids, flavonoids, phenolic compounds and other metabolites which exhibit various important pharmacological activities such as antioxidant, anti-inflammatory, anti-atherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, and antiviral activities [2]. Furthermore, ingestion of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes and other diseases associated with ageing. In recent years, medicinal plants have been extensively investigated for the treatment of various diseases and fewer side effects on its use are catching a trend all over the world [3].

Samanea saman (Jacq) Merr also called *Albizia saman* is the plant of interest which belongs to the family Leguminosae and is popularly called as rain tree. *Albizia* species is a socially significant large tropical tree which usually reaches a height of 50-80 feet with rough wrinkled bark and a symmetrical broad umbrella shaped canopy about 80 m wide, making this species a beautiful choice for a shade tree. The evergreen leaves are alternately arranged along twigs and have a prominent swelling (pulvinus) at the petiole base. Flowers are produced near the end of twigs in clusters on a green hairy stalk with many small tubular pinkish-green flowers. Mature pods are black-brown in colour measuring 10–20 cm long, 15–19 mm wide, straight or slightly curved, not dehiscing but eventually cracking irregular and filled with a sticky, brownish pulp that is sweet and edible [4-5].

The tree largely reports to possess medicinal properties to treat several ailments. The root decoction is used in hot baths for stomach cancer in Venezuela. Rain Tree is a traditional remedy for colds, diarrhea, headache, intestinal ailments and stomachache. The leaf infusion is used as a laxative in the West Indies; seeds are chewed for sore throat. The alcoholic extract of the leaves inhibits *Mycobacterium tubercle growth*. In Colombia, the fruit decoction is used as a sedative. Based on the literature survey and traditional use, this study has been initiated to investigate, validate and exploit the presence of the phytochemical constituents of the leaves and bark of *Albizia saman*. The results of the study can establish the

phytochemical constituent of Albizia *saman* and shall emphasize the commercial use of this tree for pharmaceutical companies in the production of new drugs for curing of various diseases [6-7].

MATERIALS AND METHODS

Collection of plant material

The leaves and barks of *Albizia saman* were collected from Aravind Nagar, Puduchatram of Dindigul district and the specimen were deposited and authenticated by Botanical Survey of India, Southern Regional Centre, T.N.A.U. Campus, Coimbatore.

Extraction of the plant material

The fresh plant materials were washed with running tap water and dried in shade. The dried leaves and bark of *Albizia saman* were ground to coarsely powder. The coarse powder (100 g) was then subjected to successive extraction in 600 ml of each solvent (Methanol and 50% Hydroethanol) by using Soxhlet apparatus. The collected extracts were stored and then taken up for further investigations.

Phytochemical screening

Qualitative analysis

The preliminary qualitative phytochemical screening with the bark and leaves of *Albizia saman* were done with methanol and 50% hydroethanolic extract. Screening for secondary metabolites such as alkaloids, flavonoids, coumarins, saponins, steroids, tannins, terpenoids, phenols and cardiac glycosides were done according to standard phytochemical methods [8].

Test for Alkaloids:

For the confirmation of alkaloids, Dragendroff's test was performed. To 1 ml of each extract, 1 ml of marquis reagent, 2 ml of concentrated sulphuric acid and few drops of 40% formaldehyde were added and mixed; appearance of dark orange or purple colour indicates the presence of alkaloids.

Test for Flavonoids:

Shinoda test, Lead acetate test and alkaline reagent tests were conducted for the flavonoid determination. 2 ml of each extract was added with few drops of 20% sodium hydroxide, formation of intense yellow colour is observed. To this, few drops of 70% dilute hydrochloric acid were added and yellow colour was disappeared. Formation and disappearance of yellow colour indicates the presence of flavonoids in the sample extract.

Test for Saponins:

Foam and lead acetate tests were carried out for indicating the presence of saponins. To 2 ml of each extract, 6 ml of distilled water were added and shaken vigorously; formation of bubbles or persistent foam indicates the presence of saponins.

Test for Steroids:

Liebermann-Burchard test was carried out for steroids. While performing test to 2 ml extract with chloroform. 1-2 ml acetic anhydride few drops cons. H_2SO_4 was added from the side of test tube. Steroids were indicated by reddish brown coloured ring at the junction of two layers.

Test for Coumarins:

In a test tube, 2 ml of each of the test solution were placed and covered with filter paper moistened with dilute sodium hydroxide (NaOH), then heated on water bath for a few minutes. The filter paper was examined under UV light, yellow fluorescence indicated the presence of coumarins.

