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Assessment of pharmacognostic and phytochemical standards of *Crataeva magna* (Lour) DC stem and leaf

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Abstract
The aim of the present study is to access the pharmacognostic and phytochemical parameters of stem and leaf of *Crataeva magna*. Macroscopical, microscopical, physicochemical evaluation, fluorescence analysis, preliminary phytochemical screening of phytoconstituents were determined by various extracts of stem and leaf of *Crataeva magna*. Microscopic study shows the general internal characteristics of stem and leaf. Physiochemical investigation of stem and leaf shows the total ash, acid insoluble ash, water soluble ash and sulphated ash values were 8.24 %, 3.10%, 2.56%, 10.08% and 9.48%, 2.76%, 2.28%, 10.26% respectively. In the present study it is noted that the extractive value of water is more than that in other solvents investigated. Through the preliminary phytochemical assessment the presence of alkaloid, catechin, coumarin, flavonoid, phenol, quinine, saponin, steroid, tannin, terpenoid, sugar, glycoside and xanthoprotein is revealed. Further this information will be used for pharmacological and therapeutical evaluation of the species. The same will also be assisted in standardization for quality, purity and sample identification.

Keywords: *Crataeva magna,* pharmacognostic, phytochemical, fluorescence

Introduction
Plant provides not only food, but also useful medicaments to many of our diseases. Adulteration and substitution is very common in any crude drug market. Hence correct identification of medicinal plant is essential [14].
Pharmacognostical study is the preliminary step in the standardization of crude drugs. The pharmacognostical evaluation gives precious information about the morphology, microscopical and physical quality of the crude drugs. Pharmacognostic studies have been done on many significant drugs. The resultant observations have been included in various Pharmacopoeias [17]. There are a number of crude drugs where the plant source has not yet been logically identified. Hence pharmacognostical study gives the scientific information concerning the purity and quality of the plant drugs [6].
Crataeva magna (Lour) DC. belongs to family Capparaceae. It is a high value medium sized deciduous medicinal tree of tropical climate. This is found in tropical regions of the world and also grows almost all over India, especially in the semiarid regions. Reports demonstrate the traditional systems of using medicine, such as Ayurveda and Unani [9],[3]. This plant is known to possess immense pharmacological activity, nephrotoxicity, arthritis [7] and urinary disorders [5]. In folk medicine, its stem pith is used for lactation after child birth by the tribal people of Kandhamal district of Orissa known as Eastern Ghats of India. The bark also treats urinary disorders, kidney bladder stones, fever, vomiting and gastric irritation [16],[2].
Review of literature survey and scientific data revealed that a large number of indigenous drugs have already been investigated with regard to their botany and chemical properties. However a systematic standardization including pharmacological and physic-chemical study is still lacking. Therefore the present investigation of *C.magna* is taken up to appraise certain botanical and chemical standards which would help in crude drug identification as well as in checking adulteration, if any. Further the study will greatly help in quality assurance of finished products of herbal drugs.

Methodology
Whole plant of *Crataeva magna* (Lour) DC was collected from Vellamadam, Nagercoil, Kanyakumari District, and Tamil Nadu. The plant samples were identified with the help of local flora and authenticated by Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, and India.

A voucher specimen (VOCB3636) of collected plants was deposited in the ethnopharmacological Unit, PG & Research Department of Botany, V.O. Chidambaram College, Thoothukudi District, Tamil Nadu.

Macroscopical Studies

The macroscopic characters 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 stem and leaf were cut and removed from the plant and instantaneously fixed in FAA (formalin- 5 ml + acetic acid- 5 ml + 70% Ethyl alcohol- 90 ml). Further the specimens were left in the preservative for two days; then the materials were washed in water and processed. Standard microtome techniques were followed for anatomical investigation [8]. Transverse sections of the materials were prepared. The microtome sections were stained with 0.25% aqueous Toluidine blue (Metachromatic stain) adjusted to pH 4.7 [13]. 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 current study, the powered stem and leaf were treated with different chemical reagents which include 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 HNO3+NH3. These extracts were subjected to fluorescence analysis in day light and UV light (254nm and 366nm). Various ash types and extractive values were determined by following standard methods [1].

