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Research Article

PHARMACOGNOSTICAL STANDARDIZATION OF *HUGONIA MYSTAX* L. LEAVES

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ABSTRACT

The present study discloses detailed pharmacognostical profile of leaves of ***Hugonia mystax* L**. This plant belongs to family Linaceae and an important medicinal plant in the Indian system of medicine. This species is has a restricted global distribution, occurring only in India and Sri Lanka. In the present study leaves of ***H. mystax*** were subjected for macroscopical, microscopical, physicochemical, phytochemical, fluorescence and H.P.T.L.C. analysis. The microscopical studies revealed the presence of shield shaped vascular bundle accompanied by 2 to 1 small accessory vascular bundles in the midrib region, pericyclic fibers around the vascular bundle, rubiaceous type stomata in lower region, presence of abundant rhomboidal to prism shaped calcium oxalate crystals, presence of reddish tannin content in palisade region and presence of abundant fibers. To determine extent of adulteration as well as to establish the quality and purity of drug, Physicochemical parameters like loss on drying, total ash value, acid insoluble ash, water insoluble ash, various extractive values etc., were carried out and revealed the total ash value as 8.7%, of which, 1.34% was acid insoluble ash, and 2.25% was water soluble ash and 5.8 % water insoluble. The extractive values were found to be 18.54% and 13.42% for water and alcohol respectively, which indicated higher extractive value for water compared to alcohol. Further, qualitative tests for various functional groups like Triterpenoids, alkaloids, glycosides etc., were carried out and H.P.T.L.C. profile was also established with Methanolic extract.

Key words: Hugonia mystax L. Leaves, Pharmacognostical standardization.

INTRODUCTION

In India there are about 7500 wild plants used for medicinal purposes by different tribal inhabitants. Unfortunately the descriptions provided for the identification of these plants/drugs in the literature are insufficient for the proper understanding. Usage of multitude vernacular names for single plant by tribal inhabitants pose problems in identifying the correct botanical names of medicinal plants. And, it is worst puzzled with the use of different botanical species under the same drug name. Further these are claimed to possess similar therapeutic efficacy and used as the same drug. Such drugs are today termed as controversial drugs. In such cases Pharmacognostical standardization is the only source to prevent unscrupulous commercial practice of adulterating and substituting the genuine herbal drugs, which are posing great difficulty to procure genuine medicine and popularizing the time-tested herbal-based traditional medicine. In these circumstances, pharmacognostical studies on leaves of ***H. mystax*** have been undertaken mainly with this object in view and no literature was found on this plant on the aspect of pharmacognostical studies.

This species is has a restricted global distribution, occurring only in India and Sri Lanka. Within India, it has been recorded in Maharashtra (Between Malvan and Vengurla), Karnataka (Dakshina and Uttara Kannada) Tamil Nadu

(Plain, Foothills, and almost all districts) and Andhra Pradesh (Foothills, some of the districts). It is an unexplored medicinal plant in the Indian medicinal system. According to ethnobotanical information, the leaves are used in the treatment of peptic ulcers and as anthelmintic, febrifuge, antidote and its fruits are used in diarrhoea and dysentery. Stem bark is used in the treatment of jaundice and skin diseases. The problem encountered in standardization of this medicinal plant is its identification by source.

MATERIALS AND METHODS

Fresh leaves of ***H. mystax*** were collected from the Tribes of Nellore district. Identification and confirmation were done by Department of Botany Sri Venkateswara University, Tirupathi, Andhra Pradesh, India. The voucher herbarium specimen was processed followed by standard procedure present in Jain and Rao[6]. Microscopical studies of leaf using fresh plant material were carried out with standard procedures[7-10]. During these studies T.S of the leaf, Powder microscopical studies and Maceration were done to observe peculiar characters of the leaf. Physicochemical studies like, total ash, acid insoluble ash, water soluble ash; water soluble and alcohol soluble extractive values were computed according to the methods described in Indian Pharmacopoeia[11]. Preliminary phytochemical investigation in leaf powder was performed as described in Khandelwal et al.[12] and Kokate[10]. Fluorescence analysis was carried out according to methods of Kokoski *et al.*[8]. HPTLC fingerprinting Methanolic extract was also carried out. Precoated TLC plate of silica gel 60 F (Merck) was used as stationary phase. For sample application, DESAGA sample applicator was employed. The plates were then developed in glass twin trough chamber. The developed plates were scanned using TLC Scanner 3 (CAMAG).

Macroscopical Characters of the Leaf

3-6 cm length by 2.5-3 cm. width, elliptic-obovate, obtuse or subacute, entire, reticulately veined, the veins conspicuous on both surfaces, glabrous, base tapering; petioles 1.5 mm. long, hairy; stipules lanceolate – subulate (Fig. No 01 and 02).

