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Pharmacognostical, Phytochemical investigations on whole plant of *Cleome chelidonii* Linn

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Abstract : *Cleome chelidonii* Linn. (Family: Capparaceae) is a rare plant grown as perennials throughout dry seasons and widely distributed as a weed in wet places. However, the plant having wide therapeutic properties has not been scientifically validated. In present investigation, the detailed Pharmacognostical study of *Cleome chelidonii* is carried out to lay down the standards which could be useful in future experimental studies. The study includes macroscopy, microscopy, preliminary phytochemical screening, separation and isolation of plant constituents by chromatographic methods, characterization of isolated plant constituents and fluorescence analysis, elemental analysis and spectral studies. These studies provided referential information for correct identification and standardization of this plant material and to differentiate the plant C*leome chelidonii* from other species of Cleome.
Keywords : *Cleome chelidonii* - Morphology, Histology, Phytochemical studies, Column chromatography, HPTLC.

Introduction

Herbal medicines are now in a great demand in the developing world for primary health care not because they are inexpensive but also for better cultural acceptability, better compatibility with the human body and minimal side effects. Standardization and validation is very important for the herbal drugs. Herbal medicine is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history. It was an integral part of the development of modern civilization. Primitive man observed and appreciated the great diversity of plants available to him. Much of the medicinal use of plants seems to have been developed through observations of wild animals and by trial and error. [1]

Herbal drugs constitute a major share of all the officially recognized systems of health in India. More than 70% of India's population still use these systems of medicine. Currently there is no separate category of herbal drugs or dietary supplements, as per the Indian Drugs Act. However, there is a vast experimental studies evidence base for many of the natural drugs. Evidence based herbals are widely used in the diverse systems and manufactured as per the Pharmacopoeial guidelines by a well organized industry.[2]

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The plant *Cleome chelidonii* Linn. (Family: Capparaceae) is grown as perennials throughout dry seasons and widely distributed as a weed in wet places[3].It is found in India, Myanmar, Malaysia, Indo China and Java. In Tamil it is called Neela Naikadugu[4]. However, the plant having wide therapeutic properties has not been scientifically validated.The leaves of *Cleome chelidonii* is generally known to be used for the treatment of colic, dysentery, headache, otitis, and rheumatism. The whole plant has also been found to possess multiple therapeutic properties such as its use a vermifuge, in the treatment of skin diseases including leucoderma, and with anti-inflammatory, anti microbial, anti nociceptive and anti pyretic properties[5-8].The objective of the presentwork has been aimed to investigate the Pharmacognostical and Phytochemical properties of the plant *Cleome chelidonii.*

Fig. 1 :Whole Plant of *Cleome chelidonii*

Materials and Methods

Collection and Authentication of the Plant material

The fresh plant material was collected from Tiruchirappalli, Tamil Nadu in the month of June 2019.The plant material was taxonomically authenticated for its botanical identity by Botanist, and voucher specimen deposited in herbarium of the institute. Transverse section of leaf along the midrib, stem and roots were done. The coarsely powered plant material was subjected to extraction by solvents with increasing polarityand the dried extracts were subjected to phytochemical studies using standard test procedures.

Observations

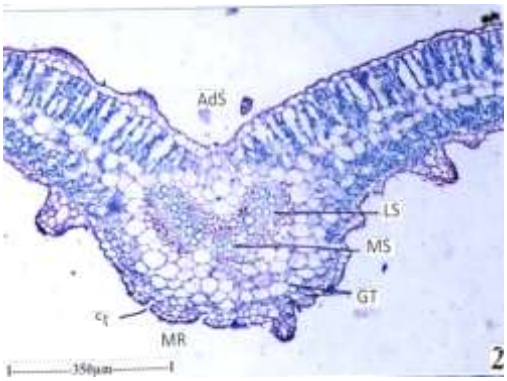
Morphology:

