

Pharmacognostic Standardisation of Heart Wood of *Combretum albidum* G.Don; An Important Ethno-Medicinal Liana

Sreekanth Sreedhar, U Prakash Kumar, *A B Rema Shree

Centre for Medicinal Plants Research, Arya Vaidya Sala, Kottakkal, Malappuram, Kerala, India

ABSTRACT

Traditional knowledge and ethno-botanical use of plants have been widely acknowledged all over the world. The tribes of Chinnar, Idukki District of Kerala are being using *Combretum albidum* G.Don as an effective remedy for jaundice, though they are aware about the use of *Phyllanthus* species against jaundice. So the validation and pharmacognostic standardisation of heart wood of *C. albidum* were carried out to establish its macro- and microscopical standards, physicochemical parameters, preliminary phytochemical investigation and TLC profiles to evaluate the characters of the plant. The dried piece of heart wood usually very hard, around 1 to 2 cm in thickness, bright yellow in colour, odourless and slightly bitter in taste. Detailed anatomy showed xylem region consists of vessels, xylem parenchyma loaded with starch grains, crystal fibers and medullary rays. Histochemical studies revealed the presence of lignin, tannin and starch grains. Physicochemical parameters like moisture content (7.82%), total ash (4.32%), acid insoluble ash (0.06%), extractive values of alcohol (6.56%) and water (7.51%) and sequential extractive values of sample in petroleum ether (0.64%), ethyl acetate (1.85%) and methanol (6.91%) were also evaluated. Preliminary phytochemical analysis indicated a high percentage of tannins, flavonoids and triterpenes. Well resolved TLC profiles were recorded in sequential extraction with petroleum ether, ethyl acetate and methanol. The above 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.

Key words: *Combretum albidum*, Histochemical study, Pharmacognostic standardisation, Physicochemical evaluation

INTRODUCTION

The Western Ghats of south-western India and the highlands of south-western Sri Lanka, together form one of the most densely populated biodiversity hotspots in the world. The Western Ghats alone harbours approximately 5,000 species of vascular plants belonging to nearly 2,200 genera; about 1,700 species (34 percent) are endemic (found nowhere else)¹. In traditional medicine a number of such plants are using, which is very effective, but the use and therapeutic efficacy of many of those plants are not yet been documented or scientifically validated.

Herbal drugs play an important role in health care programs especially in developing countries. However obstacle behind the acceptance of alternative medicines in developed countries is the lack of documentation and stringent quality control. So that the documentation and standardization of the raw materials used in herbal medicine is very essential for the worldwide acceptance of this system of medicine. Pharmacognostic standardisation, physicochemical analysis and preliminary phytochemical studies will help in identification and authentication of the genuine plant materials. Correct identification and quality assurance of plant material is indispensable to ensure reproducible quality of herbal medicine, which will contribute to its safety and efficacy. Pharmacognostic standardisation of plant material include its morphological, anatomical and biochemical characteristics are the essential parameters².

Muthuvans, Chinnar tribe of Idukki district, Kerala is using many plants for their health care and day to day ailments, but many of them are not yet been scientifically validated. This tribe is using *Combretum albidum*, commonly known as Manjakody, as an effective remedy for jaundice. Though they are aware about the use of *Phyllanthus amarus* and *P. airy-shawii* against jaundice, they prefer water extract of stem bark of *C. albidum* as a remedy for both normal and severe jaundice³. The data collected from this area revealed that it is very effective for the treatment of any liver disorders. The macroscopy, microscopy (histochemical and powder), physicochemical standards, preliminary phytochemical investigation and TLC profiles in petroleum ether, ethyl acetate and methanol extracts of stem bark were studied⁴. *C. albidum*, Buffalo calf plant in English, is a large woody climbing, deciduous shrub, up to 30 m high, Leaves opposite, decussate, ovate or ovate-lanceolate, 6-10 x 3-6 cm, thin coriaceous, 7-9 nerved, prominent below. Its distribution is restricted in semi-evergreen and deciduous forests, along river banks of Peninsular India and Sri Lanka.