Preliminary phytochemical analysis

Shade dried and powdered stem and leaf samples were successively extracted with petroleum ether, benzene, ethyl acetate, methanol and ethanol. The extracts were filtered and concentrated using vacuum distillation. For the identification of various phytochemical constituents, the different extracts were subjected to qualitative tests as per the standard procedure [11],[4].

Results

Anatomy of the leaf

The leaf consists of a prominent midrib and uniformly thick lamina. The midrib is broadly conical on the adaxial side and broadly semicircular on the abaxial side (Plate I a). The midrib is 750 µm in vertical plane and 700 µm in horizontal plane. The epidermal layers on the adaxial and abaxial sides have small squarish cells with prominent circular outgrowths on the outer tangential walls. Inner to the epidermis are two or three layers of collenchyma cells and the rest of the region has parenchymatous ground tissue.

The vascular system of the midrib consists of wide arc of several discrete segments of collateral vascular bundles. The bundles are radially elongated and cylindrical and elliptical in shape (Plate I a). There are about nine bundles with narrow space in between the bundles. The bundles have cluster of wide circular or ovate, thick walled vessels. The protoxylem vessels are directed towards adaxial side and the metaxylem elements are 30µm wide. On the lower end of the xylem segment occur large, semicircular compact phloem elements. The phloem elements are angular, thick walled and are in vertical lines.

Lateral Vein

The lateral vein is flat on the abaxial side and slightly raised on the adaxial side. The vein consists of a single semicircular vascular bundle. It consists of a cluster of wide, circular, highly thick walled vessels and about five groups of phloem element located on the lower end of the xylem.

Lamina

The lamina is smooth and even on both surfaces. It is 170 µm thick and the lamina is dorsiventral. The adaxial epidermal layer consists of fairly thick, rectangular and squarish cells with prominent cuticle. The mesophyll tissue consists of adaxial band of two horizontal rows of palisade cells. It also consists of abaxial zone of about five layers of spongy parenchyma cells. These are small elliptical vascular bundles located along the median part of the portion of the lamina. The vascular bundles are collateral bundles and have uniseriate layer of parenchymatous bundle sheath cells. The lamina is hypostomatic, the stomata occur on the abaxial epidermal layer (Plate I b). The stomata are slightly below the level of the epidermis. The guard cells have single beak shaped sharp stomatal ledges.

Leaf-margin

The marginal part of this lamina is semicircular, straight and slightly bulged. The epidermal cells along marginal part are smaller, thick walled and have thick cuticle. The interior marginal part includes compact mass of large circular thick walled cells. The leaf margin is 220 µm thick.

Petiole

The petiole is semicircular with thick short lateral adaxial wings and median wide short hump. The petiole is 1.5 mm in diameter. The petiole has undulate outline, thin prominent epidermal layer, parenchymatous ground tissue and a deep arc of many segments of vascular bundles.

The epidermal layer consists of radially oblong cells with deeply wavy thick cuticle. The ground parenchyma is homogenous. The vascular system consists of a deep arc of about nine radial oblong elliptical collateral vascular bundles. Each bundle has two or three radial rows of vessels. The vessels are circular to elliptical in outline, the vessel lumen is wide and vessel walls are thick. The vessels are 20 µm wide. Phloem occurs in thick semicircular mass of sieve-elements and parenchyma cells on the outer end of the vascular bundles, there is a thick sclerenchymatous cap.

Stomata

In paradermal section of the lamina, the epidermal cells and stomata were visible in surface view (Plate Id). The epidermal cells are polyhedral and have thick straight anticlinal walls. The stomata are sparse in distribution. The guard cells are broadly elliptical measuring 20 & 30 µm in size. The stomatal pores are narrow and slit like. The stoma is surrounded by five subsidiary cells and these cells are cyclocytic type by four radiating subsidiary cells forming actinocyclic type of stomata (Plate II e and f).

Venation pattern of the lamina

The venation is densely reticulate. The veinlets uniform in thickness. The veinlets are thick and are wavy. The vein islets are narrow. They are highly variable in shape and size. Vein-

terminatious is present in some of its vein-islets whereas, some islets are lacking the vein-terminatious. The vein terminatious is of two types: some are simple and unbranched; others are branched once or twice. The terminatious thick and wavy. Dark spherical bodies are common in the lamina. They are large with spiny surface. These spherical spiny bodies are calcium oxalate denses (Plate II a, b and c).