Test for Tannins:

To 2 ml of each extract, 10% of alcoholic ferric chloride was added; formation of brownish blue or black colour indicates the presence of tannins.

Test for Terpenoids:

Salkowski's test: To 1 ml of extract of each solvent and add 0.5 ml of chloroform followed by a few drops of concentrated sulphuric acid, formation of reddish brown precipitate indicates the presence of terpenoids in the extract.

Test for Phenols:

Ferric chloride test: To 2 ml of each extract, 2 ml of 5% aqueous ferric chloride was added; formation of blue colour indicates the presence of phenols in the sample extract.

Test for Cardiac Glycosides:

Keller-Kilian's test: To 1 ml of each extract, 0.5ml of glacial acetic acid and 3 drops of 1% aqueous ferric chloride solution were added, formation of brown ring at the interface indicates the presence of cardiac glycosides in the sample extract.

Quantitative analysis

For quantification of various phytochemical Constituent's standard procedures for estimation were performed based on Gracelin *et al*. [9]. Parameters like alkaloid, flavonoids, saponins, tannins, terpenoids, phenols and cardiac glycosides were selected as gold standards.

Estimation of alkaloid:

50 µl sample extract was made up to 5 ml with water and 2 ml of Draggendorff's reagent was added and centrifuged at 5000 rpm. To the precipitate, 2 ml of 1% sodium sulphite was added and centrifuged again. With the precipitate 2 ml of conc. nitric acid and 5ml of 3% thiourea were added and absorbance was read at 435 nm. Atropine is used as a standard material and compared the assay with Atropine equivalents (AE). The total alkaloid content was calculated from standard curve against absorbance and expressed as mg of AE/g of extract [10].

Estimation of flavonoids:

To the extract, 0.1 ml 10% aluminium chloride, 0.1 ml sodium potassium tartrate and 2.5 ml distilled water was added, vigorously shaken and incubated at room temperature for 30 mins and absorbance was read at 415 nm. The total flavonoids Content was expressed as milligrams of quercetin equivalents (QE)/g of extract [11].

Estimation of saponins:

50 µl of plant extract was added with 250 µl of water, 250 µl of vanillin reagent and 2.5 ml of 72% sulphuric acid and mixed and incubated in boiling water bath for 10 min at 60˚C. The

solution is cooled in ice cold water and read at 544 nm. Diosgenin is used as a standard material and compared the assay with Diosgenin equivalents (DQ)/g of extract.

Estimation of tannins:

To 100 µl of sample, 7.5 ml of water, 0.5 ml of phenol reagent, 1 ml of 3.5% sodium carbonate was added and made up to 10 ml and incubated at RT for 30 mins and read at 700 nm. The tannin content was expressed in terms of mg of tannic acid equivalents (TAE) /g of extract.

Estimation of terpenoids:

To 200 µl of sample extract, 1.5 ml chloroform was added and vortexed for 3-4 mins. To which 100 µl of conc. Sulfuric acid was added and incubated at RT for 2 hours. The supernatant was discarded and to the pellet, 1.5 ml of 95% methanol was added, mixed well and read at 538 nm. The terpenoids content was expressed in terms of mg of linalool equivalents (LE) /g of extract.

Estimation of phenols:

50µl sample extract was mixed with 150 µl water. To which 1 ml of Folins reagent and 1 ml of 7.5% sodium carbonate was added and incubated for 2 hours and read spectrophotometrically at 726 nm. Total phenol content was expressed as mg of Gallic acid equivalents (GAE) /g of extract.

Estimation of cardiac glycosides:

1g of sample was mixed with 5ml of aqueous methanol, allowed to stand for 10 mins and filtered. Take 1 ml of filtrate; add 1 ml of 2% 3, 5-dinitrosalicylic acid and 1 ml of 5% aqueous sodium hydroxide. Boiled for 2 mins in boiling water bath at 95-100˚C until a brick red precipitate was formed. An empty Whatman No. 42 filter paper was weighed and used for filtration. The filter paper was dried in an oven at 50˚ C till dryness and reweighed. Using the formula, cardiac glycosides was estimated.

$$\% \text{ Cardiac glycosides} = \frac{(\text{Weight of filter paper + residue}) - (\text{Weight of empty filter paper})}{\text{Weight of sample}} * 100$$

RESULTS

The study reveals the presence of phytochemicals considered as active medicinal chemical constituents. The phytochemical constituents of *Albizia saman* were qualitatively and quantitatively analyzed from leaf and bark and the results were shown in Table 1 and Figure 2 & 3. The qualitative phytochemical analysis of *Albizia saman* (leaf and bark) showed the presence of all the major phytoconstituents except steroids.

