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# Pharmacognostical studies on the stem of *Hugonia* mystax L.

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#### **Abstract**

Hugonia mystax L. is an evergreen liana distributed throughout India, in dry forest area. Ethnobotanically, the stem bark used for indigestion, inflammations in the stomach, stomach pain and vomiting. The present investigation was analyzed to evaluate the pharmacognostical studies on selected medicinal plant for the first time in this plant. The parameters such as anatomical, histochemical colour reactions, fluorescence analysis, ash values, extractive values were evaluated in the stem. The values calculated and data collected could be used for the identification of powdered drug of this taxon. Pharmacognostical analysis will help to identify authentically the drug from adulteration, substitution in the herbal market and also for the quality control in pharmaceutical industry.

**Keywords:** *Hugonia mystax* L., Pharmacognosy, anatomical, Ash value, extractive value.

#### 1. Introduction

Standardization and quality control of herbals is the process involved in the physicochemical evaluation of crude drug covering aspects, such as selection and handling of crude material, safety, efficacy and stability assessment of finished product, documentation of safety and risk based on experience, provision of product information to consumer and product promotion [1]. They are standard pharmacognostic parameters that can be used to differentiate closely related plant species or varieties with similar constituents or pharmacological activities. Standardization of crude drug is an integral part of establishing its correct identity. The quantitative determination of some pharmacognostical parameters is useful for setting standards for crude drugs [3].

The plant *Hugonia mystax* L., is a woody evergreen species belonging to the family Linaceae, locally knows as Modirakanni. Ethnobotanically, the stem barks are used for indigestion, inflammations in the stomach, stomach pain and vomiting <sup>[4]</sup>. After the scrutiny of literatures, so far scanty work has been carried out regarding pharmacognostical study of stem of selected plant. Hence in the present study, the anatomical, quantitative analysis, histochemical and physico-chemical parameters of stem of *Hugonia mystax* L. were done.

# 2. Materials and methods

# 2.1 Collection of plant material

The plant material (stem) of *Hugonia mystax* L., were collected from the Marakanam Reserve forest of Villupuram district, Tamil Nadu. The plant material was botanically identified by using the Flora of Presidency of Madras <sup>[5]</sup>. An Excursion Flora of Central Tamil Nadu <sup>[6]</sup> and the confirmation were engaged at French Institute Herbarium (HIFP), Puducherry. The herbarium specimen was prepared and deposited at the Department of Botany, Kanchi Mamunivar Centre for Post Graduate Studies, Lawspet, Puducherry for further reference (Voucher no. AV74).

# 2.2 Description

Stragling shrubs upto 3 m high. Branchlets short, horizontal, circinate hooked. Leaves simple, alternate, stipulate,  $3.8\text{-}6.3 \times 2.5\text{-}3.8$  cm, elliptic-obovate, cuneate at base, entire at margin, obtuse or subacute at apex, glabrous; veins reticulate, conspicuous on both sides, tapering towards base; petiole 1.5 cm long, hairy; stipules lanceolate-subulate. Inflorescence axillary or terminal cymes. Flowers regular, bisexual, yellow, 2.5-3.3 cm across, pedicels short. Sepals 5, free, imbricate, 7.5 mm long, ovate-lanceolate, acute at apex, fulvous pubescent. Petals 5, larger than sepals, ovate-oblong, truncate at apex, acute at apex. Stamens 10; filament short,

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Department of Plant Science, Tagore Arts College, Lawspet, Puducherry – 605 008, India. connate at base with glandular swellings. Ovary superior, styles 5, filiform; stigmas capitate. Fruit drupe, globose, 1 cm across, surrounded by persistent sepals (Fig.1).

#### a. Anatomical studies

The stem were cut in to small pieces and fixed in FAA (Formalin: Acetic acid: 70% ethyl alcohol = 5 mL: 5 mL: 90 mL) immediately after collection <sup>[7]</sup> follow series of tertiary butyl alcohol <sup>[8]</sup> and paraffin embedded specimens were sectioned with the help of rotary microtome.

The thickness of the sections was 10-12  $\mu$ m. Dewaxing of the sections was by customary procedure <sup>[9]</sup>. The sections were stained with Toluidine blue as per the standard method <sup>[10]</sup>

# b. Quantitative microscopy

The qualitative and quantitative features of stem vice: Epidermal cell number and size, stomatal number and size [11], Stomatal index [12], histochemical analysis [13], physicochemical parameters such as ash value [14], loss on drying [15], batch [16] and successive extractive value [17], fluorescence analysis [18] were followed.