As per the survey conducted in that area, it is noticed that while taking the stem bark, some part of the woody region also attached intentionally or accidentally along with the material. Therefore in the present study, pharmacognostic standardisation of heart wood of *C. albidum* was carried out to establish its macro- and microscopical standards, physicochemical parameters, preliminary phytochemical

Figure 1. *C. albidum* G. Don



A. Habit

B. Dried pieces of heart wood

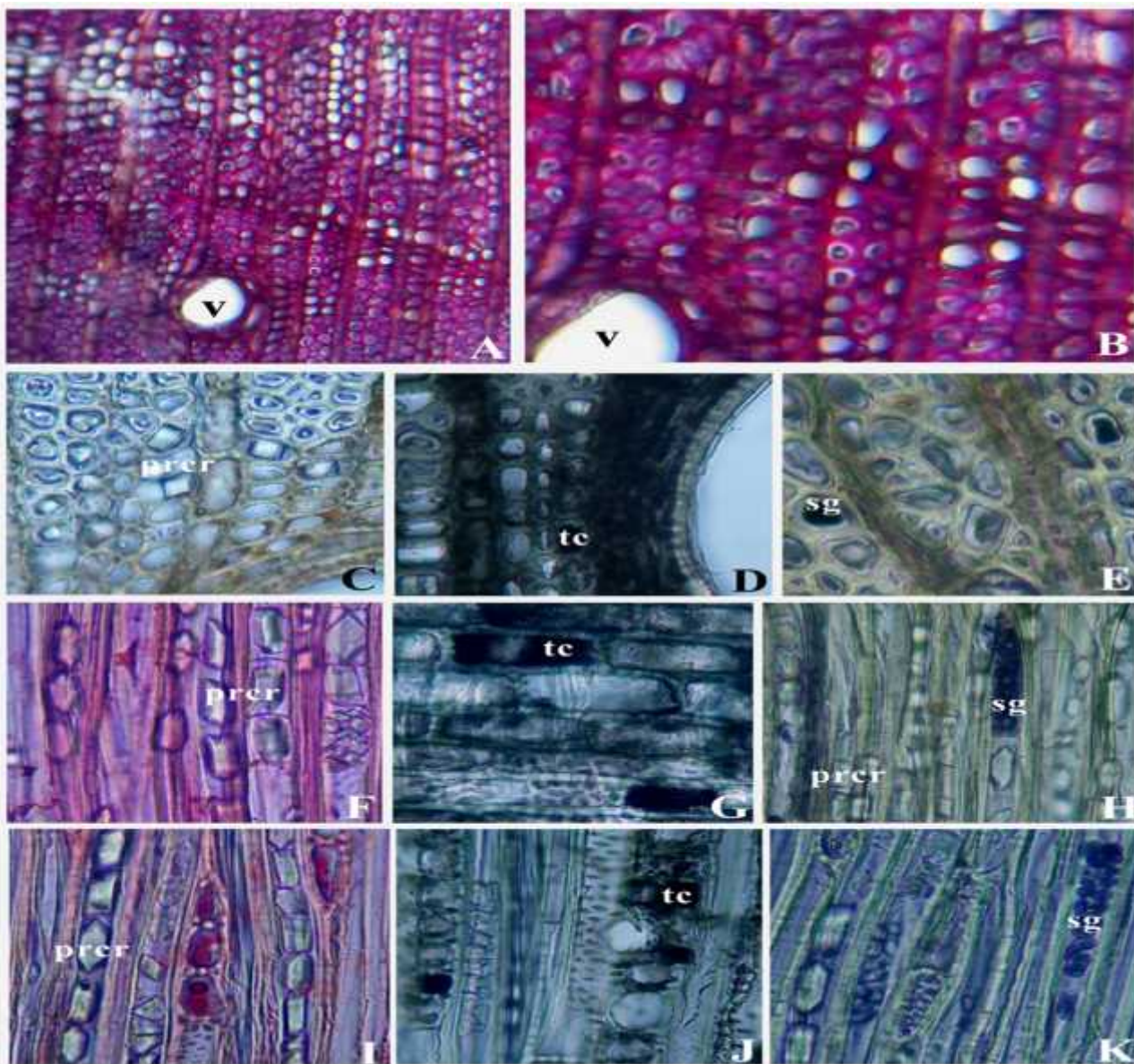


Figure 2. Microscopy of *C. albidum* heart wood. A & B TS x 100 & x 400. C, TS showing prismatic crystals of calcium oxalate x 400; D, G & J, histochemical test for tannin deposits; E, H & K, histochemical test for starch x 400. F-H, RLS x 400; I-K, TLS, Abbreviation: prcr, prismatic crystals of calcium oxalate; sg, starch grains; tc, tannin contents; v, vessel).