Height	-	0.3 - 0.9 m.
Leaves	-	1- or 3 - foliate at the top and 5- or 7-foliate at the base
Leaflets	-	obovate, 2-3 cm long, 0.5-1.5 cm wide.
Stem branches	-	striate, glabrous.
Flowers	-	4, rose coloured petals, and big mass of over 100 stamens in the center. Capsule is hairless.
Pods	-	5 - 6 cm in length.
Seeds	-	asymmetrical, spherical to oval, comma shaped,

compressed and diameter 1.5 to 2.0 mm. Brown to blackish brown, central portion paler and smooth. Cleft fairly open about 0.7 to 1.00 mm deep. Testa echinate to muricate, with rough and blunt tubercles of various heights[11]

Microscopical studies

The required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin- 5ml + Acetic acid- 5 ml +70% ethyl alcohol-90 ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary – butyl alcohol as per the schedule given by Sass, 1940. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58 – 60°C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks [12-15].



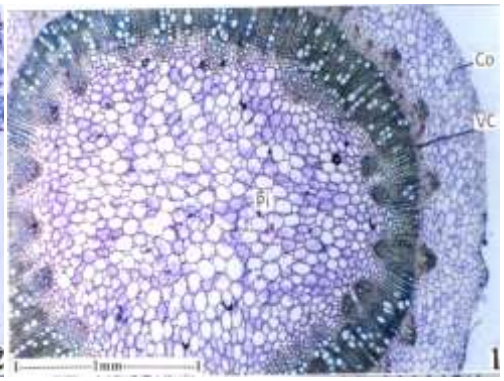
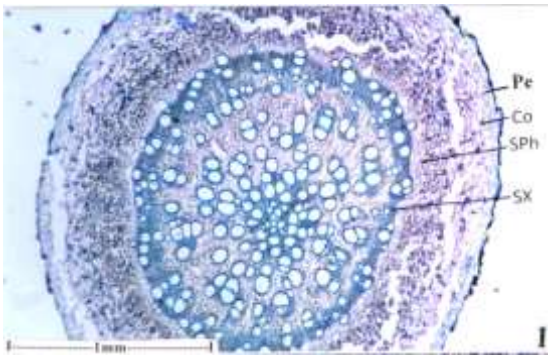


Fig. 2 : Midrib enlarged **Fig. 3: T.S of the Stem – Ground Plan**
Ads : Adaxial strand ; MR :Midrib ;LS : Lateral strand ;
GT : Ground tissue; MS : Median strand; St :Stomata; Pi : Pith; Co: Cortex



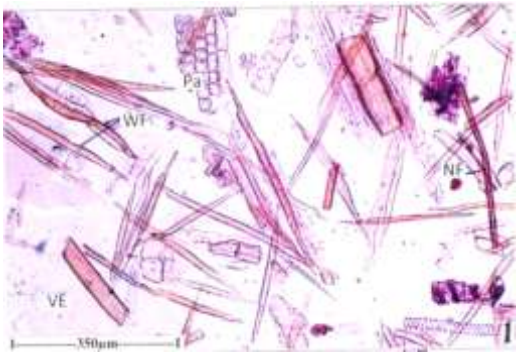
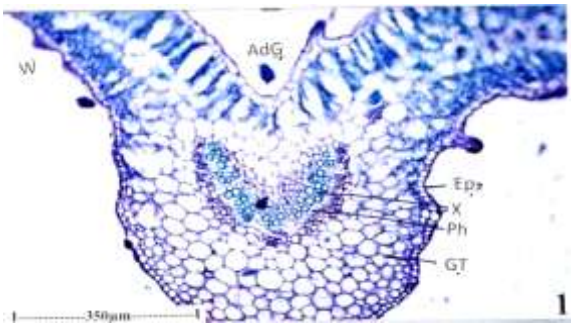


Fig. 4 : T.S of the Root – Entire View **Fig. 5 : Vessel elements and Fibres**
Co : Cortex ; Ep :Epidermis ; SPh : Secondary Phloem ; Pa : Parenchyma ;
NF : Narrow fibre ; SX : Secondary xylem ; Pe : Periderm;
VE : Vessel elements ; WF : Wide fibre



