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Comparative profile of *Hibiscus schizopetalus* (Mast) hook and *Hibiscus rosa-sinensis* L. (Malvaceae)

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Abstract
The present research was carried out to establish detailed pharmacognosy of *Hibiscus schizopetalus* (Mast) Hook and *Hibiscus rosa sinensis* L., which included macros copy, microscopy, physico-chemical parameters and fluorescence analysis of flower and leaf samples of both plants. It was observed that macroscopic and microscopic characteristics were diagnostic features and can be used for distinction and identification of these closely related plant species. Physico-chemical parameters revealed that in investigated plant species total ash value, acid insoluble ash value and water soluble ash value was observed to be higher in flower of *H. rosa sinensis* (20.35 % w/w, 4.01 % w/w, 6.00 % w/w respectively) whereas moisture content was found to be higher in *H. schizopetalus*.

Keywords: *Hibiscus schizopetalus,* Hook, *Hibiscus rosa-sinensis* L.

Introduction

Genus *Hibiscus*, with more than 300 species distributed in tropical and subtropical regions have been widely used in several formulae in traditional medicine [1]. Previous investigations of the genus Hibiscus indicated the presence of some species with useful biological and pharmacological activities [2-10].

H. schizopetalus (Mast) Hook belong to family Malvaceae The shrub is initially found in the east of tropical Africa, native of Kenya and Tanganyika. It is a common ornamental shrub cultivated in Pakistan. The plant is found in Hawaii, Florida and India [11]. *H. schizopetalus* is the allied specie of *H. rosa-sinensis* sharing the same genera *Hibiscus* but the difference in both plant is the direction of flower. Leaves of plant are alternate, ovate to lanceolate, often with a toothed or lobed margin; resemble those of *H. rosa-sinensis* leaves. Various parts of the plant used in cold, cough and to reduce fever [12-14]. According to the current literature methanolic extract of flower and leaves found significant analgesic and antipyretic potential [15].

Hibiscus rosa-sinensis L. is an ever blooming, glabrous shrub up to 5-8 feet high. Flowers are red in color, bell shaped, large in size; almost diameter is 4-6 inches. Corolla is up to 7.5cm in diameter. From the center, calyxes are almost divided, puberulous and have small stellate hairs. Petals are three times larger than calyx. Leaves are sharp green in color, oval in shape and roughly toothed. Beneath the leaves some stellate hairs are present [16]. It is an inhabitant of Southern Eastern Asia. It is mainly cultivated in Pakistan, china and India as a decorative plant to beautify their gardens [17]. It contain methyl sterculate, methyl-2-hydroxysterculate, beta – rosasterol, 2- hydroxyl sterculate, malvate, cyclopropanoids, anthocyanin, amino acids (aspartic acid and asparginin), fat, phosphorus, iron, calcium, riboflavin, thiamine, niacin, vitamin C and fibers [18-19]. This is an effective abortifacient and anti convulsant plant [20]. It is majorly used in tachy cardia, insanity and palpitation [21].The flowers and leaves have healing properties [22]. The floral part of this plant is emollient, anti-oxidant, anti-bacterial, hypotensive, refrigerant, aphrodisiac, emmenagogue, anti-inflammatory and its decoction is used in the treatment of bronchial catarrh**.** For the treatment of menorrhagia, they are used in fried form [23].

Certainly, no previous literature reported concerning the comparative evaluation of *Hibiscus schizopetalus* (Mast) Hook ad *Hibiscus rosa-sinensis* L. which encouraged us to undertake full descriptive macro and micro morphological studies of these plants. This study is of utmost important not only in finding out genuinity but also in detection of adulterants in the samples.

Materials and Methods

Plant material: The plant materials of *Hibiscus schizopetalus* (Mast) Hook and *Hibiscus rosa-sinensis* L. was collected from the private garden of Faculty of Pharmacy, University of Karachi. The identification of these plants was verified by Prof. Dr. Ghazala H. Rizwani (Meritorious), Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi. Voucher samples of the plants are kept in the Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi. Fresh and dried samples were used throughout the study.

Microscopy: Macro morphological characters of leaf and flower of *H. schizopetalus* and *H. rosa-sinensis* samples such as color, taste, odor and appearance were studied and recorded.

Microscopy: Free hand sections were used for histological studies. Quantitative microscopy was done as per the standard methods [24].