Figure 3. Powder microscopy of heart wood of *C. albidum*. **a & f**, fragment of crystal fibers; **b**, starch grains; **c**, prismatic crystals of calcium oxalate; **d & e**, fragments of bordered pitting; **g**, fragment of parenchyma cells embedded with starch grains; **h**, stone cell

Table 1: Physicochemical parameters of heart wood of *C. albidum*

Physicochemical constants (%)	Total ash	Acid-insoluble ash	Moisture content
	4.32	0.06	7.82
Extractive values (%)	Ethanol	Water soluble	
	6.56	7.51	
Sequential extractive values (%)	Petroleum ether	Ethyl acetate	Methanol
	0.64	1.85	6.91

investigation and TLC profiles to characterise the plant material.

MATERIALS AND METHODS

Collection and Authentication: The plant collected from Chinnar, Idukki district of Kerala and authenticated at Taxonomy Division, Centre for Medicinal Plants Research

(CMPR), Arya Vaidya Sala, Kottakkal, Kerala. The voucher specimen (Col. No. 5545) and raw drug (Col. No. CMPR/RD 211) were deposited at CMPR herbarium and raw drug depository respectively. One part of heart wood is preserved in Formalin: Acetic acid: Alcohol mixture (FAA) for anatomical studies and the remaining part shade dried and powdered for the estimation of physico-

Table 2. Preliminary phytochemical investigation of methanolic extract of heart wood of *C. albidum*

Test for active constituents	Tannins	Flavonoids	Triterpenes	Glycosides	Saponins	Steroids	Alkaloids
	+++	++	+	+	+	-	-

Absent; + presents

Table 3. TLC details of petroleum ether extract of heart wood of *C. albidum*

Detection/ Visualization	Under UV 254 nm		Under UV 366 nm		After derivatization	
	R _f values	Colour of the band	R _f values	Colour of the band	R _f values	Colour of the band
Track 1, 2 & 3	0.46	Grey	0.10	Blue	0.12	Greyish
	0.63	Grey	0.21	Blue	0.18	Greyish violet
	0.75	Grey	0.41	Greenish blue	0.28	Violet
			0.53	Blue	0.36	Greyish blue
					0.42	Greyish blue
					0.45	Yellow
					0.64	Violet
				0.73	Bluish grey	

Table 4. TLC details of ethyl acetate extract of heart wood of *C. albidum*

Detection/ Visualization	Under UV 254 nm		Under UV 366 nm		After derivatization	
	R _f values	Colour of the band	R _f values	Colour of the band	R _f values	Colour of the band
Track 1, 2 & 3	0.17	grey	0.17	Blue	0.05	Violet
	0.45	Grey	0.52	Blue	0.08	Greyish violet
	0.49	grey	0.91	Blue	0.15	Greyish violet
	0.53	grey			0.25	Greyish violet
	0.58	grey			0.29	Greyish violet
					0.36	Bluish black
					0.43	Greyish
					0.49	Greyish violet
					0.53	Brown
					0.56	Bluish black
				0.59	Bluish black	
				0.65	Brownish grey	
				0.73	brown	

chemical parameters and preliminary phytochemical investigation.

Pharmacognostic Standardization: The organoleptic characters such as shape, size, colour, odour, taste and fracture of heart wood were determined. Microscopic studies were done by preparing thin hand section of heart wood, cleared with chloral hydrate solution, stained with phloroglucinol-hydrochloric acid (1:1) and mounted in glycerine^{5,6}. Histochemical studies and powder microscopy were carried out to know about the inclusions and detailed anatomical characters of the material⁷.

Physicochemical Evaluation: Moisture content, total ash, acid-insoluble ash and extractive values in alcohol and water were carried out as described in Indian Pharmacopoeia⁸.

Preliminary Phytochemical Screening: The methanolic

extract of heart wood was screened for the presence or absence of the major class of compounds by standard method⁹.

