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Research Article

Standardisation of *Guazuma tomentosa* leaf

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ABSTRACT

Standardisation of leaf of *Guazuma tomentosa* is carried out to establish its macroscopic and microscopic characters and its quantitative physicochemical standards. Total ash, water soluble ash, acid insoluble ash, swelling index, extractive value (ethyl acetate, dichloromethne, alcohol and water soluble extractive value both hot and cold) were determined for physicochemical evaluations. Preliminary phytochemical screening was done to detect the presence and absence of phytoconstituents. Thin layer chromatography was carried out which play important role in assuring quality of crude drug. The drug can be identified on the basis of morphology and microscopic characters. Phytochemical screening revealed that leaf extract contain alkaloids, carbohydrate, phytosterol, resin, flavanoids, tannins, diterpenes and protein. TLC chromatogram and different phsicochemical standard has been developed. The present study on pharmacognostic standardisation, physicochemical evaluation of *Guazuma tomentosa* leaf might be useful to supplement information in regard to its identification parameters assumed significantly in the way of acceptibility of herbal drugs in present scenario.

Key words: *Guazuma tomentosa*, Standardisation, Microscopy, TLC, Pundraaksha

INTRODUCTION

Guazuma tomentosa Kunth. syn. G. ulmifolia Lamk. belonging to family Sterculiaceae is a plant native to tropical America, Ecuador and Colombia. In Sanskrit (Ayurveda) *Guazuma tomentosa* is known as Pundraaksha. Other vernacular (common) name of *Guazuma tomentosa* is mention in table: 1 and image of plant is shown in figure: 1. Despite of its enthnopharmacological uses, presently it is proven to have many therapeutic applications because of the presence of many phytoconstituents e.g. colistin, colatannins, catechins, caffeine, kaempferol, procyanidin B-2, procyanidin B-5, procyanidin C-1, tartaric acid, theobromine, xanthan gum, etc. Preclinically reported activities involve, anti diabetic, anti hypertensive, anti microbial, anti viral, anti oxidant, anti ulcer, neurological, anti secretory, cytotoxic and uterine stimulating activity.[1,2,3] Standardisation of medicinal plant is necessary because of the fact that in some cases desirable pharmacological action are not achieved because the biological action of herbal medicine is due to phytoconstituents which can vary batch to batch. The amount of phytoconstituents in a plant can vary according to age of plant, time of collection, environmental condition etc.[4,5] To overcome this problem standardized medicinal plants, plant extracts and isolated constituents can be used. Although plant is of very high impact with reference to medicinal value but there is no standard data available for identification, to insure quality of this valuable medicinal plant so an attempt has been taken to standardise leaves of *Guazuma tomentosa*.

MATERIALS AND METHODS

Plant material: The leaves of *Guazuma tomentosa* were collected in the month of September, 2011 from the Veermata jijabai bhosale udyan Byculla, Mumbai and get authenticated at Chinmaya College of Sciences, BHEL, Haridwar. The specimen of herbarium of plant is kept in domain of Pharmacognosy and phytochemistry, School of Pharmaceutical science, Lovely Professional University. The leaves were washed with tap water followed by drying under shade (temperature 30-40°C). Dryied leaves were grinded to coarse powder and packed in suitable container for further study.

Morphological / Organoleptic properties: Macroscopic studies of leaves were done visually and evaluated for organoleptic properties.[6]

Microscopic characterisation: Transverse section of leaf

Fig. 1: Leaf of Guazuma tomentosa

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Table 1: Vernacular (Common) names of *Guazuma tomentosa*

Language / Region	Vernacular name
Bengali	Nipaltunth
English	Baster cedar, Honey fruit tree, Musket tree
Guajarati	Bhadraksha
Kannada	Bhadrakshi mara, Bucha rudrakshi
Malayalam	Rudraksham, Uttharaksham
Oriya	Debodura
Sanskrit	Pundraaksha, Rudraakshi
Tamil	Rudrasam, Tenbachai, Thenmaram, Tubakki
Telegu	Rudraksha

