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Short Communication

PHARMACOBOTANICAL, PHYSICOCHEMICAL AND PHYTOCHEMICAL CHARACTERIZATION OF *HIBISCUS PUNCTATUS DALZ.* (MALVACEAE) LEAVES: A COMPREHENSIVE PHARMACOGNOSTICAL STUDY

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ABSTRACT

Systematic Study was carried out to determine pharmacognostic characteristics of *Hibiscus punctatus Dalz.* which is a plant species from Malvaceae family found in western ghats of Maharashtra, Gujarat, Rajasthan and Punjab region of India. In this study all macroscopic, microscopic as well as physicochemical parameters of *Hibiscus punctatus Dalz.* are determined as per WHO Guidelines. Transverse sections of leaves have shown the presence of Upper and Lower Epidermis, Collenchyma, Ground Tissues, Mesophyll, Trichomes and Sclerenchyma in it. Phytochemical investigation revealed the presence of flavonoids, alkaloids and glycosides in ethanolic extracts of *Hibiscus punctatus Dalz.* leaves while the aqueous extracts have reflected the presence of carbohydrates, tannins, steroids and flavonoids in it. Physicochemical parameters such as ash value, extractive value, moisture content and fluorescent characteristics of leaf powder were also determined. In Present Study, we report the Pharmacognostic data and presence of flavonoids indicates high potential of this plant which is used as medicine for various diseases.

Keywords: *Hibiscus punctatus Dalz.,* Pharmacognostic Study, Flavonoids, Phytochemical Investigation

1. INTRODUCTION

Novel drug discoveries have shifted focus from synthetic models and compounds to natural products of plant origin. This is because scientists now believe that Lead molecules would be more probably available in plants and other natural resources which are yet to be fully explored [1]. Since long, a large number of plants are well documented in ancient medical literature of India. Indian Traditional system of medicine is based on effective use of plants for the therapeutic and medicinal purposes. These plants typically from western ghats of India are widely known across the globe for their medicinal purposes [2]. *Hibiscus punctatus Dalz.* is one of the medicinal plant species which are found exclusively in western ghats of various states such as Maharashtra, Gujarat, Rajasthan and Punjab of India. It is erect under shrub which is about 0.3-1 m tall annual plant. The Stems are woody, branches pubescent with stellate hairs. Leaves of *Hibiscus punctatus Dalz.* are alternate, broadly ovate to orbicular, about 2.5-10 x 1.5-6 cm across, base cordate to truncate, 5-7 veined, midrib usually with obscure nectarines, entire or 3 lobed, margins crenate-undulate to coarsely serrate, middle lobe apex longer, stellate hairy both above and beneath, petiole stellate hairy, about 1-7 cm long, stipules linear lanceolate, about 4-8 mm long. Inflorescence of this plant is usually axillary, solitary, or in terminal subpanicles, by the reduction of the upper leaves. Flowers are bisexual, pedicel slender, stiff stellate hairy and tubercled hairy, jointed near apex, about 1.5-6.5 cm long, epicalyx 8-10, base slightly connate, about 5 mm long, calyx 5 lobed, distinctly nerved, campanulate, lobes deltoid-lanceolate, base connate, apex acute to acuminate, valvate, sometimes with nectarines, persistent, densely stellate tomentose, about 1 x 0.3 mm across, corolla showy and large, yellow, white, pink, campanulate. Staminal column is usually shorter or almost as long petals, base wide, filaments apex truncate or 5-dentate, anthers basifixed, throughout or in the upper half. Ovaries are superior, five locular, axile placentation. Fruit are capsule shape, globose-cylindrical, about 1 cm across, apex acute, dehiscing loculicidally, densely stellate pubescent. Seeds are reniform or subglobose, about 2 mm across, muricated, brownish black [3]. *Hibiscus punctatus Dalz.*

leaves have been used by different cultures as a remedy for several conditions. Egyptians used hibiscus tea to lower body temperature, treat heart and nerve diseases, and as a diuretic to increase urine production. In Africa, tea was used to treat constipation, cancer, liver disease, and cold symptoms. It is also known to have anti-snake venom properties [4]. Because the Plant *Hibiscus punctatus Dalz.* is exclusively available in Maharashtra region and was unexplored, we hereby report about the pharmacognostic study of this species [5]. This study will be surely beneficial towards the standardization of the species and it will also be useful in determining the future scope in determination of its potential chemical constituents.