TLC profile: Powdered heart wood, 10 g was extracted by refluxing with petroleum ether, ethyl acetate and methanol (50 ml x 3) sequentially for a period of 30 minutes each and the combined extract of each were filtered and concentrated to 10 ml. Apply 10 µl each extract as bands at a height of 10 mm from the base of a 5 x 10 cm precoated silica gel Aluminium plate 60 F₂₅₄ using CAMAG Automatic sampler (ATS4) and developed up to 80 mm from the base of the plate in an Automatic Developing Chamber (CAMAG ADC2) using the mobile phase hexane: ethyl acetate (9:1) v/v for petroleum ether, chloroform: ethyl acetate: formic acid (8:2:1) v/v for ethyl

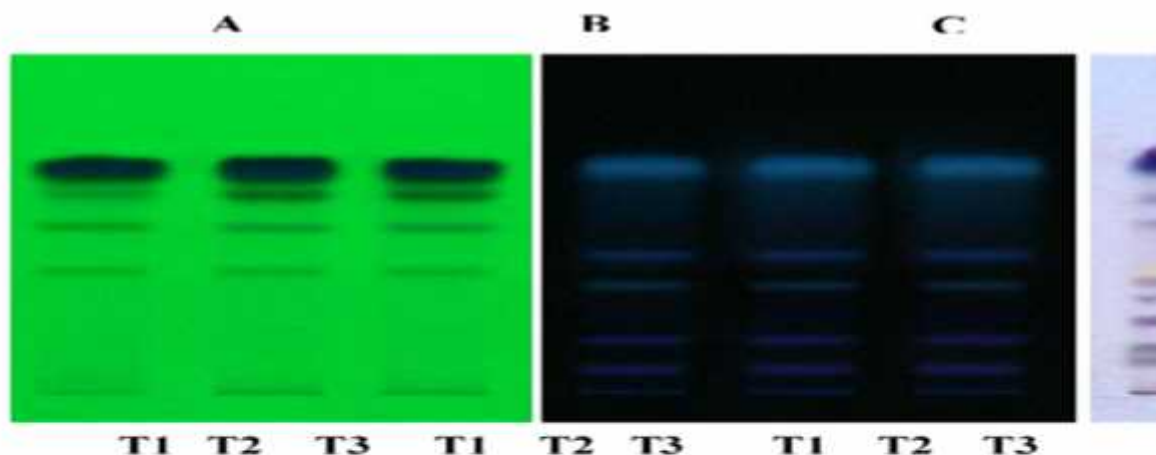


Figure 4. TLC details of petroleum ether extract of heart wood of *C. albidum*. A. UV 254 nm; B. UV 366 nm; C. Dervatized with ANS.

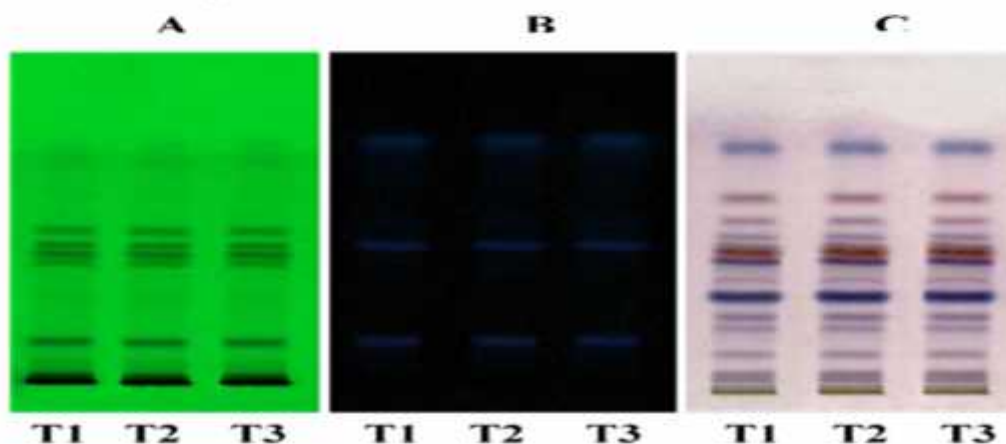


Figure 5. TLC details of ethyl acetate extract of heart wood of *C. albidum*. A. UV 254; B. UV 366; C. Dervatized with ANS.

