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PHARMOCOGNOSTICAL AND PHYTOCHEMICAL EVALUATION OF LEAF OF *PLUMERIA RUBRA*

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ABSTRACT

Objective: To study detailed pharmacognostic profile and preliminary phytochemical investigation of the leaves of *Plumeria rubra* commonly known as Temple tree a small fugitive artistic tree belongs to the family Apocynaceae. The leaves of *Plumeria rubra Linn* used traditionally in Ayurveda in ulcer, leprosy, inflammation, rheumatism, bronchitis, cholera, rubifacient, cold and cough, sedative and analgesic. They are used as a poultice in swelling. Plant is found in throughout hotter parts of India and in its tropical regions. **Methods:** Leaf of *Plumeria rubra was* studied by Macroscopical, Microscopical, Quantitative microscopy, Physicochemical, Phytochemical analysis of leaf powder of the plant and other methods for standardization recommended by WHO. **Results**: Macroscopically, the leaves are simple, alternative, Entire undulate margin, Elliptic, obovate, oblanceolate in shape, Banchidodrome, pinnate, in venation, green in colour, characteristic odour, bitter taste,15-30 cm in long, clustered at the end of the branches Microscopically, the leaf showed the presence of palisade mesophyll, covering trichome, adaxial phloem, adaxial epidermis, paracytic stomata, laticifer cells, Ground tissue having angular parenchymatic cells, angular laticifers cells, Collateral vascular strand, uniformly thick lamina, are the diagnostic features noted from anatomical study. The investigations also included leaf surface data; quantitative leaf microscopy. Physiochemical parameters such as loss on drying, extractive values and ash values were also determined. Preliminary phytochemical screening showed the presence sterols, carbohydrates, flavonoids, protein, alkaloids, cyanogenetic glycosides, tannins, saponins, mucilage and volatile oils. **Conclusions:** The results of the study can serve as a valuable

source of information and provide suitable standards for identification of this plant material in future investigations and applications.

KEYWORDS: *Plumeria rubra*, Ayurveda, Macroscopy, Microscopy Phytochemical evaluation.

INTRODUCTION

The importance of medicinal and aromatic crops in the national economy and their potential for the rapid growth of phyto pharmaceuticals, perfumery and allied industries in India has been emphasized from time to time. Medicinal plants belong to the oldest known health care products that have been used by mankind all over the world in the form of folklore medicines or traditional or ethnic medicine.[1] The world Health organization (WHO) estimates that about 4 billion people, 80% of the world population presently use herbal medicine for some aspect of primary health care.[2] In almost all the traditional medicine, the medicinal plants play a major role and constitute the backbone of the traditional medicine.[3] Indian Materia Medica includes about 2000 drugs of natural origin almost all of which are derived from traditional system and folklore practices. Medicinal plants are inextricably inter-twined with the rich history, culture and culinary tradition of India. India has a rich and glorious ethno medical heritage.[4] Medicinal plants are also used by the codified systems of medicine such as Ayurveda, Siddha, Unani, Chinese and Tibetian systems of medicine.[5] Medicinal plant research continues to be fruitful approach for the search of new drugs. The endurance of herbal medicine may be explain often without side effects both on the illness and its symptoms. Various latest technological development has lead to increased accuracy in Estimation, Purification, Separation and Determination of principle and therapeutically active constituents in crude drugs. *Plumeria rubra* commonly known as Temple tree a small fugitive artistic tree belongs to the family Apocynaceae. It is a small deciduous tree with thick branches and copious milky juice; bark corky, fissured. Leaves 15-30 cm long, oblanceolate, thick. Flowers 5 cm across, white with yellow centre, in terminal peduncled cymes. It is commonly known as ‘graveyard flower’ in English and ‘Golainchi’ in Hindi Kshirachampa in Sanskrit and Perungali in Tamil.[6] It is native of Mexico and cultivated in gardens throughout India. It is also grown in sacred groves. Various parts of the plant are useful as medicine. In Ayurveda it is used in malarial fevers, antiseptic and stimulant.[7] The leaves of *Plumeria rubra Linn* used in ulcer, leprosy, inflammation, rheumatism, bronchitis, cholera, rubifacient, cold and cough.[8] *Plumeria rubra Linn* plant tradionally used for the treatment of diarrhea,

dysentery and typhoid.[9] Pharmacognostic studies on leaves are not adequate necessitating the present investigation. The current work aims to contribute in solving the problems of controversial drugs prevalent in Ayurveda besides helping in laying down pharmacopoeial standards. Therefore, keeping above view in mind various Macroscopic, Histological and physiochemical and quantitative microscopical studies and Preliminary phytochemical investigation of leaves of *Plumeria rubra* was carried out in present study.