Physiochemical Analysis: Physico-chemical parameters were also determined such as total ash value, acid insoluble ash value, water-soluble ash value, moisture content (loss of weight on drying) of powdered sample according to the standard methods [25].

Determination of moisture content: About one gm of leaf powder was weighed in a silica crucible and placed in oven at 105°C for a period until constant weight was obtained. Loss in weight was recorded as moisture content (w/w %).

$$\% \text{ of moisture content} = \frac{\text{Loss in weight of powder sample} \times 100}{\text{Weight of powder sample}}$$

Determination of ash values

Total ash value

Leaf powder (3gm) was weighed in a silica crucible, to make sample free from carbon, ignited till red hot by gradually increasing flame and this step was repeated until constant value was obtained. Then total ash was calculated (w/w %) by using the following formula.

$$\% \text{ of total ash value} = \frac{\text{Weight of total ash} \times 100}{\text{Weight of powder sample}}$$

Acid insoluble and water soluble ash values

The total ash (which was obtained by above mentioned method) was mixed with 25mL of 2N HCl (for determination of acid insoluble ash value) and mixed with 25mL of water (for determination of water soluble ash value) and boiled for 5 min. The resultant material was collected on ash less filter paper, washed with water following the step of drying. The material was than subjected to ignition and weighed. Finally the acid insoluble and water-soluble ash values were calculated (w/w %) with the help of following formulas.

$$\% \text{ of acid insoluble ash value} = \frac{\text{Weight of total ash -weight of acid insoluble ash}}{\text{Weight of powdered sample}} \times 100$$

Fluorescence analysis: The fluorescence behavior of the powder drug in the visible light and ultraviolet light were carried out by soaking the powder in different reagent solutions and viewing under the light of required wavelength in a UV chamber [26-27].

Results and Discussion

Plants have been a richest source of maintaining good health among the inhabitant of developing countries while in developed countries a major hindrance by which herbal drugs were not frequently practiced is their lack of standardization data. For the preparation of botanical drug, a first and bit important step is the morphological study which is helpful in correct identification of a specific source from which initial material have been obtained because the adulterants are very much similar with the right source and responsible for producing a number of harmful effects. Afterwards its microscopic studies confirmed the purity of herbal drugs [28-29].

Hibiscus schizopetalus (Mast) Hook have showy flowers features recurred, 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). Leaves of *H. schizopetalus* are alternate, ovate to lanceolate, often with a toothed or lobed margin; resemble those of *H. rosa – sinensis* leaves (Table 1).

Hibiscus rosa-sinensis L. carries red auxiliary or in terminal solitary flowers around the year. The leaves are simple, alternate, cauline, petiolate, stipulate. Lamina is simple, ovate to oblong- lanceolate in shape having acuminate apex, symmetric base and hairy surface, having entire margin in the lower half part and being dentate in the upper half one, green in colour and the upper surface is darker than lower one. It measures 2 - 7cm long and 2 - 4cm broad. The venation is pinnately reticulate and the midrib and big veins are prominent on the lower surface than the upper one (Table 1).

Table 1: Macro morphology of *Hibiscus schizopetalus* and *Hibiscus rosa-sinensis*

Name of plant	Part Used	Color	Taste	Odor	Appearance
Hibiscus schizopetalus	Flower	Pinkish Red	Insipid	No Characteristic odor	Flower are pink to red splited petals, hanging towards the ground
	Leaf	Green	Slightly Bitter	Characteristic odor	Leaves are green, ovate to lanceolate, often with a toothed or lobed margin
Hibiscus rosa sinensis	Flower	Red	Sour	Slightly present	It is shiny red pentapetalus flowers having vertical lines arranged in twisted manner
	Leaf	Dark green	Slightly Bitter	Characteristic odor	Leaves are bright green, short petiolated andovate

Micromorphology

Leaf of *H. schizopetalus*: Leaves are covered by a thick cuticular layer coexist 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 1-3 layered and present just below the columnar palisade parenchyma cells. Intracellular spaces 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 1).

Leaf of *H. Rosa sinensis*: A transverse section of the leaf is biconvex. The upper and lower epidermis consists of polygonal cell carrying glandular and non-glandular trachoma's. Both upper and lower epidermis covered with smooth cuticle. The lamina has dorsiventral structure with one row of upper palisade being discontinuous in the midrib region. The midrib is prominent on the both surfaces showing subepidermal collenchyma's, cortical tissues and large central collateral vascular bundle. Cluster crystals of calcium oxalate are scattered in the cortical tissue as well as in the mesophyll. Secretory glands and mucilage cells are also, present in the parenchymatous tissues. The stomata are anisocytic type. The endodermis is well differentiated containing starch granules (Figure 2).