Table 1: Qualitative Phytochemical Analysis of *Albizia saman* leaf and bark extracted with Methanol and 50% Hydroethanol Solvent

Phytochemicals	Leaf extract		Bark extract	
	50% Hydroethanol	Methanol	50% Hydroethanol	Methanol
Alkaloids	+	+	+	+
Flavanoids	+	+	+	+
Coumarin	+	+	-	-
Saponin	+	+	+	+
Steroids	-	-	-	-
Tannins	+	+	+	+
Terpenoids	+	+	+	+
Phenols	+	+	+	+
Cardiac glycosides	+	+	+	+

+ = Present, - = Absent

Quantitative Phytochemical Estimation of bark and leaf extract

A comparison between the methanolic and 50% hydroethanolic extract of bark and leaves were done. Figure 1 and 2 shows the results of the quantitative estimation of the bark and leaf extracts. The bark extract revealed cardiac glycosides to be in the highest order with 1004.2 µg/g present in both methanolic and hydroethanolic extract. This was followed by phenol 800.341 µg/g of GAE, saponins 414.57 µg/g of DE, flavonoids 175.33µg/g of QE, terpenoids 72.417µg/g of LE and tannins 39.85 µg/g of TAE. Use of 50% Hydroethanol gives a better phytochemical extraction result than methanol in the bark of *Albizia saman.*

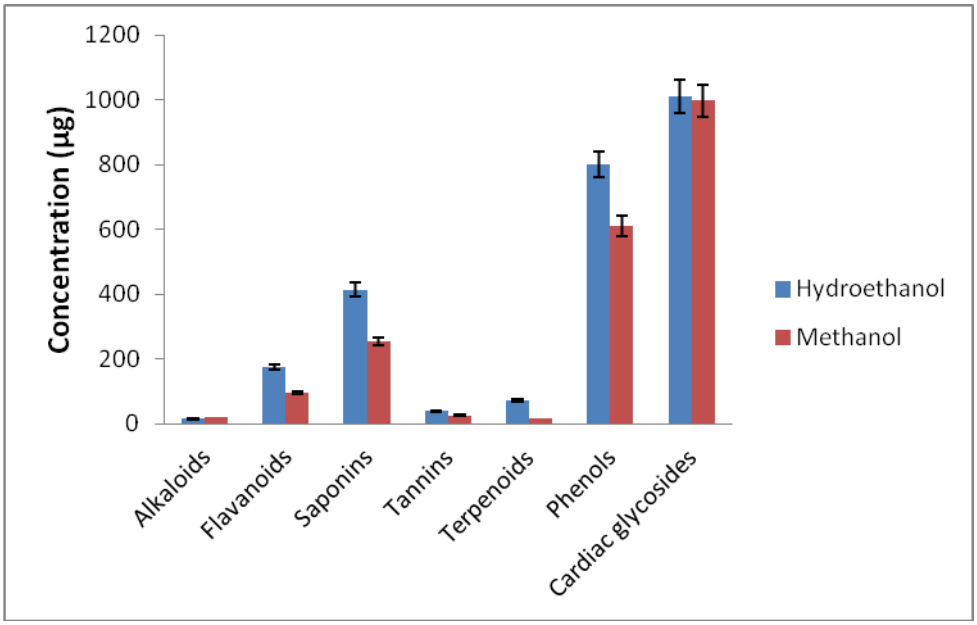

Figure 1: Quantitative phytochemical analysis of bark extract in *Albizia saman*

All the values were performed in triplicates and the results were expressed as Mean ± S.D.

Observing the phytochemical quantity in leaf extract of *Albizia saman*, it was observed that methanolic leaf extract gave better results than hydroethanolic extract. Similar to bark extract, leaf extract also had high amount of cardiac glycosides 997.5 µg/g. Other phytochemicals namely phenols 615.9 µg/g of GAE, flavonoids 578 µg/ g of QE, saponins 264 µg/ g of DE and terpenoids 105 µg / g of LE were found to be in good quantity in the leaves extract as seen in fig.2

To summate our investigation on the bark and leaf extracts of *Albizia saman* under study, 50% hydroethanolic extract is of good use in bark extraction and methanolic extraction is best suited for leaves in obtaining phytochemicals. Both the sources of the tree (bark and leaves) of *Albizia saman* were found to contain a good concentration of cardiac glycosides, flavonoids, phenols, saponins and terpenoids.