Stem

The stem is circular in transactional view with even surface. The stem is 6 mm thick. The stem consists of thick, lignified epidermal layer, thick cortical zone, rhizome of secondary phloem and thick hollow cylinder of secondary xylem. There is a wide parenchymatous piths in the centre (Plate III a).

The epidermal cells are rectangular in radial plane and highly thick walled and lignified. The epidermis is 50 µm thick. The cortical zone is heterocellular and comprises parenchymatous ground tissue and isolated several islands of sclerenchyma cells (Plate III b). The cortical zone is absupthy stop and is followed by secondary phloem cylinder (Plate III c). The secondary phloem zone is 70 µm thick. It consists gradial, compact lines of rectangular cells and wide, dilated phloem rays. The phloem elements are wide and thick walled. The elements include sieve elements and wide parenchyma cells.

Secondary xylem cylinder is 120 µm thick. The tissue includes vessels and xylem fibres. The vessels are either solitary or in short radial multiples. The vessels vary in cross sectional outline from circular to ovate and polyhedral. The vessels are wide and thick walled. They are 35 µm in diameter. Xylem fibres are angular in transactional outline, thick walled with wide lumens. The fibres are in compact vertical lines (Plate III d).

Powder microscopic studies

The powder preparation was mounted on a slide after warming powder to remove air from the powder was studied after stained with dilute safranin. The following inclusions were recorded.

- Fragments of adaxial (upper) epidermis was seen in the powder. The adaxial epidermal cells are polyhedral in outline with thick straight anticlinal walls. The cells have thin several parallel cuticular lamellatious. The adaxial epidermis is apostomatic (without stomata) (Fig. 16.1).
- Abaxial epidermis (lower epidermis) (Fig 16. 2, 3): The lower epidermal cells are polyhedral with thick, straight anticlinal walls. The epidermis is densely stomatiferous. The stomata are prominent and circular in outline. The stomata are cyclocytic type. Each stoma is surrounded by one or two circles of cells with four subsidiary cells in each cycle (Fig. 16.3). The guard cells are 20 µm in diameter.
- Fibres (Plate IV a, c): Libriform fibres are abundant in the powder. The fibres are tapering and pointed at the ends. The cell walls are thick and lignified. The cell lumen is fairly wide. No pits are wident on the cell walls. The fibres range in length from 300-700-900 µm. They are 15-20 µm thick.
- Sclereids (Plate IV b): The sclereids are long cells with lignified walls and simple circular pits. The cell walls are thicker and the cell lumen is wider. These are dense simple circular pits. The cell walls have canal like narrow pits. The sclereids are up to 190 µm long and 20 µm wide.
- Vessel elements (Plate d, e): Narrow, cylindrical vessels elements are common in the powder. The vessel elements have dense, multiseriate bordered pits on their lateral walls (Fig. 20.1, 2). Some of the vessel elements have short pointed tails at their ends. The end walls have wide circular perforations. The perforations are either horizontal or oblique. The vessel elements are 190-220 µm long and 40-50 µm wide.
- Periderm cells (Fig. 20.3): Broken pieces of the periderm are also seen in the powder. The periderm fragments consist of square shaped thick walled cells. They are arranged in compact parallel series. The cells are 40 square µm in size.

Physicochemical Constants

The result of the ash and extractive values of leaves and stem of *C.magna* are depicted in table 1a and 1b. The total ash content of the powdered leaves and stem of *C.magna* are 8.24 and 9.48% respectively. The extractive value of water is more than that in other solvents investigated in the present study.

