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PHARMACOGNOSTIC AND PHYTOCHEMICAL STUDIES ON *HOLARRHENA ANTIDYSENTERICA* (ROTH) WALL. EX A. DC

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ABSTRACT
Holarrhena antidysenterica (Roth) Wall. Ex A. DC belongs to the family Apocynaceae, is commonly known as kuda in Marathi. Plant parts like Root, stem, bark and seeds of *H. antidysenterica* are reported to be used in many traditional systems of medicine including Ayurveda and Unani. However, there are no reports of medicinal application of flowers and fruits of this plant which are in use as wild vegetable in most of the part of central India. Hence, the study is undertaken to understand pharmacognostic and phytochemical characters of *H. antidysenterica*. In the microscopic studies, T.S. of stem shows outermost thick cuticle layer and epidermis made up of thin rounded and iso-diametric cells. Surface of leaves showed the presence of trichomes and anisocytic type of stomata, internally cup shaped vascular bundle in midrib region, spongy parenchyma and palisade cells are also present. Physicochemical parameters such as total ash, moisture content, dry matter content, fluorescence analysis have been studied. Phytochemical evaluation revealed the presence of flavanoids, steroids, volatile oils, alkaloids, glycosides, and saponins. 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: Medicinal plants, pharmacognosy, phytochemistry, *Holarrhena antidysenterica.*

INTRODUCTION

Holarrhena antidysenterica (Roth) Wall. Ex A. DC (Syn: *H. pubescens* Wall. Ex G. Don), a member of family Apocynaceae, is commonly called as Kuda or Pandhra Kuda in Marathi. The plant is found in tropical and subtropical regions of Asia and Africa. In India, it is reported throughout the country, especially in deciduous forests of tropical Himalayas, at altitudes ranging from 900 to 1250 m^2.The plant is widely cited in literature for various medicinal preparations especially dysentery related matters which signifies from its species name also i.e. antidysenterica. The use of various parts of *H. antidysenterica* viz. bark, root, stem and seeds in medicine are reported in many traditional systems including Ayurveda and Unani. Based on those reports various pharmacological studies had been conducted and confirmed by many researchers. Various parts of *H. antidysenterica* have been reported to possess antibacterial activity (Jolly and Mechery 1996; Ganapathy *et al.*, 2008). The bark has been reported to possess stringent antidiarrheal properties (Chopra *et al.*, 1982), while leaves are used to cure scabies (Prajapati *et al.*, 2004). The seeds of this plant are also used in hyperglycemic condition (Mana *et al.*, 2010). Though the literature on therapeutic values of *H. antidysenterica* are available in large, however, there are no reports of medicinal application of flowers and fruits which are also in use as wild vegetable in some part of Vidarbha *i.e*. Central India. Even there are no reports regarding their nutritional analysis. The tribes of these regions collect flowers and fruits from forest in young condition and cooked as a dish. Hence, a study is planned to understand pharmacognostic and phyto chemical analysis of *H. antidysenterica* with special emphasis on flowers and fruits.

MATERIALS & METHODS

Collection of plant material

H. antidysenterica is available in most of the part of Gondia district. Its flowering occurs in the month of April - July and fruiting immediately after that i.e. from May to August. Mostly plants are observed in bushy habit while at some places it is in tree form also. Leaf, stem, fresh flowers and fruits of *H. antidysenterica* were collected from forest area. The plant is identified by using flora of Maharashtra and flora of Marathwada (Singh and Karthikeyan 2000, Naik 1998). Specimen of herbarium sheets are deposited at Department of Botany, D.B. Science College, Gondia Herbarium. The Collected stem and leaves are used freshly for anatomy and stomatal studies, while flowers dried at room temperature under shade. The dried flowers then crushed in the form of powder with the help of mixer grinder and stored in air tight container, where as the other part of the plant were collected from the forest area and stored in 4% formalin for further use (Fox H, Frank B. Johnson *et. al*, 1985).

