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Research Article

PHARMACOGNOSTICAL EVALUATION OF LEAF OF *HOLIGARNA GRAHAMII* (WIGHT) KURZ: AN ENDEMIC PLANT TO WESTERN GHATS

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ABSTRACT

The Pharmacognostical investigations of leaf of *Holigarna grahamii* (Wight) Kurz (Anacardiaceae) were carried out in terms of macroscopic and microscopic characters, Powder behavior and fluorescence analysis. The dried leaves powder was subjected to Soxhlet extraction using petroleum ether, chloroform, methanol, ethanol and aqueous etc. as a solvent for preliminary phytochemical analysis. A phytochemical test reveals maximum concentration of Phenols, flavones, tannins, alkaloids

Keywords: Pharmacognosy, phytochemical, *Holigarna grahamii* (Wight) Kurz.

INTRODUCTION

Holigarna grahamii (Wight) Kurz is large tree up to 35 m tall, belonging to family Anacardiaceae. *H. grahamii* is Endemic to Western Ghats, common from South Sahyadri to Central Sahyadri and rare in North Sahyadri. Canopy trees in semi-evergreen to wet evergreen forests at low elevations (between 200 and 700 m). Plants of this genus *Holigarna* have been known to cause 'Rhus' type contact dermatitis (Chopra et al 1958). Allergenic agent in contact dermatitis from *Holigarna ferruginea* March. (Srinivas et. al 1987).

Ethnopharmacognosy is the scientific study of crude drugs used by different ethnic or cultural groups. Wild plants play vital role in rural people of India. Pharmacognosy means knowledge of drug. It is applied branch of science, in which number of scientific disciplines for solving the problem pertaining to identity, purity, quality and preservation of drugs from plants. Plants from Anacardiaceae family are used in indigenous system of medicine for their antiarthritic, antibacterial, antioxidant properties. They are used to treat cough, cold, inflammation, tumor, cancer, skin diseases. The present study deals with morphology, anatomy, powder behaviour, fluorescence analysis and preliminary phytochemical analysis of leaves of *H. grahamii*.

MATERIALS AND METHODS

The leaves of *H. grahamii* were collected from Phonda and Amboli areas. The identification of the plant was done by using relevant literature. Leaves were shade dried and made into fine powder with mechanical grinder.

Macroscopic Characters

The morphological and taxonomical observations were made and the characters were described in technical terms. (according to the method of Trease & Evanse 1972).

Microscopic Characters

For microscopic studies, free hand sections were taken, stained in safranin, mounted in glycerin and photographs were taken. (Metcalf & Chalk 1950).

Stomatal index

Stomatal index is the percentage which the number of stomata forms to the total number of epidermal cells, each stomata being counted as one cell. Stomatal index can be calculated by using following equation. (Salisbury, 1928)

$$I = \frac{S}{E + S} \times 100$$

I = Stomatal index, S = No. of stomata per unit area,
E = No. of epidermal cells in the same unit area.

Powder behavior and Fluorescence analysis

Leaf powder treated with different chemical reagent and Fluorescence characteristics of leaf under UV light were examined (Kokoski *et al* 1956 and Chase & Pratt 1949). The observed results are given in the Table No.1 and 2 respectively.

Preliminary Phytochemical analysis

The Leaf materials were air dried under so as to prevent decomposition of active principle and made fine powder by using mechanical grinder. Then leaf powder used for powder behavior, fluorescence and phytochemical analysis. Leaf powder was extracted using Water, Chloroform, Methanol, Ethanol, Pet ether as a solvent. 20 gram of dried leaves powder was weighed and put in a cheese cloth and subjected to extract successively with 200 ml methanol in Soxhlet extractor until the extract was clear. All the extracts were condensed and preserved in refrigerator in air tight bottles until further use.

RESULT AND DISSCUSSION

Macroscopic Characters

Leaves of *H. grahamii* is simple, oblanceolate, rigidly coriaceous, triangular above the middle, acute or acuminate, glabrous and shining above. Main nerves 20-30 pairs, prominent and pilose beneath. Petiole is stout with spur like appendages. (fig. 1b)

Microscopic Characters

Transverse section of leaf consist upper epidermis and lower epidermis (fig. 1c). Some of epidermal cells elongate to form simple, multicellular trichomes (fig. 1f). Below the upper epidermis columnar, elongated, compactly arranged single rows of Palisade cells. Spongy parenchyma is found throughout tissue and is composed of loosely arranged spherical parenchyma cells. In transverse section of petiole shows presence of clustered crystal which is transparent spot. T.S of petiole is oval to somewhat rectangular in shape (fig.1d) The secretion duct is present in both transverse section of leaf and petiole. On lower side Anomocytic stomata is present (fig.1e). While in *Holigarna ferruginea* March the leaf shows the cluster crystal in mesophyll tissue, anomocytic stomata and thick walled, multicellular, uniseriate covering trichomes. In leaf also showed presence of schizogenous secretary ducts in the midrib and lamina region. (Nayak. *et al*. 1993)

Stomatal Index

The stomata are present on lower surface of leaf. The type of stomata is Anomocytic. The Stomatal index is 16.66/sq.mm.

Powder & Fluorescence study

The leaf powders are treated with various chemicals exhibited various colours in the Short and Long wavelength under UV light. For example when the powder was treated with aqueous 1 N HCl the leaf powder exhibited varied brown and black colour under 254nm & 366nm wavelength respectively in UV light likewise all the results are depicted in Table 1 & 2.

