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PHARMACOGNOSTIC AND PRELIMINARY PHYTOCHEMICAL STUDY OF *LAGERSTROEMIA SPECIOSA* LEAVES

Vijayaraghavalu Sai Saraswathi*, Dhakshanamurthy Thirumalai, Pothula Kusal Yadav and Muniswamy Saranya

Pharmaceutical Chemistry Division, Department of Chemistry, School of Advanced Sciences, VIT University, Vellore- 632014 India

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ABSTRACT

Natural products have provided important therapeutic use in several areas of medicine. The Leaves of *Lagerstroemia speciosa* were reported to possess good Anti-Diabetic activity. This study deals with microscopical analysis, different extraction process, preliminary phytochemical analysis and physicochemical parameters like Ash value, Loss on drying, Extractive value, which could be used to prepare a monograph for the proper identification of the plant. Microscopical analysis showed the leaf is dorsiventral, hypostomatic, xeromorphic and thick-coriaceous. It has prominent midrib and lateral view. To identify the major compounds, the two extraction process, Soxhlet and decoction extraction procedures were adopted. Based on the literature review, four different solvents like Aqueous, 80%Ethanol, Methanol, Ethyl acetate were used for extractions. These Extracts were subjected to preliminary phytochemical analysis which reveals the presence of Tannins and Terpenoids. Powdered leaves were also subjected to fluorescence analysis using different chemicals, among them aqueous sodium hydroxide showed good fluorescence green color. Physicochemical parameters results were compared with the standard value and their results are reported.

KEYWORDS: *Lagerstroemia speciosa*, microscopically analysis, phytochemical analysis.

***Corresponding Author**

Mrs. V. Sai Saraswathi, M. Pharm., Asst. Prof., Pharmaceutical Chemistry Division, School of Advanced Sciences, VIT University, Vellore-632014 India Email: saisaraswathi@gmail.com, v.saisaraswathi@vit.ac.in

INTRODUCTION

Lagerstroemia speciosa (Lythraceae), which is called as banaba, is a common tree in Philippines. Banaba leaves have been traditionally used over thousands of years as folklore treatment by the native Indians and more recently used by the Japanese, mostly as tea preparation. Banaba leaves has the ability to reduce blood sugar level and its "Insulin like principle" made it popular herbal decoction and with others in many formulations for controlling blood sugar and weight loss[1].

Lagerstroemia speciosa have been previously reported to have hypoglycemic activity by reducing fasting blood glucose of streptozotocin-induced Diabetic rats. Apart from Hypoglycemic activity[2-6] Banaba leaf also possesses Antioxidative[7], Anti- Inflammatory[8], Anti-Obesity[9], Anti-fibrotic[10] properties. From the literature review, the present investigations were designed to meet the quality control standards which include Macroscopical, Microscopical study, phytochemical analysis and physicochemical analysis.

MATERIALS AND METHODS

Plant material collection and authentication

The leaves of *Lagerstroemia speciosa* were collected from the Garden of VIT University, Vellore, Tamil Nadu, India, in the month of May-July 2011. It was authenticated by the botanist, Dr. P. Jayaraman, Department of Botany, PARC, Tambaram, Tamil Nadu, India.

Processing and extraction of Sample

The fresh Leaves were collected for the microscopical analysis. For the extraction process, the matured leaves of *Lagerstroemia speciosa* were identified, collected and shade dried (Processing) up to 20-25 days; care should be taken by avoiding contact with the sunlight. The leaves are crushed into powder by mixer. Defatting is done by immersing the leaf powder in to petroleum ether for more than 12hrs by regular shaking. The defatted

leaves were used for extraction, by using two different methods: namely, Decoction and hot continuous soxhlet extraction and four different solvents (Aqueous, 80% Ethanol, Methanol, Ethyl acetate) were used.

PHARMACOGNOSTIC STUDIES

Plant Description

Lagerstroemia speciosa is a medium- sized tree growing to 20m tall, with smooth flaky bark. The leaves are deciduous, oval to elliptic, 8-15cm long and 3-7cm broad with an acute apex. The flowers are produced in erect panicles 20-40cm long, each flower with six white to purple petals 2-3.5cm long.

