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Pharmacognostical Study on *Hiptage benghalensis kurz*.

D. SATHIS KUMAR*, T. PRAVEEN KUMAR, J.M. ANVESH, G. KIRAN KUMAR and DAVID BANJI

Department of Pharmaceutical Analysis, Nalanda College of Pharmacy, Nalgonda-508 001, India

*Corresponding author: E-mail: satmpdina@yahoo.co.in

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This paper deals with the pharmacognostical evaluation of the *Hiptage benghalensis kurz*. Morphology of the entire plant has been studied with the aim to aid pharmacognostic and taxonomic species identification using light and confocal microscopy, WHO recommended physico-chemical, morphological and histological parameters presented in this paper. This study may be proposed as parameters to establish the authenticity of *Hiptage benghalensis kurz* and can possibly help to differentiate the drug from its other species/varieties.

Key Words: *Hiptage benghalensis kurz*, Microscopy, Macroscopy, Preliminary chemical tests.

INTRODUCTION

Hiptage benghalensis (L.) Kurz belongs to the family malpighiaceae, it ranges in nature from a large woody vine (*i.e.*, liana) climbing into the canopy of forests to a shrubby plant. It distributes throughout the warmer parts of Maharashtra, Konkan, Karnataka and other parts of India and North of South America[1,2]. The bark, leaves and flowers of *H. benghalensis* are aromatic in nature. The leaves are useful in chronic rheumatism, skin diseases, asthma. Juice of leaves is used as insecticidal and treatment of scabies[3]. The fruits are used to treat diarrhea, diabetes, dysentery, liver disorders[4]. The ethanolic extract is used as CNS depressant and antihypotensive[5]. The stem and bark contains various chemical constituents like β-sitosterol, octacosanol, friedelin, epifriedelinol, α-amyrin, hiptagin[6]. In view of its importance of *H. benghalensis* in traditional and modern system of medicine, it was though worthwhile of develop quality standard for the same. As for as botany (morphology) and chemistry of this plant concerned, large number of scientific data is available but a systematic standardization study is still lacking. Hence, in the present investigation an attempt has been made to explore microscopic characters, physico-chemical parameters, phytochemical constituents.

EXPERIMENTAL

Collection and authentication: Plant material of *H. benghalensis kurz* was collected from Tirupati, India and authentified by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, S.V. University, Tirupati, India. Plant was dried in the shade and ground into uniform powder using milling machine.

For the microscopic studies, transverse sections were prepared and stained[7,8]. The leaves were boiled separately with saturated chloral hydrate solution for surface studies and quantitative microscopical observation of leaf. The selected whole plant of *Hiptage benghalensis kurz* was subjected to organoleptic microscopic (essential for powdered crude drugs consist of the fragments of cells in the form of recognizable tissues and the study of surface constants like fibers, lignified vessel, phloem fibers, calcium oxalate crystals, starch grains, *etc.*)[9-11] and physico-chemical values such as the percentage of total ash, acid-insoluble ash, water-soluble ash, sulphated ash, water and alcohol soluble extractives, crude fiber content and foreign matter were calculated as per the Indian pharmacopoeia[12-14] and fluorescence analysis of crude powder were estimated using various chemical and organic reagents.

Determination of stomatal index: Leaf fragments were observed under microscope for the presence and quantification of epidermal cells, stomata (type and distribution), palisade cells, vein islet number and vein let termination number. Stomatal index was calculated as the percentage of number of stomata present per number of epidermal cells and each stoma was counted as one cell.

Preparation of extracts: The extracts of leaves of *Hiptage benghalensis kurz* were prepared by successive soxhlation with various solvents. The shade dried leaf powder was packed in thimble kept in the soxhlet apparatus and extraction was allowed to run successively using the solvents, petroleum ether (60-80 ºC), chloroform, ethyl acetate and ethanol. Finally, the marc was dried and macerated with chloroform-water for 24 h to obtain the aqueous extract. Each extract was concentrated by evaporating the solvent on the water-bath and the obtained

extracts were weighed. The physical characteristics and percentage yield of various extracts were tabulated.

Phytochemical screening: All the extracts were subjected to preliminary phytochemical screening for the detection of various chemical constituents. The presence or absence of different phytoconstituents *viz.* proteins, steroids, alkaloids, sugar, tannins, glycosides and flavanoids, *etc*., were detected by usual prescribed methods.

RESULTS AND DISCUSSION

Macroscopic studies: The leaf of hiptage is simple and in pairs along the stems on short stalks (*i.e.* petioles). These leaves are relatively large (6-20 cm long), are usually somewhat elongated in shape (*i.e*., lanceolate to ovate-lanceolate) and have long-pointed tips (*i.e.,* attenuate apices). The flower of hiptage is fragrant and appears as compact clusters. Each flower cluster can contain 10-30 flowers. The individual flowers have five rounded petals 1-2 cm long. These petals are white or with pink shade.

