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PHARMACOGNOSTICAL CHARACTERIZATION OF THE BARK OF THE PLANT *PLUMERIA RUBRA.*

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ABSTRACT

To investigate the physico-chemical parameter, preliminary phytochemical screening, fluorescence and Thin Layer Chromatographic analysis of the barks of the plant Plumeria rubra (Apocynaceae). The barks of the plant Plumeria rubra were studied by morphology, preliminary phytochemical screening, and florescence analysis of powdered drug. Other physicochemical parameters were also performed as per WHO guidelines. The results of physic-chemical parameters such as loss on drying, ash values and extractive values, fluorescence analysis, preliminary phyto-chemical screening and TLC are described in this study.

The present information on the pharmacognostic evaluation of the plant drug Plumeria rubra delivered the qualitative and quantitative parameters serve the important information for the identity and to determine the quality and purity of the plant material in the future. It also signify the important information of the closely related other species and varieties.

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INTRODUCTION

Medicinal plants play a significant role in providing primary health care services to rural people and are used by about 80% of the marginal communities around the world [1-4]. Medicinal plants have a long-standing history in many indigenous communities and continue to provide useful tools for treating various diseases. The practices of traditional medicine are based on hundreds of years of belief and observations, which predate the development and spread of modern medicine [5].

At present, there is a worldwide movement or assessing the plant resources which are of medicinal and economical value and importance. Researchers are focusing mainly on ethnobotanical & ethnomedicinal investigations to fulfil the increasing demand of herbal products. In the last few decades there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and

developed countries because of their natural origin and less side effects [6]. WHO estimate that, about 80% of the population in the developing countries depends directly on plants for its medicine [7, 8]. In India 2,000 medicinal preparations used are of plant origin. India has a rich heritage of traditional medicine and the traditional health care system have been flourishing for many centuries. Herbal drugs play an important role in health care programmes especially in developing countries. However, a key obstacle, which has hindered the acceptance of the alternative medicines in the developed countries, is the lack of documentation of research work carried out on traditional medicines and stringent quality control [9, 10]. With this backdrop, it becomes extremely important to make an effort towards standardization of the plant material to be used as medicine.

Recently, many international authorities and agencies, including the World Health Organization, European Agency for the Evaluation of Medicinal Products and European Scientific Cooperation of phytomedicine, US Agency for Health Care Policy and Research, European Pharmacopoeia Commission and Department of Indian System of Medicine have started creating new mechanisms to induce and regulate quality control and standardization of botanical medicine. For Ayurvedic medicine and other traditional medicines, newer guidelines of standardization are required, and thus pharmacognostic evaluations of medicinal plant/herbal formulation promoted by WHO. This will be a major step in the development of new generation standardization of botanical medicines [11].

Pharmacognosy is a simple and reliable tool, by which complete information of the crude drug can be obtained . Today with the present surge of interest in the phyto-therapeutics, the availability of genuine plant material is becoming scarce. Since crude plant drugs form the basis for the manufacture of numerous medicinal preparations, accurate determination of drug identity forms an essential part of its study. It becomes extremely important to make an effort towards standardization of the plant material as medicine. The process of standardization can be achieved by stepwise pharmacognostic studies [12, 13].

The North-East region of India (22^0-29^0 N; 89^0-97^0 E) comprises the Sikkim and the seven sister states namely Assam, Arunachal Pradesh, Nagaland, Meghalaya, Mizoram, Manipur and Tripura. This region of India has several hill ranges interspersed with valleys and is by large sparsely populated. Nearly 40% of the total geographical area of this region is covered by evergreen forest. A large no of people belonging to various groups of the north-eastern region of India still practicing their own traditional heath care systems.

Plumeria rubra is a roadside tree of this region belongings to the family Apocynaceae and commonly known as Champa. The old tree (9-10 years) is 20-40 ft in high and has grey bark covered with. The different parts of the trees have multi-disciplinary aspect to human. The plant material is widely used as purgative, remedy for pain, fever, diarrhoea and cure for itch. The milky juice is employed for the treatment of inflammation. The excessive doses of the latex derived from *Plumeria acuminate* (a close variety to P. *rubra*) are poisonous and the root is a violent cathartic. The essential oil from the flowers possesses antifungal activity. In Unani practice, the medicinal herb is used to treat tumours and rheumatic pains [14].