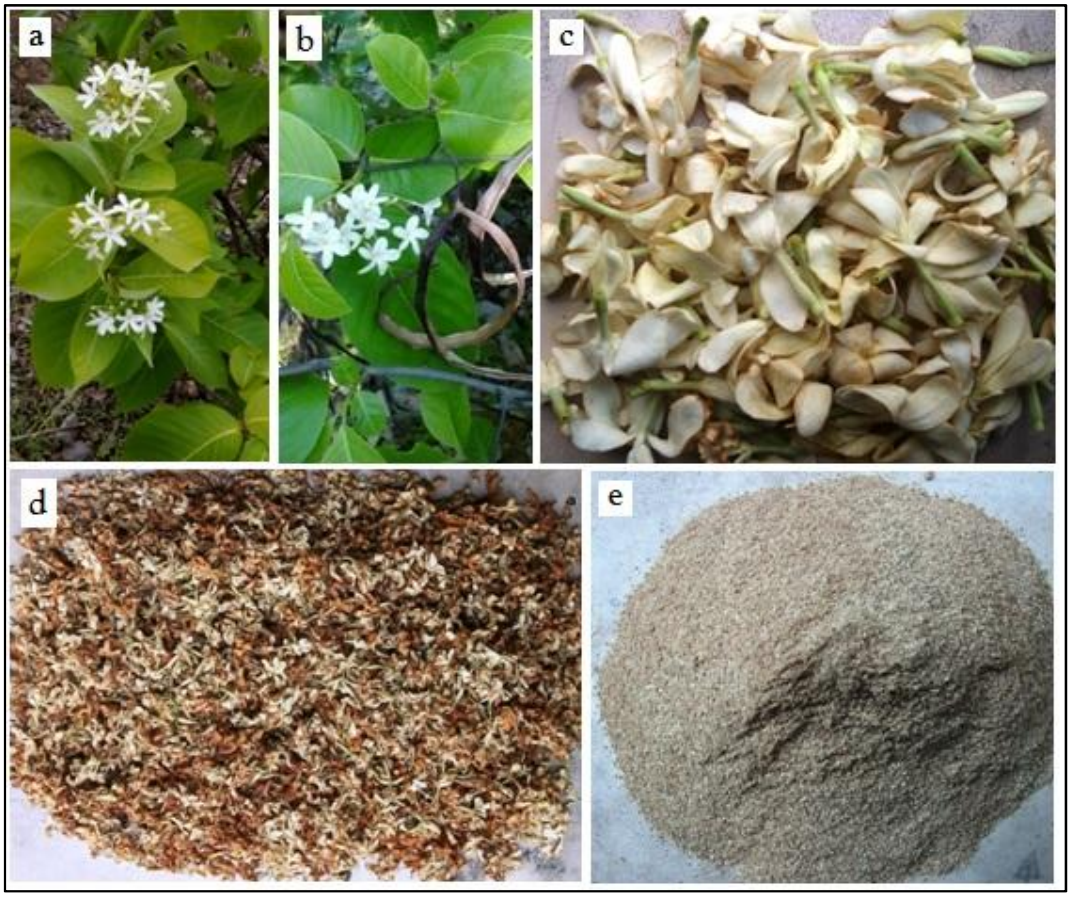

FIGURE 1:- *Holarrhena antidysenterica* a) Whole Plant with flowers, b) Whole plant with flowers and fruits, c) Fresh Flowers, d) Dried Flowers e) Powdered flowers

Microscopic characterization

The microscopic studies were carried out by free hand sectioning of leaves and stem and stained with 0.5% Toluidine. These semi-permanent preparations are studied for different microscopic characters. The micro-photography at different magnifications was performed with the help of Carl Zeiss Primo Star Microscope with Axiocam 105 color camera. **(**Sass 1998, and Annonymous 1986).