Table 2: Microscopic cellular fragments of *Hibiscus schizopetalus* and *Hibiscus rosa-sinensis*

	Hibiscus schizopetalus		***Hibiscus rosa sinensis***	
	Flower	**Leaf**	**Flower**	**Leaf**
Chloral hydrate 10%	Clusters of starch granules, glandular trichomes, simple fibers, epidermal cells, pollen grains	Phloem fiber, xylem vessels, spongy parenchyma, epidermal cell, thick lignified cell	Groups of pits, sclerids, uneven distribution of color pigments in couple of fibers with needle shape calcium oxalate crystals, Pigmented fragments with Calcium oxalate	Simple fiber, crystals of calcium oxalate, xylem vessels, spongy parenchyma, epidermal cell, glandular and non-glandular trichomes
Glycerine 50%	Calcium oxalate, pollen grain	Thin oil cell, fiber	Group of lignified stone cells, Cellular fragment containing sclerids, Calcium oxalate and stone cells	crystals of calcium oxalate, glandular and non-glandular trichomes
Iodine 5%	Clusters of starch granules, pollen grains	Fiber with lignin, unicellular trichomes	Lignified fiber, group of stone cell, starch grains with color	Parenchymatous cell, epidermal cell

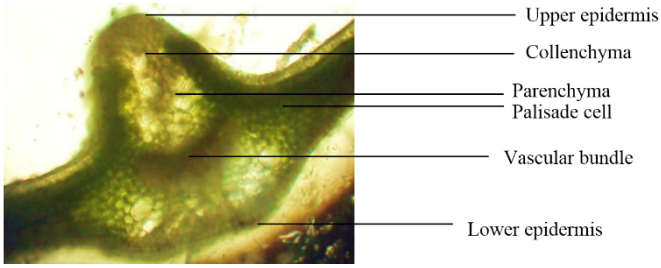

Fig 1: Transverse section of *H. schizopetalus* leaf

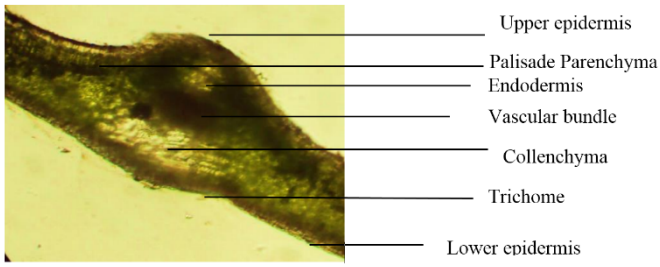

Fig 2: Transverse section of *H. rosa sinensis* leaf

Flower of *H. schizopetalus*

The pedicel: The pedicel of flower is covered with epidermis cells, radial vascular bundle are present in irregular manner, center of the pedicle containing parenchymatous tissues (Figure 3).

The Petal: In histological examination of transverse section of flower petals of *H. schizopetalus* (Mast) Hook showed prominent, swollen and sequential arrangement of longitudinal cells when observed under microscope. Starch containing cells were also seen in the petals (Figure 4).

The gynoecium: The ovary is pentalocular containing two seeds in each ovule, placenta is marginal, and ovary is covered with thick ovary wall, possessing multiple layers of outer cell (Figure 5).