Table 1a: Ash values of the powdered leaves and stem of *Crataeva magna*

S. No	Type of Ash	% of Ash values	
		Leaf	Stem
1.	Total ash value of powder	8.24 ±0.10	9.48±0.12
2.	Water soluble ash	3.10±0.04	2.76±0.02
3.	Acid insoluble ash	2.56±0.02	2.28 ±0.01
4.	Sulphated ash	10.08±0.13	10.26±0.08

Table 1b: Extractive values of the powdered leaves and stem of *Crataeva magna*

S. No	Nature of the Extract	% of extractive values	
		Leaf	Stem
1.	Petroleum ether	6.12 ± 0.03	7.54± 0.04
2.	Benzene	6.35 ± 0.05	6.66± 0.03
3.	Chloroform	8.48 ±0.06	8.58 ±0.05
4.	Acetone	9.50 ±0.04	9.66 ±0.02
5.	Methanol	9.28 ± 0.11	9.38± 0.14
6.	Ethanol	10.11 ± 0.13	10.24± 0.10
7.	Water	10.56 ±0.10	11.06 ±0.18

a All values are mean of triplicate determinations ± Standard error

Fluorescence analysis

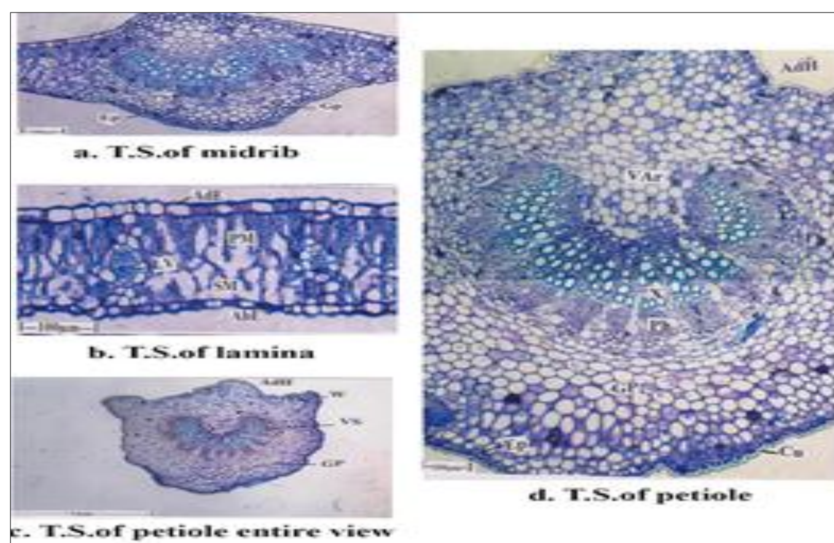
The results of fluorescent analysis of leaves and stem of *C. magna* are shown in table 2 and 3. The leaf powder of *C. magna* shows the characteristic fluorescent green colour treated with 1N aqueous NaOH, 50% H_2So_4, conc. H_2So_4, 50% HNO_3, NHO_3 + NH_3, 40% NaOH+10% lead acetate, ferric chloride, chloroform and acetone. The stem powder of *C. magna* shows the characteristic fluorescent green colour treated with 1N aqueous NaOH, conc.H2So4, 40% NaOH + 10% lead acetate, ferric chloride, chloroform, benzene, methanol and acetone.

