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Pharmacognostic and Phytochemical Evaluation of *Holarrhena antidysenterica* Wall.

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ABSTRACT

Holarrhena antidysenterica Wall. is an important plant employed in various indigenous systems of medicine against several diseases, and almost every part of the plant has diverse medicinal properties. The current communication provides a detailed account of the pharmacognostic investigation carried out on *H. antidysenterica*. The study includes macro- and micromorphological characters of leaf, quantitative leaf microscopy, fluorescence study of powder, physicochemical studies and preliminary phytochemical aspects. The results of the study could be useful in setting some diagnostic indices for the identification and preparation of a monograph of the plant.

Keywords: fluorescence study, pharmacognosy, physicochemical studies, quantitative leaf microscopy

INTRODUCTION

Holarrhena antidysenterica Wall. (Apocynaceae), commonly known as "Kutaja", is an important plant used in indigenous systems of medicine as remedy for bronchitis, hematuria, spermatorrhoea, epilepsy, asthma, piles, leprosy, eczema, diarrhea, fevers and jaundice (Bhattacharjee 2000; Guha Bakshi *et al.* 2001).

Various parts of *H. antidysenterica* have been reported to possess antibacterial activity (Jolly and Mechery 1996; Sujan Ganapathy *et al.* 2008). The bark has been reported to possess astringent and antidiarrheal properties (Chopra *et al.* 1982). Leaves of the plant are used to cure scabies (Prajapati *et al.* 2004).

However, a major constraint which has hindered the acceptance of alternative medicine is the lack of documentation and stringent quality control with this backdrop; it is of prime importance to make an effort towards standardization of the plant material. The process of standardization is achieved by step wise pharmacognostic studies (Ozarkar 2005) which in turn help to identify and authenticate plant material.

In view of its diverse medicinal applications and in order to ensure the quality of its supply, especially at a time in which adulteration and substitution prevail on the crude drug markets of India, the present communication deals with a detailed pharmacognostic evaluation of *H. antidysenterica*.

MATERIALS AND METHODS

The first step in standardization of herbal drugs is the correct identification of the plant. The plant was authenticated by Dr. Y. L. Ramachandra, Department of Biotechnology, Kuvempu University, Shankaraghatta. The specimen of the plant has been submitted to the Department of Biotechnology for future reference (Voucher specimen number YLR 204).

Fig. 1 ***Holarrhena antidysenterica*** **leaves (A), flowers (B), tree (C).**

Leaf macroscopy

The following characters for the fresh leaves were noted: size and shape, colour, surfaces, venation, presence or absence of petiole, the apex, margin, base, lamina, texture, odour and taste (Wallis 1985; Evans 2002).

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Leaf microscopy

Transverse sections of the fresh leaves through the lamina and the midrib were mounted in glycerine and observed under a compound microscope. The presence/absence of the following was observed: epidermal cells, stomata (type and distribution) and epidermal hairs (types of trichomes and distribution) (Anonymous 1986).

The fresh plant material (i.e., stem bark, leaf and inflorescence) was collected locally from Bhadra Wildlife Sanctuary, Karnataka (Southern India) in the month of May. The collected sample was dried under shade, packed in a paper bag and stored at ambient temperature until use.

The colour changes of the powdered samples with respect to different chemical reagents on the basis of different chemical constituents was observed in daylight and ultraviolet light as per the methods described by Chase and Pratt (1949) and Kokoshi *et al*. (1958).

The percentage of physicochemical values viz., moisture content, total ash, acid insoluble ash and water-alcohol-soluble extractives were calculated according to the methods described in the Indian Pharmacoepia (Anonymous 1966). The percentage of tannin was also determined using a Jenway-6035 spectrophotometer (Anonymous 1984).

The preliminary phytochemical analysis of petroleum ether, chloroform, ethanol extracts was carried out using the methods as described in Harborne (1984), Trease and Evans (1989), Kokate *et al.* (1998), Khandelwal (2005).