Microscopical Analysis

The fresh plant specimens for the proposed study were collected from-VIT university campus. Care was taken to select healthy plants and normal organs. The required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml). After 24hrs of fixing, the specimens were dehydrated with graded series of tertiary- Butyl alcohol (TBA) as per the schedule[11]. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60^{0}C), until Tertiary Butyl Alcohol solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12 µm, dewaxing of the sections was by customary procedure[12]. The sections were stained with Toluidine blue as per the method published[13]. Since Toluidine blue is a polychromatic stain. The staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink color to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. Wherever necessary sections were also stained with safranin and fast-green and IKI (for starch)

For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid were prepared. Glycerin mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with NaOH and mounted in glycerin medium after staining. Different cell component were studied and measured.

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic units. For normal observation bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard Anatomy books[14].

EXTRACTION

1. Decoction Extraction

Decoction[15] is a liquid preparation made by boiling the herbs in water. 25 gms of defatted leaves were taken in to a 500 ml beaker and subjected to decoction extraction by using with different solvents (250ml). It was heated on a boiling water bath for 30 minutes and filtered. The excess of solvent were removed by simple evaporation technique. The extracts contain the active ingredients.

2. Soxhlet Extraction

Extraction process is done by "CONTINUOUS HOT PERCOLATION METHOD" by using soxhlet apparatus. 25 gm of defatted leaf powder is carefully packed in to the soxhlet apparatus and extraction is done by using different solvents like Aqueous, 80% Ethanol, Ethyl acetate and Methanol. After extraction the excess solvent is removed by Simple distillation method. After distillation the solvent is recovered and stored. The crude extracts were weighed and used for the further steps.

Phytochemical Analysis

The phytochemical analysis[16-18] and fluorescence analyses were carried out based on the standard protocol.

Crude Drug Evaluation

Ash values of a crude drug

Ash values are helpful in determining the quality and purity of a crude drug, especially in powder form.

A. Determination of Total Ash

Weigh accurately about 3 g of the powdered drug in a tarred silica crucible. Incinerate the powdered drug by gradually increasing the heat until free from carbon and cool, keep it in desiccators. Weight the ash and calculate the percentage of the total ash with reference to the air dried sample.

B. Determination of Acid-Insoluble Ash

Boil the total ash obtained as above for 5min with 25 ml of dilute hydrochloric acid. Filter and collect the insoluble matter on ash less filter paper, wash the filter paper with hot water, ignite in tarred crucible, cool and keep in desiccators. Weigh the residue and calculate acid-insoluble ash of *Lagerstroemia speciosa* leaves with reference to the air-dried drug.

2. Determination of Moisture content (Loss on drying)

4gm of coarsely powered leaves of *L. Speciosa* were taken in tarred china dish. Dried in an oven at 105°C cooled in desiccator and watch[19]. After that the loss were recorded as moisture. The procedure was continued for atleast 2 common readings.

3. Extractive Value

The alcohol and water soluble Extractive value[20] were carried out based on the standard procedure.

a. Determination of Alcohol soluble extractive

Macerate about 4gm accurately weighed coarsely powdered of leaves of *L. Speciosa* with 100ml of alcohol (90%) into a stoppered flask for 24hrs, shaking frequently during first 6hrs. Filter rapidly through filter paper taking precaution against excessive loss of alcohol. Evaporate 25ml of alcoholic extract to dryness in a tarred flat bottomed shallow dish. Dry at 105°C, kept in desiccator and weigh.

b. Determination of Water soluble extractive

Macerate about 4gm accurately weighed coarsely powdered of leaves of *L. Speciosa* with 100ml of water into a stoppered flask for 24hrs, shaking frequently during first 6hrs. Filter rapidly through filter paper. Evaporate 25ml of aqueous extract to dryness in a tarred flat bottomed shallow dish. Dry at 105°C, kept in desiccator and weigh.

