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Standardization of *Hibiscus Schizopetalus* (Mast) Hook according to World Health Organization (WHO) guidelines

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Hibiscus schizopetalus **(Mast) Hook (Malvaceae) is a shrub with spreading or usually drooping branches, attaining a height up to 13 feet (4 m) and found in east of tropical Africa. It is also a common ornamental shrub cultivated in Pakistan. The pharmacognostic standards for the flower and leaves of *H. schizopetalus* were done for the first time and this could be useful as quality control parameter in future. The leaves and flower of the plant was studied for standardization, including examination of morphological, anatomical, microscopic characters, histochemical reactions, fluorescence characters and ash values. The morphological studies revealed that flowers are pink to red in color and leaves are green in color with characteristic odour and slight bitter taste. Pulverized samples of leaves and floral parts of the plant treated with different chemical reagents showed the presence of alkaloids, steroids and triterpenoids more prominently. The present investigation has stated important standardization parameters for *H. schizopetalus* which is a new entry as medicinal aid, providing immense help in authenticating the plant material.**

Key words: *Hibiscus schizopetalus* (Mast) Hook, pharmacognostic evaluations, fluorescence characters, ash values.

INTRODUCTION

Plants are utilized extensively as raw drugs for many formulations in traditional as well as modern systems of medicine. To check the genuineness of the raw drugs and to detect adulteration of these materials, detailed pharmacognostic study of all materials used in formulation is required for authentication. Therefore, an extensive anatomical and phytochemical screening is also needed for each drug material used in the formulation to avoid any ambiguity and such a study will serve as a reference for further studies (Vaibhav and kamlesh, 2007).

In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing

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to its natural origin and lesser side effects. In older times, Vaidyas, Chinese and Unanis used to treat patients on individual basis, and prepared drugs according to the requirement of the patients and condition of the disease, but the scene has been changed now. As the expansion for the exploration of new plant resources, herbal medicines are being manufactured on a large scale in mechanical units, where manufacturers are facing many problems such as availability of good quality raw material, authentication of raw material, availability of standards, proper standardization methodology of drugs and formulations and quality control parameters (Agrawal, 2000; Agrawal, 2005; Ali et al., 2005).

According to World Health Organization (WHO) guidelines investigation of drug material and its safety profile is usually carried through their organoleptic and anatomical features that is, macroscopic and microscopic, physicochemical, chromogenic, chromatographic profile, fluorescence analysis of constituents, chemical behavior of powder drug material. Moreover foreign matter, ash value, pesticidal residues, microbial counts were also detected in this regard (Prajapati et al., 2003).

The genus *Hibiscus* comprises about 275 species in the tropics and subtropics. With attractive and colorful flowers, and are widely planted as ornamentals and are used in traditional medicine (Dasuki, 2001). It is one of the least examined specie of this genus. *Hibiscus schizopetalus* (Mast) Hook (Malvaceae) is a shrub with spreading or usually drooping branches, attaining a height up to 13 feet (4 m) found in east of tropical Africa. It is also a common ornamental shrub cultivated in Pakistan. Coral Hibiscus, Chinese Hibiscus, Japanese lantern, Fringed Hibiscus (English), Tanglong (Malay), Arana (Spanish) are its common names. From the vast literature survey we come to know that no local name has been reported for *H. schizopetalus* so we named it Taskeen -e- Gurhal (Urdu). Colombians use the infusion of flower to treat cold and cough (Yasin, 1979; Jalan, 2002).

The pharmacognostic standards for the flower and leaves of *H. schizopetalus* were done for the first time and could be useful as quality control parameter in future. Morphological and anatomical studies will enable us to recognize this flora.

MATERIALS AND METHODS

Plant material

Fresh flowers and leaves of *H. schizopetalus* was collected from the premises of University of Karachi, Pakistan, in the month of July, 2009. Morphological parts of plant were washed with distilled water to remove dirt and dried under shade separately. The plant was identified and authenticated by Prof. Dr. Surriya Khatoon, Ex. Chairperson, Department of Botany, University of Karachi, Pakistan. A voucher specimen of the plant (No. 082) has been deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Pakistan.