Microscopical Characters

T.S of the leaf is dorsiventral in structure and plano convex towards lower side and plano concave towards upper side (Fig.No.41). Centrally large well developed shield shaped vascular bundle is present accompanied by 1-2 small well developed accessory vascular bundles on either side of the main bundles (Fig.No.21, 41 and 42). Both upper and lower epidermis well developed, covered by thick cuticle, towards the lower region 1 to 2 layered collenchymatous cells are present (Fig.No.42) and 4 to 6 layered parenchymatous cells are present (Fig.No.42). Parenchymatous cells show small starch grains and abundant reddish tannin content (Fig.No.42). Vascular bundle is accompanied by prominent pericyclic fibers (Fig.No.37). Towards the upper region 1 to 2 layered collenchymatous cells and 3 to 4 layered parenchymatous cells are present.

T.S through laminar region shows well developed mesophyll tissue with 2 to 3 layered palisade layers (Fig.No.43) and 1 to 2 layered spongy parenchymatous layers (Fig.No.43). Some of the palisade tissue shows reddish brown tannin content of cells (Fig.No.43). Smaller veins are represented by small vascular bundles covered by sclerenchymatous sheath (Fig.No.43). More stomata are confined to lower side and are of rubiaceous type (Fig.No.40). Abundant rhomboidal to prism shaped calcium oxalate crystals and clustered calcium oxalate crystals are present (Fig.No.44).

Diagnostic Characters

1) Presence of well developed shield shaped vascular bundle accompanied by 2 to 1 small accessory vascular bundles in the midrib region
2) Presence of reddish tannin content in parenchymatous region of midrib portion
3) Presence of reddish tannin content in palisade region
4) Presence of pericyclic fibers around the vascular bundle
5) Presence of rubiaceous type stomata in lower region
6) Presence of abundant rhomboidal to prism shaped calcium oxalate crystals

Powder microscopy of Leaf

Leaf powder dark green in colour, coarse to touch, smell agreeable, tastes slightly sour, when treated with chloral hydrate solution and water following fragments of different tissues were observed under the microscope.

Fragments of abundant stomata with epidermal cells (Fig.No.7 and 10), fragments of xylem vessels with simple pits (Fig.No.9 and 18), fragments of thin walled elongated parenchymatous cells (Fig.No.13), fragments of epidermal cells (Fig.No.17 and 20), fragments of Vessels with helical thickenings (Fig.No.16), fragments of thin walled epidermal cells with reddish tannin content (Fig.No.19), fragments of epidermal cells with rubiaceous type of stomata (Fig.No.12 and 40), fragments of broad xylem vessel, medium sized with intervascular pitting (Fig.No.18), fragments of epidermal cells with mucilage and Parenchyma cells (Fig.No.18), fragments of parenchyma cells with reddish tannin contents (Fig.No.15), parenchyma cells with clustered calcium oxalate crystals (Fig.No.11), abundant rhomboidal to prism shaped calcium oxalate crystals (Fig.No.9) and abundant fibers (Fig.No.5 and 8).

Diagnostic characters

1) Presence of rubiaceous type of stomata
2) Presence of abundant medium sized xylem vessels with intravascular pittings
3) Presence of thin walled elongated parenchymatous cells
4) Presence of abundant Rhomboidal to prism crystals
5) Presence of abundant fibers
6) Presence of reddish tannin contents in the parenchymatous cells

Fluorescence study: Fluorescence analysis were studied and recorded in Table No.1.

Table 1: Behavior of the Drug (Leaf Powder) with Different Chemical Reagents

S.N0.	Treatment	Colour observations Under		
		Ordinary light	U.V Light	
			255nm	365nm
1.	Powder +Distilled water	Light Green, mucilaginous with foamy nature	Dark Green	Light Green
2.	Powder + 5%Aqueous FeCl	No change	Black colour	Black colour
3.	Powder + Glacial acetic acid	No change	No change	No change
4.	Powder + 5% HNO_3	No change	No change	No change
5.	Powder + N/10 Iodine Solution	Blue colour	Dark Blue	Blue colour
6.	Powder + Con HCl	Light Green	Dark Green	Dark Green
7.	Powder + Con H_2SO_4	Black	Black	Black
8.	Powder + Ammonia solution	Light green	Dark Green	Light green
9.	Powder + 5% Aqueous NaOH	No change	Light green	Light green
10.	Powder + 5% Aqueous KOH Solution	Green	Black	Dark Green

Physico-chemical details

Ash values of a drug give an idea of the earthy matter or the inorganic composition and other impurities present along with the drug [07, 11, 13-15]. The results are given in Table-2.