Epidermal cells, stomatal morphology, trichome distribution, clearing of stem with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid <sup>[7, 8]</sup>. The histomorphological measurements were taken with the help of linear micrometer in a calibrated Binocular microscope. For the quantitative values, the numbers of cells were determined with reference to the field under higher magnification. These readings were brought to one square millimeter. For each category, a total of 25 readings were taken. Mean values and the values of standard error for each character were calculated from these readings.

## 3. Results

# 3.1 Anatomical studies

## a. Stem epidermal cells and stomatal morphology

Epidermal cells are rectangular to rhomboidal, stomata are seen one to four in one focul field and they are oriented towards the longitudinal axis of cells. Stamata is exclusively paracytic type (Fig. 2). Young stem peeling showed numerous trichomes, both glandular and nonglandular hairs (Fig. 3). Shaggy glandular trichomes, uniseriate nonglandular trichomes and secretory cavity are visible on epidermal peeling.

#### b. Transverse section of Stem

The stem is circular with shallow irregular fissures. It is  $1.9\,$  mm thick. The periderm is very thick comprising a homogeneous, phellem cells are thin walled and suberised of  $100\,\mu m$  thickness and about six layers of narrow phelloderm cells. The cortex narrow parenchymatous; the cells are compressed at certain regions.

The inner boundary of the cortex is a thick continuous sclerenchyma cylinder which enclosed the vascular cylinder included sclereids and fibres; crushed phloem and intact phloem follow the sclerenchyma cylinder. The secondary phloem is 130  $\mu m$  thick. It consists of outer tangential blocks collapsed phloem where the cells are compressed in to dark cylinders. Inner phloem is non collapsed phloem with intact cells which are in radial files.

The secondary xylem cylinder is  $100 \mu m$  thick. It consists of solitary diffuse vessels, thick walled fibres and wide straight

rays. The xylem fibres and xylem rays are prominent. The vessels are found to be filled with some amorphous deposition which occludes the vessel lumen. The vessels are oval to circular thick walled and up to 30 µm wide (Fig. 4).

# c. Crystals and starch grains

Calcium oxalate crystals are common in the cortex and starch grains in the pith. The crystals may be druses or prismatic crystals. They occur in the periderm and sclerenchyma cylinder of the cortex. Starch grains are located in the pith. They are simple, circular grains with concentric hilum. The starch grains are up to  $12 \mu m$  wide (Fig. 5).

#### ii. Stem bark

# (i) Transverse section of stem bark

The stem bark has very thick, deeply fissured periderm. It is unequal in thickness around the stem. The periderm is 800  $\mu m\text{-}1.1$  mm thick. The periderm is heterogenous. It includes only phellem and phelloderm is warting. The phellem tissue consists of several layers of tabular cells; the cells are in regular parallel rows and are suberised. At frequent intervals there are 35 mini layers of cells which run tangentially in the phellem. These layers are one or two cells thick; the cells are similar to the phellem cells. But the cell walls are called phelloids (Plate 8). The phellem and phelloid cells are 30-40  $\mu m$  in tangential plane and 10-40  $\mu m$  in tangential plane and 10-40  $\mu m$  in radial plane (Fig. 6, 7).

# Secondary phloem

The secondary phloem is the major portion of the bark. It is differentiated in to outer wider zone of collapsed phloem and narrow inner zone of noncollapsed phloem. Noncollapsed phloem comprises several tangential bands of sclerenchyma blocks and crushed sieve elements and intact parenchyma cells. The sclerenchyma blocks are two or three layers thick and 150-250  $\mu m$  wide. The collapsed phloem also includes thick, dark tangential streaks which are crushed sieve elements.

Noncollapsed phloem zone is very narrow zone having intact sieve elements small parenchyma cells and narrow rays. The sclerenchyma bands are also not present in the noncollapsed phloem zone. In the noncollapsed regions, the sieve elements are narrow polygonal cells with fairly thick walls with the companion cells located at one of the corners of the sieve elements.