2. MATERIAL AND METHODS

2.1. Plant Material and Authentication

Hibiscus punctatus Dalz. leaves were collected from Janai-Malai Hills of Satara District of Maharashtra. Authentication of collected plant material was carried out at Department of Botany, Yashwantrao Chavan Institute of Science, Satara. Voucher Specimen (HPUNCT-1) was deposited for future reference [6].

2.2. Morphological Evaluation

Morphological evaluation was carried as per WHO guidelines [7]. Important characteristics of plants such as Surface of Leaves, Colour, Odour, and Taste were evaluated.

2.3. Microscopic Evaluation

Transverse sections (T.S.) of *Hibiscus punctatus Dalz.* leaves were taken using sharp section blade. T.S. were kept in suitable media and then mounted on glass slide with the help of soft brush. T.S. were observed under Photographic Microscopes under Normal and Polarized Lights. Transverse Sections of leaves were studied for different microscopic characters like epidermis, xylem, phloem, parenchymatous cells, Calcium Oxalate crystals, Starch grains etc. Photographs of all microscopic characters were captured, printed, labeled and stored for further referencing [6, 7].

2.4. Powder Microscopic Study

Preliminary examination and behavior of powder with different chemical reagents was carried out and microscopic examination was performed on treatment with different reagents like phloroglucinol, conc. HCl, ruthenium red, iodine solution and acetic acid. These are the reagents that are often used for detection of various microscopic components like xylem, phloem, calcium oxalate crystals, starch grains etc. Leaves of *Hibiscus punctatus Dalz.* were collected and shade dried. Dried leaves were powdered using a mixer grinder (Bajaj Ltd) and used further for microscopic evaluation. Powder was also treated separately with different chemical reagents and was observed under the microscope [6, 7].

2.5. Physicochemical analysis

Physicochemical parameters of *Hibiscus punctatus Dalz.* leaves powder such as ash values, extractive values, and moisture content (loss on drying) were determined according to methods prescribed in official books such as Indian Pharmacopoeia and the WHO guidelines on quality control methods for medicinal plant materials. Extractive values of *Hibiscus punctatus Dalz.* leaf powder were determined by using different solvents viz. water and ethanol. Five gram dried powder of leaves were placed in a glass-stopper conical flasks containing different solvents. All the flasks were placed in a water bath shaker for 6 hours with frequent shaking, and allowed to stand for 18 hours. After 18 hours each extract containing different solvents were filtered and 25 ml of filtrate from each conical flask was dried at 105 °C for 6 hours and extractable matter of air-dried material was calculated.

The total Ash value was determined by burning ground leaf powder of *Hibiscus punctatus Dalz.,* accurately 2.0 gm of powder was weighed in a previously ignited and tared silica crucible and it was ignited by gradually increasing the heat to 500-600 °C until it was red-white which indicate the absence of carbon. It was cooled in desiccators and weighed. The content of total ash in mg per gm of air-dried material was calculated. The Acid insoluble ash was determined by adding 25ml of hydrochloric acid (~70g/l) in to crucible containing 2.0gm of total ash. The crucible was covered and boiled for 5 min. The watch-glass was rinsed with 5.0 ml of hot water and this liquid was added to the crucible. The insoluble matter was collected on an ash less filter-paper and washed with hot water until the filtrate was neutral. The filter-paper containing the insoluble matter to the original crucible was dried on a hot-plate and ignited to constant weight. The residues were allowed to cool in suitable desiccators for 30 minutes and then weighed. The content of acid-insoluble ash in mg per gm of air-dried material was calculated. The water soluble ash was determined by adding 25ml of water in to the crucible

containing 2.0gm of total ash and it was boiled for 5 minutes. Insoluble matter was collected on an ash-less filter-paper, washed with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding 450 °C. The content of water-soluble ash in mg per gm of air-dried material was calculated by subtracting the weight of this residue in mg from the weight of total ash. The water soluble ash value was calculated. Loss on drying study was carried out by using hot air oven. The powder was taken in porcelain dish and weight of empty porcelain dish was noted. After that 1 gm of powder was taken and allowed for 2 hours at 105 °C for drying. The loss on drying was calculated [7-9].