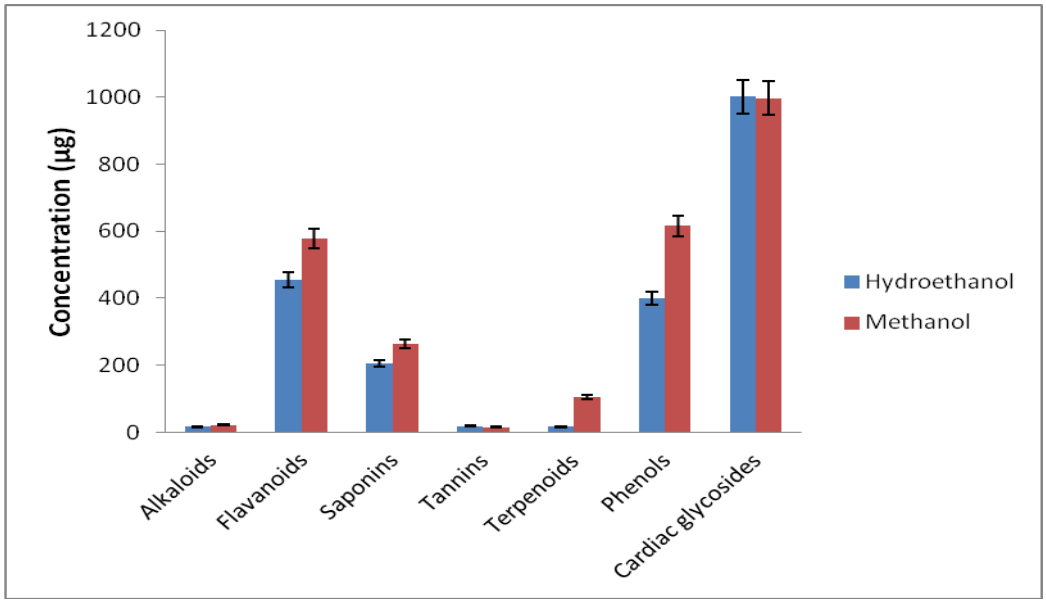

Figure 2: Quantitative phytochemical analysis of leaf extract in *Albizia saman*

All the values were performed in triplicates and the results were expressed as Mean ± S.D.

DISCUSSION

Plants play important roles in discovery associated with new beneficial therapeutic agents and have received significant focus because of their bioactive substances like antioxidants, hypoglycemic and hypolipidemic factors. India has a prosperous record associated with applying different potent natural herbs and plant based components regarding management of different diseases. Plants have invariably been exemplary source of drugs and a number of currently available drugs happen to be derived directly or indirectly from them.

According to Braunwald *et al*. [12], cardiac glycoside has been used in treatment of congestive heart failure due to its direct action which increases the force of myocardial contraction. They also explained that in the vascular system cardiac glycoside acts directly on the smooth muscles. Their effects on neural tissues and indirect effect on electrical activities of the heart and vascular resistance as well as capacitance are equally reported [12]. Both the leaf and bark extracts of *Albizia saman* were shown to contain high amount of cardiac glycosides which could be exploited for their medicinal properties.

Flavonoids tend to be most commonly known with regards to antioxidant nature. They are transformers which alter the body biochemical reactions to carcinogenic chemicals, viruses, and things that trigger allergies. Many plants display their characters for anticancer, anti-

inflammatory, antibacterial and anti-allergic nature [13], and could be useful in therapeutic roles [14].

Phenolic compounds are some of the most widespread molecules among plant secondary metabolites, are known to act as natural antioxidants [15]. Additionally, they serve as flower pigments; act as constitutive protection agents against invading organisms.

Saponins are extensively utilized in veterinary vaccines because their character as an adjuvant and helps in the improvement of immune response. Many of them are useful in intracellular histo-chemistry staining permitting antibody access to intracellular protein molecules. The results extracted from our research are usually in agreement with the studies associated with other workers in the same field [16-17].

CONCLUSION

The results of the above study revealed that the methanolic and hydroethanolic extracts of the bark and leaf samples of *Albizia saman* showed to contain major phytochemical constituents making the plant of high medicinal importance. The future perspective demands the isolation and identification of the active principle and encourages further research of its potential bioactive compounds for the evolution of preventive health care without any harmful side effects. It may be concluded that *Albizia saman* shall be considered as a promising plant with various therapeutic properties and can be further explored in curing various diseases.

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Conflict of interest

No Conflict of interest for the above work.

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