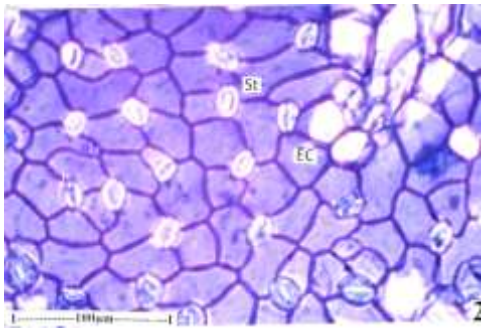


Fig. 6 : T.S of basal (Proximal) part of the petiole **Fig.7 : Paradermal sections of the abaxial epidermis Showing stomata.**
Ec : Epidermal Cell ; AW : Antielinal wall ; GC : Guard Cells

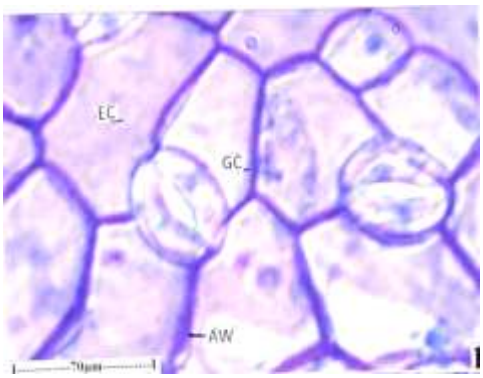


Fig. 8: Paradermal sections of the abaxial epidermis

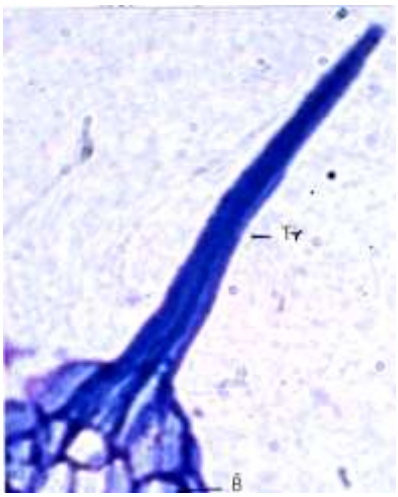


Fig. 9 : One Trichome enlarged Showing Anomocytic Stomata

Results

Table 1. Data showing the Quantitative Microscopy for leaves of *Cleome chelidonii*[16]

S.No.	ameters	an ± SD
1.	n islet number	3 ± 1.3302
2.	n termination number	92 ± 2.0932
3.	Stomatal number (Upper epidermis)	5 ± 2.1090
4.	matal number (Lower epidermis)	6 ± 2.1118
5.	matal index (Upper epidermis)	08 ± 0.6881
6.	matal index (Lower epidermis)	52 ± 0.6554
7.	sade ratio (Upper epidermis)	3 ± 3.1964

Table 2.Data showing the Physico Chemical Standards of whole plant powder of Cleome chelidonii[17-21]

S.No.	Parameters	Mean(%)w/w ±SD
1.	Total Ash	10.3±0.0774
2.	Acid in Soluble Ash	1.7±0.1897
3.	Water soluble Ash	2.2±0.1897
4.	Sulphated Ash	4.6±0.2645
5.	Loss on Drying	1.86±0.0729
6.	Alcohol Soluble Extractive	1.28±1.5938
7.	Water Soluble Extractive	0.82±0.0412
8.	Crude Fibre Content	11.26±0.4715

Table 3. Fluorescence Analysis of Extracts and Drug powder of *Cleome chelidonii*[22]

Reagents	**Chloroform extract**		**Drug powder**	
	DL	**UVL**	**DL**	**UVL**
Extract as such	Yellow	Yellow	-	-
1 N Sodium Hydroxide (aqueous)	Yellowish brown	Dark green	Pale green	Yellowish green
1 N Sodium Hydroxide (alcohol)	Yellow	Yellow colour	Green	Fluorescent green
1 N Hydrochloric Acid	Brown	Green colour	Brown	Light green
50% Nitric Acid	Greenish yellow	Yellow colour	Green	Fluorescent green
50% Sulphuric Acid	Green	Yellowish green	Green	Fluorescent green
Methanol	Light green	Green	Green	Fluorescent green
Ammonia	Greenish yellow	Dark green	Green	Yellowish green
Iodine	Brownish yellow	Yellowish brown	Brown	Light green
Ferric Chloride	Greenish brown	Yellow	Green	Light green

