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Pharmacognostical studies on Alstonia venenata R. Br.-an ethnomedicinal plant

S.Sutha[1], V.Kalpana Devi[2], P.Tresina soris[2], A.Maruthupandian[2] and V.R. Mohan[2]*

[1]Department of Medicinal Botany, Govt. Siddha Medical College, Palayamkottai, Tamil Nadu, India.

[2]Ethnopharmacology unit, Botany research Lab, V.O. Chidambaram College, Tuticorin, Tamil Nadu, India.

Corres. author: vrmohan_2005@yahoo.com

Abstract: The genus *Alstonia* finds a prominent place in different Indian systems of medicine. The different ethnic communities in India have used different species of *Alstonia* in the treatment of various human ailments. Establishment of pharmacognostic profile of the leaves of *A. venenata* will assist in standardization of quality, purity and sample identification. Pharmacochemical characterizations like physicochemical constant, fluorescence analysis, preliminary phytochemical analysis were carried out in the leaf as per standard procedures. Macroscopic and microscopic features of leaves were carried out. These findings should be suitable for inclusion in the proposed pharmacopoeia of Indian medicinal plants.
Key words: *Alstonia venenata*, pharmacognostic evaluation, pharmacopoeia.

Introduction

The genus *Alstonia* belongs to the family Apocynaceae. It includes totally 43 species of which two species namely, *A. scholaris* (L.) R. Br. and *A. venenata* R. Br. are represented in South India[1, 2]. These two species can be identified with their habits, shape and texture of the leaves, fruit size and papilla of the seeds. When these plants are used in herbal formulations, their botanical identity needs to be established beyond any ambiguity.

The genus *Alstonia* finds a prominent place in different Indian systems of medicine. The different ethnic communities in India have used different species of *Alstonia* in the treatment of various human ailments[3, 4, 5, 6]. Kanikkar tribals of Kalakad-Mundanthurai Tiger Reserve Sanctuary, Tamil Nadu, boiled the fresh leaves of *Alstonia venenata* in neem oil over a low flame until the oil extracts the complete essence of the drug. The affected part is massaged with the lukewarm oil, specifically, in the direction from the limbs to the upper portion applying soft presence. Hot water bath is administered after few hours, until there is relief from the rheumatic complaints. [7]The fruits are stated to posses tonic and anthelmintic properties and are reported to used as a remedy for impure blood, syphilis, insanity and epilepsy[8].

The *Alstonia venenata* leaf is collected from the wild sources and varied in constituents and efficacy due to the geographical diversity. Improper

collection and storage condition lead to the deterioration of the raw material. Keeping in view the above mentions problems, it was essential to standardize the leaves of *Alstonia venenata* for the establishment of quality and identify the profile of the drug for the purpose of safety monitoring and overall quality assurance of the drug. There is no report in literature regarding the standardization of *Alstonia venenata* leaves. Therefore, in the present investigation an attempt has been made to standardize *Alstonia venenata* leaves by using macroscopic and microscopic characters, physicochemical constant, fluorescence analysis and preliminary phytochemical analysis.

Materials and Methods

The plant materials were collected from the well grown plant found in the natural forest of Kalakad-Mundanthurai Tiger Reserve Forest, Western Ghats, Tirunelveli, Tamil Nadu, India. Identification and confirmation were done by Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. Voucher specimens were deposited in the Ethnopharmacology unit, Botany Research Laboratory. V.O. Chidambaram College, Tuticorin with the number VOCB 3323.

Macroscopical Studies

The following macroscopic characters for the fresh leaves like surface, shape, size, venation, phyllotaxy, length of the petiole, length of the leaf etc were noted.

Anatomical Studies

For anatomical studies, the required samples of leaf were cut and removed from the plant and immediately fixed in FAA (formalin- 5 ml + acetic acid- 5 ml + 70% Ethyl alcohol- 90 ml). The specimens were left in the preservative for two days; then the materials were washed in water and processed further. Standard microtome techniques were followed for anatomical investigation[9]. Transverse sections of the materials were made. The microtome sections were stained with 0.25% aqueous Toluidine blue (Metachromatic stain) adjusted to pH 4.7 [10]. Photomicrographs were taken with NIKON trinocular photo micrographic unit.