Macroscopic evaluation

In the present study, the powder of crude drug was investigated for its macroscopic characteristics that is, colour, odour, taste (Gowda et al., 2009).

Histological evaluation

Cellular sequences in the flower and leaves of plant were examined by making permanent slides of the transverse sections of plant. Procedure was followed by staining and glutening, there after the complete histology was observed under electronic microscope (Brain and Turner, 1975; Wallis, 1985).

Microscopy of pulverized samples

Flower and leaves of the plant were first clean and then dried under shade separately. Then each dried material was ground in electric grinder separately to get pulverized material. This powder material was used for powder microscopy. The powdered drug was separately treated with glycerin 50%, chloral hydrate solution 10% and iodine solution 5% (Iyengar, 1974; Johansen, 1940). The results were registered by botanical illustration.

Preliminary phytochemical screening

Preliminary phytochemical screening of powdered samples with different chromogenic reagents was studied to detect the presence of phytoconstituents with color changes under daylight by reported method (Chase and Pratt, 1949).

Histochemical color reactions

Histochemical color reactions were carried out on the leaf transverse sections by the reported methods (Trease and Evans, 1986; Kokate, 1994). The transverse section was treated with different reagents and then observed under electronic microscope. Color in the specific histological zone indicated the presence of constituent in the leaves.

Fluorescent studies

A pinch of dried and powdered plant material was taken in a clean test tube with about 10 ml of solvent like saturated picric acid, concentrated nitric acid, 50% HCl, 80% sulphuric acid, acetic acid, acetone, 1 N NaOH, 5% w/v ferric solution, N/20 iodine solution, diethyl ether and ethyl acetate. All the tubes were shaken well and incubated for about 30 min. The colors of the drug solutions thus obtained were observed for their characteristic color reaction under the visible light and ultra violet light (256 and 366 nm) and were recorded (Kokashi et al., 1985; Brindha et al., 1990).

Physicochemical evaluation

Total ash, acidic-soluble ash and water soluble of dried powdered of flower and leaf of plant were determined following the reported method (Anonymous, 1996, 2002).

Loss on drying (LOD)

Pulverized samples of both flower and leaves (2 g) were taken in

separate petri dish and kept in oven at 105°C for 2 h. After that, cooled in a desiccators and the losses in weight were recorded in each case. The LOD was calculated by the giving equation:

$$\text{Loss on drying } (\%) = \frac{A-B}{W} \times 100$$

Where, W = weight of sample (g); A = weight of the sample before drying (g); B = weight of the sample after drying (g).

Moisture content

Moisture content was determined after calculating the dry matter weight of samples. It was calculated by:

$$\%\ \text{Dry Matter weight (DM)} = \frac{A-B}{B} \times 100$$

Where, % DM = Percentage of dry matter weight of the sample, A = weight of sample before drying (gm); B = weight of sample after drying (g).

Then moisture content can be calculated as:

% MC = 100 - % DM

Total ash

Two grams of dried and pulverized plant material was taken in the pre-weighed clean sintered silica crucibles. Then, they were incarnated by gradual increasing of the temperature (400 to 5000°C) in the muffle furnace for 6 h. The crucible was cooled to room temperature in a desecrator and the weight of ash content was weighed in electronic digital balance.

Acid- insoluble ash

The total ash content of the plant material thus obtained was boiled for 15 min, after adding 25 ml of 25% (v/v) HCl and was allowed to cool. It was filtered through a Whatman filter paper No. 44 (ash less). The insoluble ash thus retained on filter paper along with paper was ignited in a preweighed sintered crucible (1000°C).