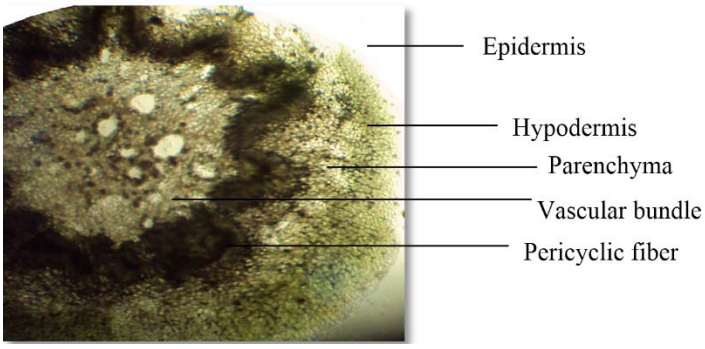

Fig 3: Transverse section of *H. schizopetalus* pedicle

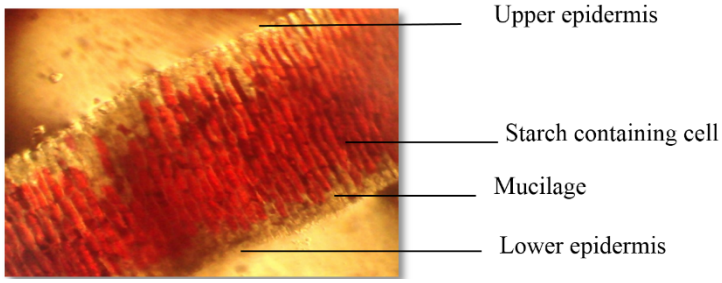

Fig 4: Transverse section of *H. schizopetalus* petal

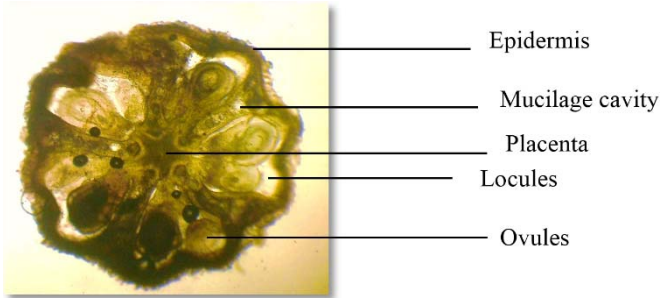

Fig 5: Transverse section of *H. schizopetalus gynoecium*

Flower of *H. rosa-sinensis*

The pedicel: A transverse section of the pedicel is more or less rounded in outline. It shows an outer, hairy epidermis followed by the cortex which is formed of 1-2 rows of hypodermal chlorenchymatous cells, 1-2 rows of collenchymatous cells and 3-4 rows of parenchymatous cells. The pericycle is represented by intermittent groups of lignified fibers. The vascular tissue is formed of continuous ring of collateral vascular bundle enclosing wide parenchymatous pith with small groups of per medullary phloem. Cluster crystals of calcium oxalate, secretory glands and mucilage cells are present in parenchymatous cells (Figure 6).

The Petal: A transverse section of the petal shows an inner and outer epidermis enclosing homogenous mesophyll. The parenchymatous cortex traversed by small collateral vascular bundles alternating with large mucilage cavities. The mesophyll is homogenous formed of almost rounded parenchymatous cells with thin wall and narrow intercellular space. The cortex consists of almost rounded, thin walled parenchymatous cells with small intercellular spaces (Figure 7).

The gynoecium: A transverse section of the pentacarpellary ovary is almost rounded in outline showing five locules, each locule contain two ovules. The ovary showing an outer and inner epidermis enclosing a parenchymatous mesophyll in between. The mesophyll is formed of rounded, moderately thin-walled parenchyma with small intercellular spaces. It is traversed by five vascular strands and mucilage cavities. Each vascular strand is composed of a xylem showing few spiral vessels and a phloem formed of thin-walled cells (Figure 8).

