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Phyto Pharmacognostic Study of *Lagerstroemia speciosa* - An Analytical Study

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ABSTRACT

Lagerstroemia speciosa (L.) Pers (Hindi - Jarul, Taglog - Banaba) a member of Lythraceae family is found all over India, specially in Bengal, Assam and Deccan Peninsula. The leaves of *L. speciosa* is widely used for lowering blood sugar levels in Philippines, Japan and Taiwan. Pharmacognostical investigation of leaves of *L. speciosa* was done by evaluating its morphological, microscopical studies, Physicochemical and phytochemical parameters. *Rasa Nirdharana* was done. Microscopical study revealed presence of epidermal cells contained clusters of rosette aggregate calcium oxalate crystals and few cells were mucilaginous. Lower epidermis showed the presence of anomocytic stomata. Physical constants of leaf powder showed, loss on drying - 3.8%, total ash - 6.7%, acid insoluble ash - 1.039%, water soluble extractive value - 8.88%, alcohol soluble extractive value - 4.49% and pH - 5.75. Preliminary phytochemical study revealed the presence of alkaloids, flavonoids, saponins, triterpenes, tannins, proteins and iron. *Rasa Nirdharana* confirmed the leaves are *Kashaya* in *Rasa*. The Pharmacognostical study was useful for authentication of leaves of *Lagerstoemia speciosa*.

Key words: *Lagerstroemia speciosa, Pharmacognostic Study.*

INTRODUCTION

Lagerstroemia speciosa is salubrious tree, traditionally used to lower the Blood sugar levels. Its high content of Corosolic acid, a pentacyclic triterpene makes it a potent anti-diabetic drug.[1] *Lagerstroemia speciosa* (L.) Pers is the plant species of Lythraceae family. Pride of India, Rose of India, Queen crape myrtle, Queen of flowers, Queens flower are the common names which represents alluring and colourful flowers. It is known as Jarul in Hindi and Banaba in Tagalog (A language of Philippines). Pride of India or Tamhan in Marathi is the State flower of Maharashtra.[2] L. speciosa is a semi- deciduous, small to medium sized tree with fluted trunk and flaky bark which is native Tropical Southern Asia.[3] It is an ornamental tree planted along road sides, gardens, parks and yards. The leaves, fruits and bark of the plant are widely used as a traditional medicine in Philippines, Japan and Taiwan.

Banaba is one among 69 herbal plants promoted by Philippines Department of Health. In Vietnam young leaves are used as green leafy Vegetables, matured fruits and old leaves are used as Etheno-medicine for lowering blood glucose.[4] Authenticity and purity are the major attributes for quality control and standardisation.

Rasa concept described in Ayurveda is a pharmaco-dynamic principle and serves as a tool to explain *Panchabhoutika* constitution of a drug. Hence *Rasa Nirdharana* has been done and an attempt has been made to authenticate and standardise the drug through Pharmacognostic evaluation.

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MATERIALS AND METHODS[5],[6]

Plant material

Botanically identified *Lagerstroemia speciosa* leaves were collected from the trees in Sanjeevini Herbal Garden of Government Ayurveda Medical College, Bengaluru in the month of April-May.

Macroscopic and Microscopic analysis

Leaf was studied macroscopically for important identification points i.e. odour, taste and texture. For microscopical studies, a transverse section was prepared and stained. Microscopy of powder was investigated according to method of Kokate (2010).

Physiochemical analysis

Physiochemical studies such as moisture content, total ash, foreign matter, Loss on drying, acid insoluble ash, Water soluble extractive value, alcohol soluble extractive value and pH value were determined as per WHO guidelines on quality control method for medicinal plants.

Phytochemical screening

Phytochemical screening was carried out using the standard procedures.

High performance liquid chromatography (HPLC)

HPLC analysis of terpenoids

The analysis was made (Waters model no. 550; Waters Corp., Milford, MA, USA) on C18 column (symmetry, 4·6mm×250mm) in isocratic mode with the mobile phase methanol and 0.1% formic acid in the ratio 92:8 with the RP-HPLC C-18 column at a flow rate of 1mL/min. The standard tannic acid with the concentration 0.4mg/mL and sample (10mg/mL) were dissolved in mobile phase and 20µL was injected and the elution was monitored at 210nm. The amount of elution was monitored at 210nm. The amount of terpenes present in the sample was estimated using the appropriate formula.

