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PHARMACOGNOSTICAL AND PRELIMINARY PHYTOCHEMICAL CHARACTERIZATION OF TENDER LEAVES OF COMBRETUM LATIFOLIUM BLUME - A FOLKLORE MEDICINAL PLANT

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ABSTRACT

Objective: The study is undertaken with the intention to document the pharmacognostic characters in order to ascertain identity, quality and authenticity of the crude drug. Further the Phytochemical class predominantly to which the plant belongs will be of great importance contributing to its biological activity and therapeutic use. The tender leaves of *Combretum latifolium*(*C. latifolium*)Blume. are used to render the immune system strong. Combretum species are extensively used in traditional medicinal system from inflammation to diuretic. The present study is a step to unleash its phytochemical characterization which may be responsible for its biological activity. Pharmacognostic characters will serve the drugs identity and authentication. **Materials and methods:** Sample of crude plant material were collected around Udupi area and its identity was thus confirmed by botanist. Macro-microscopic study of the preserved specimen in the fixative was conducted in the department of Pharmacognosy and preliminary phytochemical test and HPTLC fingerprint of the leaf extract was carried out as per testing protocols. **Results and Discussion:** The unique microscopic characters of this leaf is arc shaped collateral vascular bundle which is encircled by pericylicfibre. Trichomes were found to be absent. Standardization parameters such

as moisture content (8.97±0.01), total ash (5.83±0.16), acid insoluble ash (0.10±0.00), water soluble ash (3.19±0.01), alcohol soluble extractive value (7.79±0.01), water soluble extractive value (27.06±0.00) are conducted as a part to prove quality standards proposed by the guidelines. **Conclusion:** Microscopic characters, standardization parameters, HPTLC will serve as a fingerprint revealing various phytoconstituents pertaining to this plant material. Phytoconstituents belonging to chemical groups such as alkaloids, carbohydrates, steroids, tannins, flavonoids, phenols, coumarins, resins were found to be present through qualitative screening tests. Proximate analysis involving total fat, fibre, protein, carbohydrates will appraise the total calorific value calculated by the formula (Carbohydrate × 4) + (Fat × 9) + (Protein × 4).

Keywords: *Combretum latifolium*, Densitometry, FAA

INTRODUCTION

The folklore informers are the bridge between the traditional Ayurvedic knowledge and new Ayurvedic research scholars. These healers are silently serving the society without any advertisements. The extra pharmacopoeial drugs used by such people which are not mentioned in any texts book of *Ayurveda* has to be studied in detail and bring them to mainstream practice for the healthy society. *Combretum latifolium* (*C. latifolium*) Blume**.** is one such which is used by the traditional people in Udupi District.*C. latifolium* belongs to combretaceae family, commonly called as *chagarukudi* in Kannada. It is fairly common in forests of Western Ghats. It is a large scandent shrub. Leaves are broadly elliptic or obtuse, shortly acuminate at apex, round at base, glabrous. Flowers pale yellow, Fruits ovoid, with 4 membranous wings.[1] The tender leaves of this plant are used to increase the immunity of the children and cure the common ailments of children. It is also used in worm infestation.[2]Hence by observing these utilities of this plant, a pharmacognostical study- macro and microscopical and preliminary phytochemical study has been carried out in the present investigation.

Materials and methods

Collection of samples: Samples of Combretum latifolium Blume. were collected from Karje, Bramhavara and Kokkarne region of Udupi district during July and August month 2018. The authenticity of plant was confirmed by expert Botanist. Botanical characters were also compared with flora of South Canara.

Preservation of sample: The collected samples were air dried and packed and preserved in airtight containers at SDM Centre for Research in Ayurveda and Allied Sciences, Udupi for pharmacognostical & phytochemical studies. For microscopic examination sample was preserved in fixative solution FAA (Formalin 5 ml + Acetic acid – 5 ml + 70% Ethyl alcohol – 90 ml) for more than 48 h.

Macroscopy: The external features of the test samples were documented using Canon IXUS digital camera. The macroscopic features were compared to local flora for authentication.