Transverse section of the leaf: Leaf is dorsiventral. Lamina portion shows upper epidermis, which is singled layered having rectangular cells and cuticularized. Surface shows unicellular 2 - 3 trichomes. Mesophyll consists of double layers of palisade parenchyma and 4 - 5 layers of loosely bound spongy parenchyma. Midrib portion shows both upper and lower epidermis which is continuous. Upper convex surface is grooved and below each groove, it shows 6 - 8 layers of collenchymas which are followed by double layers of embedded palisade parenchyma. Vascular bundles are arc shaped. Xylem is lignified and phloem is non-lignified (Fig. 1).

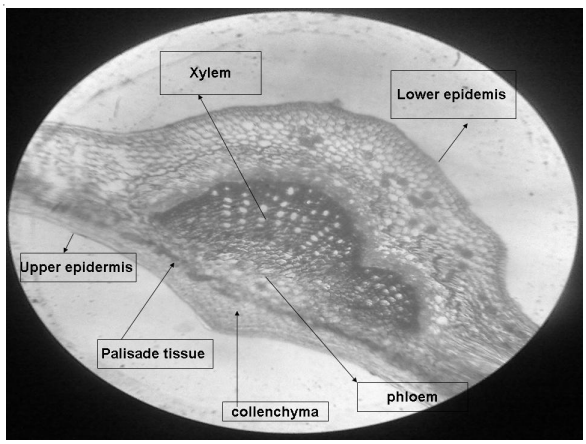
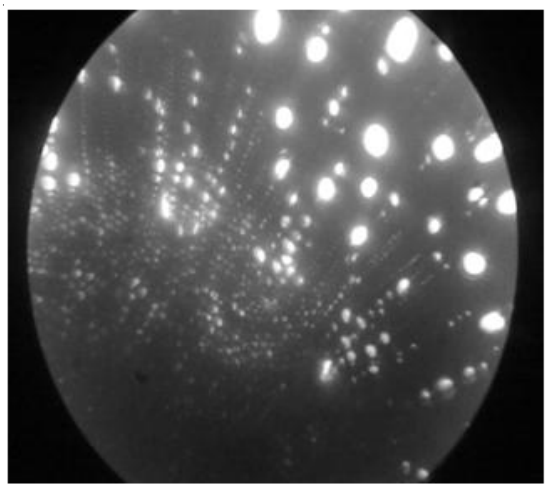


Fig. 1. Transverse section (T.S.) of leaf of *Hiptage benghalensis*.

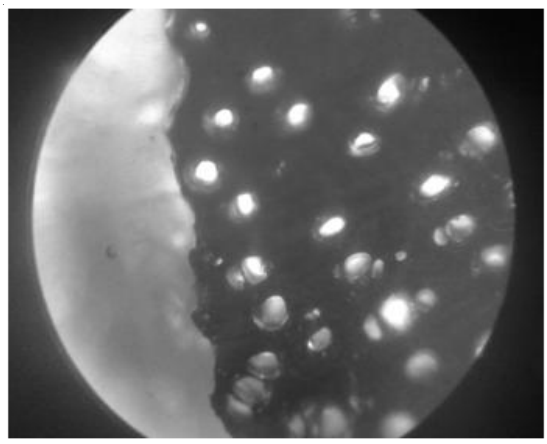
Transverse section of stem: It shows single layer of epidermal cells covered with cuticle, below the epidermis there is multilayered cortex followed by vascularbundle layer (consists of xylem and phloem). The innermost layer is pith or medulla which consists of spherical lignified cells (Fig. 2).

Stomata: The stoma was a type of paracytic (rubiaceous or parallel celled) with two subsidiary cells around the guard cells, the long axis of which is parallel to that of stoma.

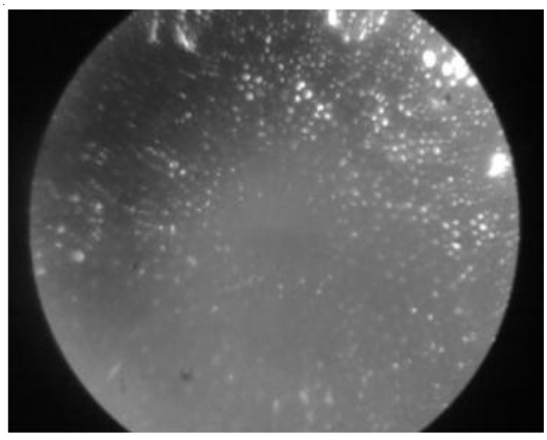
Powder microscopy: Microscopical observation of the powder was performed and the photographs were taken, the observation shows calcium oxalate crystals (monoclinic type of crystals of about 1-3 microns length and width), unicellular covering trichomes (of length 6-8 microns and width 1-4 microns), lignified xylem vessels (of length about 8-26 microns and width 3-12 microns), minute, small spherical, individual starch grains (of about 1-3 microns), pitted, lignified fibres (of length 14-27 microns and width 1-4 microns) were found as shown in the Fig. 3.