Evaluation of drug means confirmation of its identity and determination of its quality and purity and detection of nature of adulteration. The evaluation of a crude drug is necessary because of these main reasons i) biochemical variation in the drugs ii) detoriation due to treatment and storage, and iii) substitution and adulteration, a result of carelessness, ignorance or fraud. Due to advancement in the chemical knowledge of crude drugs, at present, evaluation also includes method of estimating active constituents present in the crude drug, in addition to its morphological and microscopic analysis. With the advent of separation techniques and instrumental analysis, it is possible to perform physical evaluation of a crude drug, which could be both of qualitative and quantitative in nature [15].

The present study on this plant was undertaken to determine the pharmacognostical standards for evaluating the plant material, various investigations like organoleptic parameter, various physico-chemical evaluations like ash value, extractive value, loss on drying (LOD) and phyto-chemical screening test. Thin layer chromatography profiling and fluorescence analysis of powdered crude drug were carried out and some salient qualitative as quantitative parameter were mentioned.

MATERIAL AND METHOD:

Plant material

The stem barks of *Plumeria rubra* (Family - Apocynaceae) were collected from campus garden of Dibrugarh university, Assam, India in the month of June 2012. The plant was identified and authenticated taxonomically by Dr. B.K. Sinha at National Botanical survey of Shillong, India. A voucher specimen DU/PM/2012/8 of the collected sample was deposited in the institutional herbarium and departmental museum for future reference.

Plant profile

Taxonomical classification [16]

Kingdom- *Plantae*
Class- *Magnoliopsida*
Subclass- *Asteridae*
Order- *Gentianales*
Genus- *Plumeria*
Species- *Rubra*
Family- *Apocynaceae*
Tribe- *Plumeria*
Botanical Name- *Plumeria rubra*

Vernacular name [17]

- Assamese : Golanchi
- Bengali : Gulancha, kat champa
- Hindi : Chameli
- Gujrati : Dholo champo
- Marathi : Khairchampa
- Tamil : Ilattalari

Reagent and Chemicals

All reagents and chemicals used for pharmacognostic evaluation and phyto-chemical screening were analytical grade obtained from SRL Chemical, Rankem, Otto, Himedia Pvt. Ltd. India.

Organoleptic evaluation and macroscopic evaluation [18, 19]

Various sensory parameters of the plant material (such as colour, odour, size, shape, and taste) were studied by organoleptic evaluation.

The bark is morphologically studied for its size, shape, surface, fracture and configuration. The macroscopy of crude drug includes its visual appearance to the naked eyes and its sensory characteristics. Simple microscope of magnification 10xs was used for the perception of special structural features such as:

- Size and shape of the drug,
- Colour and external marking
- Fracture and degree of uniformity of the particles.
- Surface appearance by reflected light.

Physico-chemical evaluation

Physico-chemical parameters such as the percentage of loss on drying (LOD), total ash, acid insoluble ash, water-soluble ash were determined as per the Indian Pharmacopoeia guidelines [20]. Water and alcohol soluble extractive were estimated by cold maceration according to the method prescribed by WHO [21]. All the parameters were taken in triplicate and the result that was obtained presented as mean ± standard error of mean (SEM).

Fluorescence Analysis [22, 23]

A small quantity of dried and finely powdered bark sample was placed on a grease free microscopic slide and added 1-2 drops of freshly prepared solution, mixed by gentle tilting the slide and waited for 1-2 minutes. Then the slide was placed inside the UV viewer chamber and viewed in day light, short (254 nm) and long (365 nm) ultraviolet radiations. The colours observed by application of different reagents in various radiations were recorded.