Table 2: Fluorescence analysis of the powdered leaf of *Crataeva magna*

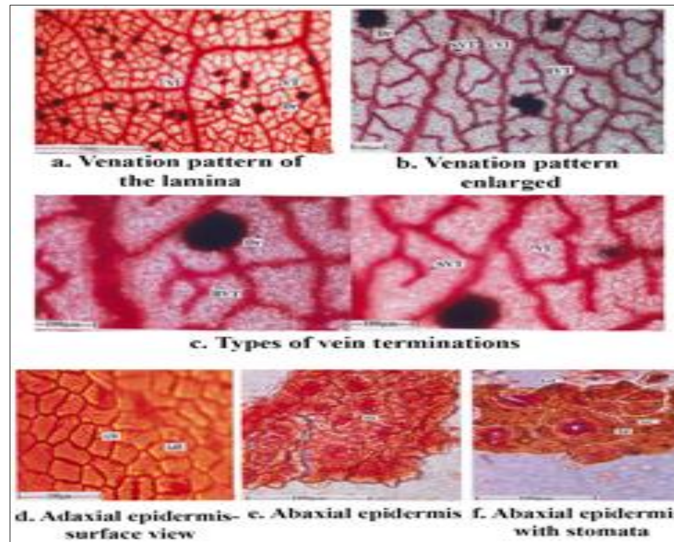
Treatment	Under Day Light	Under UV light	
		245 nm	365 nm
Powder as such	Pale green	Green	Black
Powder + 1N Aqueous NaOH	Yellowish green	Fluorescent green	Black
Powder + 1N Alcoholic NaOH	Greenish yellow	Fluorescent green	Dark blue
Powder + 1N HCL	Green	Dark green	Black
Powder + Conc. HCL	Green	Yellowish green	
Powder + Conc. H_2SO_4	Brown	Green	Dark blue
Powder + 50% H_2SO_4	Yellowish green	Fluorescent green	Black
Powder + Con.HNO_3	Yellowish green	Yellowish green	Dark green
Powder + 40% NaOH + 10% Lead Acetate	Yellowish green	Fluorescent green	Dark green
Powder + Acetic acid	Green	Yellowish green	Dark green
Powder + Ferric Chloride	Green	Fluorescent green	Dark green
Powder + Chloroform	Yellowish green	Fluorescent green	Dark green
Powder + Benzene	Green	Fluorescent green	Dark green
Powder + Petroleum ether	Green	Yellow	Blue
Powder + Methanol	Green	Dark green	Dark green
Powder + Ethanol	Yellowish green	Greenish yellow	Black
Powder + acetone	Green	Fluorescent green	Black
Powder + HNO_3 + NH_3	Green	Green	Black
Powder + 50% HNO_3	Green	Greenish yellow	Black

Table 3: Fluorescence analysis of the powdered stem of *Crataeva magna*

Treatment	Under Day Light	Under UV light	
		245 nm	365 nm
Powder as such	Pale brown	Green	Dark blue
Powder + 1N Aqueous NaOH	Yellowish green	Fluorescent green	Dark black
Powder + 1N Alcoholic NaOH	Yellowish green	Green	Black
Powder + 1N HCL	Yellow	Green	Black
Powder + Conc. HCL	Brown	Greenish yellow	Dark brown
Powder + Conc. H_2SO_4	Yellowish green	Fluorescent green	Black
Powder + 50% H_2SO_4	Yellowish green	Greening yellow	Dark blue
Powder + Con.HNO_3	Dark green	Yellowish green	Dark brown
Powder + 40% NaOH + 10% Lead Acetate	yellow	Fluorescent green	Black
Powder + Acetic acid	Yellowish green	yellow	Dark green
Powder + Ferric Chloride	Dark green	Fluorescent green	Dark green
Powder + Chloroform	Pale brown	Fluorescent green	Dark green
Powder + Benzene	yellow	Fluorescent green	Dark brown
Powder + Petroleum ether	yellow	Yellowish green	Black
Powder + Methanol	Yellowish green	Yellowish green	Black
Powder + Ethanol	Yellowish green	Yellowish green	Dark green
Powder + acetone	yellow	Fluorescent green	Dark green
Powder + HNO_3 + NH_3	Pale brown	Green	Black
Powder + 50% HNO_3	yellow	Green	Dark green

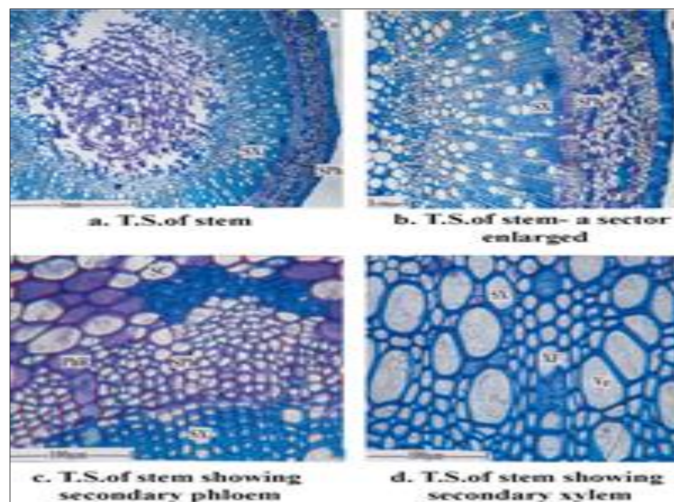


Ep-Epidermis; GP-Ground Parenchyma; X-Xylem; Ph-Phloem;
AdE- Adaxial Epidermis; AdH-Adaxial Hump; VAr- Vascular Arc;
Cu-Cuticle; W- cell wall; VS-Vascular strand