Physicochemical and Fluorescence Analysis

These studies were carried out as per the standard procedures [11]. In the present study, the powered leaf was treated with various chemical reagents like aqueous 1N sodium hydroxide, alcoholic 1N sodium hydroxide, 1N hydrochloric acid, 50% sulphuric acid, concentrated nitric acid, picric acid, acetic acid, ferric chloride and concentrated HNO_3+NH_3 These extracts were subjected to fluorescence analysis in day light and UV light(254nm and366nm). Various ash types and extractive values were determined by following standard methods [12].

Preliminary Phytochemical Analyses

Shade dried and powdered leaf samples were successively extracted with Hexane, Chloroform, Ethanol and Water. The extracts were filtered and concentrated using vacuum distillation. The different extracts were subjected to qualitative tests for the identification of various phytochemical constituents as per the standard procedure [13, 11].

Results

Macroscopic features of the Leaf

Whorls of 3-6, membranous, 10-20 by 2-4.5 cm oblong to lanceolate, very finely acuminate, base much tapered; main nerves numerous, very close, parallel, slender, uniting in an intra-marginal nerve, midrib strong; petioles 1.3-2.3 cm long, but obscure owing to the decurrent leaf-blade.

Microscopic features of the leaf

Midrib (Plate I, 1)

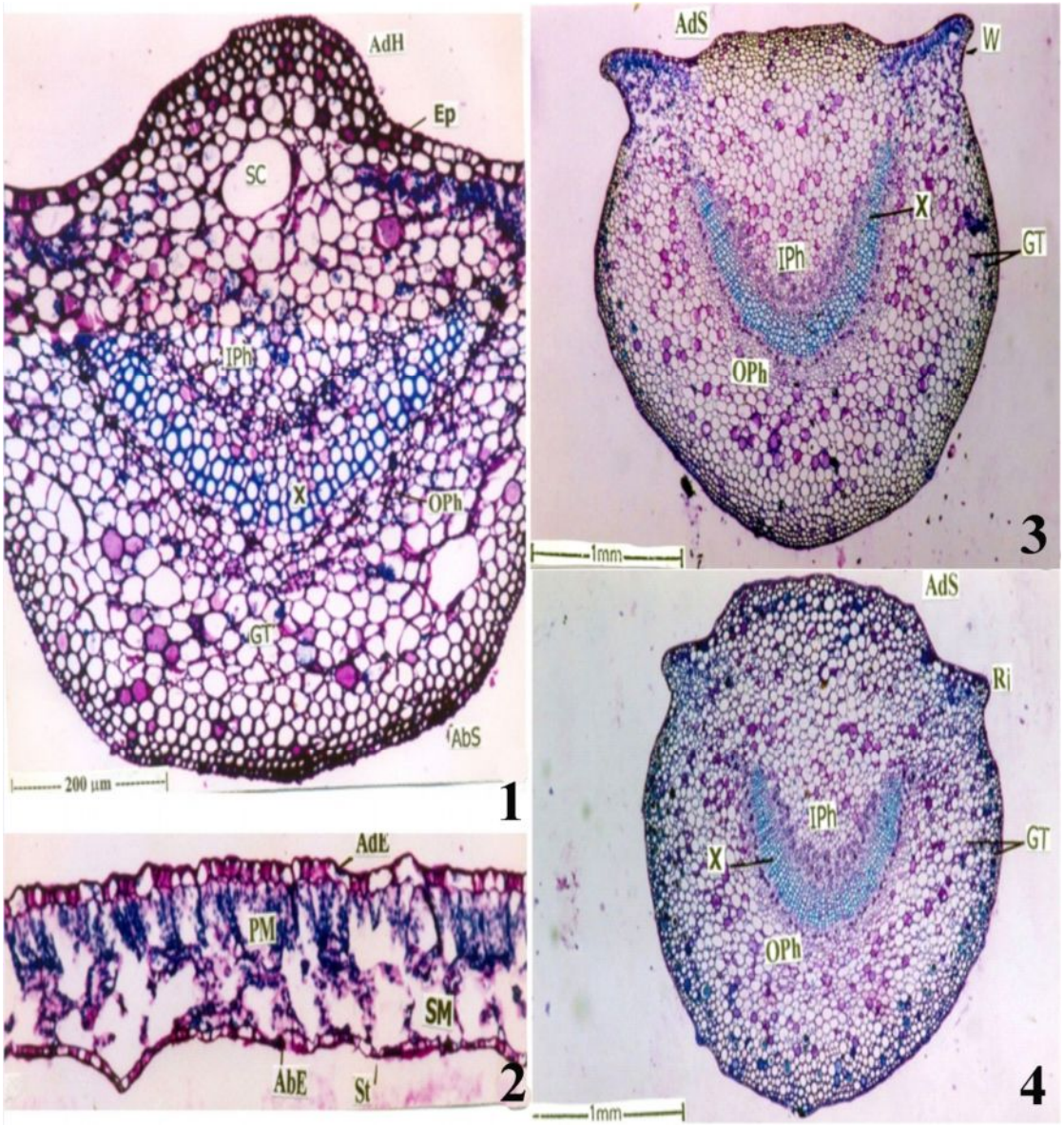
The midrib has wide and short adaxial hump and broadly hemispherical abaxial part. It is 650 µm in vertical plane; the adaxial hump is 250 µm wide in horizontal axis; the abaxial part is 600 µm wide. The midrib has thin epidermal layer consisting of small squarish thick walled cells. About three or four layers of sub-epidermal cells are collenchymatous. Rest of the ground tissue consists of parenchymatous, compact cells. Wide secretary canals with uneven outline are sparingly seen in the ground tissue. The vascular tissue occurs in a single wide arc of bicollateral strand. It consists of several radial parallel lines of thick walled angular xylem elements; phloem occurs in small clusters both on the abaxial and adaxial parts of the xylem band.