RESULT

Transverse Section of *Lagerstroemia speciosa* Leaves

The leaf is dorsiventral, hypostomatic, Xeromorphic and thick-coriaceous. It has prominent midrib and lateral view (fig 1.1 & 1.2) The Midrib is planoconvex with flat adaxial side and semicircular abaxial part. It is 450 µm thick; the abaxial semicircular part is 250 µm, wide. The epidermis of the midrib is thin with small squarish thick walled cells and smooth cuticle. The ground tissue in the abaxial part is thick walled, compact, small parenchyma cells. The vascular strand of the midrib is single, large comprising a circular bundle of narrow, thick walled xylem elements and abaxial are of thin phloem elements (fig.2.1). A thick layer of 4 or 5 cells of sclerenchyma encircles vascular strand which extends into thick vertical adaxial pillar (fig.1.1).

The lateral vein is also plano-convex with flat adaxial side and short, less prominent abaxial cone (fig.1.2).Similar to the midrib the lateral vein also consist of a thin layer of epidermis, compact thick walled ground tissue and circular collateral vascular bundle. A thick layer of bundle sheath fibers surrounds the vascular bundle which extends into an adaxial pillar. The lateral vein is 350 µm thick Lamina (fig 3.1) the lamina is 250 µm thick. It consists of fairly wide adaxial epidermal layer of thick walled tabular cells; the abaxial layer is thin with narrow, thin walled cells.

The stomata area located only on the abaxial epidermis. The mesophyll tissue is differentiated into adaxial thick zone of palisade cells and abaxial zone of spongy parenchyma. The pallisade part comprises two layers of narrow, cylindrical vertical, less compact layers of columnar cells; the palisade zone is 100-120 µm in height. The spongy parenchyma consists of vertical rows of small spherical cells with wide air-chambers. Veinlets of different sizes are seen all along medium part of the mesophyll tissue. Leaf-margin fig (3.2) the leaf margin is slightly bent down and conical in shape. The epidermal layer is prominent and the cells are squirish with prominent cuticle. The mesophyll tissue undifferentiated and these 4 or 5 layers of compact parenchyma cells. The marginal part is 100 µm thick Crystals (fig.2.2).

Calcium oxalate crystals are wide spreaded in the lamina. The crystals are druses or sphaerocrystals. They are spherical bodies, comprising many minute pointed crystals. The druses occur in side wide, circular mesophyll cells. Only one crystal occurs in a cell. Apart from the mesophyll cells, the druses also occur in the cells that surround the vascular strands. The mesophyll druses are 30 µm wide; the druses in the bundle sheath cells are 10 µm wide.

The phytochemical results and fluorescence analysis were tabulated as below in table no. 1, 2 and 3 respectively. The crude drug evaluation results were tabulated in table no. 4.

DISCUSSION

Microscopic evaluation is an indispensable tool for identification of medicinal herbs and is one of the essential parameter in modern monograph. From the results of microscopical analysis, the calcium oxalate crystals were found to be widely spread on the lamina. From the above preliminary analysis for both the extractions, tannins and triterpenoids were found to be predominant. Also in fluorescence analysis, with 1N sodium hydroxide it showed good fluorescence green color, this preliminary analysis would serve as valuable information for the scientist engaged in research on the medicinal properties of this plant. Studies on physiochemical constants can serve as a vital source of information for the quality control of the crude drug. Also the Physicochemical analysis states that the results were found to be within the standard limits.

The above screening would help us in future to prepare the formulation and routine assessment; this will help the quality control and the safer use of the crude drug.