Rasa Nirdharana of *Lagerstroemia speciosa* (L.) Pers

Nipata Method and Taste Threshold method were carried out.

Method

1. Nipata Method: Separate proforma was given to each volunteer.

The sample (*Lagerstroemia speciosa* patra churna) was served to twenty healthy volunteers and requested to note the taste they felt in the separate proforma.

2. Threshold Method

General procedure for Threshold Test:

- Authentic herbal sample (churna) was taken
- Distilled Water was taken as solvent system since jala having Avyaktha rasa
- Concentration/ Dilution steps were set Eg. 1/3, 1/9 etc.
- Dilution of the sample were done
- The numbering of dilution was given from the start dilution no.1
- The sample size was taken as 20
- The serially assessment of the dilution was taken into consideration
- Appropriate Instructions were given to the Healthy volunteers

Solution Preparation

- The solution for the threshold was prepared by adding 10 gm of the drug in 100 ml of distilled water, stirred well and kept for 12 hours. This solution was used to pursue taste threshold of the drug.
- The procedure was carried out in Dravyaguna Department of Government Ayurveda College Bangalore. After 12 hours the solution was filtered and taken.
- Dilution was made by adding 10 ml of filtrate of infusion in 25 ml of water which is numbered as 10:25, ...10:100 dilution, likewise further dilutions were made with the difference of 25 ml, till the taste gets disappeared.

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Method of Taste Threshold Detection

- The volunteers were asked to refrain from tea, coffee or any food items before half an hour of the experiment.
- They were instructed to rinse the mouth with water. Each volunteer was subjected for tasting 5 ml of each dilution starting from higher concentration to lower concentration.
- They were advised to note down their expressions.
- The observations obtained were noted down in designed proforma based on memory about different tastes of the individuals.

Table 1: Rating scale of taste description.

Responses	(numerical values used in analysis but not shown to subjects)
1.	Same as water
2.	Doubtful if pure water
3.	A very faint taste can't say
4.	A very faint taste (mention the name of the taste)
5.	A faint taste (mention the name of the taste)
6.	A weak taste (mention the name of the taste)
7.	Clear taste (mention the name of the taste)

RESULTS

Macroscopic characteristics

In sensory evaluation appropriate parameters like taste, odour, size, shape and colour of the leaves and leaf powder were studied. The leaves are approximately 11 to 20cm long and 7 to 12 cm wide and are ovate in shape. The leaves are smooth. There are 10 to 15 pairs of side veins, looped at the margin and quite prominent below. The petiole was 1 cm long. Old leaves are orange-red colour. The flowers are 5 to 7.5 cm in diameter and bright purple in colour. The fruit is 1.5 to 2.5 cm in size and globose in shape. The odour was slightly characteristic and the taste was slightly bitter. Leaf powder is green in colour and rough to touch, smell agreeable with astringent in taste.

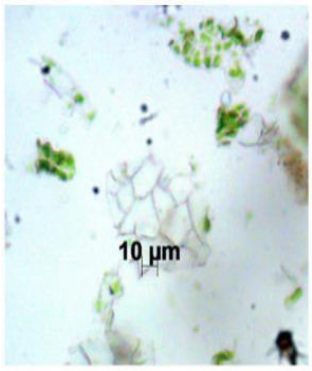
Microscopic characteristics

The transverse section of the leaf showed parenchyma, collenchyma, xylem, phloem and parenchyma containing calcium oxalate crystals. The epidermal cells were rectangular to round in shape, some cells contained clusters of rosette aggregate calcium oxalate crystals and some cells were mucilaginous. Cells of the upper epidermis are larger than lower epidermis. Lower epidermis showed the presence of anomocytic stomata. The mesophyll was well differentiated and composed of double palisade layer.

Powder microscopy

The cells observedin powder microscopy are epidermal parenchyma,vessels scalariform thickening, thick walled parenchyma, fibers, Rosette calcium oxalate crystals, vessels with reticulate thickenings and mesophyll.