Phytochemical Screening: [18, 24]

The dried and powdered barks were subjected to preliminary phyto-chemical screening for qualitative detection of phytoconstituents. The dried and coarsely powdered sample (50 gm) was

extracted successively with petroleum ether (60-80^{0}C), chloroform, ethyl acetate, and methanol in a soxhlet extractor by continuous hot percolation method. Each time before extracting with the next solvent of higher polarity the powder drug (marc) was dried below 50^{0}C for 10 minutes. Each extract was concentrated by distilling off the solvent, which was recovered subsequently. The concentrated extracts were evaporated to dryness and the extracts obtained with each solvent were weighed. The colours of extracts were observed. The successive extract, as mentioned above, were subjected to various qualitative phyto-chemical test for the identification of chemical constituents present in the plant material.

Thin Layer Chromatographic Analysis [25]

The chromatographic methods which are presently available, Thin Layer Chromatography are widely used for the rapid analysis of drugs and drug preparations. There are several reasons for this.

- The time required for the demonstrations of most of the characteristic constituent of a drug by TLC is very short.
- In addition to qualitative detection, TLC also provides semi-quantitative information on the major active constituent of a drug or drug preparation, thus enabling an assessment of drug quality.
- TLC provides a chromatographic drug finger print. It is therefore suitable for monitoring the identity and purity of drugs and for detecting adulterations and substitutions.
- With the aid of appropriate separation procedures, TLC can be used to analyze drug combinations and phytochemical preparations.

Preparation of Bark Extract

a) Methanolic extract: One gram-powdered drug is mixed thoroughly with 1 ml of 10% ammonia solution and extracted for 10 minutes with 5 ml methanol under reflux. The filtrate is in concentrated and used as spotting agent for TLC analysis.

b) Successive solvent extract: The dried bark powder was extracted with chloroform, ethyl acetate and methanol by hot percolation method. Successive, after filtration the resultant extracts were collected in a clean dry glass beaker. The liquid extracts were heated over water bath to evaporate the solvent for 30-40 minutes. The semi-solid extract is taken for TLC analysis and before applying spots the extract were diluted with little amount of chloroform, ethyl acetate and methanol respectively.

Stationary Phase

Silica gel G, particle size 10 – 40 µm applied as a thin layer on a clean glass plate support and activated (110^{0}C for 30 minutes) just before use.

Mobile Phase

Quantity – 50 ml for each.

a. The mobile phase was ethyl acetate and toluene in the ratio of 9:1 (S-1). In addition, Ethyl acetate, Formalin, Methanol in the ratio of 9:0.5:0.5 (S-2).
b. The solvent system was Formalin : Methanol in the ration of 4:1 in case of S-3 and the other one was Ethyl acetate, Formalin, Methanol in the ratio of 8.0:1.0:1.0 (S-4).

Development Method

Development of chromatogram:

The extracts were spotted on the plates with the help of fine bore capillaries and chromatogram was developed in chromatographic chamber using different solvent systems in a room temperature. The time required for the development varied from 10-20 min (for 19.5/9.5 cm plate). After completion of run the plates were removed from the chamber, allowed to dry in air, and visualized followed by iodine chamber. The R_f values of the spots were calculated and recorded. Retardation factor is the ratio of distance travelled by the solute to the distance travelled by the solvent.

$$R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Visualization

a. After development the TLC plate, initially three and two spots were visualized in iodine chamber for the methanolic extract.

b. In case of S-3 solvent system the chloroform and ethyl acetate extract shown two spots for each and the methanolic extract shown three spots respectively. At the last choosing S-4 solvent system the chloroform extract shown three spots and the other two solvents (ethyl acetate & methanol) shown two spots for each.

Documentation

The R_f value were measured correctly and carefully and the chromatogram was presented in the table number of 4 & 5.

General appearance – Single or branched and entire or longitudinally sliced stems.
Colour- Light grey
Odour – Characteristics
Taste- Astringent
Shape- Exfoliating.

Physico-Chemical Evaluations

The values of all determinations are summarized in table 1. In this evaluations the amount of water soluble ash is lesser than acid insoluble ash, where as the amount of total ash was nearly double of acid soluble ash.

RESULTS AND DISCUSSION:

Macroscopic Evaluation and Organoleptics

The characters recorded are described below.