Stomata study

Small sections of the leaves were taken for epidermal piling and stained with safranin, mounted with glycerin and observed under different magnification by compound microscope. The stomata size was determine by Ocular Micrometry. Microscopic observations like the density of stomata was determine as number of stomata per square millimeter and the stomatal index was calculated as the number of stomata per square mm divided by number of stomata plus number of epidermal cell per mm square multiplied by 100 (Abdul Rahaman 2009).

Fluorescence analysis

The flowers part of the plant contains some secondary metabolites like phenolic compound, flavonoids and alkaloids. Hence, the powder of flower may show different fluorescence when treated with respective reagents and observed under visible and UV light. The observations were recorded in the tabular form in results (Sethiya 2016).

Proximate Analysis

The total moisture content of the fresh flowers was obtained by drying it in the hot air oven at about 100^0 C till constant weight is obtained, whereas the total ash content was determined by igniting the dry flower material at about 500-700^0 C in muffle furnace (Kokate 1994 and Thimmaiah 1999).

Preliminary phytochemical screening

The qualitative phytochemical analysis was carried out by chemical tests. The powder prepared from the dried flower material was used for the preliminary phytochemical screening. For this extracts are prepared by successive cold extraction method (Handa *et. al*, 2008). The detection of phytoconstituents like flavonoids, steroids, saponins, alkaloids, tannins, glycosides and phenolic compounds were one by using procedure given by various workers (Khandelwar *et. al*, 2001; Kokate 1994, Thimmaiah 1999).

Phytochemical quantification

The quantitative phytochemical analysis was done by using standards methods given by many scientists. The total phenolics of flower was determined with the help of Folin-Ciocalteu reagent by comparing Catechol as a standard phenolic compound similarly total protein and soluble sugar were determine by Bradford's method and Anthrone method respectively whereas the alkaloids were determine by gravimetric method (Thimmaiah 1999).

RESULTS AND DISCUSSION

1. Microscopic characterization

a. Microscopic character of stem

The stem is nearly circular in cross section outline. It consists of an outer most cuticles layer which covers single layer epidermis. In young stem, trichomes are observed which are absent in old stems. Epidermis is followed by fairly wide cortical zone. The epidermal layer consists of cylindrical shaped continuous layer of cells. The cortex regions consist of thin walled parenchyma in the inner side and collenchymas in the outer side. In the pericycle region sclerenchyma cells arrange in bundle form. The vascular zones consist of outer phloem cells which are readily arranged and covers xylem patch. It includes outer metaxylem and inner protoxylem. The wood parenchyma is also present in between the protoxylem and pith.