Table 2: Physicochemical Parameters (% W/W)

S.No	Reaction Name	Values
1.	Total ash	8.7%
2.	Acid insoluble ash	1.34%
3.	Water soluble ash	2.25%
4.	Water insoluble ash	5.8%
5.	Moisture content (LOD) at 110 C	18.12%
6.	Water soluble extractive values	18.54%
7.	Alcohol soluble extractive values	13.42%

Preliminary phytochemical tests: These tests revealed the presence of flavonoids, alkaloids, carbohydrates, proteins and tannins listed in Table No-3

Table 3: Preliminary Phytochemical Tests

S.No	Chemical test	Ethanol Extracts	Petroleum Ether Extracts	Chloroform Extracts
1.	**Triterpenoids**			
	a)Leibermann buchard test	-	-	-
	b)salkowsky test	-	-	-
2.	**Flavonoids**			
	a)Lead acetate test	+	-	+
3.	**Glycosides**			
	a)Baljet test	+	+	+
	b)Legal test	+	-	-
4.	**Steroids**			
	a) Leibermann buchard test	-	-	-
	b)Salkowsky test	-	-	-
5.	**Alkaloids**			
	a)Dragendroffs test	+	-	+
	b)Hagers test	-	-	+
	c)wagers test	-	-	-
	d) Mayers test	-	-	-
6.	**Saponins**			
	Foam test	-	-	-
7.	**Carbohydrates**			
	a)Molishcs test	+	+	+
	b)Fehlings test	-	-	+
8.	**Proteins**			
	Biuret test	+	+	+
9.	**Tannins**			
	Ferric chloride test	+	+	+

H.P.T.L.C. analysis

Extract of samples was spotted on silica gel "G" plate as shown in Fig. No.41 and developed the plate using Toluene: Ethyl Acetate: methanol (7: 2: 1) mobile phase shows various spots under UV 366nm at Rf values mentioned in the table. Densitogram represents the separation of peaks of *H. **mystax*** **L.** leaves (Fig.No.42.).

Table 4: Peak List of *H. mystax* Leaf Powder at 366nm

Peak no.	Y-Pos	Area	Area (%)	Height	Rf value
1.	10.2	710.60	73.7	262.86	0.02
2.	20.6	5.50	0.6	3.84	0.16
3.	24.9	14.35	1.5	8.53	0.22
4.	37.7	31.81	3.3	12.67	0.40
5.	47.2	6.19	0.6	4.21	0.53
6.	50.1	11.62	1.2	5.34	0.57
7.	55.7	79.36	8.2	31.88	0.65
8.	62.9	7.24	0.8	4.37	0.75
9.	71.8	30.66	3.2	13.80	0.87
10.	77.1	67.25	7.0	23.60	0.95

DISCUSSION

During this study the microscopical, anatomical, physico-chemical and preliminary phytochemical analysis was performed in the leaf material. HPTLC profile was also established with Ethyl acetate and Methanolic extract. This anatomical study brought to light the diagnostic features that revealed a characteristic pattern of arrangement of the cellular components of ***H.***

mystax leaves. The fluorescence characters of powdered drug play a vital role in the determination of quality and purity of the drug material. In the present study, powder treated with various reagents shows characteristic fluorescence at 255 nm and 365 nm wavelength. To determine extent of adulteration as well as to establish the quality and purity of drug, ash values were calculated. Total ash was found to be 8.7%, of which, 1.34% was acid insoluble ash, and 2.25% was water soluble ash 2.5 % and 5.8 %water insoluble. The extractive values were found to be 18.54% and 13.42% for water and alcohol respectively, which indicated higher extractive value for water compared to alcohol. The moisture content was found to be 18.12%. Preliminary phytochemical screening revealed the presence of, Flavonoids, Alkaloids, Saponins, Carbohydrates, Proteins and Tannins. HPTLC fingerprint was established with Ethyl acetate and Methanolic extract. The pharmacognostical and phytochemical screening on the leaves of ***H. mystax*** furnish useful information for identification and authentication of plant. It can also assist as an important source of information to insure the identity and to determine the quality and purity of the plant material in future studies.

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Fig.No.1. Leaves and Fruits of *H. mystax* L.