Transverse section of stem showing xylem



Transverse section of stem showing cortex and xylem



Transverse section of stem showing pith

Fig. 2 Transverse section of stem

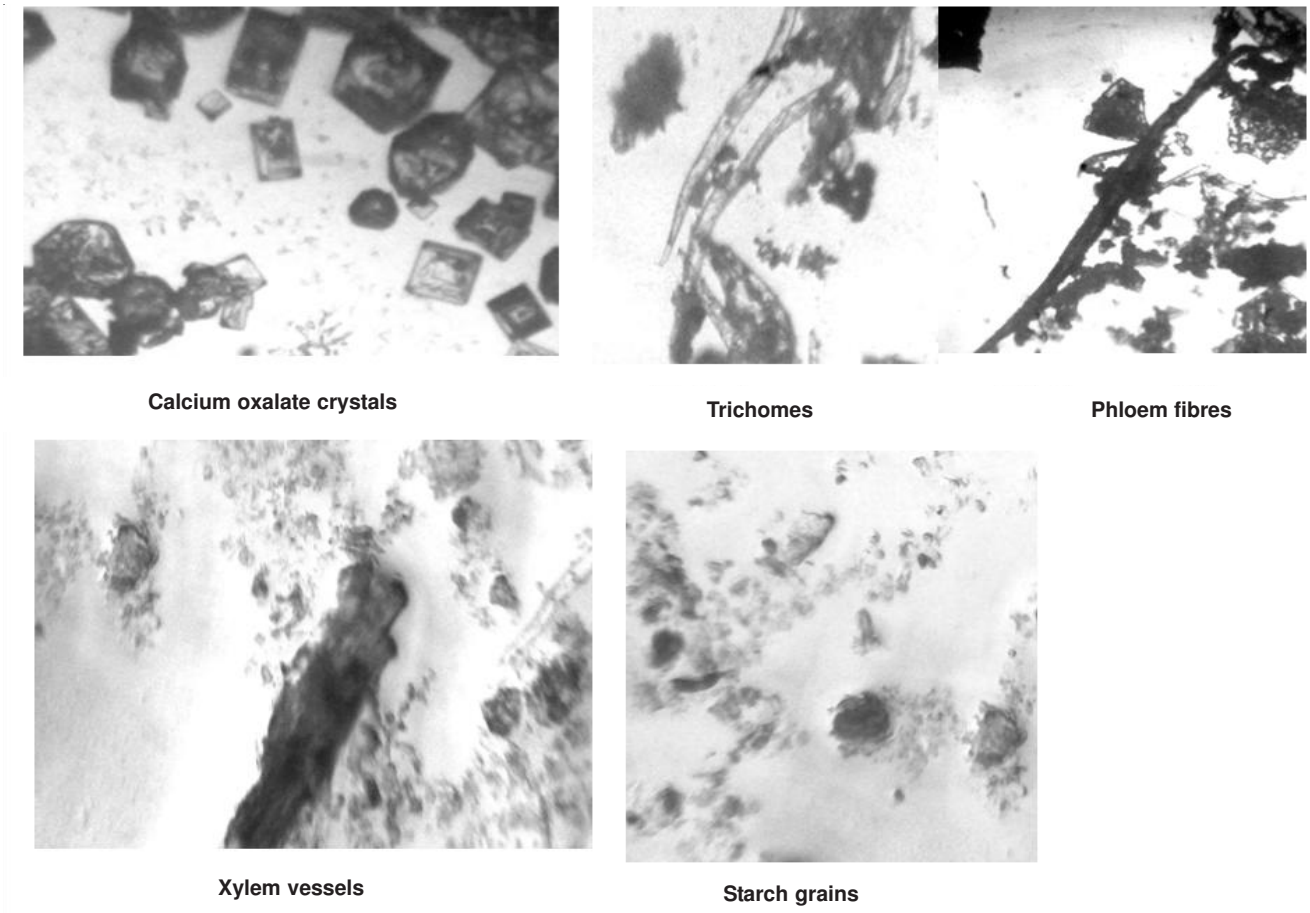


Fig. 3. Powder analysis

Physico-chemical properties: The physico-chemical characters of powdered drug of whole plant of *Hiptage benghalensis kurtz* such as the percentage of total ash, acid-insoluble ash, water-soluble ash, sulphated ash, alcohol soluble extractives, moisture content and foreign matter were presented in Table-1. The fluorescence analysis of the powdered drug of *Hiptage benghalensis kurz* in various solvents and chemical reagents was performed under normal and ultraviolet (UV) light (Table-2).