DL - Day Light UVL -UV Light

Table 4. Elemental Analysis of *Cleome chelidonii*[23-25]

S. No.	**Parameters**	**Amount**
1.	Organic Carbon (%)	4.58
2.	Total Nitrogen (%)	1.79
3.	Total Phosphorus (%)	0.87
4.	Total Potassium (%)	4.28
5.	Total Sodium (%)	0.69
6.	Total Calcium (%)	5.23
7.	Total Magnesium (%)	3.91
8.	Total Sulphur (%)	0.52
9.	Total Zinc (ppm)	4.53
10.	Total Copper (ppm)	1.29
11.	Total Iron (ppm)	56.32
12.	Total Manganese (ppm)	12.54
13.	Total Boron (ppm)	0.12
14.	Total Molybdenum (ppm)	0.16
15.	Heavy Metals (ppm)	Nil

Table 5. Quantitative estimation of drug powder of *Cleome chelidonii*

S.No.	**Parameters**	**Total amount in mg/kg**
1.	Total alkaloids	0.79
2.	Total Flavanoids	2.54
3.	Tannin	0.79
4.	Lignin	0.87
5.	Glycosides	0.06

Phytochemical Studies

Table 6.Data showing the preliminary phytochemical screening of *Cleome chelidonii*[16]

Phytoconstituents	Petroleum ether extract	n-hexane extract	Chloroform extract	Acetone extract	Methanol extract	Aqueous extract
Alkaloids	(-)	(-)	(+)	(+)	(+)	(-)
Carbohydrates	(-)	(-)	(+)	(+)	(+)	(-)
Glycosides	(-)	(-)	(+)	(+)	(+)	(-)
Flavonoids	(-)	(-)	(+)	(+)	(+)	(-)
Phytosterols	(+)	(+)	(+)	(+)	(+)	(+)
Fixed oils and Fats	(-)	(-)	(+)	(-)	(-)	(-)
Saponins	(-)	(-)	(-)	(-)	(-)	(-)
Phenolic	(-)	(-)	(+)	(+)	(+)	(+)
Lignins	(+)	(+)	(+)	(+)	(+)	(+)
Proteins and free	(+)	(+)	(+)	(+)	(+)	(+)
Gums and	(-)	(-)	(-)	(+)	(-)	(+)

(+) Positive **(-) Negative**

Table 7.Thin Layer Chromatography of Chloroform extract of *Cleome chelidonii*[26-29]

S.No.	Phytoconstituents	Mobile phase	Detecting agent	No. of spot	R_f value
1.	Alkaloids	Benzene: Ethanol (9`:1)	Dragendorff's reagent	1	0.34
2.	Glycosides	Toluene: Ethylacetate (7:3)	Anisaldehydesulphuric acid	2	0.37 0.74
3.	Flavonoids	Petroleum ether : Ethylacetate (2:1)	Iodine vapour	4	0.56 0.85 0.89 0.95
4.	Steroids	Benzene: Ethyl acetate (9:1)	Vanillin in sulphuric acid	3	0.1 0.75 0.78
5.	Essential oil	Chloroform	Vanillin in sulphuric acid	2	0.48 0.73