(containing midrib and lamina portion) was cut and stained with phluoroglucinol and hydrochloric acid. Further powder microscopy of dried leaves powder was also performed.[6]

Preliminary phytochemical screening: Ethyl acetate, dichloromethane and alcoholic extract of *Guazuma tomentosa* was prepared by soxlet extraction. Aquos extract was prepared by refux method. All the extracts were subjected to phytochemical screening for qualitative analysis for presence and absence of secondary metabolite.[7,8,9]

Physicochemical parameter: Triplicate reading of each physicochemical parameter were taken and value are presented as Mean ± Standard error mean (SEM)

Determination of Swelling Index: The swelling index is the volume in ml taken up by the swelling of 1g of plant material under specified conditions. The plant material was reduced to fineness passing from sieve no. 22 and was accurately weighed 1g into a 25 ml glass-stoppered measuring cylinder. Water (25 ml) was added and shaken thoroughly after every 10 min for 1 hr. Then the mixture was allowed to stand for 3 hr at room temperature. The volume was measured in ml occupied by the plant materials. The mean value of the individual determinations was calculated related to 1g of plant material.[10]

Swelling index = (Final volume – Initial volume / Final volume) X 100

Determination of extractive value: Cold and hot extractive value was determined using ethyl acetate, dichloromethane, alcoholic and water.

Hot extractive value: Coarsely powdered air-dried material 4 g was accurately weighed and placed in glass-stoppered conical flask. 100 ml of solvent was added and total weight including the flask was noted. It was shaken well and allowed to stand for 1 hr. Reflux condenser was attached to the flask and gently boiled for 1 hr, cooled and weighed. Original total weight was readjusted with the solvent specified in the test procedure. It was then shaken well and filtered rapidly through a dry filter. The 25 ml of filterate was transferred to a tared flat-bottomed dish and evaporated to dryness on a water bath. Then dried at 105°C untills constant weight, cooled in a dessicator for 30 min and then weighed without delay. Percentage hot extractive value was calculated using following formula [10].

% hot extractive value = (extract obtained / plant material taken) X100

Cold extraction value: Coarsely powdered air-dried material 4 g was accurately weighed and placed in glass-stoppered conical flask. It was macerated with 100 ml the solvent specified for the plant material concerned for 6 hr, shaken frequently and then allowed to stand for 18 hr. Filtered rapidly taking care not to lose any solvent, 25ml of filterate was transferred to tared flat-bottomed dish and evaporated to dryness on a water bath. Dried at 105°C untills constant weight, cooled in a dessicator for 30 min and weighed without delay. Percentage cold extractive value was calculated using following formula.[10]

% cold extractive value = (extract obtained / plant material taken) X100

Determination of Ash value: The ash remaining after ignition of medicinal plant materials is determined by three different methods which measure total ash, acid insoluble ash and water soluble ash.[10]

Total ash: Weigh accurately 1g of finely powdered leaves and placed in a previously ignited and tarred crucible. The material was spread in an even layer and was ignited by gradually increasing the heat to 550^0C until it turns white, indicating the absence of carbon. It was cooled in desiccators for 30 min and weighed without delay. The content of total ash was calculated using following formula.[10]

% Total ash = (Ash obtained after calcination / Plant material taken) X 100

Acid insoluble ash: To the crucible containing total ash, hydrochloric acid (25 ml) was added. It was covered with watch glass and allowed to boil for 5 min. The watch glass was rinsed with hot water (5 ml) and this liquid was added to the crucible. The insoluble matter was collected on ash less filter paper and washed with hot water until the filtrate was neutral. The filter paper containing insoluble matter was transferred to the original crucible. It was dried on a hot plate and ignited to constant weight. The residue was cooled in a dessicator for 30 min and weighed without delay. The content of acid insoluble ash was calculated using following formula.[10]