RESULTS

Brief taxonomic description of the plant

H. antidysenterica is a dwarf tree (**Fig. 1**) 10 m tall, with milky white latex. Leaves are thin, ovate, up to 30 cm long, nerves are conspicuous and leaf stalk small. Flowers appear in large terminal branch, white and fragrant. Fruits are linear, slender, cylindrical up to 45 cm long and 1 cm thick, dark grey with white specks. Seeds are 1 cm long (Bhattacharjee 2000).

Macroscopically the leaf (**Fig. 2**) is simple, lanceolate, opposite phyllotaxy, sub-petiolate to petiolate (3 to 12 mm), margin undulate, glabrous, acute apex, unicostate reticulate venation, and average leaf size is 26.10 cm ± 2.72 (length) and 10.20 cm ± 0.55 (breadth). The leaves have green upper surface and pale green underneath, with characteristic odour and taste.

Microscopically, the cells of the epidermis (**Fig. 3**) consists of straight anticlinal walls, anisocytic type of stomata, ranging from 20 to 28 µm in length and 15 to 23 µm in width and present only on the lower surface. A few unicellular trichomes (**Fig. 4**) present only on veinlets ranging from 28 to 56 µm long and 5 to 6 µm width were also observed. A transverse section of the leaf through the midrib revealed the presence of upper epidermis with straight anticlinal walls and elongated palisade cells underneath. There are loosely arranged spongy parenchyma cells on lower epidermis of smaller cells. The quantitative values of leaves are presented in **Table 1**.

Fig. 2 ***Holarrhena antidysenterica*** **leaf, adaxial and abaxial surfaces.**

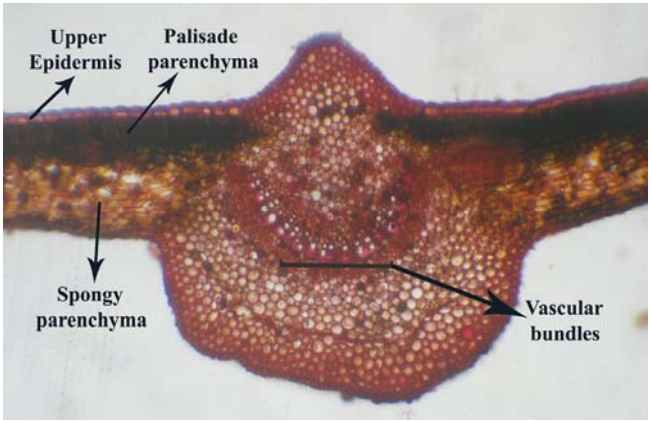

Fig. 3 Cells of the epidermis.

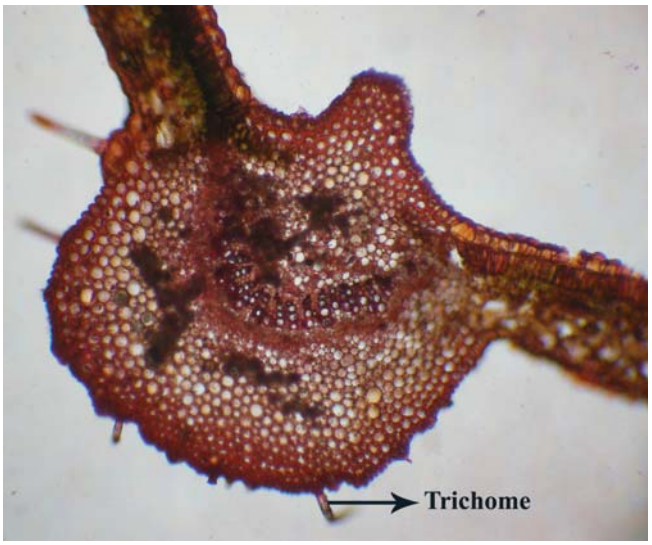

Fig. 4 Unicellular trichomes on veinlets.