Microscopy: Sample was preserved in fixative solution. The fixative used was FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml). The materials were left in FAA for more than 48 hours. The preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained with safranin. Transverse sections were photographed using Zeiss AXIO trinocular microscope attached with Zeiss AxioCam camera under bright field light. Magnifications of the figures are indicated by the scale-bars. Physicochemical analysis of Combretum latifoliumBlume. powder was tested for pharmacopoeial constants like loss on drying at 105oC, total ash, acid insoluble ash, alcohol soluble extractive, water soluble extractive as per standard protocol.[3]Preliminary phytochemical investigation was done to detect the presence of alkaloids, steroids, carbohydrates, tannin, flavanoids, saponins, triterpenoids, coumarins and phenols in alcoholic extracts of Combretum latifoliumBlume.[4]

HPTLC: 1g of *Combretum Latifolium* Blume. powder was extracted with 10 ml of alcohol. 4, 8 and 12µl of

the above extract were applied on a pre-coated silica gel F_{254} on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate (7.0: 3.0). The developed plates were visualized in short UV, long UV and then derivatized with vanillin sulphuric acid and scanned under UV 254nm, 366nm and 620nm. Rf, colour of the spots and densitometric scan were recorded. [5, 6]

Nutritional values assessment of *Combretum Latifolium* Blume: Determination of fat, fibre, protein and carbohydrate was done using standard procedures.[7, 8]