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Table 1: PHYTOCHEMICAL ANALYSIS OF SOXHLET EXTRACTION

Test for	Aqueous Ex	80% Ethanol Ex	Ethyl acetate Ex	Methanol Ex
Proteins	--	--	--	--
Carbohydrates	++	+	+	++
Glycosides	--	--	--	--
Alkaloids	++	++	++	++
Terpenoids	+++	+++	+++	+++
Saponins	+	--	--	+
Tannins	+++	+++	+++	+++
Phytosterols	+	+	+	+
Flavanoids	+	++	++	++
Gums & mucilage	--	--	--	--

Note: (-): Absence; (+): Mild; (++): Moderate; (+++): Potent

Table.2: PHYTOCHEMICAL ANALYSIS OF DECOCTION EXTRACTION

Test for	Aqueous Ex	80% Ethanol Ex	Ethyl acetate Ex	Methanol Ex
Proteins	--	--	--	--
Carbohydrates	+++	++	+	++
Glycosides	--	--	--	--
Alkaloids	++	++	++	++
Terpenoids	+++	+++	+++	+++
Saponins	+	--	--	+
Tannins	+++	+++	++	++
Phytosterols	++	+	+	+
Flavanoids	+	++	++	++
Gums & mucilage	--	--	--	--

Note: (-): Absence; (+): Mild; (++): Moderate; (+++): Potent

Table 3: FLUORESCENCE ANALYSIS

Dry leaf powder	Color at Dry light	Color at Shot UV light	Color at Long UV light	Color at Fluorescence light
Normal powder	Light green	Light green	Light green	Light green
Powder +1N NaOH (Aq)	Greenish brown	Brown	Reddish brown	Fluoresces green
Powder +1N HCl	Light green	Dark green	Dark green	--
Powder +50% H_2SO_4	Light green	Green	Light green	Mild Fluoresces
Powder + 1N NaOH (Aq)	Fluoresces green	Mild Fluoresces	Fluoresces green	Fluoresces green

Table 4: PHYSICOCHEMICAL ANALYSIS

Test	Result	Limits
1. Ash value: A. Total ash value	0.966	< 5.0 As per USP
B.Acid-insoluble ash	0.775	< 2.0 As per USP
2. Loss on Drying	3.586 g	NMT 8%
3. Extractive Value: a. Alcohol Soluble b. Water soluble	0.088g	NLT 60% w/w
	0.103g	NLT 70% w/w
4. pH of 1% w/v Solution	4.8	4-6

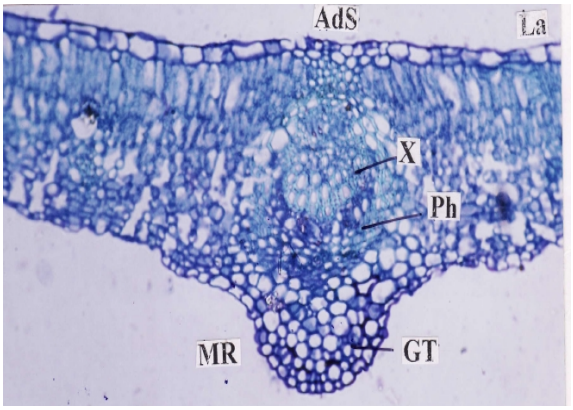

Fig.1.1: Ts of leaf through midrib

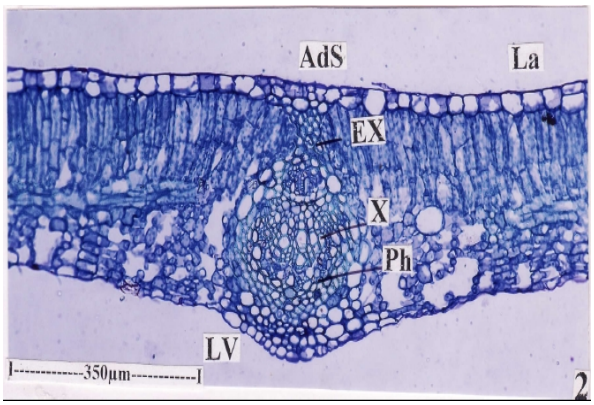

Fig.1.2: Ts of leaf through lateral view

(Ads: Adaxial side. Ex: Extension of bundle sheath sclerenchyma; La: Lamina; GT: Ground tissue; LV: lateral vein; MR: Midrib; ph: Pholem; X: Xylem).

