September 2010 ISSN : 0974 - 7508 Volume 6 Issue 3

Trade Science Inc.

Natural Products

An Indian Journal

Full Paper

NPAIJ, 6(3), 2010 [107-113]

Comparative pharmacognostic studies in *Calotropis spp*.

G.M.Vidyasagar*, Bharathi Halu

Department of Postgraduate Studies and Research in Botany, Gulbarga University, Gulbarga - 585 106, Karnataka, (INDIA)

E-mail : gmvidyasagar@rediffmail.com; gmvidyasagar@gmail.com

Received: 29th April, 2010 ; Accepted: 9th May, 2010

ABSTRACT

The paper presents the comparative pharmacognostical studies of *Calotropis spp*, a traditional medicinal plant. It has been used as a folk medicine in many countries as stomachic, tonic, cough, catarrh, purgative etc. The pharmacognostic evaluation included organoleptic, microscopic, macroscopical and microscopical characters, physico-chemical constants, quantitative microscopy parameters, extractive values with different solvents, fluorescence analysis of extracts, its reaction after treatment with chemical reagents under visible light and UV light. Preliminary phytochemical screening and physicochemical properties have been studied.

KEYWORDS

Pharmacognosy;
Calotropis spp.;
Microchemical;
TLC.

INTRODUCTION

In the present scenario world is turning towards natural medicines for better alternative to allopathic drugs. However a key obstacle, which has hindered the acceptance of the alternative medicines in the developed countries, is the lack of documentation and stringent quality control. There is a need for documentation of research work carried out on traditional medicines.[1] The beneficial medicinal effect of plant materials typically result from the combination of secondary products present in the plant. The medicinal actions of the plants are unique to particular plant species or groups are constituent with this concept as the combination of secondary products in a particular plant is taxonomically distinct.[2] According to the World Health Organization[3], the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken.[3] *Calotropis* is an important traditional medicinal plant. Two species of *Calotropis C. gigantea and C. procera*, both of which are reported to be used in distinct way to cure various diseases (TABLE 1). Keeping this in view, pharmacognostic standardization of the Calotropis spp was undertaken as no work have been done in this respect to differentiate between the species. The species of *C. gigantea* has flowers ranging from purple to white colour. In traditional medications people suggest that white flowered is preferred over purple. For this reason they were considered as two different samples and further work was carried out considering three samples viz, *C.procera, C.gigantea* white flowered, *C.gigantea* purple flowered further represented as R, W, P in the tables.

MATERIALS AND METHODS

The fresh materials of Calotropis were collected in the month of December 2008 from Gulbarga university

Full Paper

TABLE 1 : Ethnomedical information for calotropis spp.[4-10]

Plant spp	Part	Uses
Calotropis procera	--	Boils, remove thorns from the body.
	Root	anti dote for snake bite,
	Root, bark, latex, flower, leaves, juice	Abortifacient, antidote in scorpion sting, dropsy, eczema, epilepsy, fever, insect bite, laxative, leprosy, rheumatism, ringworm, scorpion bite, skin diseases, small pox, sores, spleen, complaints, stomach, diseases, swell, tooth ache, tooth worms, wounds, swell of mouth
	Root	malaria
	Root	herpes
	Leaves	sprain
	Flowers	cough, asthma, cold
	Root bark	dysentery, cough, skin disease, cutaneous, infection, elephantiasis
Calotropis gigantea	Juice	purgative, tooth ache
	Flower and milky juice	Asthma, malaria
	Latex, leaves, root, bark, juice, flower	Abortifacient, anasarca, anti-fertility, asthma, body ache, boils, burns, carbuncle, child birth, dropsy, dysentery, ear complaints, epilepsy, eye complaints, guinea-worm, hydrophobia, injuries, intestinal worms, leprosy, purgative, rheumatism, ringworm, stomach ache, syphilis, tetanus, tooth ache, worms in gum and wounds

campus. These were identified, confirmed and authenticated at The Department of Post Graduate Studies and Research in Botany, Gulbarga University, Gulbarga. The collected fresh materials were washed and used for study of organoleptic and microscopic characteristics. The powder of dried leaves was used for the determination ash values, extractive values and phytochemical investigations.