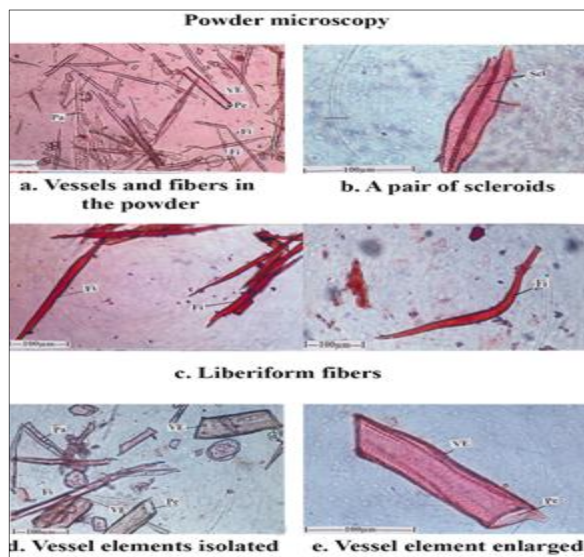
a. Venation pattern of the lamina; b. Venation pattern enlarged; c. Types of vein terminations; d. Adaxial epidermis-surface view; e. Abaxial epidermis; f. Abaxial epidermis with stomata

Dr- Druses; VI- Vein Islet; VT- Vein termination; SVT- Simple vein termination; BVT- Branched vein termination; AW- Anticlinal wall; AdE- Adaxial Epidermis; St- Stoma; GC- Guard Cells; SC- Subsidiary cells



a. T.S.of stem; b. T.S.of stem- a sector enlarged; c. T.S.of stem showing secondary phloem; d. T.S.of stem showing secondary xylem

Co- Cortex; EP- Epidermis; Pi- Pith; SX- Secondary xylem; SPh- Secondary Phloem; Sc- Sclerenchyma; PhR- Phloem Ray; XF- Xylem fibers; Ve- Vessel



Powder microscopy

a. Vessels and fibers in the powder; b. A pair of scleroids; c. Liberiform fibers; d. Vessel elements isolated; e. Vessel element enlarged

VE- vessel elements; Fi-Fibers; Scl-scleroids; Pe-Perforation; Pa- Parenchyma

Preliminary phytochemical analysis

The result of preliminary phytochemical screening of leaves and stem of *C.magna* are presented in table 4. The methanol and ethanol extracts of the leaves and stem of *C.magna* show the presence of alkaloid, catechin, coumarin, flavonoid, phenol, quinone, saponin, steroid, tannin, terpenoid, sugar, glycoside and xanthoprotein.

Table 4: Preliminary phytochemical screening of powdered leaves and stem of *Crataeva magna*

Tests	Petroleum Ether		Benzene		Ethyl acetate		Methanol		Ethanol	
	Leaf	stem	Leaf	stem	Leaf	stem	Leaf	stem	Leaf	stem
Alkaloid	+	+	+	+	+	+	+	+	+	+
Anthraqinone	-	-	-	-	-	-	-	-	-	-
Catechin	-	-	-	-	+	+	+	+	+	+
Coumarin	+	+	+	+	+	+	+	+	+	+
Flavonoid	+	+	+	+	+	+	+	+	+	+
Phenol	+	+	+	+	+	+	+	+	+	+
Quinone	-	-	-	-	+	+	+	+	+	+
Saponin	-	-	+	+	+	+	+	+	+	+
Steroids	-	-	+	+	+	+	+	+	+	+
Tannin	+	+	+	+	+	+	+	+	+	+
Terpenoids	-	-	+	+	+	+	+	+	+	+
Sugar	+	+	+	-	-	+	+	+	+	+
Glucoside	+	+	+	-	-	+	+	+	+	+
Xanthoprotein	+	-	+	+	-	+	+	+	+	+
Fixed oil	+	+	+	+	+	+	+	+	+	+

Discussion

Assessment of standards in crucial measure for detection of sample quality, purity, authentication and also anthology of quality control of crude drugs. Microscopy is one of the simple and cheap methods to start with establishing the correct identity of the source materials. First time, in this study the pharmacognostic and phytochemical evaluation was carried out for the leaf and stem of *C.magna*. The present study may enable to identify the crude drug.