% Acid insoluble ash = (Acid insoluble material / Plant material taken) X 100

Water soluble ash: To the crucible containing total ash, water (25 ml) was added and boiled for 5 min. The insoluble matter was collected on ash less filter paper and washed with hot water. It was ignited in a crucible for 15 min at a temperature not exceeding 450°C. The weight of this residue in mg was subtracted from the weight of total ash. The content of water soluble ash was calculated using following formula.[10]

% water soluble ash = (Water soluble material / Plant material taken) X 100

Chromatographic evaluation

Thin layer chromatography

Test Sample

Prepared extract is dissolved in respective solvent to make conc of 1mg/ml.

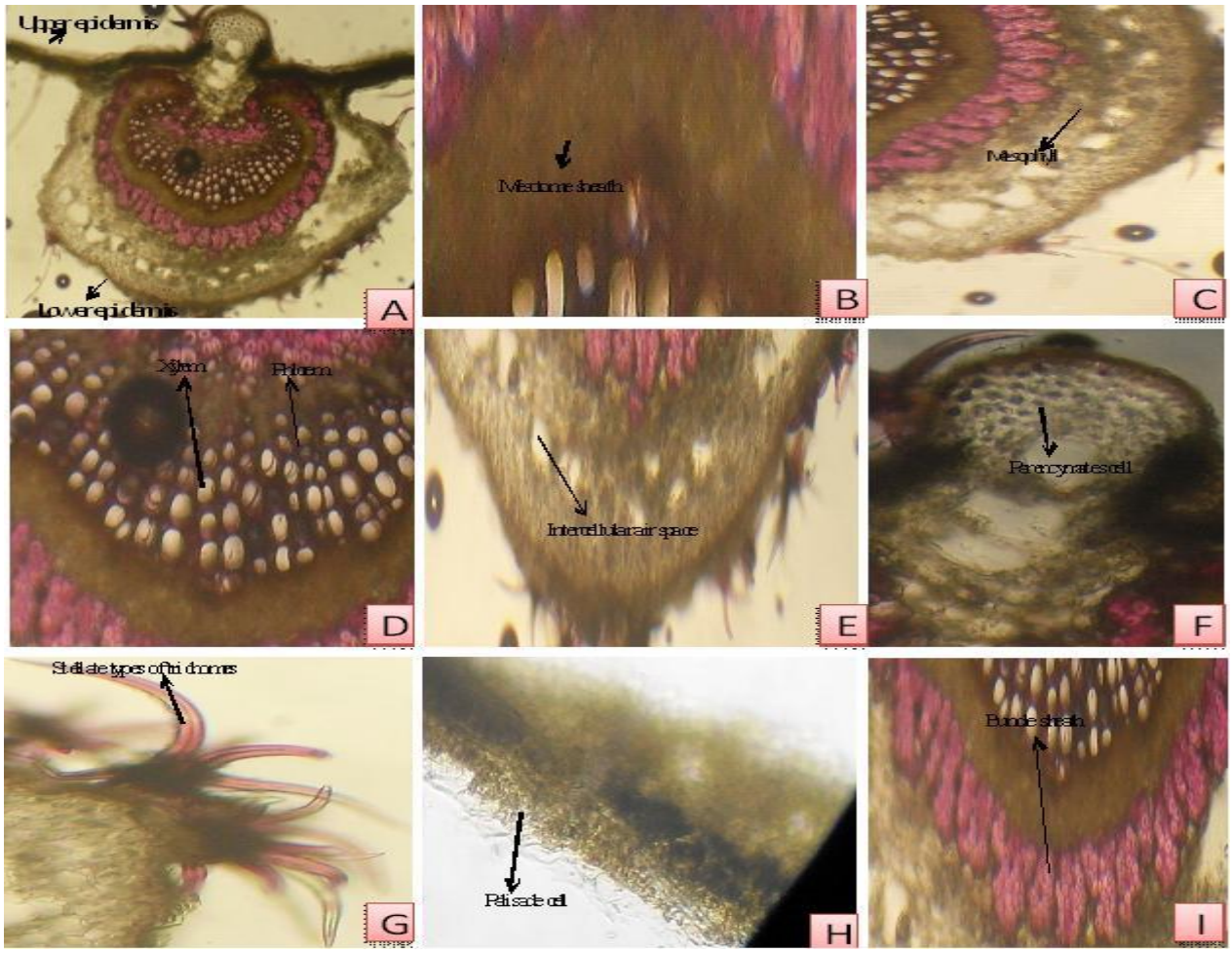

Fig. 2: T.S. of leaf of Guazuma tomentosa

Fig. 3: Powder Microscopy of Guazuma tomentosa

Stationary phase:

Silica gel precoated aluminium sheets (5x5cm) (Silica gel 60F-254) of thickness 0.2 mm were used.

Mobile Phase

Chloroform: Acetone: Formic acid in ratios of 75:16.5:8.5

Application of Spot

Applied manually with a Capillary Tube.

Development of Chromatogram

The development chamber was washed, dried and then saturated with mobile phase. The plate was then introduced in developing solvent. The chamber was made air tight and allowed to stand until 75% front was developed. The chromatogram was taken out and heated in oven at 105^0C for 5 minutes. The spot ware seen under visible and in UV light (366nm).[11,12]

Calculation of R_f value

R_f values of the spots were calculated using following formula

R_f = Distance travelled by solute/ Distance travelled by solvent front

RESULTS