Morphological studies

The macroscopical characters of various features were observed and tabulated in TABLE 2.

Microscopical studies

Fresh materials were collected for the microscopical studies. Anatomical and Quantitative studies were carried out.

TABLE 2 : Morphological features of the samples

Part	Observations	
	P and W	R
Height	2-3 mt	2.5-6 mt
Flower	Purple, white	Purple lilac
Fruit	Follicle recurved	Sub globose to obliquely ovoid
Seed	Ovate	Flat obovate
Leaves	Obovate to oblong, 10-20 x 3-8 cm, sessile, astipulate	Obovate to oblong, 5-28 x 2.5-15 cm, Sub sessile, astipulate
Arrangement	Opposite	Opposite
Venation	Reticulate	Reticulate
Apex	Pointed	Pointed
Margin	Entire	Entire
Surface	Glabrous	Glabrous
Petiole	Petiolate, cylindrical	Petiolate, cylindrical
Leaf Base	Symmetrical, cordate	Symmetrical, cordate

Morphology of flowers

Full Paper

Anatomy

Microscopic sections of stem, petiole and leaf were made by preparing thin free hand sectioning. Numerous temporary and permanent mounts of the double stained microscopical sections of the specimens were made and examined microscopically.

The comparative anatomy of all three samples revealed that anatomy of the spp to be the same.

Quantitative investigation

Quantitative leaf microscopy was done by taking leaf peel. The palisade ratio, stomata number, stomatal index, vein – islet number and veinlet termination number were carried out on epidermal strips and tabulated in TABLE 3.

TABLE 3 : Leaf constants for *Calotropis*

Leaf constants	P	W	R
Stomatal index			
Upper epidermis	0.095	0.096	0.062
Lower epidermis	0.123	0.138	0.106
Vein –islet number	84	102	73
Vein-termination number	30	30	17

Powder analysis

Microscopical examination of leaf powder

The powdered plant material also was observed for characterization.

The plant material was also used for microscopical observation of fibers. The plant material was macerated and observed for fibres according to Schultze's method[11].

Preliminary examinations of leaf powder

The powder was examined for its various characteristics and tabulated in TABLE 4.

TABLE 4 : Powder characteristics

Preliminary examination of powder	P	W	R
Colour	Green	Brownish green	Green
Odour	Odourless	Odourless	Odourless
Taste	Tasteless	Tasteless	Tasteless
Texture	Coarse	Course	Course
Mucilage	Absent	Absent	Absent

Phytochemical screening of the leaf powder

The powdered material was screened for the presence or absence of different phytoconstituents viz steroids, tannins, starch, alkaloids, flavanoids, proteins, anthroquinone glycosides which are tabulated in TABLE 5.

TABLE 5 : Different phytoconstituents in powdered leaf

Reagents	Colour/ppt	Constituent	P	W	R
Conc.sulphuric acid	Reddish	Steroids	+	+	+
Aqueous Ferric chloride solution	Blackish	Tannins	+	+	+
Iodine solution	Blue	Starch	-	-	-
Picric acid solution	Yellowish	Alkaloids	+	+	+
Aqueous Mercuric chloride solution	Brownish	Alkaloids	+	+	+
Aqueous Silver nitrate	No change	Anthroquinone glycosides	-	-	-

TABLE 6 : Fluorescence analysis of leaf powder of with different chemical reagents

Reagents	Normal light			U.V. light		
	P	W	R	P	W	R
Benzene	Brsh gr	Bk gr	Pr gr	Int rdsh or	Rds pnk	Int rds gr
Chloroform	Brsh gr	Lt gr	Lt pr gr	Lt rd or	Lt pnk	Yls pnk
Ethyl acetate	Int prt gr	Int prt gr	Prt gr	Int saffron	Grsh pnk	Or red
Ethanol	Lt prt gr	Int prt gr	Prt gr	Lt or pnk	Brsh gr	Or
Water	Trb ash	Brsh gr Tt	Brsh gr Tt	Lt ind blu	Lt gr	Gr Tt
1N Hcl	Trb ash	Brsh Tt	Brnsh Tt	Ind blu	Trb blk	Trb ash
1N NaoH in methanol	Ylsh gr	Prt gr	Prt gr	Lt ash	Blksh grn	Int brn
50% HNO3	Brsh gr	Ylsh brn	Yls brn	Gr	Fl gr	Fl gr
50% H2SO4	Grsh brn	Bksh gr	Int brn	Ash	Or gr	Or gr