Results and Discussions

Figure 1: Macroscopy of *C latifolium*

Fig 1a. Macroscopy of *C.latifolium*Fig 1b. Leaf enlarged

Figure 2: Microscopy of *C. latifolium*

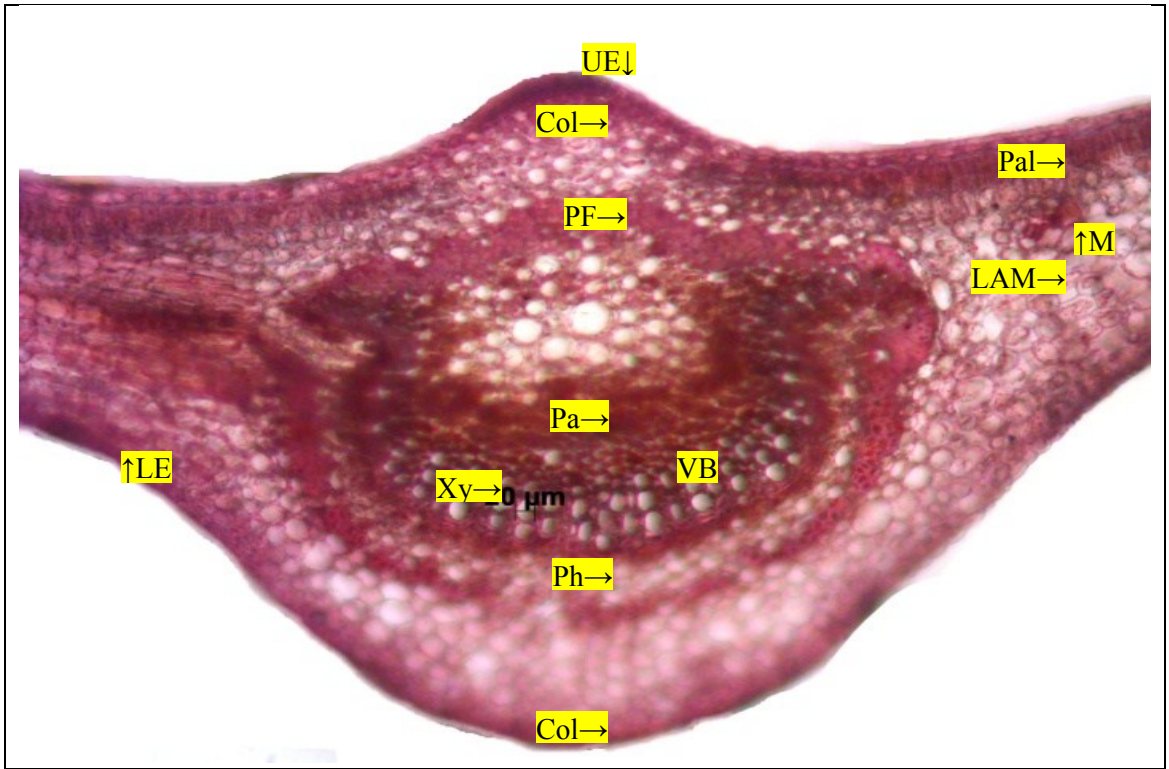

Fig 2a. T.S of leaf

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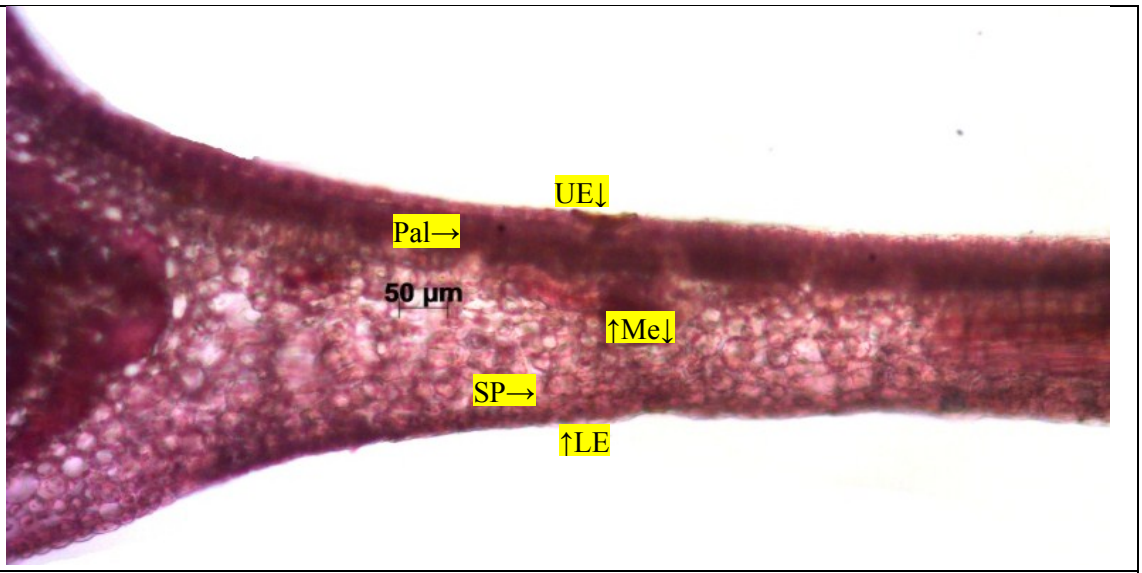

Fig 2b. T.S of lamina

Col – collenchyma; LAM – lamina; LE – lower epidermis; Me – mesophyll; MR – midrib; Pa – parenchyma; Pal – palisade; PF – pericyclic fibres; Ph – phloem; RC – rosette crystal; SP – spongy parenchyma; UE – uppr epi-dermis; VB–vascular bundle; Xy – xylem.