Leaf can be identified on the basis of morphological study (Fig: 1) as it is distinct with acuminate apex, serrate margin and unequal cordate base. The colour, odour and taste of leaf are dark green, unpleasant and astringent respectively. The average width and length of leaf is 9.5 and 19.2 cm respectively. The outer surface is slightly rough and brittle.

Transverse section (T.S.) of leaf of *Guazuma tomentosa* (Fig: 2) shows (A) Upper and Lower epidermis. Upper and lower epidermis was single continues layer with polygonal cells (B) Mesotome sheath is situated in

Table-2: Phytochemical screening of different extract of *Guazuma tomentosa* leaf

Phytochemical	Ethyl acetate extract	Dichloromethane extract	Ethanol extract	Aqueous extract
Alkaloids				
Wagner's test	+	+	+	-
Hager's test	+	+	+	-
Mayer's test	+	+	+	-
Dragendroff's test	+	+	+	-
Carbohydrates				
Molisch's test	-	-	+	+
Fehling's test	-	-	+	+
Glycosides				
Borntrager's test	-	-	-	-
Modified borntrager's test	-	-	-	-
Saponin				
Froth test	-	-	-	-
Phytosterols				
Salkowski's test	+	+	+	-
Liebermann Burchard's test	+	+	+	-
Fixed oil & Fats				
Stain test	-	-	-	-
Resins	+	+	+	-
Phenols				
Ferric chloride test	+	+	+	+
Flavonoids				
Alkaline reagent test	+	+	+	+
Lead acetate test	+	+	+	+
Diterpenes				
Copper acetate test	-	-	+	+
Tannins				
Gelatin test	+	+	+	+
Protein & amino acid				
Ninhydrin test	_	_	+	+

between vascular bundle and bundle sheath (C) Mesophyll: Palisade cell single layer, compact oval in shape (D) Vascular bundle : Contain Xylem & phloem, Xylem 5-7 Non- lignified & Phloem is lignified (E) Intercellular air space in present in mesophyll (F) Parencymatous cell: Parencymatous cell present above vascular bundle is oval or circular in shape (G) Trichomes: The epidermal cell gets modified in to stellate types of trichomes (star shaped), numerous in number and lignified (H) Palisade: Single layer, cylindrical in shape (I) Bundle sheath is single layer lignified shape Irregular.