2. MATERIALS AND METHODS

Collection and Authentication

Plumeria rubra leaf was collected, from in and around Palakkad, Kerala, India and authenticated by taxonomist and the plant authenticated specimen is deposited in the Department of Pharmacognosy Sanjo College of Pharmaceutical studies, Palakkad. Authentication specimen number is SCPS/P.COG/007/2017 the fresh leaves were kept for shade drying. Dried specimen was powdered using mechanical grinder and passed through 60 mesh sieve to get the powder of desired coarseness. Powdered material was preserved in an air tight container.

Pharmacognostic Standardization

Organoleptic characters such as shape, size, colour, odour, taste of Leaf was determined. Microscopic studies was carried out by preparing thin hand section of leaf with Chloral hydrate solution, stained with Phloroglucinol-hydrochloric acid (1:1) and mounted in glycerine.[10] Histochemical studies and powder microscopy were carried out to know about the inclusions and detailed anatomical characters of the material.[11]

Quantitative microscopy and Physico-chemical Evaluations

The vein islet number, vein terminal number, stomatal number, stomatal index were determined on fresh leaves using standard procedure.[12,14] The parameters were done to evaluate the proceedings of total ash; water soluble ash; acid insoluble ash and sulphated ash were calculated as per Indian Pharmacopoeia.[15] Extracts of the powdered leaf was prepared with different solvents for the study of extractive value. Fluorescence analysis was also carried out for the powder and for extract as per standard procedure.[16]

Extraction of Plant material

For preliminary phytochemical analysis, extract was prepared by weighing 1kg of the dried powdered leaf was subjected to hot successive continuous extraction with different solvents

as per the polarity, petroleum ether,benzene,chloroform, ethanol and aqueous. The extracts were filtered in each step using Whatman filters paper. The filtrate was concentrated using a rotary evaporator at low temperature (40-45^{0}C) and pressure. The presence or absence of the primary and secondary Phytoconstituents was detected by usual prescribed methods.[17,18]

Powder analysis

Preliminary analysis of the powder of the leaf powder of *P.rubra* with different chemical reagents was carried out microscopically.[19,20]

RESULTS

Macroscopical characters

Macroscopically, the leaves are simple, alternative, Entire undulate margin, Elliptic, obovate, oblanceolate in shape, Banchidodrome, pinnate, in venation, green in colour, characteristic odour, bitter taste, 15-30 cm in long, clustered at the end of the branches Flowers are 5 cm across, white with yellow centre, in terminal peduncled cyme. Fruits shape is elongated, does not attract wild life, inconspicuous and not showy, no significant litter problem, persistent on the tree. It has a thick succulent trunk and sausage like blunt branches covered with a thin grey bark. The branches are somewhat brittle and when broken, ooze a white latex, that can be irritating to skin and mucous membrane. 20-60 winged seeds are contained in a 17.5 cm (7 inches) pod.

Fig. 1: Habitat of the plant.

Fig. 2: Dorsal View of the leaf.

Fig. 3: Ventral view of the leaf.

Histological characters

The detail and systemic Pharmacognostical evaluation would give valuable information for the future studies.

Leaf

The midrib of the leaf is plano convex and fairly thick. It is 400 micrometer thick and 450 micrometer wide. The adaxial epidermis of the midrib is flat with vertically elongated slightly papillate cells. The abaxial epidermis is thin with small, thick walled papillate cells. The ground tissue is parenchymatous with compact, angular parenchymatous cells. Some of the ground cells are slightly wider and angular which are laticifers or latex secreting canals (Fig 4). The vascular strand is single small and collateral (Fig 5). It includes a few groups of xylem units with 3 or 4 xylem elements arranged in radial rows. The xylem elements are highly thick walled and angular in outline. Phloem elements occur both on the lower and upper part.