# (ii) T. L. S. view of stem bark

The phloem rays appear as wide, high spindle shaped bodies. L.S. of phloem through the collapsed phloem region shows wide rays. In the noncollapsed phloem region the rays are comparatively narrow; the rays are 3 to multiseriate, very wide and very high, homocellular, vertically oblong and thin walled. The rays are 700-900  $\mu$ m in height and 100-300  $\mu$ m wide. The axial parenchyma cells occur in vertical rows; the cells elongated and thin walled (Fig. 8).

# **Crystal distribution**

Calcium oxalate crystals are abundant in the bark. The crystals are prismatic type. They are located in the vertical parenchyma strands closely associated with the phloem sclerenchyma.

The crystals in vertical rows one crystal is seen in each cell (Fig. 9).

#### Stem wood

The stem wood is light brown, very hard, heavy, no specific odour or taste.

#### 1) Transverse section of stem wood

The wood has no growth rings; the vessels are diffusing porous; they are solitary, rarely in multiples of two. The vessels are circular, wide and thick walled they are 20-50  $\mu m$  in diameter. Axial xylem parenchyma cells are paratracheal aliform. The parenchyma cells forms thick sheaths around the vessels extend laterally in to short wings. The xylem fibres are heavily thick walled and lignified with narrow lumen. The walls are lignified; xylem rays are thin and straight. The ray cells are darkly stained. The rays are 13  $\mu m$  (Fig.10).

# 2) T.L.S. view of stem wood

The structure of the rays and their seriation are seen in the T.L.S. view. The xylem rays are 13 seriate. The rays are thin and very high. They are hetero cellular; the cells in the middle of the rays are short; those at the margin are vertically elongated. The rays are 250-350  $\mu m$  in length. The breadth of the rays is 30-50  $\mu m$ . The vessels

are linear or straight (Fig.11).

## 3) R.L.S. view of stem wood

In R.L.S. view the xylem rays are seen in horizontal layers, lying at right angles to the vertical system of fibres and vessels. The cells of the ray in middle portion are square shaped and those which are at the upper and lower ends at vertical oblong. The ray cells are thick walled and lignified. The lateral walls of vessels have dense bordered pits. They are elliptical, multiseriate and alternate (Fig. 12).

#### 2. Quantitative Microscopy

In stem, Quantitative microscopical analysis observed such as epidermal cell size was  $28\pm1.1\times20\pm1.0~\mu m$ , epidermal cell number was  $6875\pm180.2/mm^2$ , stomatal size was  $32.3\pm2.0\times20.4\pm1.0~\mu m$ , stomatal number was  $38.4\pm3.1/mm^2$ , stomatal index was  $0.56\pm0.04$ , number of glandular hairs was  $69.3\pm7/mm^2$ , glandular hair size was  $47\pm3.0\times26\pm1.0~\mu m$ , number of non-glandular hairs was  $98\pm17.2/mm^2$  and non-glandular hair size was  $152\pm15.0\times26.0\pm1.1~\mu m$  (Table 1).

Table 1: Quantitative microscopical analysis of stem

S. No.	Various parameters of sten	1	Size (µm) and Number (mm²)
1	Epidermal cell size	(L)	28 ± 1.1
1		(B)	$20 \pm 1.0$
2	Epidermal cell number		$6875 \pm 180.2$
3	Stomatal size	(L)	$32.3 \pm 2.0$
3		(B)	$20.4 \pm 1.0$
4	Stomatal number		$38.4 \pm 3.1$
5	Stomatal index		0.56±0.04
6	Glandular hair number		$69.3 \pm 7.0$
7	Glandular hair size	(L)	$47 \pm 3.0$
,		(B)	$26 \pm 1.0$
	Non-glandular hair number		98 ± 17.2
8	Non-glandular hair size	(L)	$152 \pm 15.0$
o		(B)	$26.0 \pm 1.1$

 $\overline{(L)} = \text{Length}; (B) = \text{Breath}$ 

All the values are expressed as mean  $\pm$  S.E.M.

# 2. Powder Microscopic observation

The stem powder includes larger number of fibre sclereids and fibres. These cells have thick, secondary walls narrow lumen and tapering ends. The fibre sclereids are characterized by wide and dense canal like simple pits. The fibre sclereids are 600-700  $\mu m$  long and 30  $\mu m$  thick. The fibres thin walled and are more than 700  $\mu m$  long.