Image 1: Powder microscopy of *Lagerstroemia speciosa* (L.) Pers.



Epidermal parenchyma

Vessels scalariform thickening

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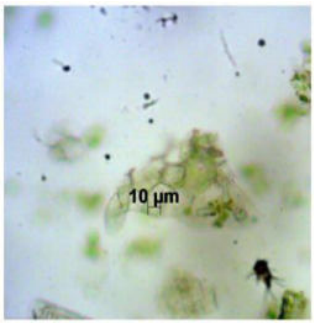
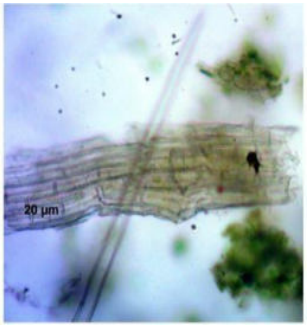
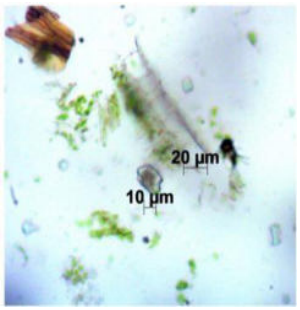
	
Thick walled parenchyma	**Fibres**
	
Rosette crystals and vessels with reticulate thickenings	**Mesophyll**

PHYSICOCHEMICAL ANALYSIS

Physical Constants of *Lagerstroemia speciosa* leaves Powder

Physical constants such as Foreign matter, loss on drying, total ash, acid insoluble ash, water soluble extractive value, alcohol soluble extractive value and pH were estimated and are presented in the table.

Table 2: Physical Constants of Lagerstroemia speciosa leaves Powder

Parameters	*Lagerstroemia speciosa*
Foreign matter	Nil
Loss on Drying	3.84 %
Total Ash	6.7%
Acid Insoluble Ash	1.039%
Water Soluble Extractive Value	8.88%
Alcohol Soluble Extractive Value	4.49%
pH Value	5.75

PHYTOCHEMICAL ANALYSIS

Phytochemical analysis of Organic and Inorganic Constituents of *Lagerstroemia speciosa* (L) Pers. leaves.

Alkaloids, Flavonoids, Saponins, Triterpenes, Tannins and Proteins are the organic phytochemicals present in both aqueous and alcoholic extracts and Iron was the Inorganic Constituent present in *Lagerstroemia speciosa* leaves.

Table 3: Phytochemical analysis of Organic Constituents *Lagerstroemia speciosa* leaves.

SN	Constituents	*Lagerstroemia speciosa*	
		Aqueous	Alcoholic
1.	Alkaloids	+	+
2.	Flavonoids	+	+
3.	Saponins	+	+
4.	Glycosides	-	-
5.	Triterpenes	+	+
6.	Tannins	+	+
7.	Carbohydrates	-	-
8.	Protein	+	+
9.	Steroids	-	-
10.	Starch	-	-

Table 4: Phytochemical analysis of Inorganic Constituents - *Lagerstroemia speciosa* leaves.

SN	Constituents	*Lagerstroemia speciosa*
1.	Iron	+
2.	Calcium	-
3.	Sulphate	-

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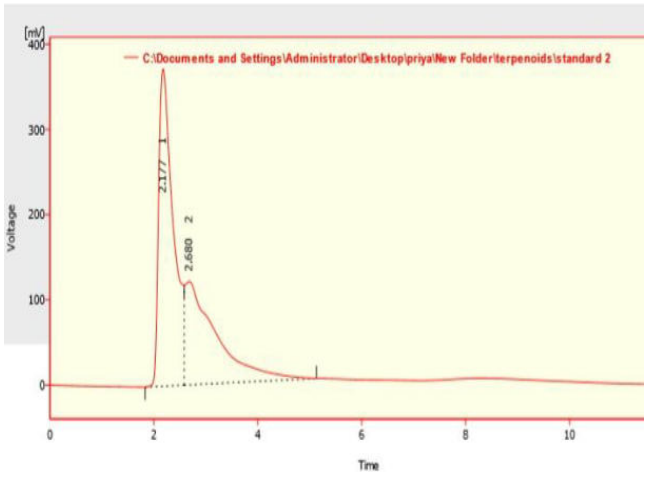
HPLC

Retention time of *Lagerstroemia speciosa pathra* (2.140) was corresponding to the retention time of standard Tannic acid (2.177). Presence of Terpenoids was observed in HPLC of *Lagerstroemia speciosa* pathra churna. Total Triterpenes in *Lagerstroemia speciosa* pathra churna was 166 mg/gm.