Lamina

The lamina is uniformly thick and even on both sides. The lamina is 250 micrometer thick (Fig 6). The adaxial epidermis of the lamina is thick and prominent with fairly wide semicircular cells. The abaxial epidermis is thin with narrow cylindrical cells. The mesophyll

tissue is distinctly differentiated into adaxial band of cylindrical, compact palisade cells. The lower part of the lamina has many wide irregular air chambers formed by reticulate filaments of spongy mesophyll cells.

Leaf Margin

The marginal part of the lamina is conical, thick and blunt. The epidermal layer of the margin consists of small highly thick walled cells (Fig 7). Inner to the marginal epidermis layer comprising compact, thick walled cell. The inner part of the leaf margin has similar structure as the lamina. The leaf margin is 180 micrometer thick.

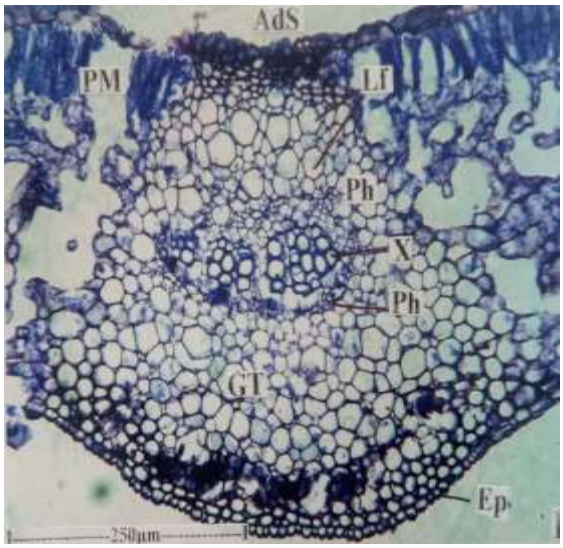

Fig. 4: T.S of leaf through midrib.

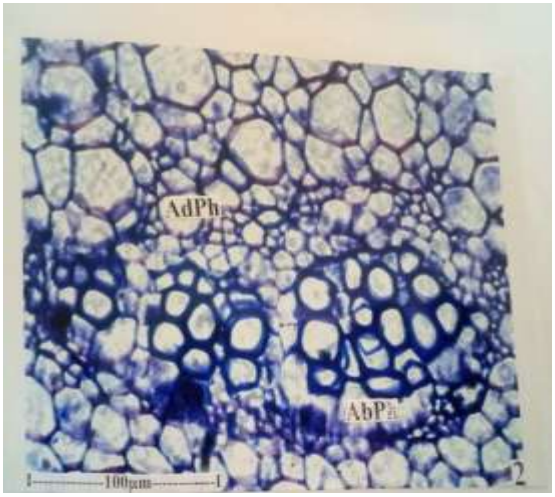

Fig. 5: T.S of Mid Rib Vascular Bundles Enlarged.

Fig. 4 and 5: PM-Palisade mesophyll, Lf-Laticifier, X-Xylem, Ads-Adaxial side, Ph-Phloem, Ep-Epidermis. Ad Ph-Adaxial Phloem, Ab Ph-Abaxial Phloem.

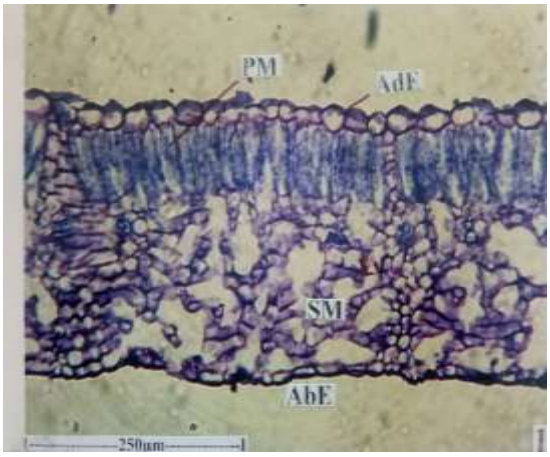

Fig. 6: T.S of lamina.