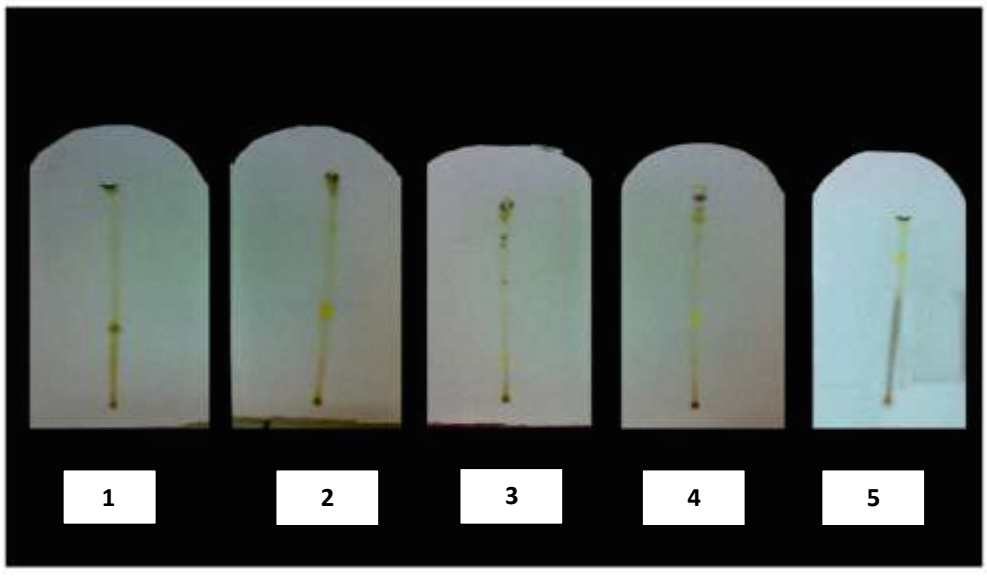


Fig.10: Thin

Layer Chromatography of Chloroform extract of *Cleome chelidonii*

1.Alkaloids ; 2. Glycosides ; 3.Flavonoids ; 4.Steroids ; 5. Essential oil

Column chromatography

The chloroform, acetone and methanol extracts of *Cleome chelidonii* were subjected to column chromatography[30-33]

An yellow crystalline compound was obtained by Column Chromatography in the fractions of Chloroform extract (Chloroform 100) and was named CCC1(*Cleome chelidonii* compound no.1)

High Performance Thin Layer Chromatography [22]

The chloroform extract of *Cleome chelidonii* was subjected to HPTLC studies

Chromatographic condition for HPTLC Finger print

The chloroform extract of whole plant of *Cleome chelidonii* was run for HPTLC studies.
The Mobile phase used was n-Hexane: Ethyl acetate: Formic Acid: Acetic acid (70:25:2.5:2.5).
25mg /ml concentration of the sample was taken and the applied volume was 2.5, 5,10 &15 µl.

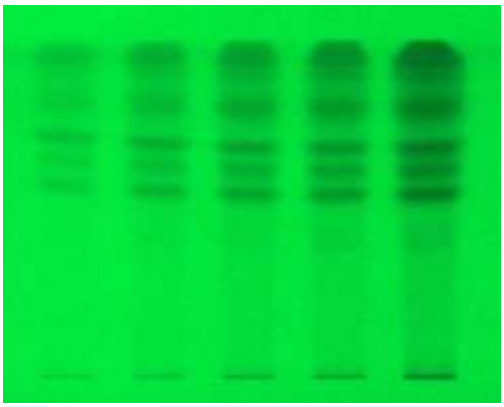


Fig.11 : HPTLC of Chloroform extract Under UV Light (254 nm)

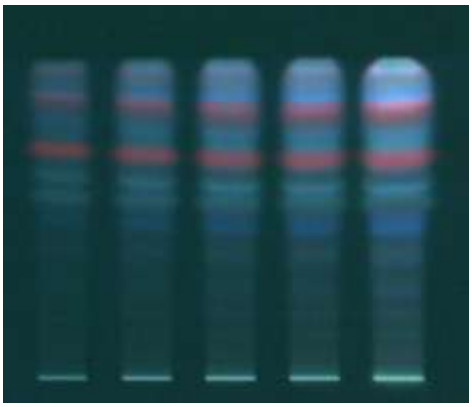


Fig.12 : HPTLC of Chloroform extract Under UV Light (366 nm)