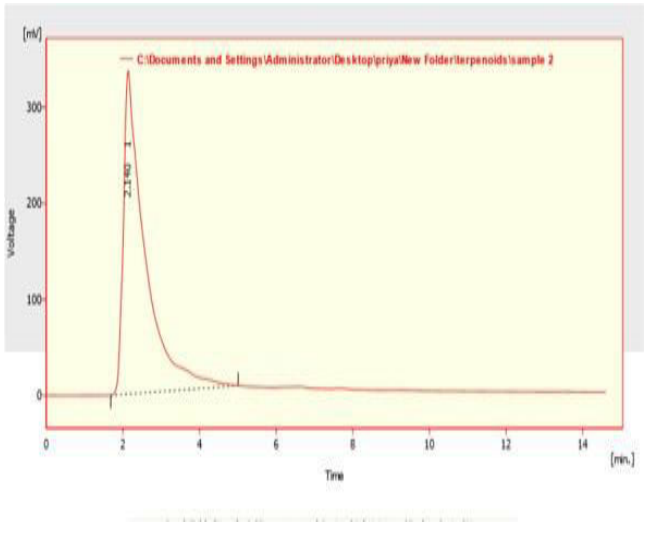
Graph 1: HPLC of Standard Tannic acid



Result Table (Uncal - C:\Documents and Settings\Administrator\Desktop\priya\New Folder\terpenoids\standard 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.177	7249.190	372.251	58.2	75.4	0.30
2	2.680	5214.871	121.283	41.8	24.6	0.61
	Total	12464.060	493.534	100.0	100.0	

Graph 2: HPLC of Lagerstroemia speciosa leaves



Result Table (Uncal - C:\Documents and Settings\Administrator\Desktop\priya\New Folder\terpenoids\sample 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.140	13220.503	336.566	100.0	100.0	0.50
	Total	13220.503	336.566	100.0	100.0	

Rasa Nirdharana

Table 5: Volunteer's experiences of *Lakshanas* of *Rasa* frequency distribution.

SN	Lakshana experienced	Lakshana type	Corresponding rasa	No. reported (N=20) with Percentage
1.	Besmears the mouth/Causes stickiness in mouth (*Vaktramanulimpati*)	Individual	*Madhura*	0
2.	Pleasant or soothing to the nose, mouth, throat, lips and tongue (*Ghrana Mukha Kanta Oshta Jihwa Prahladana*)	Individual	*Madhura*	0
3.	Causes salivation (*Aasyamaasravayati*)	Common	*Amla, Lavana, Katu*	0
4.	Cleanses the mouth (*Visadayativadanam*)	Common	*Amla, Tikta*	0
5.	Tingling sensation of teeth (*Dantanharshayati*)	Individual	*Amla*	0

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6.	Constriction or Shrinking of eyebrows and eyelids (*Akshibruvam Sankochayati*)	Individual	*Amla*	0
7.	Softens the buccal cavity (*Mardavamaa padayati*)	Individual	*Lavana*	0
8.	Burning sensation in buccal cavity and throat (*Kantakapolam Vidahati*)	Individual	*Lavana*	0
9.	Instant irritation to tongue tip (*Bhrishamudve jayati Jihwagram*)	Individual	*Katu*	0
10.	Irritation in throat and buccal cavity (*Chimichimaya ti Kantakapolam*)	Individual	*Katu*	0
11.	Secretion from nose (*Sravayati Naasikaam*)	Individual	*Katu*	0
12.	Lacrimation (*Sravayati Akshi*)	Individual	*Katu*	0
13.	Distasteful (*Arochishnu*)	Individual	*Tikta*	5 (25%)
14.	Dryness of mouth (*Vaktram Parishoshayati*)	Individual	*Kashaya*	15 (75%)
15.	Feeling of stiffness in tongue(*Jadaya tijihwa*)	Individual	*Kashaya*	15 (75%)
16.	Obstructive feeling in throat (*Kantam Badhnaati*)	Individual	*Kashaya*	15 (75%)