Table 1 Values of Physico-chemical parameters:-

Sl. No.	Parameters	Average values (%)of three replicates ± S.D of stem barks
1.	Total ash	13.33 ± 0.017
2.	Acid insoluble ash	8.65 ± 0.010
3.	Water soluble ash	2.12 ± 0.0461
4.	Water soluble extractive	4.65 ± 0.005
5.	Alcohol soluble extractive	5.32 ± 0.005
6.	Loss on drying	14 ± 0.577

Fluorescence Analysis

The results were summarized in table no 2.

Table 2. It shows fluorescence analysis of powdered barks of *Plumeria rubra:*

Powdered drug	Visible/Day light	UV 254 nm (short)	UV 365 nm (long)
Powder drug as such	Yellow	Dark brown	Yellowish brown
Powder + Methanol	brown	Brownish black	Blackish brown
Powder + 1% glacial acetic acid	Yellowish brown	Bluish black	Yellowish brown
Powder +1 M NaOH	Reddish brown	Blackish brown	Blackish yellow
Powder + dil. NH_3	Blackish brown	Brownish black	Brownish black
Powder + Conc. HNO_3	Reddish brown	Brown	Blackish
Powder+ dil.NH_3+Conc.HNO_3	Reddish brown	Brown	Bluish black
Powder +1M H_2SO_4	Reddish Brown	Brown	Greenish yellow
Powder +1M HCl	Brown	Black	Black
Powder + 10% $FeCl_3$	Blackish brown	Black	Blackish

Powder + picric acid	Yellowish brown	Bluish black	Blackish
Powder +10% Iodine	Dark brown	Black	Brownish yellow

Phytocemical screening

The results are shown in table no.3 these results represents the presence of alkaloids, carbohydrates, saponins, glycosides, tannins, flavonoids etc in the barks of P. rubra. The extractive constituents present in the different solvent extracts are tabulated in the table no 3.

Table 3: It shows phyto-chemical screenings of successive extracts of powdered barks of *Plumeria rubra*

. Constituents	Petroleum ether Extract	Chloroform Extract	Ethyl acetate Extract	Methanol Extract	Hydro-alcohol Extract
Alkaloids	-	+	+	+	+
Carbohydrates	-	_	_	+	+
Fats and oil	+	_	_	-	-
Flavonoids	_	_	_	+	+
Glycosides	_	_	+	+	+
Gum	_	_	_	_	_
Steroids	+	+	-	+	_
Proteins	_	_	_	+	+
Saponins	_	_	_	+	+
Tannins and Phenolic compounds	_	_	+	+	+

+= Present & **-** =Absent

Thin Layer Chromatographic Analysis

The barks extract showed distinct spots with different intensities in different solvent composition. The resultant R_f values were summarized in table no 4 & 5.

Table 4: TLC profile of methanolic extract of *Plumeria rubra* Stem barks.

Sl. No.	Code	Chromatography Solvent	Number of Spots	R_f Values	Visualizing Agents
1.	S-1	Ethyl Acetate : Toluene (9 : 1)	3	6.4/6.8= 0.94 5.8/6.8= 0.86 5.4/6.8 = 0.79	Iodine
2.	S-2	Ethyl Acetate : Formalin: Methanol (9 : 0.5 : 0.5)	2	6.2/6.9 = 0.89 5.6/6.9 = 0.81	Iodine

Table 5: TLC profile of successive extraction of *Plumeria rubra* Stem bark.