Stained with safranin

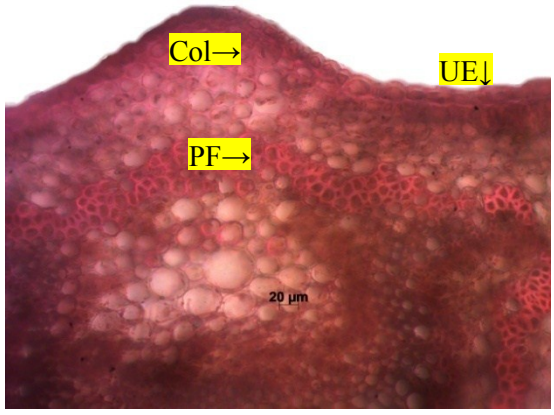

Fig 2c. Upper midrib

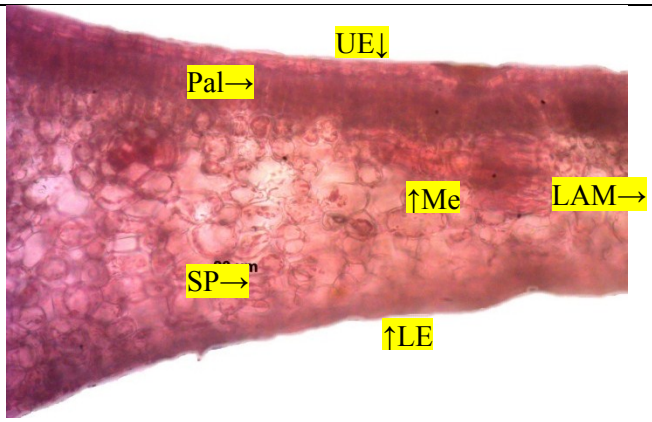

Fig 2d. lamina enlarged

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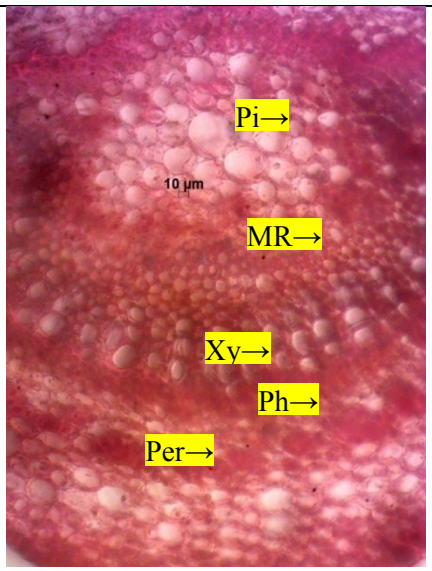

Fig 2e. Lower midrib

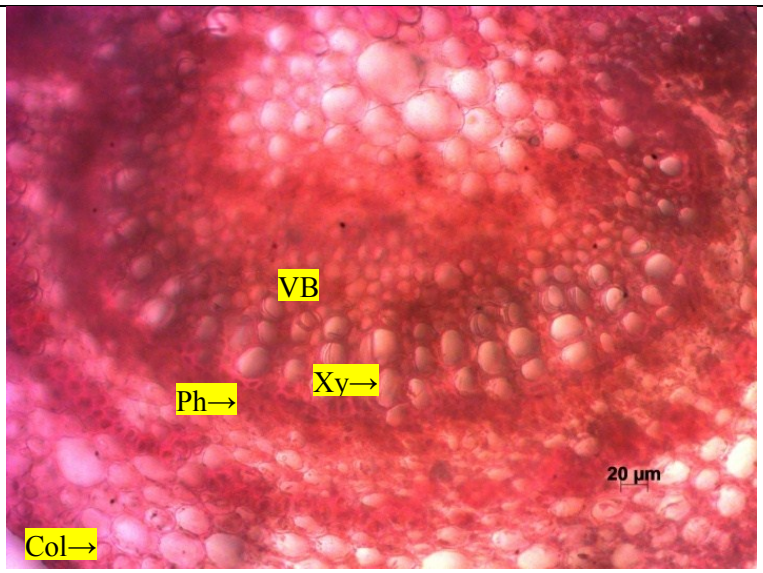

Fig 2f. Vascular bundle

Col– collenchyma; UE – uppr epidermis; LAM – lamina; LE – lower epidermis; MR – midrib; Pal – palisade; PF – pericyclic fibres; Ph – phloem; RC – rosette crystal; SP – spongy parenchyma; VB–vascular bundle; Xy – xylem. Stained with safranin

Table 1: Results of standardization parameters of leaf of *C.latifolium*

Parameter	Results n = 3 %w/w(Avg±SEM)
Loss on drying	8.97±0.01
Total Ash	5.83±0.16
Acid Insoluble Ash	0.10±0.00
Water soluble Ash	3.19±0.01
Alcohol soluble extractive value	7.79±0.01
Water soluble extractive value	27.06±0.00

Table 2: Results of preliminary phytochemical tests

Test	*C.latifolium*
Alkaloid	+
Carbohydrate	+
Steroid	+
Saponins	-
Tannin	+
Flavanoids	+
Phenol	+
Coumarins	+
Tri terpenoid	-
Amino acids	-
Carboxylic acid	-
Resins	+
Quinone	-