Fig.No.2. Branch with Flowers and Hooks

Fig.No.3. Leaf powder

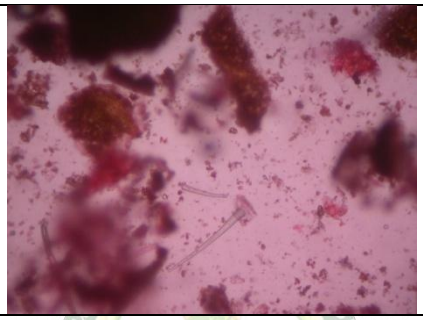


Fig.No.4. Different fragments of tissues

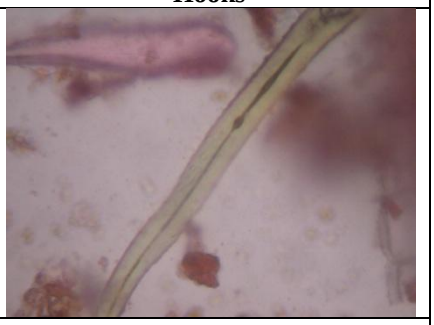


Fig.No.5. Fibers

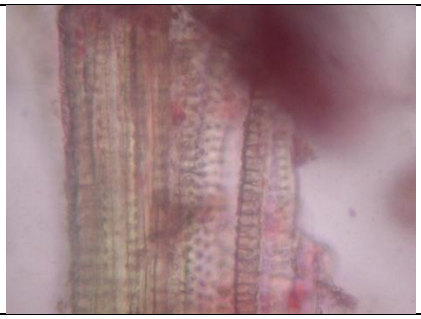


Fig.No.6. Fragments of vessels

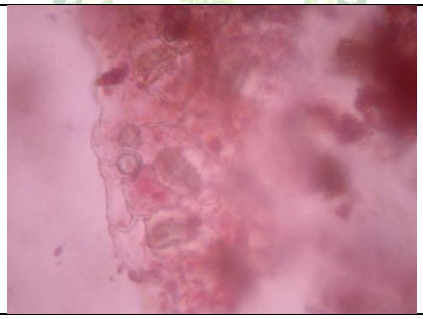


Fig.No.7.Epidermal cells with stomata

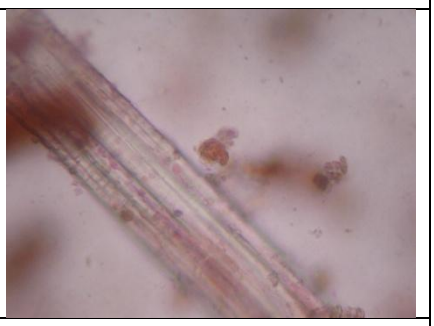


Fig.No.8.Fibers

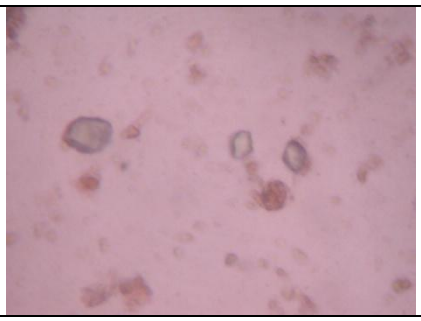


Fig .No. 9. Rambhoid shaped Calcium Oxalate crystals

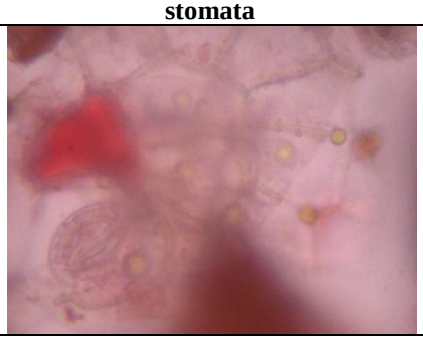


Fig.No.10. Stomata with epidermal cells

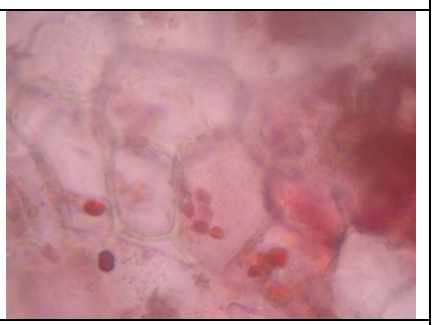


Fig.No.11. Parenchyma cells with rambhoid shaped Calcium Oxalate crystals

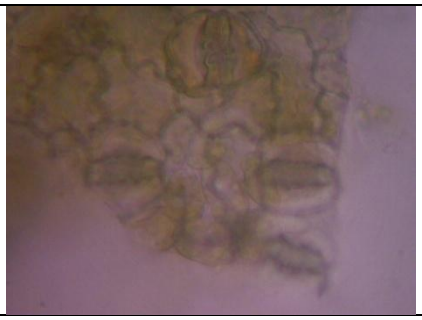


Fig.No.12. Epidermal cells with Rubiaceous type of stomata

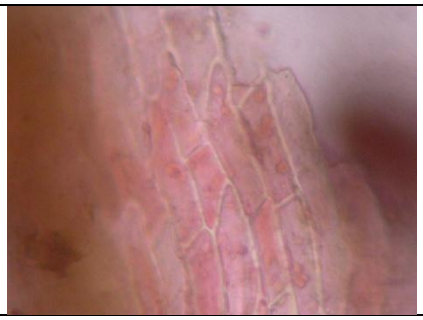