Br- brown, Gr- green, Bk- black, Pr- parrot, Int-intense, Rd- red, Or-orange, Pnk-pink, Lt-light, Yl- yellow, Ind blu-indigo blue

Fluorescence analysis of leaf powder

Fluorescence analysis was conducted using methods of Kokoski[12] and Chase and Pratt[13]. The leaf powder was treated separately with different reagents ex-

Full Paper

posed to visible, U.V light and tabulated in TABLE 6.

Physico-chemical parameters of leaf powder

Percentage of total ash, acid-insoluble ash, water-soluble ash and sulphated ash were calculated as per the Indian Pharmacopoeia. The results were tabulated in TABLE 7.

TABLE 7 : Ash values of leaf powder.

Types of ash values	Ash value(% w/v)		
	P	W	R
Total ash	3	3	4.8
Acid insoluble ash	6.4	3	5.8
Sulphated ash	5	3.6	3.8

Preliminary phytochemical analysis of leaf extracts

For the preliminary phytochemical analysis, 5 g powdered drug was extracted with ethyl acetate, chloroform, methanol .The extracts were dried and weighed. The extracts were used for various analysis. Consistency, colour & fluorescence analysis, Extractive values with different solvents, Preliminary phytochemical screening of the extracts were tabulated in TABLES 8, 9 and 10 respectively.

TABLE 8 : Consistency, colour & fluorescence analysis of different leaf extracts

Extract	Consistency			Colour in daylight			Colour in UV light		
	P	W	R	P	W	R	P	W	R
Ethyl acetate	Greasy	Greasy	Greasy	GB	GB	GB	OP	OP	OP
Chloroform	Sticky	Sticky	Sticky	TGB	TGB	TG	OP	OP	OP
Methanol	Slurry	Slurry	Slurry	GB	B	BB	PP	P	PP

GB-Greenish brown, TGB- Turbid greenish brown, B- brown, OP- Orange pink, PP- Pinkish purple, P- Purple.

TABLE 9 : Extractive values with different solvents

Sample	% Extractability in different solvents		
	Ethyl acetate	Chloroform	Methanol
P	4.4	5.2	14.0
W	4.4	4.4	13.6
R	5.4	5.8	10.4

TABLE 10 : Preliminary phytochemical screening of the extracts.

Phyto constituent	Ethyl acetate			Chloroform			Methanol		
	P	W	R	P	W	R	P	W	R
Alkaloids	-	-	-	-	-	-	+	+	+
Glycosides	+	+	+	-	-	-	+	+	+
Phenolics	-	-	-	-	-	-	+	+	+
Phyto sterols	+	+	+	+	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+	+	+	+	+

TLC profiling

TLC profiling of crude leaf extracts of ethyl acetate and chloroform was carried out to find out the approximate number of compounds and their Rf values were calculated. One dimensional Thin Layer Chromatography was performed. The solvent system used as mobile

TABLE 11 : Bands observed during TLC analysis of leaf extracts.