Powder microscopy of leaf of *Guazuma tomentosa* (Fig: 3) shows (A) Fibres with calcium oxalate crystal: Abundant, large prismatic crystals are found scattered in the powder (B) Prism: Parenchymatous cells contain single large prisms of calcium oxalate (C) Trichomes: The epidermal cell gets modified in to stellate types of trichomes, numerous in no and lignified.

Phytochemical screening: The presence or absence of phytoconstituents in ethyl acetate, dichloromethane, ethanol and aqueous extract is shown in table 2.

Physicochemical parameter: Result obtained for different physicochemical parameter is reported in table 3.

Thin layer chromatography: Chromatogram of TLC in visual lght and under UV light is shown in fig. 4. Rf value of separated constituent for DCM, ethanol and ethyl acetate extract is (0.2, 0.3, 0.34, 0.6, 0.74), (0.32, 0.56, 0.7, 0.72) and (0.16, 0.26, 0.3, 0.48, 0.56, 0.68, 0.74, 0.8) respectively.

DISCUSSION

Morphological evaluation of crude drug play important role in identification and detection of adulteration. Sometime quality of crude drug can be checked on the basis of morphology only.[13] The T.S. of crude drug allows more detail examination of a drug and it can be used to identify the organised drugs by their known histological character.[13] The T.S. of leaf of *Guazuma tomentosa* can be used as standard for authentication of this valuable medicinal plant. Powder microscopy play important role in similar way as T.S. of leaf.[13] As crude drug is blend of multiple constituent and pharmacological action of drug is due to either a constituent or due to combination of different constituent.[4] Phytochemical screening help in evaluating for presence and absence of phytoconstituents. Standardisation of medicinal plant is a complicated process so different physicochemical parameter play important role in standardisation.

Physicochemical parameter can be used as standard to ensure the quality of crude drug.[13] High ash value of leaf of *Guazuma tomentosa* shows the presence of very high

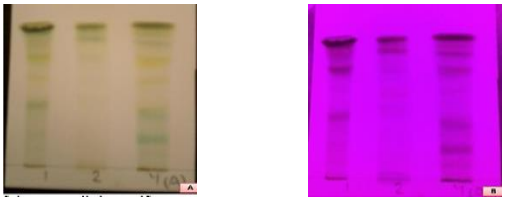

Fig. 4: TLC chromatogram of leaf extract of Guazuma tomentosa

Table 3: Physicochemical parameter of leaf of *Guazuma Tomentosa*

Parameters		Results (%)
Total ash		10.2 ± 0.125
Water soluble ash		10.1 ± 0.119
Acid insoluble ash		0.7 ± 0.015
Determination of Swelling Index		10 ± 0.115
Determination of Extractive value		
Ethyl acetate	Hot	1.8 ± 0.044
	Cold	0.84 ± 0.012
Dichloromethane	Hot	1.7 ± 0.028
	Cold	0.8 ± 0.017
Alcoholic	Hot	1.6 ± 0.032
	Cold	0.75 ± 0.011
Aqueous	Hot	4.7 ± 0.096
	Cold	3.05 ± 0.063
Determination of foreign matter		Nil
Moisture content		4.08 ± 0.096

Values are presented as mean ± SEM (n=3)

inorganic content. Lower value of the acid insoluble ash suggest the greater physiological availability of drug.[13] Extractive value give information about availability of soluble phytoconstituents in particular solvent. Alcohol soluble extractive is more as compared to aqueous extractive value suggesting alcoholic extract would be more beneficial as compared to aqueous extract for therapeutic aspect. Low value of moisture content does not promote microbial contamination as the general requirement of moisture content in crude drug is not more than 14 % (W/W).[13] The swelling index of leaf is 10% it may be due to presence of gum. Thin layer chromatography can be a valuable tool in standardisation of medicinal plant. The TLC chromatogram and Rf value of phytoconstituent is preliminary tool of evaluation of crude drug.[13-15]

CONCLUSION

The present study on pharmacognostic standardisation, physicochemical evaluation of *Guazuma tomentosa* leaf can be used as standard in regard to its identification parameters assumed significantly in the way of acceptibility of herbal drugs in present scenario.