Sl. No.	Code	Chromatography Solvent	Extracts	No. Of Spots	R_f Values	Visualizing Agents
1	S-3	Formalin : Methanol (4 : 1)	Chloroform	2	0.95 0.83	Iodine
			Ethyl acetate	2	0.96 0.67	Iodine
			Methanol	3	0.94 0.69 0.86	Iodine

2.	S-4	Ethyl Acetate : Formalin: Methanol (8 : 1.0 : 1.0)	Chloroform	3	0.89 0.62 0.59	Iodine
			Ethyl acetate	2	0.91 0.71	Iodine
			Methanol	2	0.90 0.73	Iodine

DISCUSSION:

To ensure reproducible quality of herbal products, proper control of starting material is utmost essential. Thus in recent years there has been an emphasis in standardization of medicinal plants of therapeutic potential. Despite the modern techniques, identification and evaluation of plant drugs by pharmacognostical studies is still more reliable, accurate and inexpensive means. According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken [26]. Organoleptic evaluation is a technique of qualitative evaluation based on the study of morphological and sensory profiles of whole drugs [24]. The Organoleptic studies shows the important characteristics of the drugs, the outer structure of the barks, the surface of the barks, the typical tongue sensation and the odour may screen the preliminary phytochemical constituents.

Ashing involves an oxidation of the component of the product. A high ash value is indicative of contamination, substitution or adulteration. The Total ash usually contains carbonates, phosphates, silicates which includes both physiological and non-physiological. Acid-insoluble ash usually indicates the contamination with silicon material like earth and sand. Water-soluble ash was used for the estimation of the amount of inorganic elements. Extractive value represent the extraction of any crude drug with a particular solvent yields a solution containing different phytoconstituents. The composition of these phytoconstituents in that particular solvent depends upon the nature of the drug and solvent used. By following the cold maceration method, the yield of alcohol soluble extractive is greater than water soluble extractives.

Loss on drying value is an inevitable component of crude drug which help in its preservation. The objective of drying of fresh material is to fix their constituents i.e. to check enzymatic or hydrolytic reactions that might alter the chemical composition of the drug and to reduce their weight and bulk.

The morphological studies of the bark will enable to identify the crude drug. The quantitative determination of some pharmacognostical parameters is useful for setting standards for crude drugs. These values help in the evaluation of purity of drugs.

Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Some constituents show fluorescence in the visible range in daylight. The ultra violet light produces fluorescence in many natural products (e.g. alkaloids like berberine), which do not visibly fluoresce in daylight. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence, some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation [22, 27].

Five solvents were selected for the extraction of stem bark of *Plumeria rubra* i.e. Petroleum ether, chloroform, ethyl acetate, methanol, hydro-alcohol and different phytochemical tests were performed. The petroleum ether and the chloroform extracts of stem bark showed negative response for the entire test, except sterols in the extract. However, the chloroform extracts which represents the presence of alkaloids. The ethyl acetate extract showed the presence of alkaloids, glycosides and tannins. The methanol extract showed most of the

test positive and signify the presence of maximum no. of phytoconstituents.

The presence of alkaloids, flavonoids, carbohydrates, proteins, glycosides and tannins are in the hydro-alcohol extract but the other compounds such as gum, steroids, fats & oil etc. are absence in this solvent. The preliminary phytochemical studies exhibited the presence of the maximum number of phytoconstituents in the methanolic extract of the stem bark of *Plumeria rubra.*

TLC is produced with the aim of identifying the individual substances in a mixture and also for testing purity or for separation of mixtures. The R_f value indicates the position at which a substance is located in a chromatogram. It is appropriate to regard R_f value as a guide for identification. Three different mobile phases and iodine used as visualizing agents as for consideration of TLC determination.

For methanolic extract, two solvent systems (S-1 & S-2) were chosen and resultant detection of thee & two spots were found respectively. In addition, successive extraction (table no 5) there were also two solvent systems preferred. The chloroform, ethyl acetate and methanol extracts were used as the spotting agent in both of the solvent system. The first R_f values of both of the solvent systems (spotted by chloroform, ethyl acetate and methanol extract) are proved to resembles the closely related (0.95, 0.96, 0.94 and 0.89, 0.91, 0.90). In the whole this may signify that the closely related compound mixture of compound were present in the extracted fraction.

CONCLUSIONS

The present pharmacognostic data emphasize the establishment of quality and identity of the plant *Plumeria rubra.* The qualitative and quantitative parameters serve the important information of the plant *Plumeria rubra.* These information will also be helpful to differentiate *Plumeria rubra* from the closely related other species and varieties such as *Plumeria accuminata* .

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