#### (i) Vessel elements

Vessel elements are long narrow and cylindrical. They have long or short tails at one or both ends. The perforation plate is single wide and slightly horizontal pits on the lateral walls are dense, circular and bordered. The vessel elements are  $800\text{-}950~\mu m \, long.$ 

# (ii) Xvlem ravs

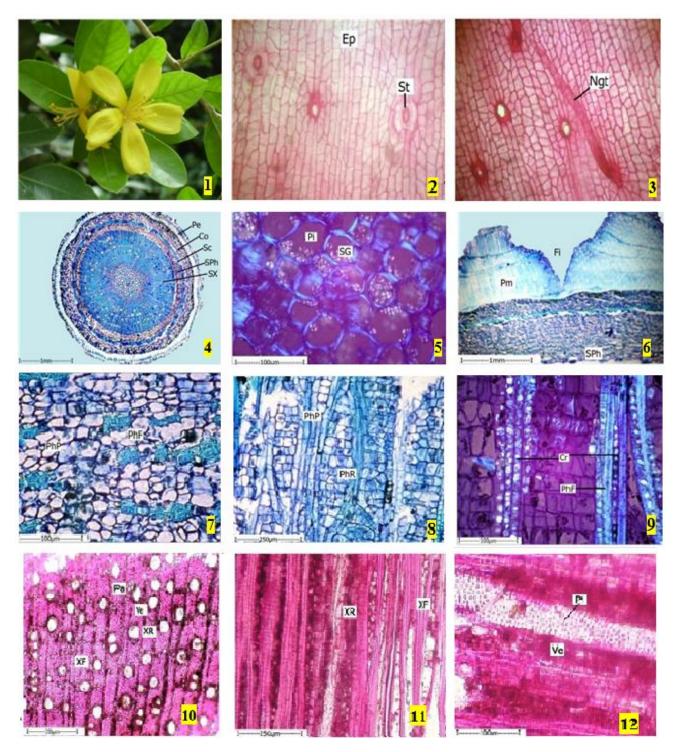
Xylem rays are seen attached horizontally on the vertical system of vessels and thick fibres. The ray cells are rectangular and thick walled. At marginal part of the ray are also seen some of cells being vertically elongated upright cells. Crystal strands are abundant

## 4. Histochemical analysis

Lignin present in the secondary phloem and xylem region, it shows deep red colour (Fig. 13). Lipids and lipoproteins stained black colour in the vascular strand and cortical cells, it stained black in colour (Fig. 14). Protein stained blue colour in the phloem region and cortical cells (Fig. 15). Starch stained black colour in the sclerenchyma, phloem and xylem tissues (Fig. 16). Tannins stained brown colour indicates moderate present and mucilage are absent (Table 2).

# **4.** Physico-chemical parameters. Ash values and Moisture content

In stem, total ash value was (5%), water soluble ash (3%), acid insoluble ash (2%), sulphated ash (2%) and moisture content (12%) respectively (Table 3).



**Plate 1:** (Fig. 1) **Plate 2:** (Fig. 2-12)

Plate – I.

[Fig 1: Hugonia mystax L. A twig with flowers]

Plate - II. Anatomical studies of stem.

[Fig 2: Epidermal cells of stem showing stomata; Fig 3: Epidermal cells showing non-glandular; trichomes; Fig 4: T.S. of stem-entire view; Fig 5: T.S. of stem with starch grains in the pith (under polarized light); Fig 6: T.S. of the stem bark showing thick periderm and deep V-shaped fissure; Fig 7: T.S. of stem bark - Inner most portion; Fig 8: Inner most portion of the bark; Fig 9: Stem bark showing crystal distributions (Crystal distribution in the phloem as seen in T.L.S. view under polarized light); Fig 10: T.S. of wood showing gross microscopic features; Fig 11: T.L.S. of wood showing ray-characters; Fig 12: R.L.S of wood - Vessels showing lateral wall pits (Co: Cortex; Cr: Crystals; Ep: Epidermal Cells; Fi: Fissure; Ngt: Non-glandular trichome; Pe: Periderm; Pm: Phellem; PhF: Phloem Fibres; PhP: Phloem Parenchyma; PhR: Phloem Rays; PPa: Paratracheal Parenchyma Pi: Pith; Pi: Pits; St: Stomata; Sc: Sclerenchyma: SPh: Secondary Phloem; SX: Secondary Xylem; SG: Starch Grains; Ve: Vessel; XF: Xylem Fibre; XR: Xylem Ray)].