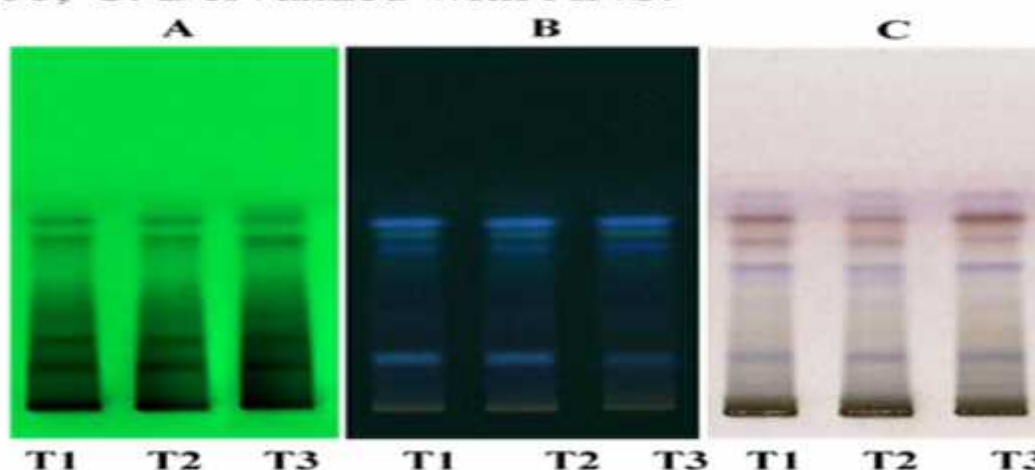


Figure 6. TLC details of methanol extract of heart wood of *C. albidum*. A UV 254; B. UV 366; C. Dervatized with ANS.
Abbreviation: T1, Track 1; T2, Track 2; T3, Track 3

Table 5. TLC details of methanol extract of heart wood of *C. albidum*

Detection/ Visualization	Under UV 254 nm		Under UV 366 nm		After derivatization	
	R _f values	Colour of the band	R _f values	Colour of the band	R _f values	Colour of the band
Track 1, 2 & 3	0.16	grey	0.19	Blue	0.11	Greyish violet
	0.26	grey	0.58	Blue	0.20	Greyish black
	0.62	Light grey	0.63	Greenish blue	0.52	Violet
	0.67	Light grey	0.67	Blue	0.61	light brown
					0.70	Brown

acetate and ethyl acetate: formic acid (9:1) v/v for methanol extract. Dry the plate in air and profile pictures were taken in CAMAG documentation visualizer attached with DXA252 camera under UV 254 nm, 366 nm and in visible light after derivatization with anisaldehyde sulphuric acid reagent (ANS).

RESULTS AND DISCUSSION

Macroscopic characters: Drug occurs in pieces of variable size and thickness, yellowish white in colour, fracture hard, odourless and slightly bitter in taste (Figure 1).

Microscopic characters: Detailed TS of heart wood showed xylem region consists of vessels with bordered pitted thickening, parenchyma filled with starch grains and tannin contents, crystal fibres embedded with prismatic crystals of calcium oxalate. Medullary rays are uni to biseriate loaded with starch grains (Figure 2). Radially longitudinally cut section showed broad lumened bordered pitted vessels, thick walled crystal fibres associated with rows of parenchymatous cells embedded with starch grains and patches of rows of medullary rays crossing these elements at places. Tangentially longitudinal cut section is identical to RLS but differ so far as the medullary rays arrangement is concerned (Figure 2).

Powder microscopic characters: showed fragments of crystal fibers, vessels with bordered pitted thickening, fragments of xylem parenchyma embedded with starch grains, stone cell; starch grains measures upto 18.50 µm and prismatic crystals of calcium oxalate as such scattered throughout the powder (Figure 3).