TABLE-1
PHYSICO-CHEMICAL PROPERTIES OF *Hiptage benghalensis kurz*

Property	Values in % (w/w)
Total ash	10.40
Water soluble ash	41.35
Acid insoluble ash	3.45
Sulphated ash	12.98
Alcohol soluble extractive	18.16
Ether soluble extractive	16.36
Water soluble extractive	23.80
Crude fiber content	66.80
Loss on drying	8.80
Foreign organic matter	0.92

Preliminary phytochemical screening: After extraction with different solvents, the residues were dried and measured. The residue obtained was 0.38, 0.7, 0.86, 3.21 and 6.08 % w/w for petroleum ether, chloroform, ethyl acetate, alcohol and water extract of *Hiptage benghalensis kurz* respectively.

TABLE-2
FLUORESCENCE ANALYSIS OF *Hiptage benghalensis kurz*

Solvents used	Daylight	UV light (254nm)	UV light (366nm)
Conc.H_2SO_4	Brown	Greenish brown	Violet
50%H_2SO_4	Greenish brown	Green	Violet
Conc. HCl	Green	Green	Violet
50%HCl	Brown	Green	Violet
Ammonia	Greenish Brown	Greenish brown	Violet black
HNO_3	Brownish orange	Green	Greenish black
50%HNO_3	Brown	Greenish brown	Greenish black
5% $FeCl_3$	Green	Greenish black	Violet
5% KOH	Greenish brown	Green	Violet black
5% NaOH	Greenish brown	Green	Violet black
1N KOH	Yellowish brown	Greenish yellow	Violet brown
1N NaOH	Yellowish brown	Green	Violet
Methanol	Greenish brown	Green	Violet black
1NMethanolic KOH	Light Greenish brown	Green	Greenish violet

The green, dark green, greenish brown and brown residues were for petroleum ether, chloroform, ethyl acetate, alcohol and water extract of *Hiptage benghalensis kurz* respectively (Table-3). All the extracts were sticky in nature. The preliminary phytochemical investigation of the chloroform and ethyl acetate extract showed the presence of carbohydrates and glycosides. Glycosides were present even in ethanolic extract. Ethanolic and aqueous extract given the positive result for carbohydrates, flavonoids and phenolic compounds. Alkaloids and saponins were present only in aqueous extract (Table-4).

TABLE-3
PERCENTAGE YIELD OF EXTRACTS OF
Hiptage benghalensis kurz

Extract	Yield (%)	Nature	Colour
Petroleum ether	0.38	Sticky	Green
Chloroform	0.70	Sticky	Dark green
Ethyl acetate	0.86	Sticky	Green
Ethanol	3.21	Sticky	Brown
Water	6.08	Sticky	Brown

TABLE-4
QUALITATIVE PRELIMINARY PHYTOCHEMICAL
STUDIES OF *Hiptage benghalensis kurz*

Extracts	Pet. ether	Chloroform	Ethyl acetate	Alcohol	Water
Carbohydrates	-	+	+	+	+
Flavonoids	-	-	-	+	+
Alkaloids	-	-	-	-	+
Amino acids and proteins	-	-	-	-	-
Phenolic compounds	-	-	-	+	+
Saponins	-	-	-	-	+
Glycosides	-	+	+	+	-

Note:+Present; -Absent

As a part of standardization study, the macroscopical examination of *Hiptage benghalensis kurz* was studied. Macroscopical evaluation is a technique of qualitative evaluation based on the study of morphological and sensory profiles of drugs. The macroscopical characters of the *Hiptage benghalensis kurz* can serve as diagnostic parameters. The extractive value, ash value, loss on drying and fluorescent analysis of whole plant extracts have been carried out. The results showed greater extractive values in hot extraction, indicating the effect of elevated temperature on extraction. Percentages of the extractive values were calculated with reference to air-dried drug. The per cent extractives in different solvents indicate the quantity and nature of constituents in the extracts. The extractive values are also helpful in estimation of specific constituents soluble in particular solvent. The fluorescence analysis of the powdered drug from the *Hiptage benghalensis kurz* in various solvents was performed under normal and UV light. All the whole plant extracts are examined in short UV (254 nm) and long UV (366 nm) to detect the fluorescent compounds.

Conclusion

The pharmacognostic parameters, which are being reported for the first time, could be useful in the identification and standardization of a crude drug. The data produced in the present investigation is also helpful in the preparation of the crude drug's monograph and inclusion in various pharmacopoeias.

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