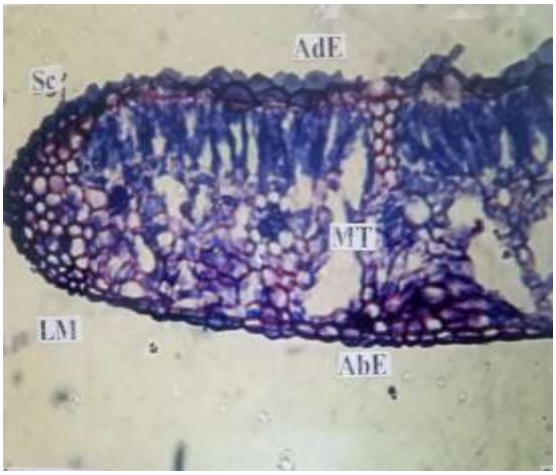

Fig. 7: T. S of midrib marginal part.

Fig 6 and 7: PM-Palisade Mesophyll, SM-Spongy Mesophyll, AdE-Adaxial Epidermis, AbE-Abaxial Epidermis, Sc-Scelerenchyma, MT-Mesophyll Tissue, LM-Leaf Margin.

Powder microscopy

Powder characteristics revealed the presence of epidermal cells with starch granules, covering trichomes, lignified fibres, epidermal cells with trichomes, paracytic stomata, vessel elements.

Quantitative microscopy

The quantitative microscopy such as vein- islet number, vein- terminal number, stomatal number and stomatal index were determined and the results were tabulated. (Table 1).

Table 1: Quantitative evaluation of the crude drug of leaf of *plumeria rubra.*

S. No	Plant constants	Values
1.	Vein islet no	15.5
2.	Vein termination no	29.33
3.	Stomatal number (upper)	16.6
4.	Stomatal number (lower)	28.66
5.	Stomatal index (upper)	6.391
6.	Stomatal index (lower)	8.835

Physico chemical features

The powdered drug was evaluated for its physico-chemical parameters like total ash values, acid insoluble ash, water soluble ash and loss on drying, and the results were tabulated (Table 2).

Table 2: Physico chemical evaluation of the crude Drug of leaf of *plumeria rubra.*

S. No	Physical Evaluation	%w/w
1.	Total Ash	6.03
2.	Acid Insoluble Ash	3.94
3.	Water Soluble Ash	2.42
4.	Loss on Drying	0.5

Fluorescence analysis of the extracts

The extracts were prepared as per their polarity in hot successive extraction technique and they were treated with reagents and the colour changes were observed under Ultra Violet light and the results were tabulated (Table 3).

Table 3: Fluorescence analysis of leaf of *plumeria rubra.*

S. No	Sample	Colour in Day Light	Colour in UV Light
1.	Petroleum ether extract	Pale green	Dark green
2.	Benzene Extract	Green	Light green
3.	Chloroform Extract	Brownish green	Green
4.	Ethanol Extract	Green	Dark Green
5.	Aqueous Extract	Brownish green	Yellowish green

Extractive values

The extracts were prepared according to the polarity and they were concentrated and their values were calculated with reference to air dried drug and the results were tabulated (Table 4).

Table 4: Extractive values of leaf of *plumeria rubra* with different solvents.

S. No	Sample	Extractability (%)
1.	Petroleum ether extract	9.5
2.	Benzene Extract	7.2
3.	Chloroform Extract	5.8
4.	Ethanol Extract	6.7
5.	Aqueous Extract	9.2

Preliminary phytochemical analysis

The leaf powder and various extracts such as petroleum ether extract, benzene extract, chloroform extract, ethanol extract and aqueous extract were subjected to preliminary phytochemical screening for their presence or absence of the constituents and the results were tabulated (Table 5).