Lamina (Plate I, 2)

The leaf has even, smooth adaxial and abaxial surfaces with prominent midrib and lateral veins. The lamina is about 120 µm thick. The adaxial epidermis is prominent comprising of squarish or cylindrical, thin walled cells. The abaxial epidermis is thin with narrowly cylindrical cells. Stomata occur on slightly raised level of the epidermis. Mesophyll consists of narrow zone of palisade cells and aerenchymatous spongy parenchyma cells. The palisade cells are short,

measuring 40 - 50 µm in height; the cells are cylindrical and less compact. The spongy parenchyma cells are lobed and interconnected with each other with wide air chambers. The lateral vein is prominent projecting on the adaxial side measuring 150 µm thick. It consists of a few vertical files of xylem elements and a small arc of phloem; the vascular bundle is surrounded by parenchymatous bundle sheath with adaxial and abaxial extensions. The marginal part of the lamina is slightly curved abaxially; the margin is semicircular with palisade and spongy tissues. The epidermal cells have short tubercles, especially on the abaxial side.

PLATE- I



1. T.S. of the midrib 3. T.S. of Petiole – tip region
2. T.S. of lamina 4. T.S. of Petiole – basal region

AbS - Abaxial side; AdS - Adaxial side; AdH - Adaxial hump; Ep - Epidermis; GT- Ground tissue; IPh - Inner phloem; OPh - Outer phloem; SC - Secretory cavity; AbE - Abaxial epidermis; AdE - Adaxial epidermis; St - Stomata; X – Xylem; Ri – Ridge; W – Wing; PM - Palisade mesophyll; SM - Spongy mesophyll.

Epidermal tissues

The adaxial epidermis is apostomatic. The epidermal cells are slightly amoeboid in shape. The anticlinal walls are wavy and fairly thick. The abaxial epidermis is stomatiferous. The stomata are predominantly paracytic with two, equal or unequal subsidiary cells lying parallel to the guard cells including the subsidiaries are wavy and thick walled. The guard cells are elliptical measuring 20 µm long and 15 µm wide.