Water-insoluble ash

The total ash obtained was boiled with 25 ml of water for 5 min. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited for 15 min at a temperature not exceeding 450°C. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water-soluble ash. Then the crucible along with the residue was weighed and the total ash and acid insoluble ash content calculated using the following formula:

$$\text{Total ash content } (\%) = \frac{Z-X}{Y} \times 100$$

Where, Z = weight of the crucible (g); X = weight of the crucible with ash (g); Y = weight of the plant material taken (g).

RESULTS

Macroscopic evaluation

Pharmacognostic studies are a straightforward and reliable tool by means of which inclusive information of the crude plant material can be obtained. Organoleptic or macroscopic evaluation of crude drug is the technique of qualitative evaluation based on the morphological and sensory profile of whole drug. Proper organoleptic examination of flower and leaves of *H. schizopetalus* were illustrated in Table 1.

Histological evaluation

Histological examination provides a tool for the determination of cellular type and shape of the sample. In histological examination of transverse section of flower petals of *H. schizopetalus* it showed prominent, swollen and sequential arrangement of longitudinal cells when observed under microscope. Starch containing cells were also seen in the petals. The pedicel of flower is covered with epidermis cells, radial vascular bundle are present in irregular manner, with center of the pedicle containing parenchymatous tissues. The ovary is pentalocular containing two seeds in each ovule, placenta is axile and ovary is covered with thick ovary wall, possessing multiple layers of outer cell (Figure 1A to D). Leaves are covered by a thick cuticular layer coexisting with anomocytic type of stomata with varying distance on both upper and lower surfaces. The upper and lower epidermis consists of a single row of barrel shaped cells in which the width and length of cells are almost equal. Palisade parenchyma cells are long and cylindrical and are rich in chloroplasts. Spongy parenchyma cells are 1 to 3 layered and present just below the columnar palisade parenchyma cells. Intracellular spaces are filled with collenchyma along with spongy cells. There is an arc shaped vascular bundle on the median region of the leaf arranged in a radial pattern (Figure 2A and B).

Microscopy of pulverized samples

Microscopic cellular fragments were visualized in three different detecting reagents (50% glycerine solution, 10% chloral hydrate solution and 5% iodine solution). Microscopic studies of the pulverized sample of *H. schizopetalus* flower in various detecting reagents showed the presence of simple fiber, calcium oxalate (rosette), glandular trichrome, clustered starch granules, epidermal cell fragments containing pollen grain and calcium oxalate, group vessels (Figure 3). Powdered leaves revealed the presence of tubular epidermis cell with anomocytic stomata, group of phloem fibers, xylem

Table 1. Macromorphological depiction of *Hibiscus schizopetalus* (Mast) Hook.

Plant picture	Plant parts	Particulars		
		Color	Odour	Taste
	*H. schizopetalus* flower	Pinkish red	No characteristic odor	Insipid
	*H. schizopetalus* leaves	Green	Characteristic odor	Slightly bitter