(+) Present; (-)Absent

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Sl No	Tests	Colour if positive	*C.latifolium*
1.	Alkaloids		
	Dragendrof's test	Orange precipitate	Orange precipitate
	Wagners test	Red precipitate	Red precipitate
	Mayers test	Dull white precipitate	Dull white precipitate
	Hagers test	Yellow precipitate	Yellow precipitate
2.	Steroids		
	Liebermann- buchard test	Bluish green	Bluish green
	Salkowski test	Bluish red to cherry red	Bluish red to cherry red
3.	Carbohydrate		
	Molish test	Violet ring	Violet ring
	Fehlings test	Brick red precipitate	Brick red precipitate
	Benedicts test	Red precipitate	Red precipitate
4.	Tannin		
	With $FeCl_3$	Dark blue or green or brown	Dark blue color
5.	Flavanoids		
	Shinoda's test	Red to pink	Red color
6.	Saponins		
	With $NaHCO_3$	Stble froth	No stable froth
7.	Triterpenoids		
	Tin and thionyl chloride test	Red	Buff color
8.	Coumarins		
	With 2 N NaOH	Yellow	Yellow color
9.	Phenols		
	With alcoholic ferric chloride	Blue to blue black, brown	Blue to blue black
10.	Carboxylic acid		
	With water and $NaHCO_3$	Brisk effervescence	No brisk effervescence
11.	Resin		
	With aqueous acetone	Turbidity	Turbidity
12.	Quinone		
	5% NaOH	Pink/purple/red	Yellow color
13.	Amino acids		
	Ninhydrine reagent	Purple color	Green color

Table 3: Nutritional (Calorific content) content of *C.latifolium*

Parameter	Results n = 3 %w/w
Total fat (%)	2.9
Total fibre (%)	1.18
Total carbohydrates (%)	95.20
Total proteins (%)	0.70

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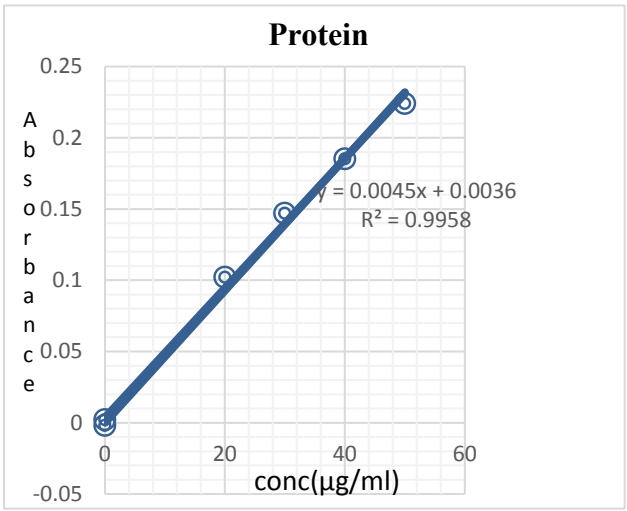
Figure 3: Standard plot of D-Glucose



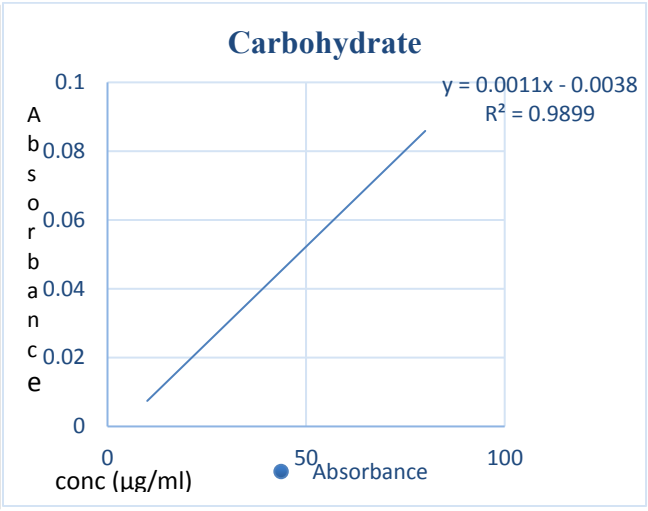
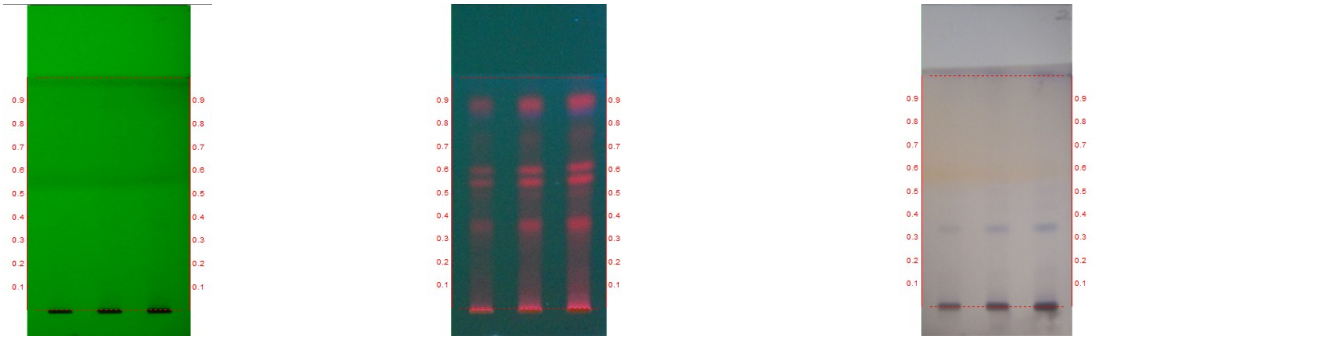