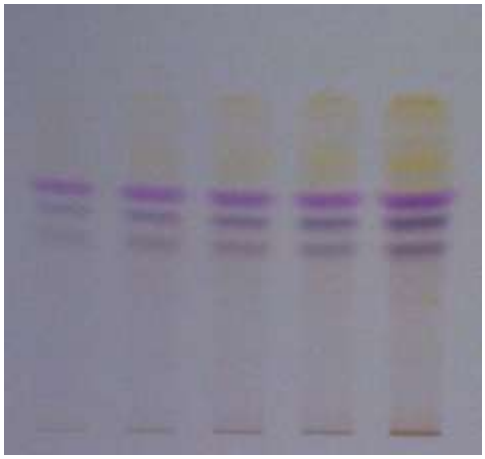


Fig. 13: HPTLC of Chloroform extract Under Visible Ligh

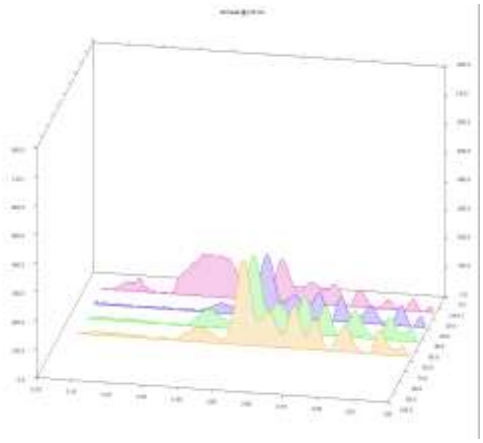


Fig. 14 : HPTLC of Chloroform extract (All Tracks at Wavelength)

Discussion

The evaluation of Pharmacognostical parameters may ensure the identity and authenticity of Plant - *Cleome chelidonii*. The leaf, petiole, stem, root of *Cleome chelidonii* were subjected to microscopical studies.

The Plant *Cleome chelidonii* Linn. is an erect herb, much branched, the leaves are 1- or 3-foliate at the top and 5- or 7-foliate at the base. The leaf exhibits dorsiventral symmetry with prominent midrib and fairly thick lamina**.**The lamina consists of fairly thick epidermal layer of spindle shaped larger cells with anomocytic stomata. In the mesophyll tissue, two horizontal rows of dilated circular hyaline cells are observed. The covering trichomes are present in the leaf. Seeds are asymmetrical, spherical to oval, comma shaped. The quantitative microscopical results were reported in Table 1.

Powder analysis of the whole plant of *Cleome chelidonii* showed the presence of wide and narrow fibres, vessel elements with perforation plate and squarish shaped parenchyma cells.

The physico chemical standard values such as total ash, water soluble ash, acid insoluble ash, sulphated ash, loss on drying, water soluble extractive value, alcohol soluble extractive value and crude fibre content were reported in Table 2. The alcohol soluble extractive values were higher than water soluble extractive values.

The fluorescence analysis of extracts and powders with different reagents were studied in day light and UV light and reported in Table 3. The results of elemental analysis and quantitative estimation of drug powder were reported in Table 4 &5.

All the extracts were subjected to preliminary phytochemical investigation. More number of phytoconstituents like alkaloids, glycosides, flavanoids, phytosterols, essential oil, proteins and aminoacids were found to be present in chloroform, acetone and methanolic extracts and the results were reported in Table 6. All the extracts were subjected to Thin Layer Chromatography (TLC) and the number of spots with its R_f values were determined and reported in Table 7.

From the results of the Thin Layer Chromatography (TLC), it was observed that more prominent spots were obtained for the Chloroform extract of *Cleome chelidonii* and was subjected to Column chromatography. The Chloroform extract of *Cleome chelidonii*gave an Yellow crystalline Compound (CCC1) in the fraction of Chloroform (100%) in the Column chromatography.

The isolated compound CCC1 was crystalline in nature, yellow in colour, odourless and bitter in taste with melting point (175^0- 177^0 C), soluble in organic solvents. The isolated compound CCC1 gave a R_f value of 0.56 when subjected to Thin Layer Chromatography which coincided with one of the R_f value obtained in the TLC of chloroform extract for flavanoids. The HPTLC Studies for the Chloroform extract were reported. The R_f value 0.56 of the TLC complied with the HPTLC studies. In future, spectral studies, Gas chromatography have been planned to identify the isolated unknown compound CCC1.

Conclusion

In the present study, the plant *Cleome chelidonii* was selected to explore the scientific information on Pharmacognostical and Phytochemical aspects. The parameters which are reported under Pharmacognostical and Phytochemical studies could be used for botanical identification of the drug in the crude form and preparation of Monograph of the plant *Cleome chelidonii.*

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