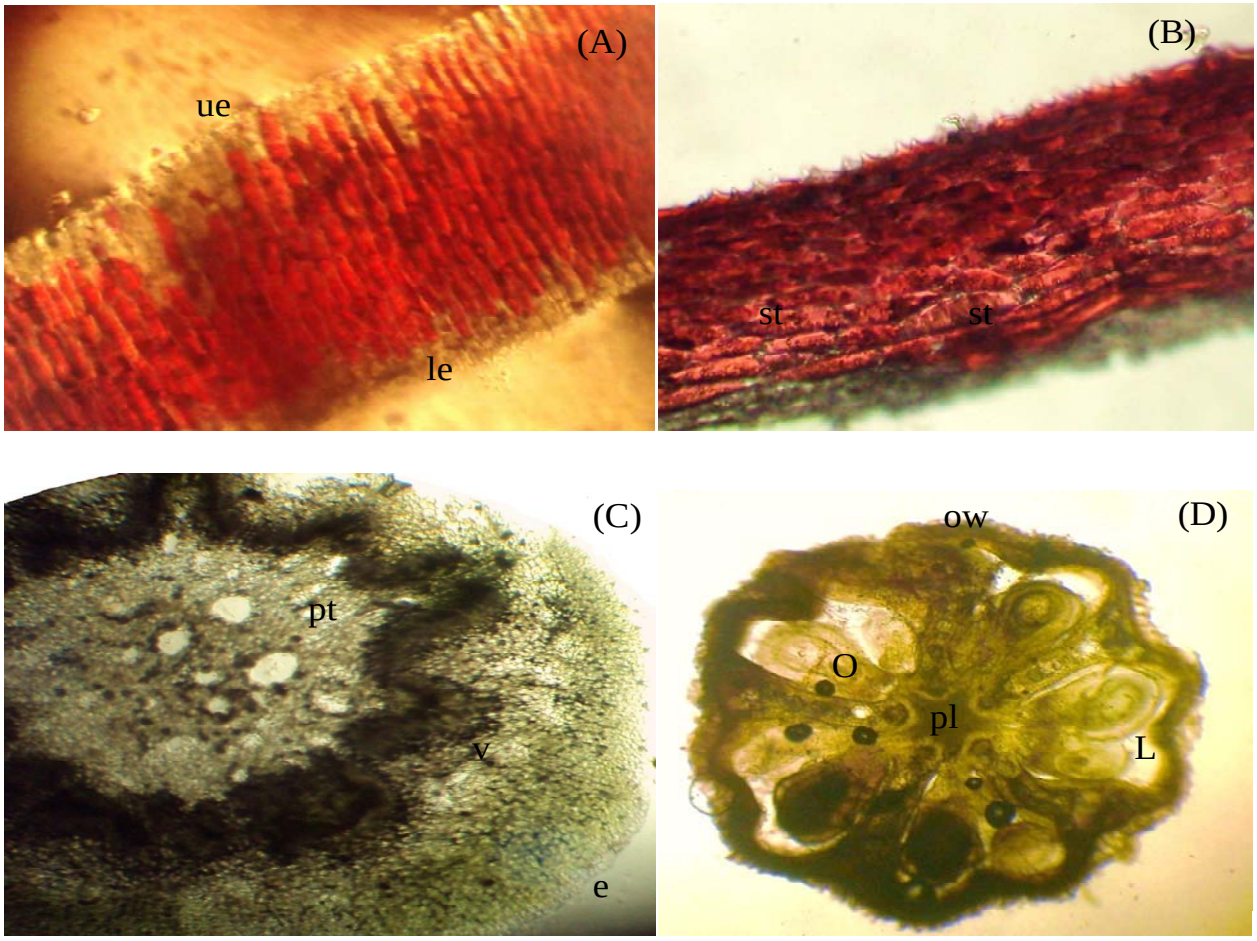

Figure 1. (A): Representative of photomicrograph of T.S. of *H. schizopetalus* flower ue: upper epidermis, le: lower epidermis, (B): Representative of photomicrograph of lateral view of *H. schizopetalus* flower showing cell with starch, (C): Representative of photomicrograph of T.S. of *H. schizopetalus* pedicle e: epidermal cells, v: vascular bundle, pt: parenchymatous tissue, (D): Representative of photomicrograph of T.S. of *H. schizopetalus* ovary ow: ovary wall, L: locules, O: ovules, pl: placenta.