Venation Pattern of the leaf

The lamina has fairly thick submarginal (intramarginal) vein. The space in between the intramarginal vein and the leaf margin has dense parallel veinlets. These marginal veins are often dichotomously branched and arise from the intra marginal venation and spread towards the margin. The intramarginal vein gives off median central veins which are parallel to each other. The central veins are obliquely horizontal and it connects the intramarginal vein and the midrib. The space between the central veins is filled with profusely branched veinlets; the veinlets are also parallel to each other and form distinct vein islets. The vein islets are obliquely rectangular, narrow and elongated. Vein terminations are distinct; they are long, slender, and straight or curved, mostly unbranched. Parallel orientation of the primary and secondary veins with reticulate veinlets is characteristic of the leaf.

Petiole (Plate I, 3&4)

The petiole does not differ much along different portions of the length. It is semicircular with flat adaxial side and two short lateral wings. It is 1.6 mm vertically and 1.85 mm horizontally. In both proximal and distal parts of the petiole, the ground tissue consists of four or five layers outer zone of collenchyma and inner zone of circular or angular compact parenchyma cells. The vascular strand is single, broadly bowl shaped and collateral. It consists of radial files of parallel, thick walled, narrow xylem elements. Phloem occurs in large, circular prominent masses on the adaxial concavity of the vascular arc; on the convex part of the arc also occurs comparatively small discrete strands of phloem. Narrow, thick walled circular laticiferous canals are seen sporadically distributed in the peripheral region of the petiole.

Physicochemical Constant

The results of the ash and extractive values of *Alstonia venenata* leaf drug powder are depicted in table 1. The total ash content of the powdered leaf is 5.34% and the extractive value of water is more than that of ethanol.

Fluorescent Analysis

The results of fluorescent analysis of leaf powder of *Alstonia venenata* are shown in table 2. The leaf powder shows the characteristic yellowish green colour treated with 1N alcoholic NaOH, HNO_3 and $HNO_3 + NH_3$ under short UV light.

Preliminary Phytochemical Screening

The result of preliminary phytochemical screening of leaf extracts of *Alstonia venenata* are presented in table 3. The ethanol extracts of the leaf shows the presence of alkaloids, terpenoids coumarin, tannin, saponin, flavonoids, phenols, anthraquinones, quinones, carbohydrate, glycosides and starch.

Table - 1: Ash Values and Extractive Values of the Powdered leaves of *Alstonia venenata*

Ash values		
S. No.	**Type of Ash**	**% of Ash**
1	Total ash value of powder	5.34 ± 0.02
2	Water soluble ash	1.28 ± 0.01
3	Alkalinity of water soluble ash	2.31 ± 0.001
4	Acid insoluble ash	1.01 ± 0.03
Extractive values		
S. No.	**Nature of the extract**	**Extractive value (%)**
1	Alcohol (Ethanol)	6.09± 0.01
2	Water (Aqueous)	7.58 ± 0.02

Table - 2: Fluorescence Analysis of the Powdered leaves of *Alstonia venenata*

Experiments	**Visible/Day light**	**UV Light**	
		254nm	**365nm**
Drug powder as such	Greenish brown	Green	Brown
Powder + 1N NaOH (aqueous)	Brown	Dark brown	Brown
Powder + 1N NaOH (alcohol)	Brown	Yellowish green	Pale yellow
Powder + 1N HCL	Pale green	Yellowish brown	Yellowish brown
Powder + 50% H_2So_4	Reddish orange	Fluorescent green	Fluorescent green
Drug powder + Nitric acid	Reddish orange	Yellowish green	Pale green
Drug Powder + Picric acid	Yellowish green	Fluorescent green	Pale green
Drug Powder + Acetic acid	Yellowish orange	Fluorescent green	Fluorescent green
Drug Powder + Ferric chloride	Brown	Green	Green
Drug Powder + HNO_3 + NH_3	Reddish orange	Yellowish green	Pale green

Table- 3: Preliminary phytochemical screening of Leaf extracts of *Alstonia venenata*