Solvent	Number of bands			Colour of bands			Rf values		
	P	W	R	P	W	R	P	W	R
Ethyl acetate	4	4	5	Ylw	Pl grn	Pl grn	0.92	0.33	0.35
				Pl drt grn	Drt grn	Drt grn	0.84	0.58	0.63
				Drt grn	Ylw grn	Pl grn	0.50	0.66	0.80
				Ylw	Drt grn	Ylw grn	0.20	0.88	0.83
				Ylw	Ylw	Drt grn	0.25	0.76	0.90
Chloroform	4	5	4	Pl grn	Ash	Ylw	0.40	0.58	0.82
				Drt grn	Ylwsh drt grn	Pl drt grn	0.58	0.49	0.63
				Ylw	Drt grn	Drt grn	0.80	0.39	0.44
					Ylw	Pl ylw	0.91	0.15	0.21
Methanol	5	6	5	Ylw	Ylw	Ylw	0.78	0.89	0.87
				Brn	Brn	Prt grn	0.61	0.73	0.68
				Prt grn	Grn	Ylw	0.46	0.51	0.55
				Ylw	Ylw	Brn	0.33	0.39	0.37
				Pl ylw	Pl ylw	Drt grn	0.16	0.28	0.17
					Ash			0.12	

Ylw-Yellow, Pl drt grn- Pale dirty green, Pl grn- Palegreen, Drt grn-Dirty green, Ylwsh drt grn – yellowish dirty green, Pl ylw- pale yellow, Brn- brown, Prt grn- parrot green, Grn- green.

phase was Toluene: Ethyl acetate [85: 15]. The extracts of Ethyl acetate, chloroform and methanol were run for TLC. The Rf values were tabulated as in TABLE 11.

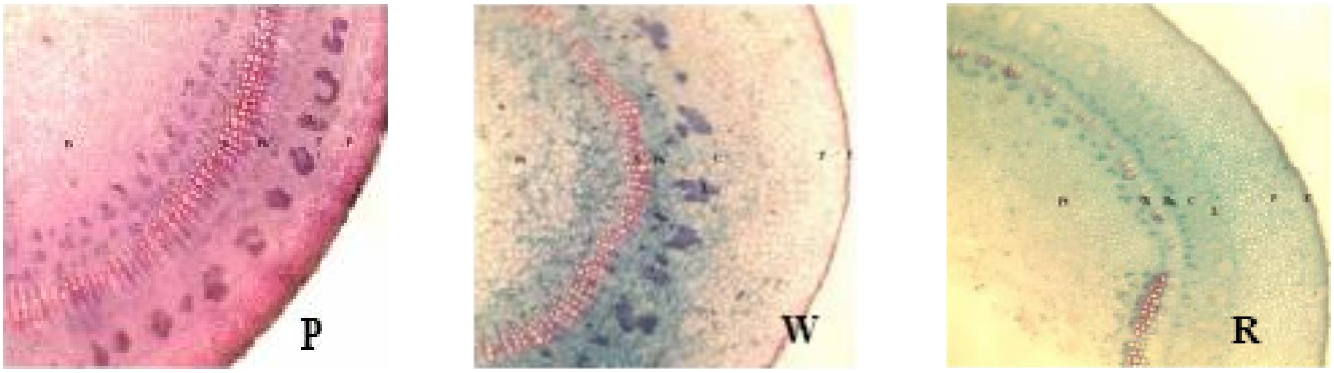
RESULTS

Anatomy

Stomatal type: Numerous anomocytic or ranunculaceous stomata are present.