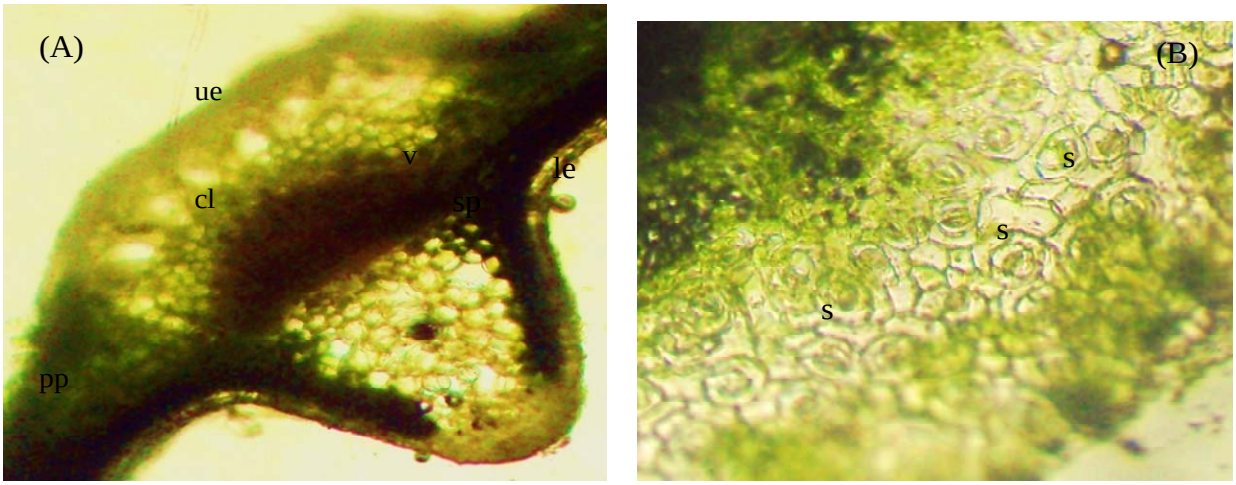

Figure 2. (A)**:** Representative of photomicrograph of T.S. of *H. schizopetalus* Leaf, cu: cuticle, ue: upper epidermis, pp: palisade parenchyma, sp: spongy parenchyma, v: vascular bundle, cl: collenchyma, le: lower epidermis, (B): Representative of photomicrograph of *H. schizopetalus* leaf showing anomocytic stomata (s).

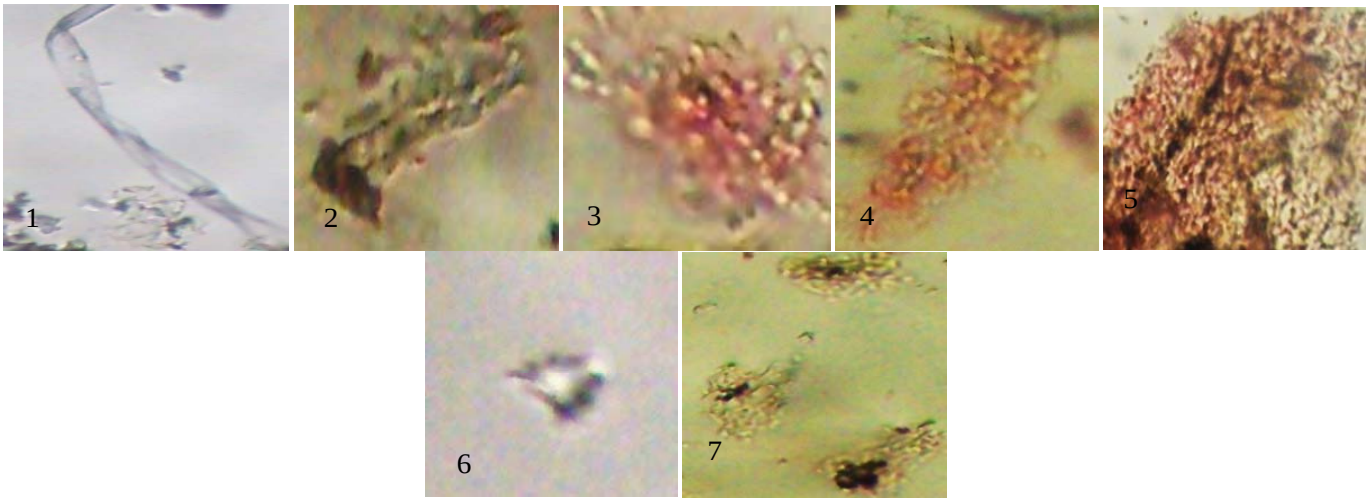

Figure 3. Microscopy of pulverized flowers of *H. schizopetalus*. 1: simple fibre, 2: glandular trichrome, 3: clustered starch granules, 4: epidermal cell fragments containing pollen grain and calcium oxalate, 5: group vessels. 6: calcium oxalate (rosette) 7: clustered starch granules.

vessels, spongy parenchyma with chlorophyll, fiber, group of fibres with lignin and unicellular trichrome (Figure 4).