Physicochemical parameters: The value of loss on drying at 110⁰ showed the moisture content present in the sample, which is 7.82%. The total ash and acid insoluble ash were found to be 4.32% and 0.06% respectively. The ash contents showed the amount of inorganic matter present in the sample and the acid insoluble ash almost within 1%, which expresses low siliceous matter present in the sample. Extractive values of ethanol and water were 6.56 % and 7.51% respectively. Sequential extractive values of sample in petroleum ether, ethyl acetate and methanol were 0.64%, 1.85%, 6.91% (w/w) respectively (Table 1). **Preliminary phytochemical investigation:** Methanol extract of heart wood showed presence of tannins, flavonoids, triterpenes, saponins and glycosides. Steroids and alkaloids were found to be absent (Table 2). Flavonoids, triterpenoids and tannins are well known for their hepatoprotective activities¹⁰⁻¹². The plant possesses a high percentage of tannins, flavonoids and triterpenes and

this may be one of the reasons behind the hepatoprotective activity of this plant.

TLC profile: Well resolved TLC profiles were recorded for the future reference and identity of the plant material. TLC profile of petroleum ether extract showed three spots under UV 254 nm, four spot under UV 366 nm and eight spots in visible light after derivatization (Figure 4 & table 3). In ethyl acetate extract, profile showed five spots under UV254 nm, three spots under UV366 nm and thirteen spots in visible light after derivatization (Figure 5 & table 4). In methanol extract showed four spots under UV 254 nm, four spots under UV 366 nm and five spots in visible light after derivatization (Figure 6 & table 5).

All the above 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 used for Jaundice by Tribals of Kerala.

CONCLUSION

In the present investigation various standardization parameters such as macroscopy, microscopy (histochemical and powder), physicochemical constants, preliminary phytochemical investigation and TLC profiles of sequential extraction of sample in petroleum ether, ethyl acetate and methanol extracts were studied, which are being reported for the first time in this plant, could be helpful in authentication and preparation of a suitable monograph for the proper identification of heart wood of *Combretum albidum* G. Don.

ACKNOWLEDGEMENT

The authors are grateful to Arya Vaidya Sala, Kottakkal for providing support to carry out this work. We are thankful to Dr. Indira Balachandran, Project Director, Centre for Medicinal Plants Research (CMPR), Arya Vaidya Sala, Kottakkal for the necessary guidance.

REFERENCES

1. <http://www.biodiversityhotspots.org/xp/Hotspots/ghats/pages/biodiversity.aspx>
2. Anonymous, *macroscopic and microscopic examination: quality control methods for medicinal plant materials*, WHO, Geneva 1998.
3. Sajeev KK, Sasidharan N. Ethnobotanical observations on Tribals of Chinnar wildlife sanctuary. *Ancient Science of Life* 1997; 16: 284-292.

4. Sreekanth Sreedhar, Prakash Kumar U, and Reema Shree AB. Pharmacognostic Analysis of stem Bark of *Combretum albidum* G. Don; an un explored medicinal plant. *Pharmacognosy journal* 2012; 4, (28):13-16.
5. Kokate CK. *Practical Pharmacognosy*. Vallabh Prakashan, Delhi, 2002, 28-98, 112-114, 123-125.
6. Evans WC, Trease and Evans' Pharmacognosy, 14th ed., W.B.Sounders Company Ltd., London, 1996, 45-546.
7. Johansen DA. *Plant Microtechnique*. (McGraw – Hill, New York, USA), 1940.
8. Anonymous, *The Indian Pharmacopoeia*, 4th ed., Vol. II, The Controller of Publications, Government of India, New Delhi, 1996, A-53, A-54, A-089.
9. Trease GE, Evans WC. *Pharmacognosy*. Williams Charles Evans as edited in 15th edition. Saunders publisher London, 2004, 137-440.
10. Manjunatha BK, Vidya SM. Hepatoprotective activity of *Vitex trifolia* against carbon tetrachloride-induced hepatic damage. *Indian Journal of Pharmaceutical Sciences* 2008; 70 (2): 241-245.
11. Das S, Sarma G. Study of the hepatoprotective activity of the ethanolic extract of the pulp of *Eugenia Jambolana* (Jamun) in Albino rats. *Experimental Research* 2009; 3 (2): 1466-1474.
12. Absar AQ, Prakash T, Tushar P, Viswanath Swamy AHM, Veeran GA, Prabhu K and Ramachandra SS. Hepato-protective and anti-oxidant activities of flowers of *Calotropis procera* in CCl₄ induced hepatic damage. *Indian Journal of Experimental Biology* 2007; 45: 304-310.