Table 6: Direct responses on *Rasa* - frequency distribution

No.	Rasa reported	Total respondents
2	*Tikta*	5 (25%)
3	*Kashaya*	15 (75%)

Among 20 Healthy Volunteers 15 (75%) members experienced the *Lakshanas* of *Kashaya Rasa* and 5 (25%) experienced the *Lakshanas* of *Tikta Rasa* with *Lagerstroemia speciosa Patra Churna*.

Table 7: Type of Taste threshold of *Lagerstroemia speciosa Pathra Churna.*

Threshold	L.S churna g/ml
Same as water	250 ml
Doubtful if pure water	200 ml
A very faint taste can't say	150 ml
A very faint taste (mention taste name)	125 ml Taste - *Kashaya Rasa*
A faint taste (mention taste name)	100 ml Taste - *Kashaya Rasa*
A weak taste (mention taste name)	75ml Taste - *Kashaya Rasa*
Clear taste (mention taste name)	25 ml Taste - *Kashaya Rasa*

The taste threshold for *Lagerstroemia speciosa Pathra Churna*, a clear taste, Doubtful if pure water, A very

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faint taste can't say, A very faint taste, A faint taste, A weak taste, Clear taste was at 25 ml/gm 75ml/gm 100 ml/gm, 125ml/gm, 150 ml/gm, 200 ml/gm, and 225 ml/gm respectively.

Discussion

Standardization of plant materials is a must for their therapeutic potentials and this can be achieved by modern pharmacognostic tools. Determining macroscopic and microscopic features are the preliminary steps towards establishing the identity and standardization of the drug. Rosette aggregate of calcium oxalate crystals and anamocytic stomata are the striking features of the drug. Physicochemical parameters are the key factors for detecting adultration and mishandling of the drugs. Moisture content in crude drug is an important factor with regards to shelf life, fungal and bacterial growth causes drug spoilage. Loss on drying should not be more than 10% and it is 3.84 in the present study which indicates the storage practice was done perfectly. Amount of admixture of foreign inorganic matter either by improper storage practices or intentional addition to disguise the appearance of the crude drug can be assessed by Ash value analysis. The total Ash value obtained for *L. speciosa* is 6.7% which is within the specified limits for crude drug. The physicochemical and Phytochemical evaluation of *Lagerstroemia speciosa Pathra Churna* was evaluated in triplicate method since the standards were not available. The results of preliminary phytochemical study and HPLC confirms the presence of essential secondary metabolites in the drug responsible for drug action. Triterpenoids, a class of bitter compounds are said to induce glucose uptake in peripheral tissue and its oxidation.[7] Thus, they help to reduce the blood sugar. Tannins are known to stimulate glucose uptake. They also help in the inhibition of α- amylase. They suppress and delay the absorption of carbohydrates and thus help in reducing Post Prandial blood sugar.[8] *Rasa Nirdhrana* stands in favour of predominance of *Kashya Rasa* followed by *Tikta Anurasa* of L. speciosa which supports *Kleda Meda Shoshana Karma* of the drug which is employed in the management of *Madhumeha*.

Conclusion

The leaves of *Lagerstroemia speciosa* belonging to family Lythraceae has been studied to give detailed reports on pharmacognostical studies like macroscopical and microscopical characters, powder microscopy, physico-chemical constants, phytochemicals, HPLC and *Rasa Nirdharana*. The anatomy of *Lagerstroemia speciosa* shows the presence of Rosette aggregate of calcium oxalate crystals and anamocytic stomata. The preliminary phytochemical study revealed the presence of alkaloids, flavonoids, saponins, triterpenes, tannins and proteins. HPLC confirms the presence of triterpens. The study helps in the identification of original drug.

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