No.	**Test**	**Reagent**	**Observation**	**Hexane**	**Chloroform**	**Ethanol**	**Water**
1	Alkaloids	Dragendorff's reagent	Orange ppt.	-	+	+	+
		Mayer's Test	White ppt.	+	+	+	+
		Hager's Test	Yellow	+	+	+	+
2	Terpenoids	Noller's Test	Pink	-	-	+	-
3	Steroid	Liebermann-Burchard's Test	Bluish green	-	-	-	-
		Salkowski Test	Red, green	-	-	+	-
4	Coumarin	10% NaOH	Yellow	-	-	+	+
5	Tannin	1% Lead acetate	White ppt.	+	+	+	+
6	Saponin	Water	Foam like froth	+	+	+	+
7	Flavonoids	Shinoda's	Reddish pink	-	+	+	-
		Zn-Hcl	Magenta	-	+	+	-
		$Fecl_3$	Blackish red	-	+	+	+
8	Quinones	H_2So_4	Red	-	-	+	+
	Anthraquinones	Borntrager's	Pinkish Red	+	+	+	+
9	Phenol	$Fecl_3$	Intense colour	-	+	+	+
10	Protein	Biuret	Violet	-	-	-	-
		Xanthoprotein	Orange	+	+	+	-
		Lead acetate	White ppt.	+	+	+	+
		Millions	White ppt.	+	+	+	+
11	Carbohydrate	Fehling's sol.	Brick red	+	+	+	+
		Molisch	Purple	+	+	+	-
12	Glycosides	Anthrone + H_2So_4	Purple colour	+	+	+	-
14	Gum	Water	No thickening	-	-	-	-
15	Starch	I_2KI_2	Red	-	+	+	+
16	Fixed oil	Press between filter paper	No oil stain	-	-	-	-

Discussion

Literature dealing with the anatomy of leaf of different species of *Alstonia* is minimal. The present study attempts a modest comprehensive investigation of the leaf of *Alstonia venenata*. Since the leaf of *Alstonia venenata* as the tribal claims has therapeutic qualities the investigation has laid down a set of anatomical features of the leaf which can employed for its botanical diagnosis. The salient features of identification of the fragmentary sample are as follows.

- Leaf dorsiventral, hypostomatic with narrow palisade zone and reticulate spongy mesophyll tissues.
- Midrib with wide adaxial hump and wide semicircular abaxial part; vascular strands single, broadly bowl shaped, bicollateral. Laticifers frequent in the ground tissue.
- Epidermal cells with papillate outer tangential walls and wavy anticlinal walls; stomatal type paracytic.
- Venation of the lamina is characteristic in having parallel primary and secondary lateral veins and rectangular horizontally oriented vein islets.
- Petiole more or less circular with two lateral short wings; vascular strand single, collateral, bowl shaped laticifers common with parenchymatous ground tissue. It shows that *Alstonia venenata* is restricted in distribution as compared with other species. Due to scarcity of the specimens of *Alstonia venenata* there is possibility of substitution or even adulteration of *Alstonia scholaris* or among other plants.

Adulteration can be checked at least at the laboratory level where microscopic standards are largely employed. Anatomical features of plants have been considered as highly dependable guide lines for diagnosis of fragmentary plant [14].Many structural features have established as specific at the species or generic level. Especially, many qualitative characters, such as petiolar vasculature, venation pattern, trichome morphology and pattern of secondary growth in stem/root are much reliable features in systematic anatomy as well as pharmaceutical studies.

In the present study on *Alstonia venenata*, farily detailed analysis was made on the anatomical features of this taxon. The results of the study provide a protocol of diagnostic features of *Alstonia venenata* which can be readily employed for diagnosis of the plant from any other simulating species of plants.

Physicochemical Constants

The physical constant evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs [15]. Equally important in the evaluation of crude drugs, is the ash value and acid-insoluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, i.e., the presence or absence of foreign organic matter such as metallic salts and/or silica[16].

The ash value of *Alstonia venenata* leaves is 5.34%. This ash value is indicative of the impurities present in the drug. Since the ash value is constant for a given drug, these values are also one of the diagnostic parameters of the drug. Sample has more water soluble ash than acid in soluble ash. The ash value is generally index of the purity as well as identity of the drug.