Fig.No.13. Thin walled elongated Parenchyma cells with reddish Taninin Contents

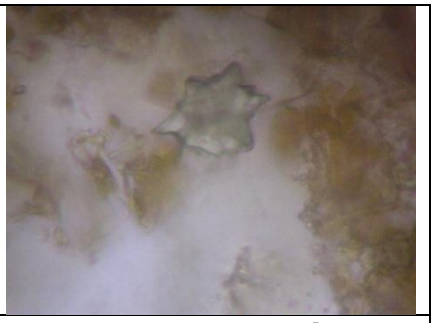


Fig.No.14. Clustered Calcium oxalate crystal

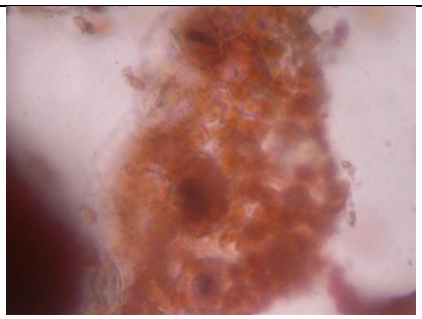


Fig.No.15. Parenchyma cells with reddish taninin Contents

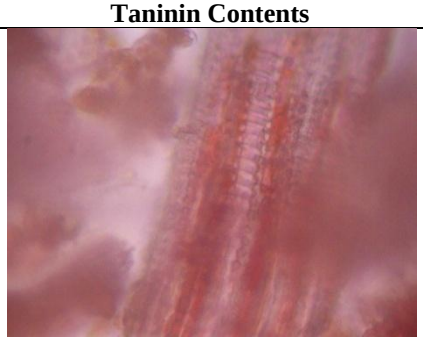


Fig. No.16. Vessels with helical thickenings

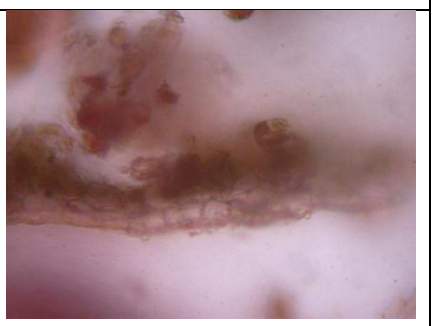


Fig.No.17. Epidermal cells with mucilage and Parenchyma cells

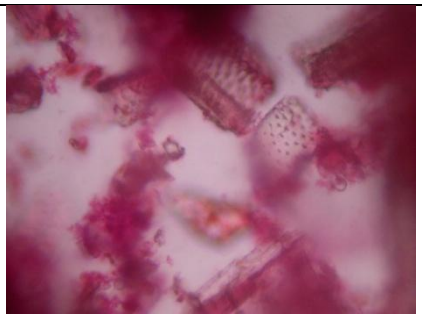


Fig.No.18. Fragments of xylem vessels

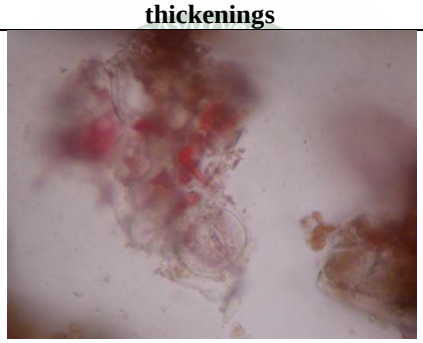


Fig.No.19. Epidermal cells with reddish contents and stomata

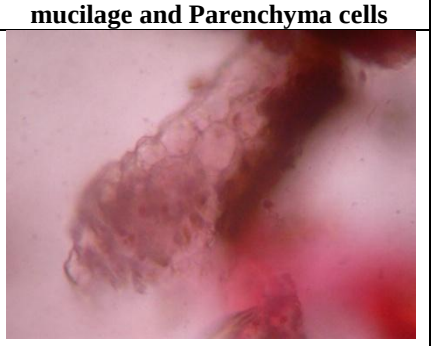


Fig.No.20. Epidermal cells with parenchyma cells

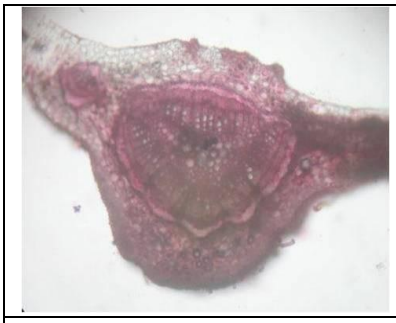


Fig.No.21. T.S. of the leaf

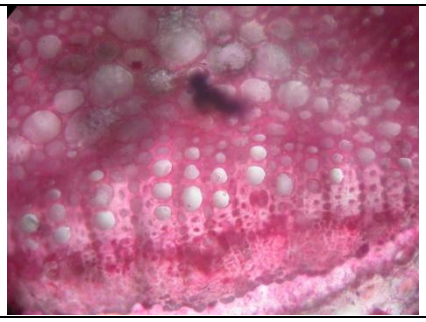


Fig.No.22. Vascular bundle enlarged.