Table 1 Quantitative leaf microscopy of *H. antidysenterica.*

Parameter	Range	Mean*
Palisade ratio	30-41	36.10 ± 1.24
Stomata number upper surface	0	0
Stomata number lower surface	61-86	71.80 ± 3.32
Stomata index upper surface	0	0
Stomata index lower surface	25.61-36.9	30.94 ± 1.38
Vein islet number	9-18	13.90 ± 0.85
Veinlet termination number	24-39	30.00 ± 1.61

*Mean value of 10 counts

Powder studies

Powder (**Fig. 5**), when treated with different chemical reagents, showed different colour reactions (**Table 2**).

Physico-chemical studies

The physico-chemical parameters of the plant material, viz., percentage moisture content, total ash, acid insoluble ash, and of the various extractives, i.e., tannins, water-alcohol solubles, were determined and the results are summarized in **Fig. 6**.

Phytochemical studies

A known quantity of dried plant material was extracted in a soxhlet apparatus with petroleum ether, chloroform and then ethanol successfully and tested for different constituent's viz. alkaloids, flavonoids, triterpenoids, sterols, quinine, saponins and glycosides. The results are presented in **Table 3**.

Fig. 5 *H. antidysenterica* powder from bark, leaves and inflorescences.

Table 2 Fluorescence behavior of *H. antidysenterica.*

Plant part	Treatment	Daylight	U.V. light
Bark	Powder (P) as such	Cream	Fluorescent yellow
	P+ 1 N NaOH in water	Blood red	Blood red
	P+ Acetic acid[1]	Dark orange	Fluorescent yellow
	P+ Alcohol[2]	Golden orange	Fluorescent light blue
	P+ 50% HNO_3[3]	Orangish yellow	Dark brown
	P+ 50% KOH[4]	Blood red	Blood red
	P+ 50% H_2SO_4[5]	Dark orange	Lavender
Leaf	Powder (P) as such	Pale green	Fluorescent green
	P+ 1 N NaOH in water	Blood red	Fluorescent blood red
	P+ Acetic acid	Olive green	Fluorescent orange
	P+ Alcohol	Olive green	Fluorescent orange
	P+ 50% HNO_3	Orangish yellow	Fluorescent green
	P+ 50% KOH	Blood red	Blood red
	P+ 50% H_2SO_4	Orangish brown	Brown
Inflorescence	Powder (P) as such	Greenish yellow	Fluorescent yellow
	P+ 1 N NaOH in water	Blood red	Blood red
	P+ Acetic acid	Light yellow	Fluorescent blue
	P+ Alcohol	Greenish yellow	Fluorescent yellow
	P+ 50% HNO_3	Orangish yellow	Fluorescent green
	P+ 50% KOH	Blood red	Blood red
	P+ 50% H_2SO_4	Orangish brown	Brown

[1] 99.8%, s.d. Fine Chem. Ltd., Mumbai.
[2] Absolute alcohol.
[3] 69-70%, Qualigens fine chemicals, Mumbai
[4] 85%, s.d. Fine Chem. Ltd., Mumbai.
[5] 97-99%, Merck Specialities Pvt. Ltd., Mumbai.

Table 3 Preliminary phytochemical analysis of crude plant extracts of *H. antidysenterica*.

Plant constituent	Stem bark			Leaf			Inflorescence		
	Petroleum ether	Chloroform	Ethanol	Petroleum ether	Chloroform	Ethanol	Petroleum ether	Chloroform	Ethanol
Alkaloids	-	+	+	-	+	+	-	+	+
Flavonoids	-	-	+	-	+	+	-	-	+
Triterpenoids	-	-	-	-	-	-	-	-	-
Sterols	+	+	+	+	+	+	-	+	+
Quinine	+	+	+	-	-	-	+	+	+
Saponins	-	-	-	-	-	-	-	-	-
Glycosides	-	-	-	-	-	-	-	-	-

+ = Present; - = Not determined.