Table 2: Histochemical analysis of stem.

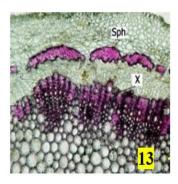
S. No.	Test	Expected colour change	Observations
1	Lignin	Deep red	++
2	Lipids and lipoproteins	Black	++
3	Mucilage	Purple	-
4	Protein	Blue	++
5	Starch	Black	++
6	Tannins	Blue	+

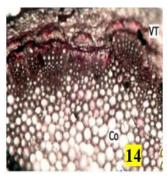
(++ = Marked present; + = Moderate present; - = Absent)

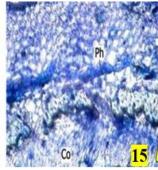
Table 3: Ash values and moisture content of stem.

S. No.	Ash value/Moisture content	Values (%)
1	Total ash	5
2	Water soluble ash	3
3	Acid insoluble ash	2
4	Sulphated ash	2
5	Moisture content	12

Plate-1(Fig. 1) Plate –II (Fig. 2-12)







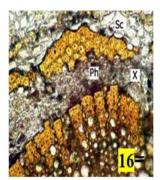


Plate 3. (Fig 13-16)

Plate - III. Histochemical analysis of the Stem

Fig 13: Lignin; Fig. 14 Lipids and lipoproteins; Fig. 15 Proteins; Fig. 16 Starch. (Co: Cortex; Ph: Phloem; Sc: Sclerenchyma; SPh: Secondary Phloem; VT: Vascular Tissue; X: Xylem).

# **Extractive values**

# Successive and batch method extractive values

Successive extractive values recorded were 4% in petroleum ether, 5% in chloroform and 7% in ethanol respectively. In batch method the highest extractive value was recorded as 16% in water followed by 14% in methanol, 13% in ethanol, 8% each in hexane, benzene, ethyl acetate and acetone respectively while 7% in chloroform and 6% in petroleum ether (Table 4)

Table 4: Extractive values of stem.

S. No.	Solvents	Values (%)
Successive process		
1	Petroleum ether	4
2	Chloroform	5
3	Ethanol	7
Batch process		
4	Acetone	8
5	Benzene	8
6	Carbon tetra chloride	9
7	Chloroform	7
8	Ethanol	13
9	Ethyl acetate	8
11	Hexane	8
12	Methanol	14
13	Petroleum ether	6
12	Water	16

# Fluorescence analysis

Stem powder, under day light, showed the green colour in acetone and ethyl acetate; light brown in ethanol and hexane; pale green colour in benzene and methanol; sandal colour in powder as such; brown colour in chloroform; yellowish green colour in carbon tetrachloride; light green colour in petroleum ether; reddish brown in water respectively. Under UV- light, showed the fluorescence green colour in acetone, benzene, carbon tetrachloride, ethanol, ethyl acetate, petroleum ether and methanol; pale green colour in powder as such and hexane; dark green colour in chloroform; dark brown colour in water respectively.

Under day light showed the reddish brown colour in 50% sulphuric acid, 10% sodium hydroxide and Fehling's solution; blackish brown colour in 10% ferric chloride and iodine solution; light brown colour in 1N HCl; yellowish green colour in 50% nitric acid; orange colour in 1% thionyl chloride respectively. Under UV-light, showed the pale green colour in 1N HCl and 1% thionyl chloride; brownish green colour in 50% sulphuric acid and Fehling's solution; yellowish green colour in 10% sodium hydroxide; fluorescence green colour in 50% nitric acid; blackish green colour in 10% ferric chloride; reddish brown colour in 5% ammonia; dark brown colour in iodine solution respectively (Table 5).