Figure 4: Standard plot of Bovine Serum Albumin (BSA)**Figure 5:** HPTLC photo documentation of ethanol extract of *C. latifolium*



Short UV Long UV Post derivatization

Track 1 - *C. latifolium* – 3µl Track 2 - *C. latifolium*– 6µl Track 3 - *C. latifolium*– 9µl Solvent system – Toluene: Ethyl Acetate: (9:1)

Table 4: R_f values of samples

Short UV	Long UV	Post derivatization
-	-	0.34 (Purple)
-	-	0.44 (Purple)
-	0.73 (F. blue)	-
-	0.83 (F. blue)	-
-	-	0.86 (Purple)
-	0.92 (F. blue)	-

*F – Fluorescent; L –Light; D – Dark

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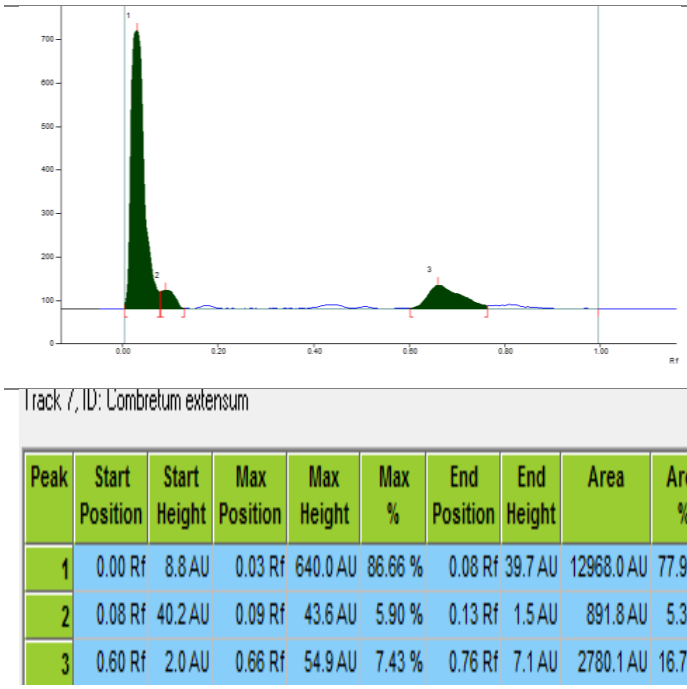
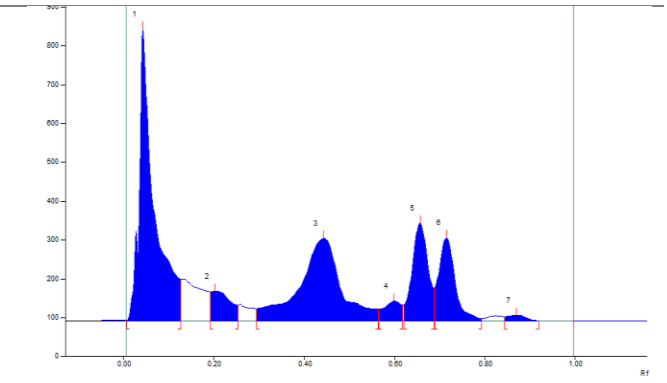