T.S. of stem

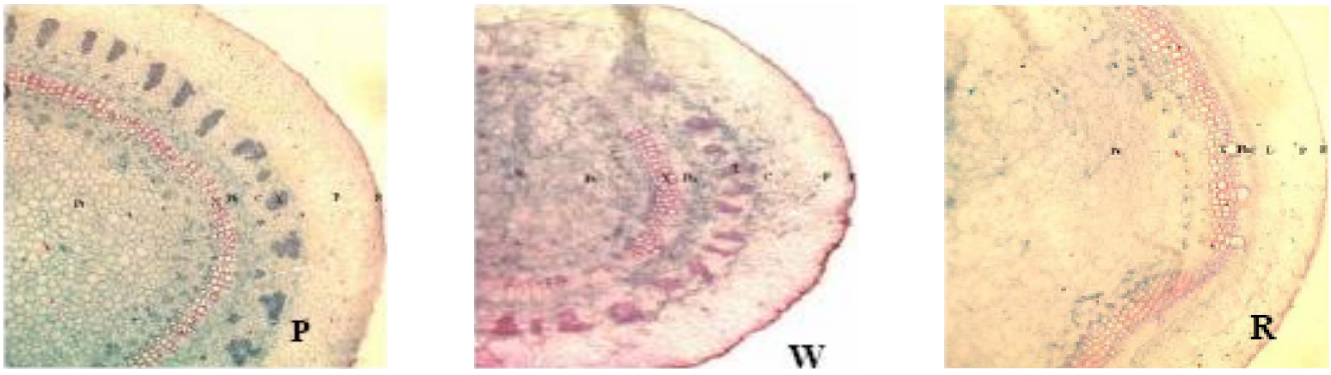
A single layered epidermis is surrounded by thick cuticle. The cells of epidermis are barrel shaped. From some of the cells develop multicellular epidermal hairs. Cortex follows epidermis which is differentiated into three regions. The outer region is parenchymatous hypodermis (20-25 layers). The middle region is sclerenchymatous (10-15 layers). Laticiferous cells arranged in groups at regular intervals in this region. The innermost layer is single layered endodermis with barrel shaped cells. Pericycle is not so distinct. Vascular system consists of primary phloem, secondary phloem, cambium, secondary xylem, primary xylem. A ring of vascular bundles is present which are conjoint, bicollateral, open and endarch. Pith is well developed with compactly arranged parenchymatous cells.



T.S. of stem

T.S. of petiole

A single layered epidermis is surrounded by thick cuticle. The cells of epidermis are barrel shaped. From some of the cells develop multicellular epidermal hairs. Cortex follows epidermis which is differentiated into three regions. The outer region is parenchymatous hypodermis (20-25 layers). The middle region is chlorenchymatous (10-15 layers). Laticiferous cells arranged in groups at regular intervals in this region. The innermost layer is single layered endodermis with barrel shaped cells. Pericycle is not so distinct. Vascular system consists of primary phloem, secondary phloem, cambium, secondary xylem, primary xylem. A ring of vascular bundles is present which are conjoint, bicollateral, open and endarch. Pith is well developed with compactly arranged parenchymatous cells.



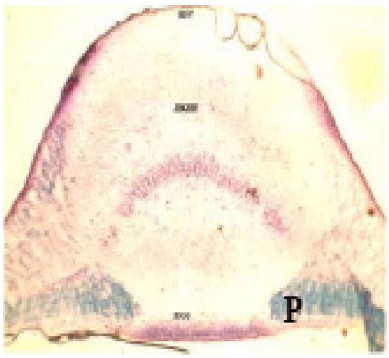
T.S. of petiole

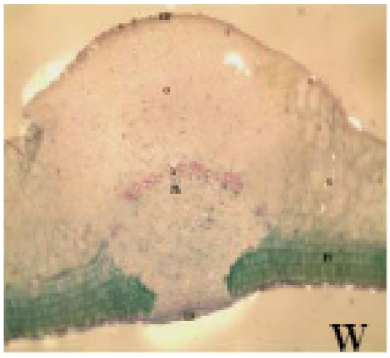
T.S. of leaf

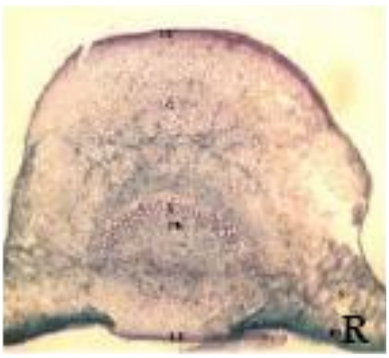
An epidermal layer is present on the upper and as well as lower surfaces. Thick cuticle present on both surfaces, the upper one being comparatively thicker. Sto-