**Table 5:** Fluorescence analysis of stem

S. No	Chamina In	Leaves	Leaves		
	Chemicals	Day light	UV light		
1	Powder as such	Sandal colour	Pale green		
Solvents	•	·			
2	Acetone	Green	Fluorescent green		
3	Benzene	Pale green	Fluorescent green		
4	Carbon tetrachloride	Yellowish green	Fluorescent green		
5	Chloroform	Brown	Dark green		
6	Ethanol	Light brown	Fluorescent green		
7	Ethyl acetate	Green	Fluorescent green		
8	Hexane	Light brown	Pale green		
9	Methanol	Pale green	Fluorescent green		
10	Petroleum ether	Light green	Fluorescent green		
11	Water	Reddish brown	Dark brown		
Reagents	•	·			
12	1NHCl	Light brown	Pale green		
13	50% H <sub>2</sub> SO <sub>4</sub>	Reddish brown	Brownish green		
14	50% HNO <sub>3</sub>	Yellowish green	Fluorescent green		
15	10% NaOH	Reddish brown	Yellowish green		
16	10% FeCl <sub>2</sub>	Blackish brown	Blackish green		
17	5% Ammonia	Dark brown	Reddish brown		
18	1%Thionylchloride	Orange	Pale green		
19	Iodine solution	Blackish brown	Dark brown		
20	Fehling's solution	Reddish brown	Brownish green		

#### Discussion

The anatomical features of the stem was characterized by the presence of more irregular fissures with thick homogenous periderm, thin walled suberised phellem cells and six layers of narrow phelloderm cells, secondary phloem with outer tangential blocks collapsed phloem, inner non-collapsed phloem, secondary xylem cylinder with solitary diffused vessels and thick sclerenchymatous layers of sclereids and fibres forms the diagnostic features of the family Linaceae [19]. As per the present studies suberized phellem, one of most important characters in anatomy which would help in authentic identity has been also reported in the barks of several plants previously such as in Cassia fistula [20], Tectona grandis [21], Viburnum erubescens [22]. The present study is also supported in the barks of Neotropical genera of Annonaceae [23]. Similarly, phelloderm was also considered as an important peculiar character for identification, hence it was 6 layered in Hugonia mystax, whereas 35-45 layered interspersed with groups of stone cells of various shapes and sizes has been reported in Cassia fistula [20]; 9-10 layered in Artocarpus hirsutus [24] but indistinguable in Mimusops elengi [25]. The observation of calcium oxalate crystals in the cortex, as druses or prismatic seen in the periderm and sclerenchyma cylinder of the cortex. Calcium oxalate crystals are of different types found in different organs or almost all parts of the plant which give protection to the plant against birds and animals form a great diagnostic value useful in correct identification of crude drugs and helps in detection of adulterants [26]. Thus calcium oxalate crystals have been reported several families as an authentic identity of Asteraceae [27-29] Cesalpiniaceae [30]; Solanaceae [31] and Vitaceae [26]. The morphology and distribution of crystals is constant in a species are under genetic control [32, 33]. Thus, the constancy of crystal type and distribution may be considered a taxonomic character for classification of species [33]. The crystal pattern is also often stable within a genus [34].

This study was aimed at identifying the crude drugs with the help of determining the presence of similarities and differences of crystals. The study also reported presence of starch grains in the pith are simple, circular grains with concentric hilum. Similarly, starch grains were reported in the root of *Oxystelma esculentum* [35] and also in the rhizome of *Lilium polyphyllum* [36].

The anatomical features of the (*Hugonia mystax*) stem bark of the species was characterized by the presence of very thick, deeply fissured, heterogenous periderm with regular parallel rows and suberised phellem and warting phelloderm, the secondary phloem with outer wider zone of collapsed phloem and narrow inner zone of noncollapsed phloem. However, suberized phellem was recorded in *Buxus wallichiana* and *Artocarpus hirsutus* [24, 37] respectively. Similarly, non-collapsed phloem was reported in *Ficus racemosa*, *F. virens*, *F. religiosa and F. benghalensis* [3]. These anatomical characters were helpful in identifying the plant material in herbal industry.

The anatomical features of stem wood vessels are largest, paratracheal aliform xylem, horizontal rays 2-5 seriate and bordered pits are abundant in the genus Hugonia [19] (Metcalfe and Chalk, 1957). Similarly, the present findings also recorded, the vessels diffused porous, solitary, paratracheal aliform xylem, lumen narrow, lignified xylem fibres. These findings were also reported previously in five Cola species [39]. In the present study of T.L.S. view showed 1-3 seriate xylem rays and straight vessels present. Whereas in Buxus wallichiana T.L.S. view of wood reported the xylem rays are narrow, biseriate and vertical rows of cells [37]. The R.L.S. view of wood has horizontal layers of xylem rays, the ray cells lying at angles to the vertical system of fibres and vessel, the vessels have dense bordered pits. However, in the family Anacardiaceae, generally vessels are fairly large and of ring porous type [40]. Moreover the fossil Anogeissisoxylon rehmaneses [41] wood character slightly

related to *Hugonia mystax* but differs in one character like growth ring is present in fossil but growth rings inconspicuous and the vessels diffused porous in the present study. These characteristic features are important in pharmacognostical research.