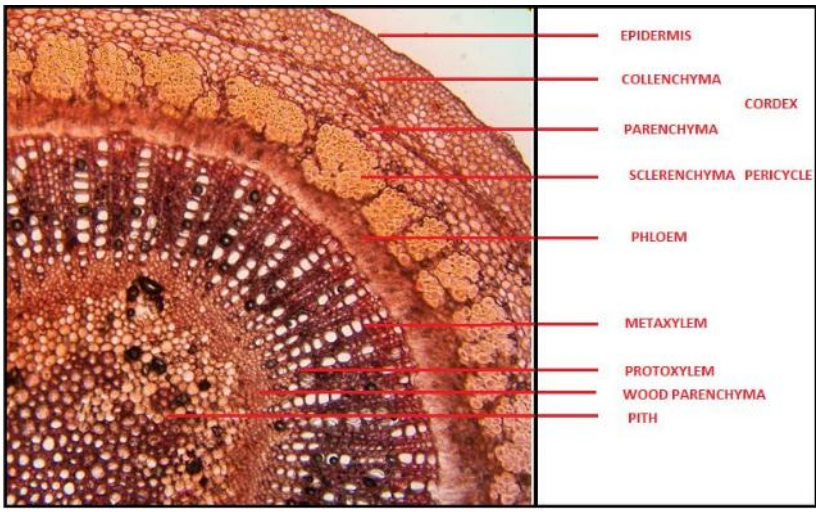

FIGURE 2: T.S. of Stem

b. Microscopic character of leaves

The transverse sections of the fresh leaf through the midrib were mounted in glycerin and observed under microscope. T.S. shows outermost single epidermis layer covered by cuticle. The epidermis is followed by cortex layer of which outer region is consist of collenchymas and inner with parenchyma cells. The refractive body like druser has observed in abundant form and some prismatic crystal were also observed in parenchyma cell of leaf. The trichomes were present in abaxial surface and adaxial hump region of leaf which was two to three cells in structure. The central area of TS showed arc shaped conjoint collateral closed vascular bundle where xylem divided in to inner protoxylem and outer metaxylem region surrounded by abaxial and adaxial phloem.

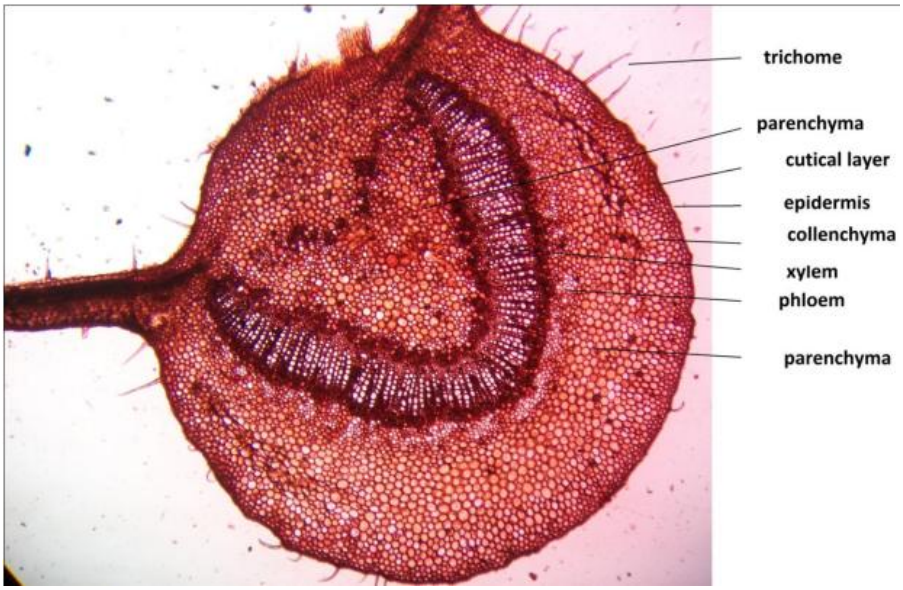

FIGURE 3: T.S. of Leaf through midrib

Stomata study

Stomata related observations were made by taking epidermal peels from both the surfaces. On the basis of occurrence of stomata, plant was hypostomatic type i.e. stomata occurred only on the abaxial surface of the leaflet. Anisocytic type stomata were observed which was 6.66u

in length and 1.38u in width. The frequency was about 70.3mm^2 stomatal index was around 30.06%. The details of frequency and stomata index are show in table 1.

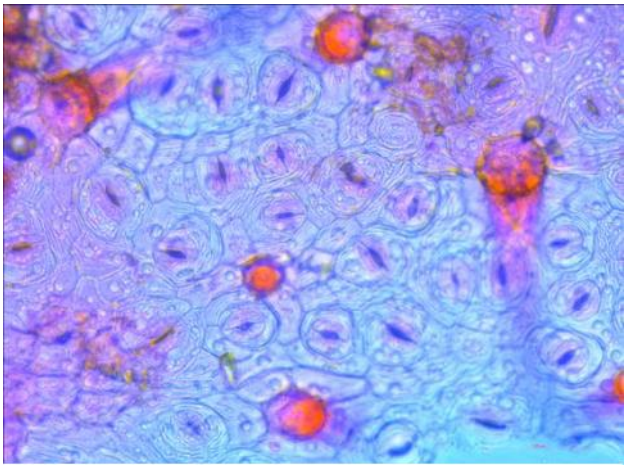

FIGURE 4: stomata, abaxial surface of the leaflet

TABLE 1: Stomatal study of plant *Holarrhena antidysenterica*

Type of stomata	Length of stomata (micro meter)	Width of stomata (micro meter)	Frequency of stomata (mm^2)	Stomata index (%)
Anisocytic	6.66 ± 2.96	1.38 ± 0.75	70.3 ± 5.5	30.06 ± 1.8