Table 5: Preliminary phytochemical tests for drug powder and various extracts of leaf of *plumeria rubra.*

S. No	Test	Drug Powder	Petroleum Ether Extract	Benzene Extract	Chloroform Extract	Ethanol Extract	Aqueous Extract
1.	Sterols	+	+	+	+	+	-
2.	Terpenoids	-	-	-	-	+	-
3.	Carbohydrates	+	-	-	-	+	+
4.	Flavanoids	+	-	-	-	+	+
5.	Proteins	+	-	-	-	+	+
6.	Alkaloids	+	-	-	-	+	+
7.	Glycosides	-	-	-	-	-	-
8.	Saponins	+	-	-	-	+	+
9.	Tannins	+	-	-	-	+	+
10.	Mucilages	+	-	-	-	+	+
11.	Volatile Oil	+	-	-	-	-	-

+ indicates positive reaction, -indicates negative reaction.

DISCUSSION

Our study has focused on examining Pharmacognostic and Preliminary phytochemical studies of *Plumeria rubra* leaves. Normalization of the macroscopic and microscopic characteristics of the *P.rubra* drug remains essential in other to identify and avoid falsification. Microscopically, the leaf showed the presence of palisade mesophyll, covering trichome, adaxial phloem, adaxial epidermis, paracytic stomata, laticifer cells, Ground tissue having angular parenchymatic cells, angular laticifers cells, Collateral vascular strand, uniformly

thick lamina, are the diagnostic features noted from anatomical study. Organoleptic characteristics are important in drugs because they play a role in the detection of adulterated or substituted drugs.[21] Thus leaves dark green in colour, emit a very fragrant and characteristic odour, bitter taste. The powdery appearance of the crushed leaves, with a coarse texture. The micrograph performed on the powder has highlighted a number of characteristic elements namely: epidermal cells with starch granules, covering trichomes, lignified fibres, epidermal cells with trichomes, paracytic stomata, vessel elements, are diagnostic substances for drugs of plant origin. These diagnostic elements are consistent with botanical standards and WHO guidelines.[22,23] The study of physicochemical parameters such as moisture content and ash values are useful as it determines the physiological and nonphysiological state of ash, this will help to determine the possibility of microbial growth and lastly contaminant or impurities. The moisture content of the drug studied had a rate of 0.5 ± 0,1, which is below 10%. This result comply with the standards established by the International Pharmacopoeia, because this water content rate, prevent oxidation reactions, fermentation and give less chance to microbial growth and contamination in drugs.[24] Therefore, for proper conservation of drugs made from the leaves of *P.rubra*, it would be desirable to use those whose water content is less than or equal to 10%. The determination of total ash gave us a rate of 6.03 ± 0.03. This value indicates the level of minerals in drugs. Insoluble ash in hydrochloric acid gave a rate of 3.94 ± 0.02. Indeed, the ash insoluble in hydrochloric acid tells us about the contamination of the drug by siliceous elements.[25] This result is in agreement with Srikanth et al.[26] who found rate of 0.97% and 0.5% respectively. The maximum extractive value was found in petroleum ether (9.5%), followed by aqueous (9.2%), Benzene (7.20%), ethanol (6.7%) Chloroform (5.8%). All the extracts of the drug was subjected to different tests for detecting the presence of various phytoconstituents present in the drug, which revealed the presence of sterols, flavanoids, alkaloids, saponin, proteins, carbohydrate, volatile oil and tannins. Preliminary phytochemical analysis indicated a high percentage of quercetine and flavonoids and this may be one of the reasons behind the sedative activity of the plant. These parameters, which are being reported for the first time in this plant, are significant towards establishing the pharmacognostic standards for future identification and authentication of genuine plant material. Though *Plumeria rubra* is a temple tre, it is a highly reputed drug used in Ayurveda. Barring the anatomical details and preliminary phytochemical screening, rest of the pharmacognostical parameters, gives us the

clue that it can be cashed economically as well to improve the standard of health in the developing countries.

CONCLUSION

WHO has emphasized the need to ensure quality control of the raw materials used for Ayurvedic medicines by using modern techniques and by applying suitable parameters and standards. In the present study various standardization parameters such as macroscopy, microscopy (histochemical and powder), physicochemical standards, preliminary phytochemical investigation, which are beingreported for the first time in this plant and could be helpful in authentication and preparation of asuitable monograph for the proper identification of *Plumeria rubra* for the future.