DISCUSSION

H. antidysenterica is currently being used in the treatment of various disease conditions without standardization. The standardization of a crude drug is an integral part of establishing its correct identity. Before any crude drug can be included in an herbal pharmacopoeia, pharmacognostic parameters and standards must be established.

The results of these investigations could therefore, serve as a basis for proper identification, collection and investigations of the plant. The macro and micro-morphological features of the leaf described, the fluorescence behaviour, quantitative leaf microscopy and physico-chemical studies are parameters that are unique to the plant and are required in its standardization. The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent (Ozarkar 2005). The preliminary phytochemical evaluation revealed the presence of several secondary metabolites which are known to possess various pharmacological effects. In last four decades the scientists are keen to evaluate many plant drugs used in medicinal folklore, due to their specific healing properties, health action and non-toxic effects (Singh *et al.* 2002).

In this dimension pharmacognostic study of *H. antidysenterica* is a substantial step and it further requires a

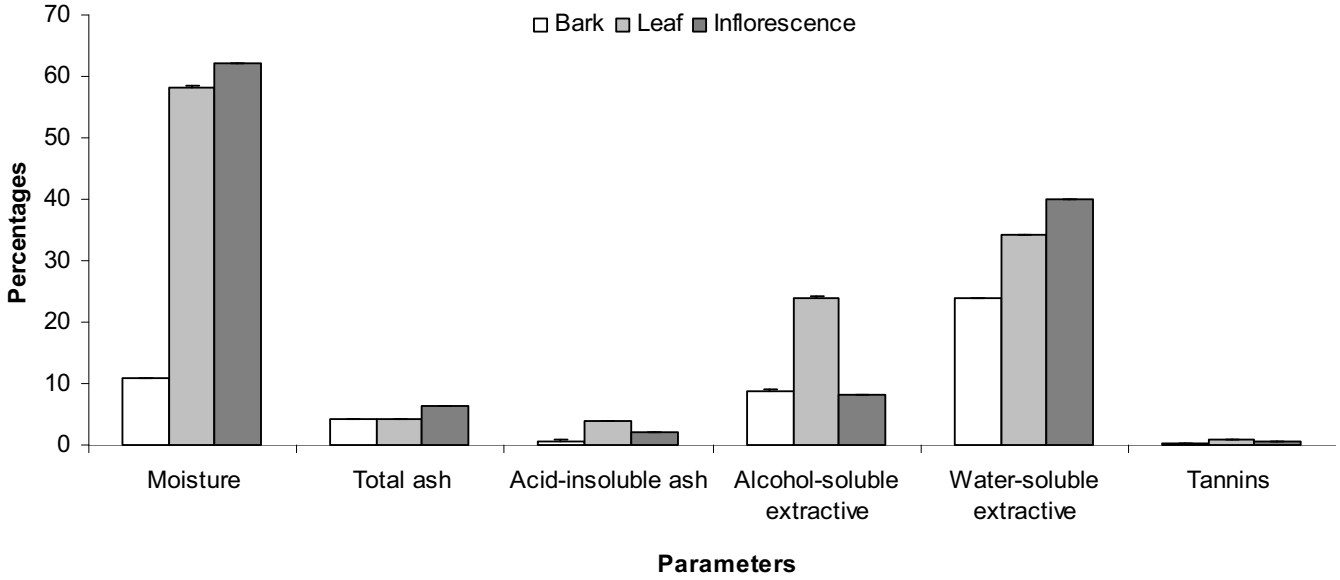

Fig. 6 Physicochemical parameters of *H. antidysenterica*.

long term study to evaluate pharmacological action as well as the therapeutic efficacy and toxicity of plant parts to establish as the drug. The pharmacognostic study of the *H. antidysenterica* has been carried out for the first time. This could also serve in the identification and preparation of a monograph on the plant.

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