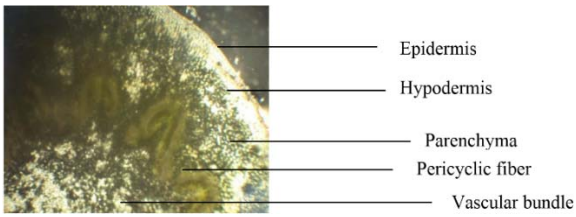

Fig 6: Transverse section of *H. rosa-sinensis* pedicle

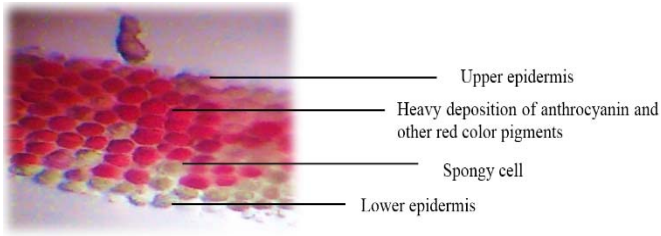

Fig 7: Transverse section of *H. rosa sinensis* petal

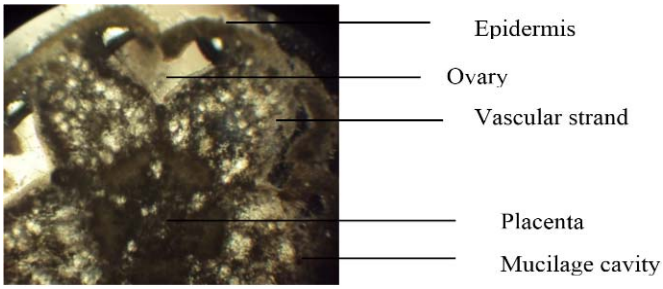

Fig 8: Transverse section of *H. rosa sinensis gynoecium*

An important tool for detection of adulterants or misleading herbal drugs is physiochemical evaluation which based on three parameters. Detection of moisture content in both plants was carried out which helps to prevent deterioration of drugs since moisture hydrolyzes many important compounds and promote growth of microorganisms [30]. In the current study total ash value, acid insoluble ash value and water soluble ash value was observed to be higher in flower of *H. rosa sinensis* (20.35 % w/w, 4.01 % w/w, 6.00 % w/w respectively) whereas moisture content was found to be higher in *H. schizopetalus*. Results obtained by physico-chemical analysis are presented in Table 3. The characteristic fluorescent colors emitted by the leaf and flower powder of plant samples after treating with various reagents under normal and U.V light (366 nm) were recorded and are presented in Table 4.

Table 3: Physiochemical analysis of *Hibiscus schizopetalus* and *Hibiscus rosa sinensis*

Plant name	Part Used	Moisture content	Total ash value	Acid insoluble ash value	Water soluble ash value
Hibiscus schizopetalus	Flower	87.64	13.72	3.92	5.00
	Leaf	97.89	18.00	3.92	6.00
Hibiscus rosa sinensis	Flower	6	20.35	4.01	12
	Leaf	4.93	7.65	0.70	6.23

Table 4: Fluorescence characters of powdered flower and leave of *Hibiscus schizopetalus* and *Hibiscus rosa-sinensis*

Plant	Treatment	Observation under	
		Ordinary light	U.V. Light (366nm)
H. schizopetalus flower	Powder as such	Purple	Black
	Powder with iodine solution (N/20)	Dark brown	Black
	Powder with 1N NaOH in water	Yellow	Brown
	Powder with 50% sulphuric acid	Marron	Black
	Powder with picric acid	Orangish red	Brown
	Powder with $FeCl_3$	Green	Brown
	Powder with 50% nitric acid	Orange	Brown
	Powder with HCl	Pink	Brown
H. schizopetalus leaves	Powder as such	Olive green	Brown
	Powder with iodine solution (N/20)	Brown	Dark Brown
	Powder with 1N NaOH in water	Yellowish green	Brown
	Powder with 50% sulphuric acid	Green	Dark green
	Powder with picric acid	Light green	Brown

	Powder with $FeCl_3$	Dark green	Dark brown
	Powder with 50% nitric acid	Brown	Dark brown
	Powder with HCl	Dark green	Black
***H. rosa sinensis* flower**	Powder as such	Dull Red	Reddish pink
	Powder with 1N NaOH in methanol	Light yellow	Colorless
	Powder with 1N NaOH in water	Reddish brown	Light greenish brown
	Powder with 50% sulphuric acid	Light brown	Pinkish brown
	Powder with chloroform	Yellow	Colorless
	Powder with $FeCl_3$	Light green	Dark green
	Powder with 50% nitric acid	Pink	Green
	Powder with water	Peach pink	Colorless
***H. rosa sinensis* leaves**	Powder as such	Light green	Blackish brown
	Powder with iodine solution (N/20)	Greenish brown	Black
	Powder with 1N NaOH in water	Brown	Black
	Powder with 50% sulphuric acid	Dark green	Black
	Powder with picric acid	Green	Greenish red
	Powder with $FeCl_3$	Brown	Black
	Powder with 50% nitric acid	Brown	Black
	Powder with HCl	Green	Black

This study presents a set of diagnostic characters of *Hibiscus schizopetalus* (Mast) Hook and *Hibiscus rosa-sinensis* L. that will help to identify the drug in fragmentary condition as well as in whole form. It is interesting to note the blooming pattern of both plants is in opposite direction. *H. schizopetalus* is bloom towards the gravity while *H. rosa sinensis* bloom in upright direction. Definitely their blooming pattern effects on the chemo pharmacological sequence. Further research could be required to prove the correlation in existence of type of chemical constituents and their pharmacological potentials.

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