2.6. Fluorescence analysis

Fluorescent characteristics of the plant powder as such and after treating them with chemical reagents were observed in daylight as well as under UV radiation. Fluorescent analyses of all the plant powders were carried out according to the standard methods. Behavior of powdered plant materials with different chemical reagents was carried out as described by standard procedures [10].

2.7. Extraction process

The extraction of dried powder of the leaf of *Hibiscus punctatus Dalz. was* performed using maceration and soxhlet extraction process. Accurately weighed, 20 gm of dried leaf powder was subjected to macerate by using different solvent media like 100 ml of Water, Ethanol: Water (50:50) and 100 ml of ethanol. This maceration process was carried out for 24 hrs. Similarly, 20 gm of leaf powder was placed in thimble (Borosil, Mumbai, MH, India), which was inserted into a Soxhlet apparatus and extracted with 200 ml ethanol. This Soxhlet extraction process was carried out for 18 hrs. After completion of maceration and Soxhlet extraction process the solvent was evaporated on rotary vacuum evaporator at a temperature 50 °C to obtain ethanolic, hydro-alcoholic and aqueous extract from maceration and ethanolic extract from Soxhlet extraction process. These extracts were stored in vacuum desiccators for further preliminary phytochemical analysis [11].

2.8. Preliminary phytochemical screening of extracts

Extracts of *Hibiscus punctatus Dalz.* were subjected to preliminary phytochemical screening for the detection of various phytoconstituents such as alkaloids, glycosides, tannins and phenolic compounds, flavonoids, steroids, saponins, proteins, amino acids, carbohydrates and triterpenoids. This examination was done by using standard procedures [12].

3. RESULTS AND DISCUSSION

3.1. Morphological evaluation

Morphological characteristics of *Hibiscus punctatus Dalz.* are mentioned in Table 1. The lamina for *Hibiscus punctatus Dalz.* leaves was found plane. The size of the leaves was in the range of about 2.5-10 x 1.5-6 cm across, shape of the leaves was ovate to orbicular, colour of the leaves was green, odour was characteristic and was bitter in taste [13].

Table 1: Morphological Characteristics of *Hibiscus punctatus Dalz.*

Characteristics	Observation
Surface of Leaves	Plane
Colour	Green
Odour	Characteristic
Taste	Bitter
Size	About 2.5-10 x 1.5-6 cm across
Shape	Ovate to Orbicular

3.2. Microscopical characterization

3.2.1. Structure of the Leaf

Transverse section of Leaves of *Hibiscus punctatus Dalz.* was found to have anomocytic type of stomata i.e. Irregular celled stomata were present. The upper and lower epidermis consists of single row of cells with both glandular and non glandular (covering) trichomes. Chloroplast rich palisade parenchymatous cells were also found. Spongy parenchymatous cells were also present in the intracellular spaces. Collenchyma along with clustered crystals of calcium oxalate was present in the section.

3.2.2. Powder microscopy

Powder analysis plays a significant role in identification of crude drug. These characters always help in the identification of right variety and search for adulterants. The microchemical test of leaf powder reveals the presence of phloem fibres, xylem vessels, spongy parenchyma and epidermal cells [15, 16].

3.2.3. Physicochemical evaluation

Results of various physicochemical parameters viz. Ash, extractive values and loss on drying are summarized in table 2 [17, 18].

Table 2 Results of Various Physicochemical Parameters of Leaves of *Hibiscus punctatus Dalz*.