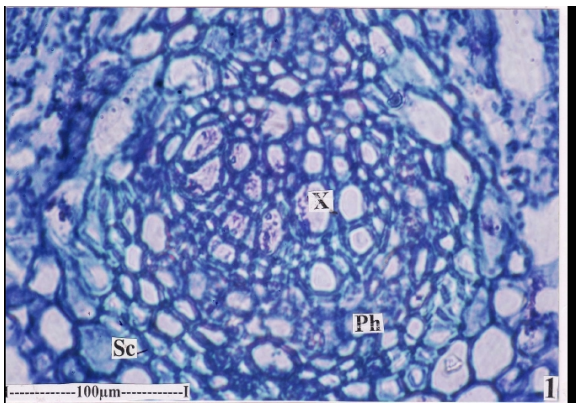

Fig.2.1: Vascular bundle of the Midrib enlarged

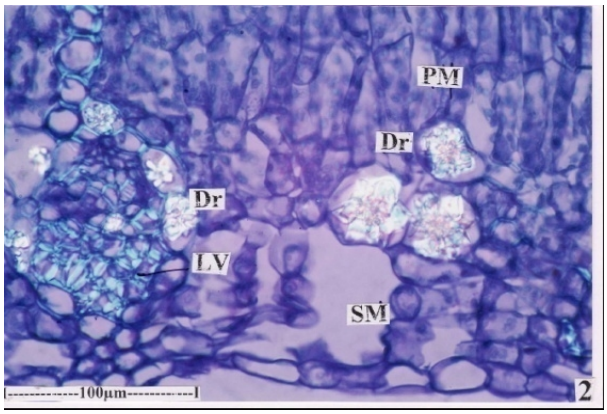

Fig.2.2: Ts of lamina shining druses (crystals)

Dr: Druses; LV: Lateral vein; PM: Palisade mesophyll; Ph: phloem; Se: sclerenchyma bundle sheath; SM: Spongy mesophyll; X: xylem).

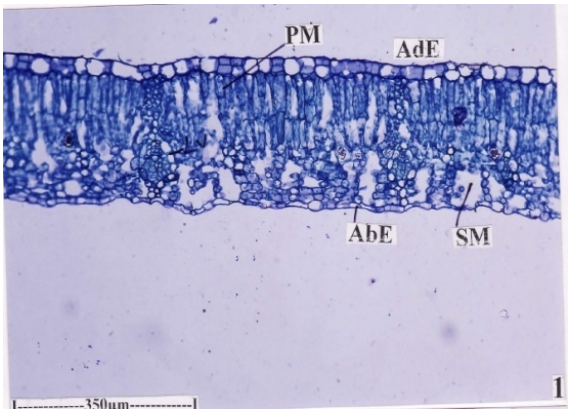

Fig.3.1.Ts of lamina

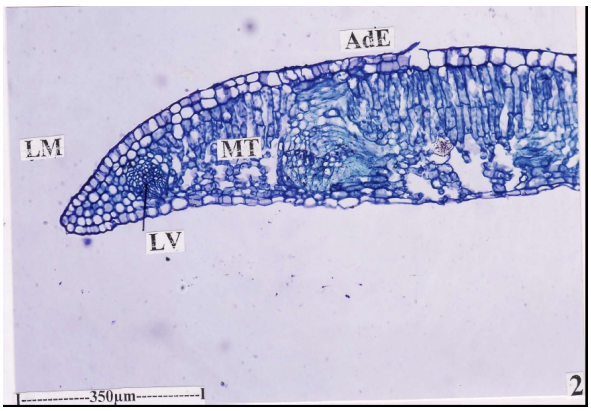

Fig.3.2: Ts of leaf margin

Abe: Abaxial epidermis; Ade: Adaxial epidermis; LM: Leaf margin; LV: Lateral Vein; Mt: Mesophyll tissue; PM: palisade mesophyll; SM: Spongy mesophyll).

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