Preliminary phytochemical screening

Pulverized samples of flower and leaves were treated with different reagents to identify the existence of phytoconstituents. Color changes or formation of precipitate were observed under daylight and the results were shown in Tables 2 and 3.

Histochemical color reactions

The histochemical color reaction usually produced information about the deposition of ergestic cell contents in specific cell type. The histochemical studies of leaves of the plant revealed that xylem contains yellow color lignin when transverse section of leaf treated with aniline sulphate with sulphuric acid. Mesophyll region showed cellulose when treated with conc. sulphuric acid and steroids and triterpenoids when color reaction performed with antimony trichloride. Starch, calcium oxalate and anthroquinone glycoside were absent. The results were

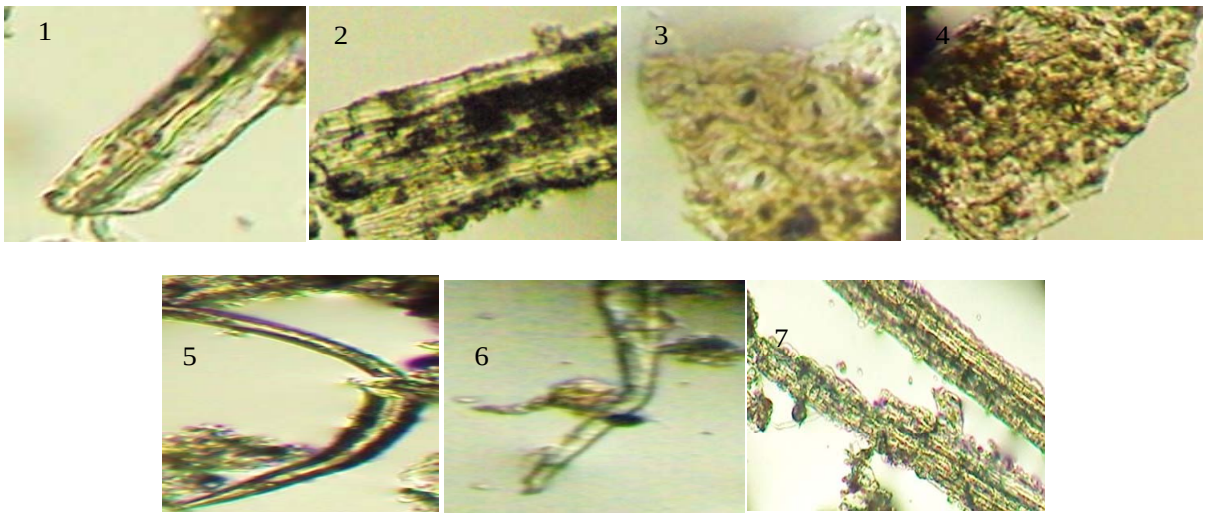

Figure 4. Microscopy of pulverized leaves of *H. schizopetalus*. 1: phloem fibres, 2: xylem vessels, 3: epidermis cell with anomocytic stomata, 4: spongy parenchyma with chlorophyll, 5: fiber, 6: unicellular trichrome, 7: group of fibres with lignin.

Table 2. Preliminary Phytochemical screening of *H. schizopetalus* powdered flower with different chromogenic reagents.