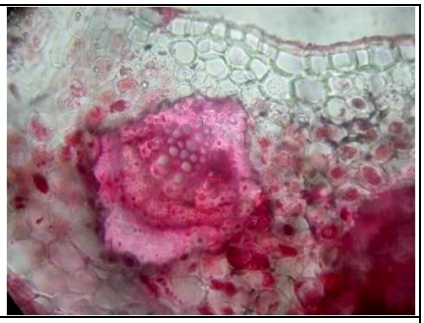


Fig.No.23. Accessory vascular bundle enlarged

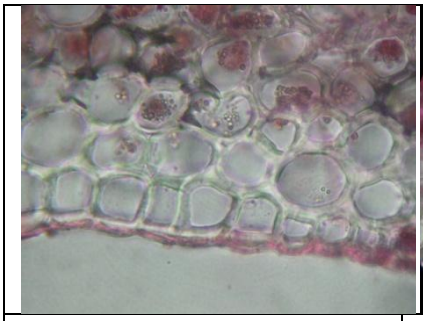


Fig.No.24. Epidermis and collenchymatous region

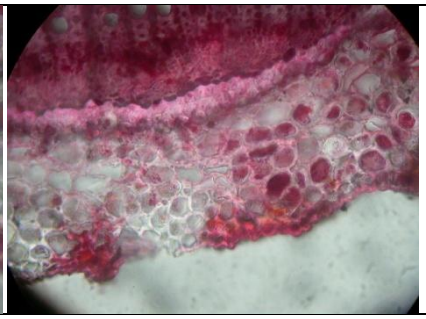


Fig.No.25. Lower portion enlarged

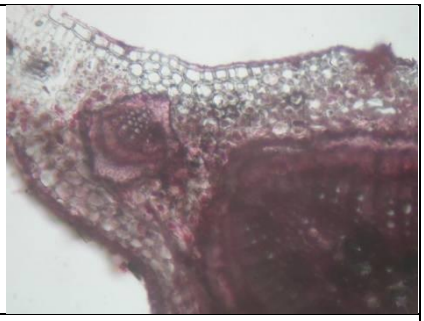


Fig.No.26. Accessory vascular bundle

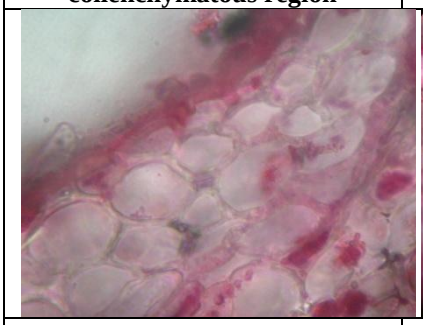


Fig.No.27. Upper epidermis enlarged

Fig.No.28. Stelar region enlarged

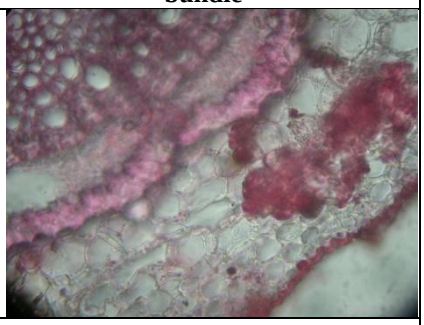


Fig.No.29. Lower region enlarged

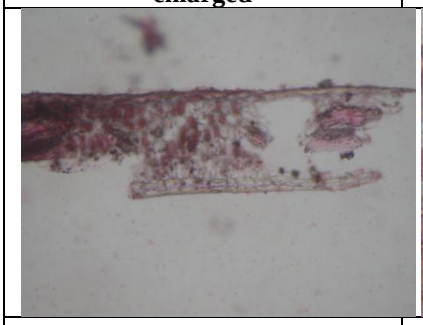


Fig.No.30. T.S. of the laminar region

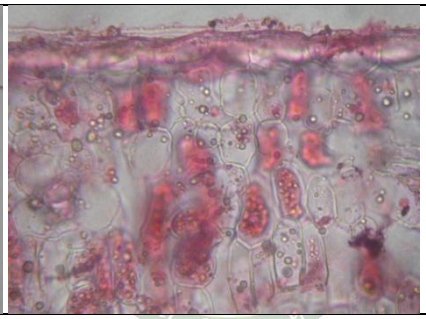


Fig.No.31. Palisade region enlarged

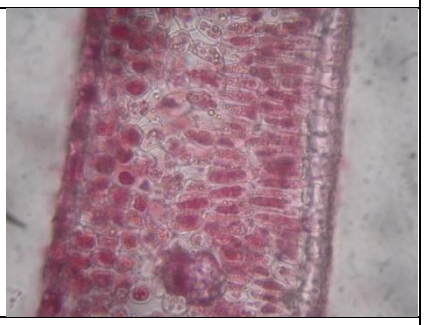


Fig.No.32. Laminar region with abundant tannin content.