(Mean ± SD; n=10)

2. Fluorescence study of powders

The dried flower powder was treated with different reagents and observed under visible and UV light to understand its fluorescence behavior. The behavior of powder under visible and UV light is due to the present of various type of secondary metabolites like alkaloids, phenolics and flavonoids. Observations are recorded in the following table no. 2.

TABLE 2: Fluorescence behavior of *Holarrhena antidysenterica* flowers

Sr. no.	Treatment	Visible light	Ultra violet light
1	Powder (P)	Pale yellow	Fluorescence yellow
2	P + 1N NaOH in water	Dark red	Dark red
3	P + Alcohol	Pale yellow	Fluorescence yellow
4	P + Acetic Acid	Yellow	Fluorescence blue
5	P + 50% HNO_3	Reddish yellow	Fluorescence yellow
6	P + 50% H_2SO_4	Brown	Brown
7	P + 50% KOH	Red	Red

3. Proximate analysis

The total moisture content was determined by drying fresh flower material at 100^o C in hot oven whereas the total ash was determined by igniting the dry flower powder at about 500 to 700^o C the remaining white powder is nothing but the ash. The total moisture of fresh flower material was 79.61± 0.29 with dry matter 20.39 ± 0.29 (where N=15). The total ash content was observed 4.27 %. The total crude fibers were 6.01% and total crude fat was 7.17% per dry material of the flowers. The details are given in table 3.

TABLE 3: proximal analysis of *Holarrhena antidysenterica* flowers.

Sr. No.	Proximal analysis	Percentage (mean ± SD; n=15)
1	Total moisture content	79.61 ± 0.29
2	Total dry matter content	20.39 ± 0.29
3	Total ash content	4.27± 0.98
4	Total Crude Fibre	6.01 ± 1.05
5	Total Crude Fat	7.17 ± 1.35

4. Preliminary phytochemical screening (Qualitative)

16 different phytochemicals were screened in five extracts starting from Non-polar Petroleum Ether to polar water. Amongst all these, water extract was found most suitable for the isolation of most of the phytochemicals. Second most useful solvent was alcohol, in which also some phytochemicals get extracted. Flavonoids were observed in all the solvents while starch was absent in all of them. The results of preliminary phytochemical screening of different solvent extracts of *H. antidysenterica* flower are shown in Table 4. The result exhibited the present and absent of some phytochemical constitutes.

TABLE 4: Phytochemical screening of *Holarrhena antidysenterica* flowers

Sr. No.	Phytochemicals	Petroleum ether	chloroform	Acetone	Alcohol	Water
1.	Anthracene Glycosides	ND	ND	ND	_	_
2.	Flavonoids	+	+	+	+	+
3.	Tannins	ND	ND	ND	_	+
4.	Cardiac Glycosides	ND	--	ND	++	ND
5.	Carotenoids	_	ND	--	--	ND
6.	Saponins	--	ND	ND	ND	++
7.	Steroids	+	_	_	+	++
8.	Volatile oils	ND	+	ND	+	+
9.	Alkaloids	ND	ND	ND	+	+
10.	Amino acids	ND	ND	ND	ND	+
11.	Proteins	ND	ND	ND	ND	+
12.	Carbohydrates	ND	ND	ND	ND	+
13.	Reducing sugars	ND	ND	ND	ND	++
14.	Starch	--	--	--	--	--
15.	Gum and mucilage's	ND	ND	ND	ND	--
16.	Fatty acids	+	ND	ND	+	--

Present +; Absent--; Not Determined – ND

5. Phytochemical Quantitative analysis

The total alkaloids were determined by gravimetric method whereas the total phenolics were analyzed spectrophotometrically by comparing with standard curve of catechol. The total alkaloid content observed in flower powder was 0.91% and total phenolics were 4.25%. The total soluble sugar was estimated by using standard curve of D Glucose and total soluble proteins were compared with bovine serum albumin. Total soluble sugar was 23.88% and soluble proteins were 3.72%. The detail results are shown in table no. 5.