S.No	Reagents	Color/ppt	Constituents	Inference
1	Picric acid	Slight ppt	Alkaloids	+
2	Conc. H_2SO_4	Reddish brown	Steroids/ triterpenoids	+
3	Aq. $FeCl_3$	No ppt	Tannin	-
4	Iodine solution	Blue color	Starch	+
5	5% Aq. KOH	Brownish red color	Anthroquinone glycosides	+
6	Spot Test	No stain observed	Fixed oil	-
7	Aq. NaOH	Yellow color	Flavonoids	+
8	Dragendroff's reagent	ppt formation	Alkaloids	+
9	Mayer's reagent	Slight ppt	Alkaloids	+
10	Aq. Lead acetate	No ppt	Tannin	-

+ = Present, - = Absent.

Table 3. Preliminary phytochemical screening of *H. schizopetalus* powdered leaves with different chromogenic reagents.

S.No	Reagents	Color/ppt	Constituents	Inference
1	Picric acid	Slight ppt	Alkaloids	+
2	Conc. H_2SO_4	Reddish brown	Steroids/ triterpenoids	+
3	Aq. $FeCl_3$	Bluish black ppt	Tannin	+
4	Iodine solution	No color change	Starch	-
5	5% Aq. KOH	No color changed	Anthroquinone glycosides	-
6	Spot Test	Stains observed	Fixed oil	+
7	Aq. NaOH	No change	Flavonoids	-
8	Dragendroff's reagent	Slight ppt	Alkaloids	+
9	Mayer's reagent	Slight ppt	Alkaloids	+
10	Aq. Lead acetate	White ppt	Tannin	+

+ = Present, - = Absent.

Table 4. Histochemical color reaction of *H. schizopetalus* leaves.

Reagents	Constituents	Color	Histological zone	Inference
Aniline SO_4 + H_2SO_4	Lignin	Yellow	Xylem	+
Phloroglucinol + HCl	Lignin	Pink	Xylem	+
Conc. H_2SO_4	Cellulose	Green	Mesophyll	+
Weak Iodine solution	Starch	-	-	-
H_2SO_4	Calcium oxalate	-	-	-
$SbCl_3$	Steroids/ triterpenoids	Reddish pink	Mesophyll	+
5% KOH	Anthroquinone glycosides	-	-	-

+ = Present, - = Absent.

Table 5. Fluorescence characters of powdered flower of *H. schizopetalus***.**

S.No	Particulars of the treatment	Ordinary light	U-V light (254 nm)	U-V light (366 nm)
1	Saturated Picric acid	OR	G	3 G
2	Nitric acid (conc.)	O	2 G	B
3	Hydrochloric acid (50%)	F	Y	B
4	Sulphuric acid (80 %)	M	3 B	Z
5	Acetic acid	Y	Y	W
6	Iodine solution (N/20)	3 B	B	3 B
7	Ferric solution (5% w/v aq. Sol)	G	B	3 B
8	1 N aq. NaOH	Y	G	B
9	Diethyl ether	G	G	B
10	Powder as such	P	B	Z
11	Ethyl acetate	2 Y	Y	B
12	Acetone	2 Y	2 G	B

Quality of colours: 2 Light, 3 Dark. Code for colours: Y= Yellow, B = Brown, Z = Black, P = Purple, G = Green, M = Marron, F = Pink, O = Orange, OR = Orangish red, W = White.

given in Table 4.

Fluorescent studies

Fluorescence analysis of flower and leaves of plant powdered revealed different color of fluorescence when treated with solvents and then observed under day light and UV light (254 and 366 nm). The observations were given in Tables 5 and 6.

Physicochemical evaluation

The physicochemical evaluation is an important parameter in detecting the adulteration in the sample. Total ash, acid-insoluble ash and water soluble ash values of leaves and flower were tabulated in Table 7.