Physicochemical Parameter	Result (% w/ w) ± S.E.M.
Foreign Matter	Nil
Ash Value	
Total Ash	9 ± 0.5
Acid Insoluble Ash Value	3.52 ± 0.15
Water Soluble Ash Value	2.7 ± 0.26
Sulphated Ash Value	5.1 ± 0.75
Extractive Value	
Alcohol Soluble Extractive Value	10 %
Water Soluble Extractive Value	16 %
Loss on Drying (Moisture Content)	27.05 ± 0.35
Foaming Index	Less than 100
Swelling Index	2.6 ± 0.5

Values are expressed as mean % ± S.E.M. (n = 6), except foaming index, extractive values. Swelling index is expressed as mean ± S.E.M. (n = 6)

3.2.4. Fluorescence analysis

The fluorescent analysis of powdered drug plays an important role in the determination of the quality and purity of the drug. In fluorescence analysis, behavior of powdered plant materials with different chemical reagents was observed. Fluorescence is exhibited by various chemical constituents present in the dried plant material. The leaf powder of the plant samples was extracted in various solvents. The fluorescence analysis was observed under ordinary visible light and also under UV light (245 nm). The fluorescence analysis of leaf powder of *Hibiscus punctatus Dalz*. showed green color under UV light when treated with NaOH and water, as well as, when the powder is used as such. Greenish black color was observed under visible light when the leaf powder was treated with $FeCl_3$, as well as, the powder without any chemical treatment. Florescent green color was observed in Ferric chloride, sulphuric acid and methanol under white light and UV light. Various colors like florescent green, yellowish green and brown were also observed under different light conditions. The fluorescence analysis results of leaf powder *Hibiscus punctatus Dalz*. are depicted in Table 3 [19].

Table 3 Results of Fluorescence Analysis of Leaf Powder

Treatment with Chemical Reagent	Observation	
	Visible Light	UV Light
Water	Green	Green
Sodium Hydroxide	Brown	Green
Methanol	Dark Brown	Fluorescent Green
Nitric Acid	Orange	Brown
Hydrochloric Acid	Brown	Dark Green
Ferric Chloride	Greenish Black	Fluorescent Green
Sulphuric Acid	Brown	Fluorescent Green
Picric Acid	Pale Yellow	Greenish Yellow
Acetic Acid	Brown	Blackish Brown
Powder As Such	Greenish Black	Green

Table 4: Preliminary Phytochemical Screening of Extracts

Phytochemicals	Ethanolic Extract	Hydroalcoholic Extract	Aqueous Extract
Alkaloids	++	++	--
Glycosides	++	+	--
Tannins	--	+	++
Starch	--	--	--
Phenolic Compounds	--	+	++
Sugars	--	+++	++
Amino Acids	--	+	+
Flavonoids	++	+++	++
Steroids	+	++	++
Volatile Oils	--	--	--

(--) Absent, (++) Present

3.3. Preliminary phytochemical screening of extracts

Phytochemicals play an important role in the treatment of different types of diseases and disorders and are still used in both traditional and modern medicine. Many of the secondary metabolites isolated from plants are used in pharmaceutical industry. Ethanolic extract of *Hibiscus punctatus Dalz.* leaves showed presence of alkaloids, flavonoids, glycosides and steroids. Hydroalcoholic extract shows the presence of alkaloids, glycoside, flavonoids, tannins and steroids while its aqueous extract showed presence of steroids, carbohydrates, tannins and flavonoids. Results are depicted in table 4 [20, 21].

4. CONCLUSION

Pharmacognostic and phytochemical investigation of *Hibiscus punctatus Dalz.* leaves was carried out. The results obtained in this study will be useful to authenticate the medicinal importance of the particular species of Hibiscus. Pharmacognostic parameters determined in present study will also be useful for establishing the pharmacopoeia standards for *Hibiscus punctatus Dalz.* Preliminary phytochemical analysis will surely be useful for further phytochemical studies and isolation of therapeutically important phytoconstituents.

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Conflict of Interest

None

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