Salient features of leaf and stem of *C. magna*

- The leaf consists of a conical adaxial part and semicircular abaxial part of the midrib.
- The vascular strand of the midrib includes a wide arc of about eight wide elliptical collateral discrete vascular bundles which compactly arranged.
- The vascular bundles have wide circular, very thick walled vessels and wide and thick semicircular phloem units.
- A thin layer of sclerenchyma cells occur on the outer end of each vascular bundle.
- The lateral veins of the leaf have single unit of collateral vascular bundle with cluster of vessels.
- The lamina is bifacial with adaxial two layered palisade cells and abaxial six layered spherical, loosely arranged spongy parenchyma cells. The leaf is hypostomatic.
- The leaf margin is semicircular, slightly dilated; the epidermal cells have highly the cuticle. Mesophyll tissue is undifferentiated.
- Petiole is circular with adaxial median hump and lateral thick short wings.
- The vascular strand is bowl shaped comprising about 13 collateral compact vascular bundles.
- Stomata are elliptical in shape and are cyclocytic type.
- Venation pattern of the lamina is densely reticulate with thick wavy veins. Vein islets have both unbranched and branched vein terminations.
- The stem consists of thick, lignified cell layers of the epidermis, parenchymatous cortex with seathed masses of sclerenchyma, thick secondary phloem cylinder and thick secondary xylem.
- Secondary xylem consists of wide thick walled vessels and xylem fibres.
- Secondary phloem forms thick distinct zone comprising sieve elements and phloem rays.
- Phloem rays are uniseriate, biseriate and multiseriate, homocellular, nonstoried and the cells ae thin walled and circular.
- Powder preparation of the leaf, stem and bark shows the following elements. Thick walled, straight anticlinal walls of the adaxial epidermis, cyclocytic stomata, abundant libriform fibres, long cylindrical vessel elements with circular multiseriate bordered piths and circular wide and wall perforations.

The ash of any organic material is composed of their non-volatile inorganic components. Controlled incineration of crude drugs results in an ash residue consisting of an inorganic material (metallic salts & silica). This value varies within fairly wide limits and is therefore an important parameter for the purpose of evaluation of crude drug [12]. The determination of ash is useful for detecting low grade products, exhausted drugs and excess of sandy or earth matter; it is more especially applicable to powdered drug. On incineration, crude drugs normally leave an ash usually consisting of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium [18]. The crude drugs be obliged their biological activity mainly due to active chemical constituents. These constituents may be in different polar, semipolar or nonpolar solvents. Total soluble constituents of the drugs in any particular solvent or mixture of solvents may be called as extractive value [15]. The extracts obtained by exhausting crude drugs are indicative of approximate measures of their chemical constitute. Taking into consideration the diversity in chemical nature and properties of contents of drugs, various solvents are used for extraction and it is in a position to dissolve appreciable quantities of substances derived [10].

Florescence analysis and behaviour of powdered drug with various chemicals/ reagents are very rapid methods to identify the doubtful specimens. In the event of lacking of physico-chemical evaluation, such methods are very important to check the adulteration.

All crude drugs are standardized for its active constituents. An extract is referring to a concentrated, well dried preparation of active constituents of medicinal crude drugs and the concept of standardised extracts definitely provides scientific validation of crude drug. Here preliminary phytochemical investigation of the leaf and stem extracts confirmed the presence of alkaloid, coumarin, catechin, flavonoid, glycoside, phenol, tannin, steroid, saponin and terpenoid.
Different diagnostic microscopical and morphological characteristics of stem and leaves and their powder form will help to differentiate the original drug and other adulteration. Different physicochemical and phytochemical investigations were performed to access the different chemical constituents which are in the possible plan for isolation and characterization.

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