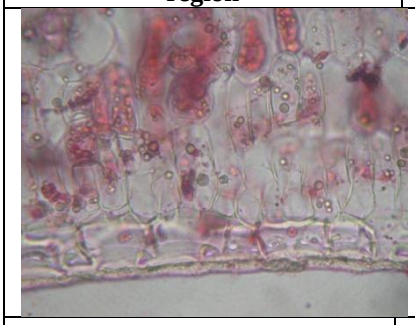


Fig.No.33. Lower region of the leaf

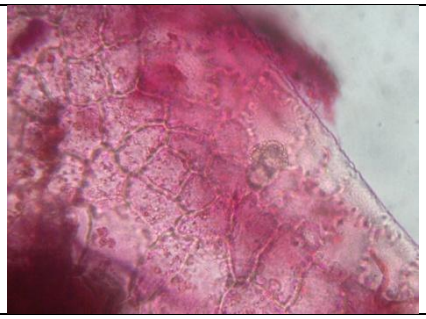


Fig.No.34. Epidermal peeling with crystals

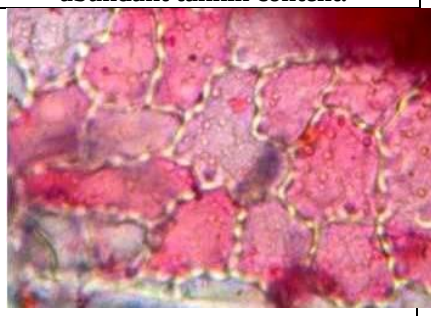


Fig.No.35. Epidermal peeling (Upper region)

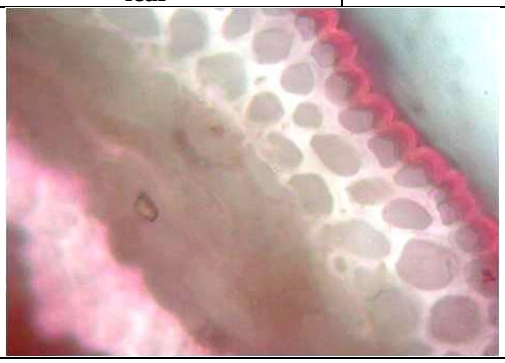


Fig.No.36. Upper region enlarged

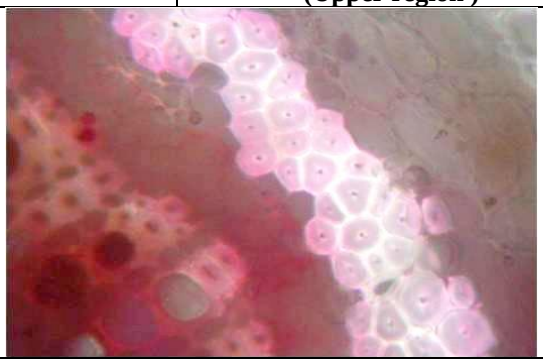


Fig.No.37. Pericyclic fibers enlarged

Leaf clearing studies of *H. mystax*

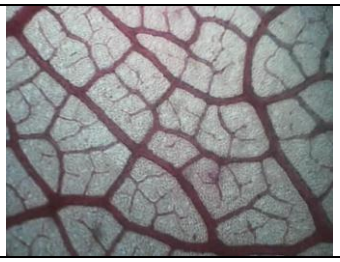


Fig.No.38. Vein islets

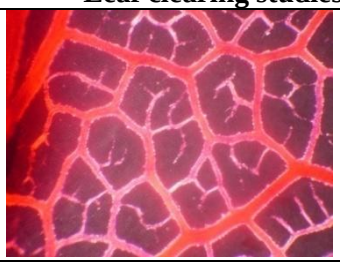


Fig.No.39. Vein islets with rambhoid shaped Calcium oxalate crystals (By Polarized Microscopical studies)