Phytochemical analysis

The phytochemical test carried out on the various extract like methanol, ethanol, chloroform, Pet ether, and Aqueous. The preliminary phytochemical screening revealed the presence of Tannin, Phenol, Alkaloids, Courmarins, reducing sugar, flavones. While the phytochemical screening in *Holigarna arnottiana* Hook f. found out the presence of alkaloids, steroids, tannins and phenolic compound, flavonoids, resins, fatty acid, gum from bark and leaves. These compounds may be responsible for the antibacterial activities of the leaf and bark extracts. (Pradeep *et. al* 2010). While in *Semecarpus anacardium* L. phytochemical study shows presence of Steroids. The alkaloid, flavonoid, terpenoid are absent. (Kantamereddi *et. al* 2010)

1. Powder Behavior

Table 1: Powder Behavior of Leaf with Different Chemical Reagent

Reagents	Colour developed in day light	Inference
Powder as such	Green	__
Powder + 1N NaOH(aq.)	Dark Yellow	Flavonoids Present
Powder + 5 % Iodine	No Change	__
Powder +40 % NaOH+Lead acetate	White ppt	Tannin Present
Powder + Mayer's Reagent	White ppt	Alkaloids Present
Powder + Conc. H_2SO_4	Dark Green	__
Powder +5 % FeCl3	Black	Tannin Present
Powder + 5% Aq. KOH	No Change	__
Powder + Aq. $AgNO_3$ (1 %)	Green	__
Powder+ Conc. HNO3+ Ammonia	Brown	Xanthoprotein Present

Powder behavior indicates the presence of tannin, Flavonoids, alkaloids, xanthoprotein.

2. Fluorescence analysis:

Table No-2: Fluorescence analysis of Leaf Powder

Treatment of powder	Visible light	Short wavelength 254 nm	Long wavelength 365 nm
Powder as such	Green	Dark Green	Black
Powder + NaOH in water	Brown	Dark Brown	Black
Powder + NaOH in Alcohol	Green	Brown	Black
Powder + 1 N HCl	Faint Green	Brown	Black
Powder + H_2SO_4	Black	Black	Black
Powder + HNO_3	Green	Dark Green	Black
Powder + 10 % HCl	Faint Green	Green	Grey
Powder + Acetone	Dark Green	Dark Green	Black
Powder + 5 % KOH	Black	Black	Black

Leaf powdered with different chemical reagent produce predominantly fluorescence effect

3. Phytochemical Analysis:

Table- 3: Preliminary Phytochemical Analysis of Leaf

Sr. No	Chemical Constituents	Extracts				
		Methanol	Ethanol	Chloroform	Pet Ether	Aqueous
1	Phenol	+++	++	++	_	+
2	Anthraquinones	_	_	_	_	_
3	Flavones	++	+	_	_	+
4	Tannins	+++	+++	++	_	+
5	Courmarin	++	_	_	+	_
6	Saponins	_	_	_	_	_
7	Alkaloids	+	++	_	+	_
8	Reducing Sugar	+++	_	+	++	+
19	Amino Acid	_	_	+	+	_
10	Glycosides	_	_	+	+	_
11	Oil	_	_	_	_	_

Phytochemical tests revels maximum concentration of alkaloids, phenols, tannins etc.

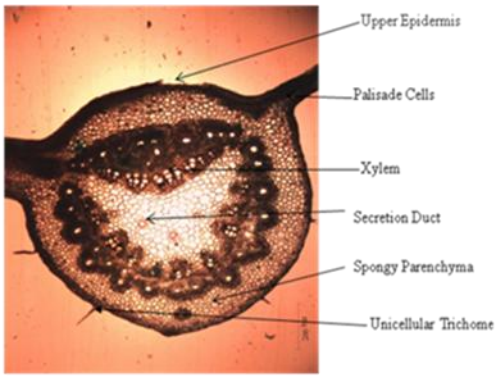
Macroscopic Study

Fig no-1

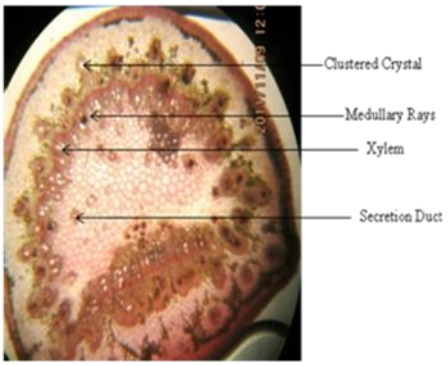
(a)

(b)

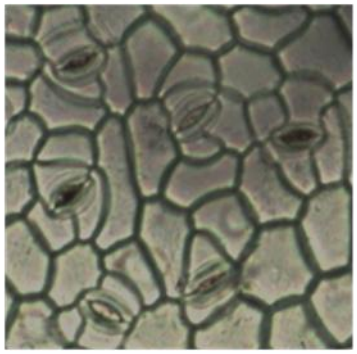
Microscopic Study:



(c)



(d)



(e)

(f)

Fig 1: a) Habit of *H. grahamii* (Wight) Kurz. b) Entire Leaf. c) T.S. of Leaf. d) T.S. of Petiole. e) Anomocytic stomata. f) Multicellular trichomes

CONCLUSION

In present investigation various parameter such as macroscopy, microscopy, powder behavior, fluorescence analysis and phytochemical screening was carried out Which could helpful in authentification of *Holigarna grahamii* (Wight) Kurz. The adulterants if any in the plant material can also easily identified by these studies.

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