Full Paper

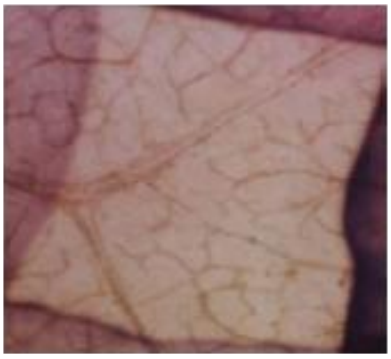
mata are present on both the surfaces. Mesophyll is differentiated into palisade and spongy parenchyma. The palisade layers are present just inner to upper epidermis and composed of elongated cells arranged in 7 layers. Parenchyma cells are present above and below vascular bundles. These cells interrupt the palisade layers and are said to be the extensions of the bundle sheath. Spongy parenchyma region is present just below the palisade and extends upto the lower epidermis. Vascular bundles are conjoint, collateral and closed. The xylem is present towards the upper epidermis and consists of vessels and xylem parenchyma. Protoxylem is present towards upper epidermis while the metaxylem towards the lower epidermis. Phloem is situated towards the lower epidermis.



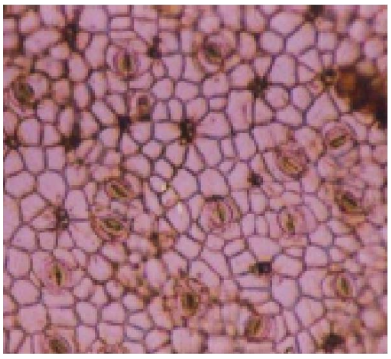




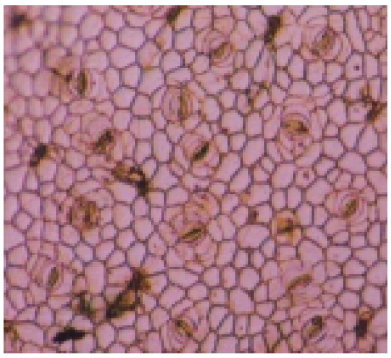
T.S. of leaf



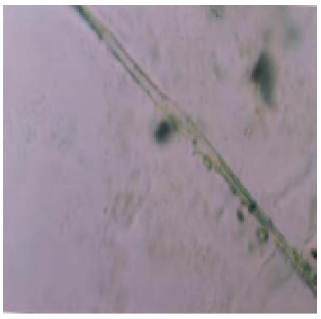
Details of veins

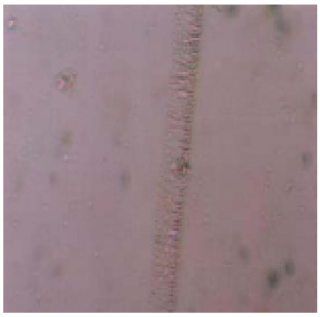


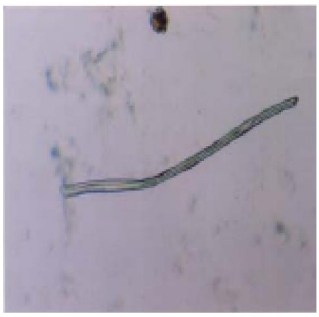
Stomatal type

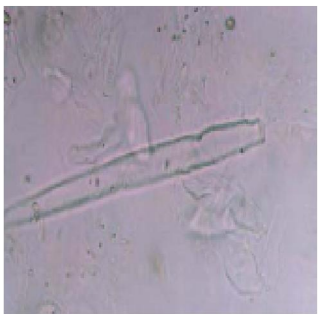


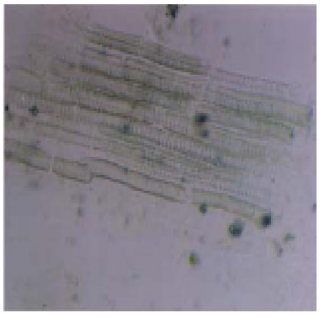
Stomatal type

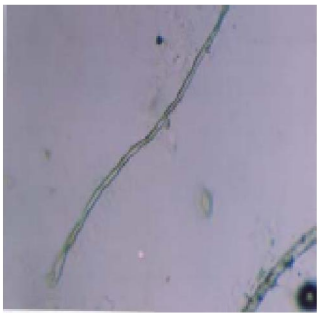


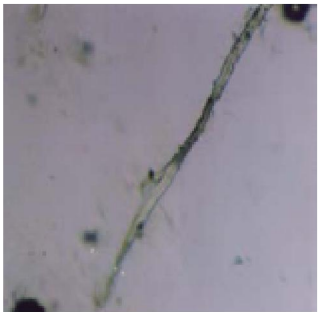












Different types of fibers

Microscopical examination of leaf powder

i) Fibers are lignified well developed sclerenchymatous from the vascular bundle region, thin, and isolated fibers measure 83 - 349 microns in length and10 - 20 microns in breadth. The fibres showed spiral thickenings.
ii) Fragments of mesophyll tissue containing vascular strands are seen good many in number.
iii) Fragments of leaf showing dorsiventral structure were also observed.

CONCLUSION

As there is no pharmacognostical work on record of this traditionally much valued drug, the present work was taken up with a view to lay down standards, which could be useful to detect the authenticity of this medicinally useful plant. Morphologically *C.procera* is like a shrub when compared to *C.gigantea* which like a small tree. The flowers of *C. procera* are morphologically distinct from *C.gigantea*. Anatomy, Phytoconstituents in powdered of all three samples were similar to each other. Therefore, we can not consider them as standard parameter. Fluorescence analysis of leaf powder of with different chemical