Fluorescent Analysis

Many phytocompounds fluoresce when suitably illuminated. The fluorescence colour is specific for each compound. A non fluorescent compound may fluoresce if mixed with impurities that are fluorescent. The fluorescent method is adequately sensitive and enables the precise and accurate determination of the analyte over a satisfactory concentration range without several time consuming dilution steps prior to analysis of pharmaceutical samples [17].

The powder from the leaves of *Alstonia venenata* fluoresced greenish brown under day light, green under short UV and browning long UV light.

Preliminary Phytochemical Analysis

Presence or absence of certain important compounds in an extract is determined by colour reactions of the compounds with specific chemicals which act as dyes. This procedure is a simple preliminary pre-requisite before going for detailed phytochemical investigation. Various tests have been conducted to find out qualitatively the presence or absence of bioactive compounds. Different chemical compounds such as alkaloids, terpenoids, steroids, coumarin, tannin, saponin, flavonoid, quinines anthraquinones, phenols and glycosides were detected in *Alstonia venenata* which could make the plant useful for treating different ailments as having a potential of providing useful drugs of human use. This is because the pharmacological activity of any plant is usually traced to a particular compound.

Therapeutically terpenoids exert wide spectrum of activities such as antiseptic, stimulant, diuretic, anthelmintic, analgesic and counter-irritant [18]. Many tannin containing drugs are used in medicine as astringent. They are used in the treatment of burns as

they precipitate the proteins of exposed tissues to form a protective covering [19]. They are also medically used as healing agents in inflammation, leucorrhoea, gonorrhoea, burns, piles and antidote [20].

Saponins, a group of natural products occur in the leaf extracts of *Alstonia venenata*. In plants, the presence of steroidal saponins like, cardiac glycosides appear to be confined to many families and these saponins have great pharmaceutical importance because of their relationship to compounds such as the sex hormones, cortisones, diuretic steroids, vitamin D etc.,[21]. From plant sapogenins, a synthetic steroid is prepared and to treat a wide variety of diseases such as rheumatoid arthritis, collagen disorders, allergic and asthmatic conditions [22].

To understand the nature of the fluorescence emission from these crude preparations under different conditions, the preliminary phytochemical analysis of these crude preparations was compared. The comparative analysis clearly showed a correlation between a compound present in it and their fluorescent behavior under different conditions. The major bioactive compounds present in these crude preparations are the coumarins, flavones, steroids, tannins, alkaloids and saponins. Coumarin especially hydroxyl amino acid derivatives like o-coumaric acid appears yellowish green in alkaline condition under short UV radiation. Flavones which are light yellow in aqueous condition under UV light turns to bright yellow under alkaline conditions. Similarly the phytosterols when treated with 50% H_2SO_4 show green fluorescence under UV light. Terpenoids especially sapogenins exhibit yellow green fluorescence under short UV light [23]. Quinine, aconitin, berberin and emetin show specific colour of fluorescence (Aconitin-light blue; berberin-light yellow; emetin-orange). Fixed oils and fats fluoresce least, waxes more strongly and mineral salts most of all [24]. Haydon, [25] studied the photophysical characters of coumarins. Hydroxyl methyl coumarin fluoresced in the 420 - 440nm when observed in different solvents with increasing polarity [26]. The fluorescence analysis of the crude drug of *Alstonia venenata* exhibited clear fluorescence behavior at different radiations which can be taken as standard fluorescence pattern.

Now a days, standardization of herbal drugs is a topic of great concern. They are subjected to variability as derived from heterogenous sources. So in the present investigation, efforts were made to provide a scientific data to standardize the plant material for further studies. The present study on micro morphological features, other physical values and chemical parameters on the leaf of *Alstonia venenata* will help to identify the correct species of the plant, since no such scientific data are available for the same.