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REFERENCES

1. Santhi R et al., Drug invention Today, 2010; 2(2): 112-144.
2. "Herbal Medicine Holistic online.com"
3. Mukherijee. K.P., "Quality Control of Herbal drugs", "An approach to evaluation of botanicals", 1st edition, New Delhi, Business Horizons, 2002; 2.
4. Narayana DBA, Katayar CK, Brindaraman N,B "Original System Search, Research or re-search, IDMA Bulletin", 29(17): 413-416.
5. Gopal.V, "Prospective in Herbal Medicine" – Global healthcare threats and opportunities Beyond 2005, Souvenir, ICIPG 2013.
6. Anon Wealth of India, Raw materials, Publications and Information Directorate, CSIR., New Delhi, 1982; 8: 164 – 65.
7. Sharma SK and Naresh Kumar, Int. J. of Pharmacy and Pharmaceutical Sciences, 2012; 54-57.
8. Indian Journal of Natural Products, Dec. 2009; 25(4): 15-18.
9. Scientia Horticulturae, July 27, 2011; 129(4): 840-843 DOI: 10.1016/j.scienta.2011.03.053.
10. Evans WC, Trease and Evans' Pharmaconosy, 14th ed., W.B. Sounders Company Ltd., London, 1996; 545-546.

11. Johansen DA. Plant Microtechnique. McGraw- Hill, New York, USA, 1940.
12. Indian Pharmacopoeia, Vol 2, Controller of Publication, Delhi, India, 1995; A-54.
13. Horbone, JB. Phytochemical methods-A guide to modern techniques of plant analysis, Chapman and Hall, London, 1998; pp 42, 129, 203.
14. Kokate. C.K., "Practical Pharmacognosy" 4th Edition, Vallabh Prakasham, Delhi, 1994; 115.
15. Wallis, T.E, "Practical Pharmacognosy" 6th Edition, London, J and A Churchill Ltd., 1955; 139-140, 173-174, 180-184.
16. Wallis, T.E., "Analytical Pharmacognosy" 3rd Edition, London, J and A Churchill Ltd.
17. Trease GE, Evans WC. Pharmacognosy. Williams Charles Evans as edited in 15th edition. Saunders publisher London, 2004; 137-44.
18. Khandelwal KR. Practical Pharmacognosy- Techniques and Experiments. Pune: Nirali Prakashan, 2002.
19. Reddy YSR, Venkatesh S, Ravichandra T. Pharmacognostical studies on *Wrightia tinctoria* bark, Pharmaceutical Biology, 1999; 37: 291-295.
20. Pratt PR, Chasse ER. Fluorescsence powder vegetable drugs in particular to development systems of identification. Journal of American Pharmaceutical Association, 1949; 38: 324-331.
21. Fouraste I. Le contrôle des plantes médicinales. Actualités Pharmaceutiques, 1990; (278): 55-58.
22. Kumar S, Kumar V, Prakash O. Microscopic evaluation and physicochemical analysis of Dillenia indica leaf. Asian Pac. J Trop Biomed, 2011; 1: 337-340.
23. Nasreen S, Radha R. Assessment of quality of Withania somnifera Dunal (Solanaceae): Pharmacognostical and physicochemical profile. Int J Pharm Sci., 2011; 3(2): 152-155.
24. Organisation de l'unité africaine/commission scientifique technique et de la recherche (OUA/CSTR). Pharmacopée africaine, méthodes générales d'analyses. Edn 1, Publisher, Lagos (Nigéria), 1998; 254.
25. Sambo MH. Etude du traitement traditionnel du diabète par une recette et les écorces de tronc de Manilkara multinervis Dub (Sapotaceae). Th Pharm., Univ.de Bamako, Mali, 2005; 125.
26. Srikanth K, Vikram G, Archana P, Rajinikanth M, Ram SN. Pharmacognostic and hytochemical investigations in Strychnos potatorum Linn. F. J of Pharm and Phyt, 2013; 2(4): 46-51.