Track 7, ID: Combretum extensum

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	8.8 AU	0.03 Rf	640.0 AU	86.66 %	0.08 Rf	39.7 AU	12968.0 AU	77.93 %
2	0.08 Rf	40.2 AU	0.09 Rf	43.6 AU	5.90 %	0.13 Rf	1.5 AU	891.8 AU	5.36 %
3	0.60 Rf	2.0 AU	0.66 Rf	54.9 AU	7.43 %	0.76 Rf	7.1 AU	2780.1 AU	16.71 %

Figure 6: Densitometric scan at 254nm



Track 7, ID: Combretum extensum

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	1.1 AU	0.04 Rf	752.3 AU	47.76 %	0.13 Rf	07.7 AU	18095.9 AU	37.42 %
2	0.19 Rf	75.2 AU	0.20 Rf	76.4 AU	4.85 %	0.25 Rf	40.2 AU	2440.8 AU	5.05 %
3	0.30 Rf	32.0 AU	0.44 Rf	213.0 AU	13.52 %	0.56 Rf	30.1 AU	14063.7 AU	29.08 %
4	0.57 Rf	30.0 AU	0.60 Rf	51.3 AU	3.26 %	0.62 Rf	41.0 AU	1419.9 AU	2.94 %
5	0.62 Rf	41.4 AU	0.66 Rf	252.2 AU	16.01 %	0.69 Rf	84.2 AU	6356.1 AU	13.14 %
6	0.69 Rf	85.8 AU	0.72 Rf	214.5 AU	13.62 %	0.79 Rf	5.6 AU	5537.8 AU	11.45 %
7	0.85 Rf	11.0 AU	0.87 Rf	15.4 AU	0.98 %	0.92 Rf	0.3 AU	448.6 AU	0.93 %

Figure 7: Densitometric scan at 366nm

DISCUSSION

Macro-microscopy of *C.latifolium* was carried out in order to ascertain its true identity it is one of the cheapest and simple method available till date. The macroscopical character serves as diagnostical parameter. Morphological and histological study will enable to identify the crude drug. There were no trichomes found, upper epidermis is continuous throughout the lamina and midrib. Below the epidermis are present a layer of elongated palisade cells and loosely arranged spongy parenchyma. In the midrib there are 2-3 layers of closely arranged collenchyma cells arranged below the upper epidermis and above the lower epidermis. Vascular bundle is arc shaped, collateral with xylem towards upper epidermis and phloem towards lower epidermis. A band of pericycle surrounds the vascular bundle. Ash value of a drug gives us fair idea of earthy silicacious matter, inorganic composition and other impurities present along with the drug. Extractive values are primarily useful for determination of exhausted or adulterated drugs. Extractive values are also useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent.

Preliminary phytochemical analysis indicates the presence of alkaloid, carbohydrate, steroid, tannin, flavonoid, phenol, coumarin and resins.

HPTLC photo documentation under short UV showed no bands, long UV showed 3 bands (all fluorescent blue) and following post derivatisation there were 3 bands (all purple) were evident (Figure 5). Densitometric scan of the sample at 254nm showed presence of 3 peaks at Rf 0.03(77.93%), 0.09 (5.36%) and 0.66(16.71%)depicted in (Figure 6), at 366nm showed 7 peaks with major peaks at 0.04(37.42%), 0.44 (29.08%), 0.66(13.14%) and 0.72(11.45%) in (Figure 7). The nutritional value assessment unlocks its potential as rich source of energy derived from carbohydrate as leaf itself is manufacturing unit. Fiber content of the leaf owe to protect the intestinal flora.

CONCLUSION

This study is undertaken with a view to lay down standards which could be useful to detect the authenticity of this medicinally useful to detect the authenticity of this medicinally useful plant. Microscopic study and physicochemical standards are useful to substantiate and authenticate the drug.

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Conflicts of Interest: Nil

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Figures

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Tables [Table 1], [Table 2], [Table 3], [Table 4]

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