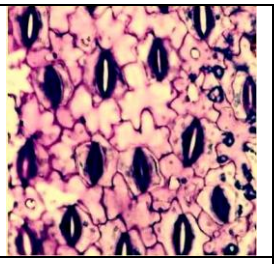


Fig.No.40. Rubiaceous type of Stomata

Leaf Transfersections with microtome

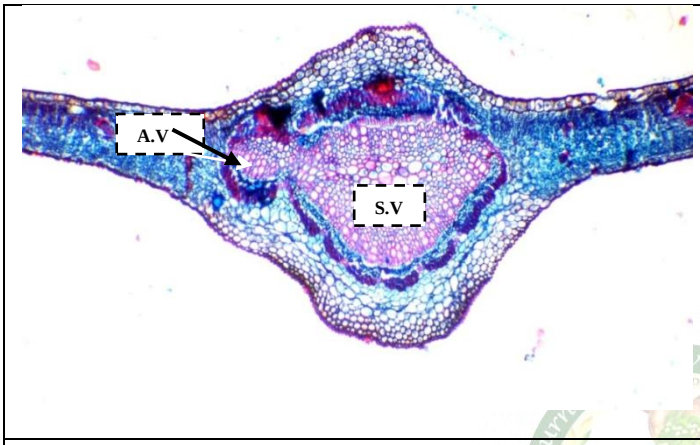


Fig.No.41. T.S of the leaf showing laminar region, Midrib region vascular bundle, upper and lower epidermis S.V.: Shield shaped vascular bundle ; A.V: Accessory vascular bundle

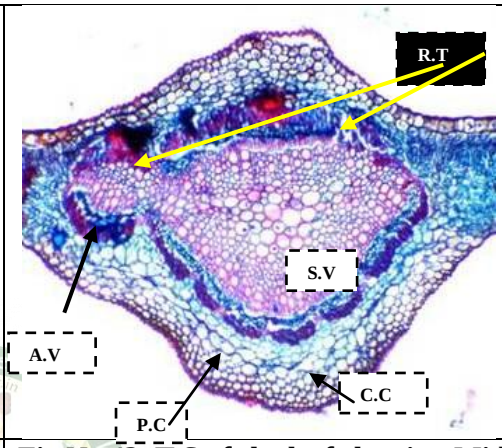


Fig.No.42. T.S of the leaf showing Midrib region vascular bundle, upper and lower epidermis 10xX10x: S.V.: Shield shaped vascular bundle ; A.V: Accessory vascular bundle; C.C: 2 layered collenchymatous cells; P.C: Parenchymatous cells ; R.T: abundant reddish tannin content

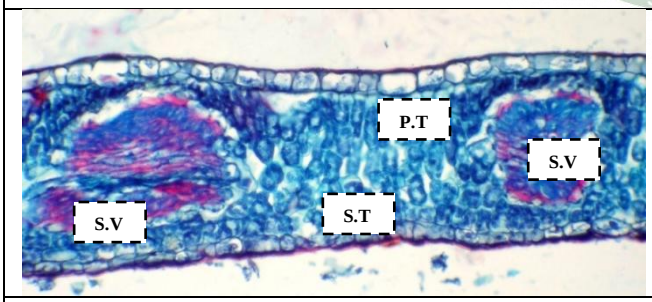
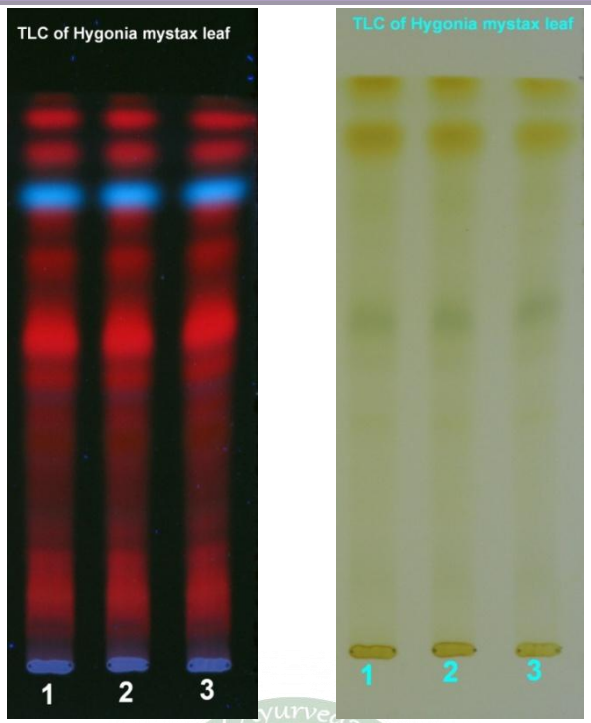


Fig.No.43. T.S of the leaf showing laminar region: P.T : 2 to 3 layered palisade tissue; S.P: 1 to 2 layered spongy parenchymatous layers; S.V: Smaller veins are represented by small vascular bundles covered by sclerenchymatous sheath

Fig.No.44. Presence of clustred and rambhoid shaped Calcium oxalate crystals in laminar region (By Polarized Microscopical studies)

Finger Print T.L.C. of Methanolic Extract of *H. mystax* Leaves Applied in Triplicate as Track 1, 2 & 3 (Fig.No.41.)



UV 366nm

Under Iodine Vapors

Solvent system: Toluene: Ethyl Acetate: Methanol = 7: 2: 1
Spots: Ten under UV 366nm
Solvent Run: 81mm

Fig.No.42. Densitogram of *H. mystax* Leaf Powder at 366nm