DISCUSSION

This is the first report on pharmacognostic studies of *H. schizopetalus* (Mast) Hook. The plant have showy flowers, features recurved, fringed, pink to red petals and a long slender pendent staminal column with no characteristic odor. The specific epithet of flower is its divided petals (*schizo* meaning split and *petalus* meaning petal). Flower is axillary, solitary and pendulous. The petals are 4 to 6 cm long and 2 to 3 cm broad, pinkish, with pink or red streaks, laciniate, recurved. Pedicle 8 to 15 cm long, articulated in the middle. Epicalyx segments 5 to 8 cm very short, 1 to 2 mm long. Calyx is tubular, 1 to 1.5 cm long and irregularly 2 to 5 lobed. Staminal column is 8 to 10 cm long. The flowers are followed by oblong, cylindrical seed capsules, 3 to 4 cm long and 1 cm across. Leaves are green in colour, 2 to 7 cm long and 1 to 5 cm broad, elliptic, sharply serrate, entire

Table 6. Fluorescence characters of powdered leaves of *H. schizopetalus*

S.No	Particulars of the treatment	Ordinary light	U-V light (254 nm)	U-V light (366 nm)
1	Saturated Picric acid	LG	G	B
2	Nitric acid (conc.)	B	2 G	3 B
3	Hydrochloric acid (50%)	3 G	G	Z
4	Sulphuric acid (80%)	G	G	3 G
5	Acetic acid	LG	B	O
6	Iodine solution (N/20)	B	3 B	3 B
7	Ferric solution (5% w/v aq. Sol)	3 G	G	3 B
8	1 N aqueous NaOH	YG	G	B
9	Diethyl ether	LG	2 G	O
10	Powder as such	LG	G	B
11	Ethyl acetate	G	G	O
12	Acetone	LG	G	B

Quality of colours: 2 Light, 3 Dark. Code for colours: YG = Yellowish Green, B = Brown, Z = Black, G = Green, O = Orange, LG = Olive Green.

Table 7. Physiochemical characteristics of flower and leaves of *H. schizopetalus.*

S.No	Particulars	% w/w	
		***H. schizopetalus* flower**	***H. schizopetalus* leaves**
1	Loss of weight on drying	7.84	10.78
2	Moisture Content	87.64	97.89
3	Total ash	13.72	18.00
4	Acid insoluble ash	3.92	3.92
5	Water soluble ash	5.00	6.00

n = 3; *dry weight basis.

below. Petiole is short 0.5 to 2 cm long (Yasin, 1979).

Alkaloids, steroids and triterpenoids were present in the powdered samples of flower and leaves. While tannins and fixed oil were absent in flower. The presence of these phytoconstituents makes the plant useful for treating different ailments and providing useful herbs of human consumption. Microscopically leaves and flower of *H. schizopetalus* is characterized by calcium oxalate (rosette), glandular trichrome, clustered starch granules, epidermal cell fragments containing pollen grain and calcium oxalate, group vessels, anomocytic stomata, group of phloem fibers, xylem vessels, spongy parenchyma with chlorophyll.

Fluorescence analysis is used for the characterization of the crude drug. Fluorescence is the phenomenon exhibited by the presence of chemical constituents present in the plant material. Some chemical constituents show fluorescence in the visible range (daylight) while some are not visibly fluorescent in daylight (Pimenta et al., 2006). Physicochemical evaluation signifies the purity and quality of the plant material. Moreover the total ash of a crude drug material reflects that care be taken in the preservation. Acid insoluble ash reflects the calcium oxalate contents in the material.

The less value of moisture content could prevent bacterial, fungal or yeast growth. In the present investigation, considerable amount of total ash was noticed that can employ as quality parameter for the evaluation of any adulteration (Chase and Pratt, 1949; Yi-Zeng et al., 2004). These studies help in identification and authentication of the plant material.

Conclusion

Establishing standards is an integral part for the correct identity and quality of a crude drug. The majority of the information on the identity, purity and quality of the plant material can be obtained from its macroscopy, microscopy and physiochemical parameters. The present

investigation has stated important standardization parameters for *H. schizopetalus* which is a new entry as medicinal aid provide immense help in authenticating the plant material. Further work aiming towards tracing out of phytochemicals present in it and pharmacological activities are in progress.

Conflict of interests

The author(s) have not declared any conflict of interests.

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