reagents varied, Ash values of leaf also showed variations. Consistency of leaf extracts was same but variations were observed in colour & fluorescence analysis. Preliminary phytochemical screening of the extracts was also similar. The TLC profile showed significant variations in their constituents. The most important aspect observed in this work was that *C.gigantea* with flower colour i.e., white and purple varied in their phytoconstituents as revealed by TLC profile. This point to be considered because some of the traditional healers investigated prefer white flowered *C.gigantea* over purple flowered *C. gigantea*. Further work on these two samples of *C.gigantea* would help to explore the better one scientifically rather than considering them as same sample.

ACKNOWLEDGEMENT

The first author is thankful to University Grants Commission, South Campus of Delhi University, Benito Juarez Marg, New Delhi for providing Rajiv Gandhi National Fellowship for SC/ST research students.

REFERENCES

[1] S.A.Dahanukar, R.A.Kulkarni, N.N.Rege; Ind.J.Pharmacol., **32**, 81-118 **(2000)**.
[2] M.Wink; 'Introduction Biochemistry, Role and Biotechnology of Secondary Products'. In: M.Wink, Ed., Biochemistry of Secondary Product Metabolism. CRC Press. Boca Raton, FL, 1-16 **(1999)**.
[3] World Health Organization; Quality Control Methods for Medicinal Plant Materials, WHO Library, Geneva, 1-115 **(1998)**.
[4] M.K.Rai, A.K.Pandey, D.Acharya; Journal of Non-timber Forest, **7(3/4)**, 237-241 **(2000)**.
[5] Ch.Muhammad Ishtiaq, M.A.Khan, Wajahat Hanif; Asian Journal of Plant Sciences, **5(2)**, 390-396 **(2006)**.
[6] Ethnomedicinal Uses of Medicinal Plants in Varanasi Sl, http://www.biomedcentral.com/content/supplementary/1746-4269-3-35-S1.pdf
[7] S.D.Rout, H.N.Thatoi; Ethnobotanical Leaflets, **13**, 379-87 **(2009)**.
[8] S.D.Jagtap, S.S.Deokule, P.K.Pawar, A.M.Harsulkar; Ethnobotanical Leaflets, **13**, 98-115 **(2009)**.
[9] Mithun Sikdar, Uzzal Dutta; Ethno-Med., **2(1)**, 39-45 **(2008)**.
[10] E.Acharya (Siwakoti), B.Pokhrel; Our Nature, **4**, 96-103 **(2006)**.
[11] O.P.Sharma, Shivani Dixit; Experiments and Techniques in Plant Sciences. Pragati Prakashan, ISBN: 81-7556-364-8, 90-91.
[12] J.Kokoski, R.Kokoski, F.J.Salma; J.Am.Pharm. Ass., **47(10)**, 715-717 **(1958)**.
[13] C.R.Chase, R.J.Pratt; J.Am.Pharm.Ass., **38**, 324-333 **(1949)**.