References

1. Gamble, J.S., (Rep. Ed.) The Flora of the Presidency of Madras 1957. II: 578-1346.
2. Mabberley, D.J., The Plant-Book. A portable dictionary of the vascular plants. 2005.Cambridge University Press.P. 267.
3. Sankar, R., Singh, V.K. and Rawat, M.S., Medicinal plants from Dibang Valley (A.P.) – Social Forestry and Afforestation B.M.E.B.R. 1993 XIV, pp. 144-149.
4. Pant S.C. and Pandey, G., Ethnobotanical studies on medicinal flora in tharu tribal pockets in Kumaon region in Uttar Pradesh. 1995. B.M.E.B.R. XVI pp. 1-10.
5. Prasad, P.N., Jabadhas, A.W.and Janakiammal, E.K., Medicinal plants used by the Kanikkars of South India. *J. Econ. Tax. Bot*. 1987. 11: 149 -155.
6. Sur, P.R., Sen, R., Halder, A.C. and Bandyo - padhyay, S., Observation on the Ethnobotany of Malda - West Dinajpur Districts, West Bengal II *J. Econ. Tax. Bot.* 1990.14: 453-459.
7. Sutha,S., Indian Journal of Traditional Knowledge Vol-9(3) July 2010. pp 502-509.
8. Wealth of India – Raw materials I- A.
9. Johanson, D.A., Plant Microtechnique. Mc Graw Hill Book Co**;** New York. 1940. pp. 523.
10. O'Brien, T.P., Feder, N. and Mc Cull, M.E., polychromatic staining of plant cell walls by toluidine blue-O. *Protoplasma* 1964. 59: 364-373.
11. Lala, P.K., Lab Manuals of Pharmacognosy, CSI Publishers and Distributors, Culcutta, 5 th (Ed) 1993.
12. Anonymous., Indian Pharmacopoea, Government of India, Ministry of Health and Family Welfare, The Controller of Publications, Civil Lines, Delhi – 110 054.1996.Vol.I & II.
13. Brindha, P., Sasikala, P. and Purushothaman, K.K., Pharmacognostic studies on merugan n kizhangu. Bull. Med. Eth. Bot. Res. 1981, 3, 84-96.

14. Metcalf, C.R and Chalk, L. Anatomy of the Dicotyledons Clarendon press,Oxford. 1950. Vol.I and II.
15. African Pharmacopoeia. General Methods for Analysis Ist ed.2: (OAU/STRC).Lagos. 1986. p. 123.
16. Musa, K.Y., Katsayal, A.U., Ahmed, A., Mohammed, Z. and Danmalam, U.H. Pharmacognostic investigation of the leaves of *Gisekia pharmacioides*. African J. Biotech. 2006, 5, 956-957.
17. Pimenta, A.M., Montenegro, M.C., Ara Ujo, A.N. and Mart'inez, J. C.,Application of sequential injections analysis to pharmaceutical analysis. J. Pharm. Biomed. Anul. 2006, 40, 16-34.
18. Gokhale, S.G., Kokate, C.K. and Purohit, A.P., A text Book of Pharmacognosy, Nirali Prakashan, Pune.2003.
19. Handa, S.S. and Kapoor, V.K., Pharmacognosy Vallabah Prakashan Publications, New Delhi. 1992. pp. 51.
20. Ali, M. Text book of Pharmacognosy. CBS Publishers and Distributors, New Delhi, 1994. pp 405.
21. Evans, W.C., and Saunders, W.B. (Eds.). Trease and Evan's Pharmacognosy Tokyo.2001, pp. 1-579
22. Claus, E.P. Pharmacognosy. (Ed.). Lea and Febiger, Philadelphia 1956, pp.1- 697.
23. Horborne, J.B. Phytochemical methods. Chapman & Hall, New York. 1976, pp. 1- 288.
24. Evans, W.C., Trease and Evans' Pharmacognosy. Singapore, Harcourt Baraco and Company Asia Pvt. Ltd. 1996, pp. 1-437. 25. Haydon, S.C., Spectroscopic letters. 1975, 8, 815.
26. Chaltopudhyay, N., Mallick, A. and Sengupt, S., Photophysical studies of hydroxyl methyl coumarin: A new fluorescent chemosensor for Zinc and Nickel ions in water. J. Photochem. Photobiol. . 2006, 177, 55-60.

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