CONFLICT OF INTEREST

The authors declares that there is no conflict of interest.

REFERENCES

1. Sharma M, Yashwant, Prasad SB. Ethanomedicinal, Hepatoprotective Activity of Guazoma Tomentosa Leaf Extracts Against CCl4 Induced Liver Damage in Rats. Int. J. Current Pharmaceutical Review and Research, 2013; 4: 128-138.
2. Kirtikar KR, Basu BD. Indian Medicinal Plants. 1st ed. Dehradun, India International book publisher:1999.p.381-382.
3. Felipe A, Rincao VP, Benati FJ, Linhares R, Galina KJ., Toledo D, et.al. Antiviral Effect of Guazuma ulmifolia and Stryphnodendron adstringens on Poliovirus and Bovine Herpesvirus Biol pharm bull 2006: 29: 1092-95.
4. Verma H, Prasad SB, Yashwant, Singh H. Herbal Drug Delivery System: A Modern Era Prospective. Int J Current Pharma Rev Res 2013; 4 : 88-101.
5. Prasad SB, Aeri V, Yashwant. Role of traditional and alternative medicine in treatment of ulcerative colitis. Int J pharmacog phytochem res 2013; 5: 248-253.
6. Prasad SB, Sharma A. Pharmacognostic and phytochemical evaluation of Convolvulus plauricaulis ". Inventi Rapid: Planta Activa , Vol. 2012 [cited 2013 Mar 03] http://inventi.in/Article/ppa/134/12.aspx.
7. Gandhi S, Shinde P, Avalaskar A. Phytochemical Investigation and Antimicrobial Activity Testing of Phyllanthus fraternus Leaves Webster. Int J Pharmacognosy and Phytochemical Research 2014; 6(3); 444-448.
8. Sharma GN, Dubey KS, Sati N, Jyotsana S. Phytochemical Screening and Estimation of Total Phenolic Content in Aegle marmelos Seeds. Int. J. of Pharmaceutical and Clinical Research 2011; 3(2): 27-29.
9. Rajan M, Kumar VK, Kumar PS, Venkatachalam T, Anbarasan V. Pharmacognostical and Phytochemical Studies of The Leaves of Albizia Odoratissima (L.F) Benth. International Journal of Pharmacognosy and Phytochemical Research 2011;3(3): 47-55.
10. Kumar GVS, Anusha N, .Ramadevi D. Pharmacognostic and Preliminary Phytochemical Studies on Leaf Extracts of Chloroxylon swietenia. International Journal of Pharmacognosy and Phytochemical Research 2014; 6(3); 492-498.
11. Sredhar S, Kumar UP, Rema Shre AB. Pharmacognostic Standardisation of Heart Wod of Combretum albidum G.Don; An Important Ethno-Medicinal Liana. Int. J. of Pharmacognosy and Phytochemical Research 2013; 5(2); 106-12.
12. Wagner H, Bladt S. Plant drug analysis: a thin layer chromatography atlas. 2nd ed. India: Berlin: Springer:1996.
13. Rungsung W, Dutta S, Mondal DN, Ratha KK, Hazra J. Pharmacognostical Profiling on the Root of Rauwolfia Serpentina. Int. J. of Pharmacognosy and Phytochemical Research 2014; 6(3): 612-616.
14. Deshpande SG, Kasture VS, Gosavi SA, Bhalke RD, Ajage RK, Inamke SR, Kolpe JB, Jadhav GP. Pharmacognostic Evaluation of Polyherbal Marketed
15. Formulation. International Journal of Pharmacognosy and Phytochemical Research 2014; 6(3): 588-592.