Stomatal size of leaves is smaller than the stem but the stomatal number is higher in leaves than stem shows significantly [42]. Moreover the same findings also recorded in *Podocarpus lambertii* [43]. All these parameters are first report in *Hugonia mystax* would be very useful in identifying the crude drug in herbal industry in order to avoid substitution and adulteration in herbal industries.

Stem powder was characterized by larger number of wide and dense canal like simple pitted fibre sclereids, thin walled fibres, long narrow and cylindrical vessel elements, rectangular and thick walled xylem rays, abundant crystal strands in sclerenchyma cells and prismatic type crystals. Moreover xylem vessels and fiber sclereids were present in *Cissus quadrangularis* [44], also investigated the same results in the powder microscopic observation in *Brassica oleracea* [45]. The present study significantly shows horizontal pits on the lateral walls are densely arranged in the stem powders of *Hugonia mystax*. Whereas it is absent in the plant *Buxus wallichiana* [37]. These anatomical features are useful in identification of plant in order to seperate the plant from adulterants and substitutions during herbal drug quality control.

Histochemical analysis is highly essential that will aid the botanist to locate chemical substances and its properties in terms of tissues, cells and cell parts [7, 13]. The similar studies on stem of *H. mystax* confirmed the presence of various plant metabolites in plant tissue forming as additional evidence to support that the selected species. *H. mystax* are relatively high in bioactive secondary compound and are thus likely to hold promise for drug discovery [46]. Adequately, tannin and lignin were present in the plant parts of *Cissus quadrangularis* [44]; *Laurencia obtuse* [47] and also reported in *Cassia tora* [48]. Thus the histochemical study of the stem indicates the first report to support the presence of the histochemical compounds in *H. mystax* contained maximum plant metabolite such as lignins, lipids and lipoproteins, mucilage, proteins, tannins and starch.

The total ash values were higher than the water soluble ash acid insoluble ash and sulphated ash values in stem. The total ash usually consists of carbonates, phosphates, silicates and silica, which include both physiologic ash and non-physiologic ash. A high ash value is indicative of contamination, substitution, adulteration, or carelessness in preparing the crude drug for marketing [49]. Evidently, the total ash value is more or less similar comparable to the other woody liana species like *Smilax zeylanica* [50], *Caesalpinia bonduc* [51], *Ventilago calyculata* [52] and *Tinospora cordifolia* [53]. Thus all the physicochemical parameter of plant drugs reported here for the first time, is an important for detecting adulteration or improper handling of drugs which would be useful in standardization of herbal drugs.

In successive extraction, lower extractive values were observed in petroleum ether solvent, medium values were recorded in chloroform solvent while higher values were recorded in ethanol solvent in stem. But in batch process, variations in extractive values were observed. In stem, water solvent showed more extractive values compared to other solvents followed by methanol, ethanol, carbon tetrachloride,

benzene and acetone. Lower values are recorded in hexane, petroleum ether solvents. Similar study was also reported in *Solanum torvum*, *Crotalaria juncea* and *Mimusops elengi* <sup>[54, 55, 25]</sup> respectively.

Fluorescence analysis is one of the pharmacognostical procedures useful in the identification of authentic samples and recognizing adulterants [56]. In the present study, the fluorescence analysis was examined in stem in their powdered form or in different solvents and reagents. Although, in most of the cases the actual substances responsible for the fluorescence properties has been identified, the merits of simplicity and rapidity of the process makes it a valuable analytical tool in the identification of plant samples and crude drugs [57]. Several works on this aspect were reported in various plants to standardization of herbal drugs in quality control [45, 58]. Thus the present study with detailed fluorescence analysis of the coarsely powdered drugs of H. mystax under UV and day light reported here for the first time would be very useful aid to confirm authenticity and to check adulterants in crude form.

#### Conclusion

Thus the present pharmacognostical studies on the stem of *Hugonia mystax* generated many parameters which aid to detect the adulteration, substitution and authentically identification of crude drugs. In the above discussion we concluded that the pharmacognostical studies of stem drugs of *Hugonia mystax* could be successfully used for the authenticity of the powdered drugs of this taxon.

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