TABLE 5: Estimation of phytochemicals of *Holarrhena antidysenterica* flowers

Sr. No.	Phytochemicals	Mg % (mean ± SD; n=10)
1	Total alkaloids	0.91± 0.28
2	Total phenolics	4.25 ± 0.397
3	Total soluble sugar	23.88 ± 4.31
4	Total soluble protein	3.72 ± 1.36

CONCLUSION

In the present work, pharmacognostic and phytochemical standardization of *H. antidysenterica* is carried out as per the standard guidelines. The flowers of *Holarrhena* are used as vegetables in the central India, showed the presence of various important bioactive compounds, which may contain many Pharmacological activities. This work hints the potentials of *Holarrhena* flowers which are needed to tested pharmacologically. If these activities tested positive then the flowers of *Holarrhena* could be considered as one of the Nutraceuticals. Further, the results of this study may help in standardization, identification and in carrying out further research on this plant based drugs.

REFERENCES

Abdul Rahaman, A.A. (2009) Stomata complex types, stomata density and the stomatal index in some species of Dioscorea. Arch.Biol. Science, Vol. 61, 847-851.

Annonymous (1986) General methods for analysis, Vol. 2. Scientific publications Logas 1-5,137-149.

Chopra, R.N., Chara, I.C., Handa, K.L. and Kapur, L.D. (1982) Indigenous drugs of India, Academic Press, New Delhi, India, 342.

Fox H., Frank, B. Johnson, John Whiting, and Peter P. Roller (1985) Formaldehyde Fixation. The Journal of Histochemistry and Cytochemistry. Vol. 33(8); 845-853.

Ganapathy, S.P.S., Sudeep, H.V., Padmalatha Rai and Ramachandra, Y.L. (2008) Screening of crude extracts of *Holarrhena antidysernterica* Wall against clinically important pathogenic strains. Medicinal and Aromatic Plants Science and Biotechnology 2, 68-71.

Jolly, C.I. and Mechery, N.R. (1996) Comparative pharmacognostical, physicochemical and antibacterial studies on seeds of *Holarrhena antidysernterica* Wall and *Wrightia tinctoria* R. Br. Indian Journal of Pharmaceutical Sciences 58, 51-53.

Khandelwar, K.R., Pawar, A.P., Kokate, C.K. and Gokhale, S.B. (2001) Practical pharmacognosy Vol. 1. Nirali Prakashan, pune 19-153.

Kokate (1994) Practical pharmacognosy. Vallabh Prakashan, New Delhi.15-30.

Naik, V.N. (1998) Flora of Marathwada. Vol. I & II, Amrut Prakashan, Aurangabad.

Prajapati, N.D., Purohit, S.S., Sharma, A.K. and Tarun Kumar (2004) A Handbook of Medicinal plants. Agrobios publishers, Jodhpur, India. Pp.273 .

Sass, J.E. (1998) Elements of Botany Microtechnique. Mc Graw Hill and compony, New York.

Sethiya, K., Brahmbhat, K., Chauhan, B. and Mishra, S. H. (2016) Pharmagogonostic and phytochemical

investigation of *Ensete superbum* (Roxb.) Cheesman Pseudosten. Indian Journal of Natural Product and Resources, l. 7, pp. 51-58.

Singh, N.P., Karthikeyan, S. (2000) Flora of Maharashtra State: Dicotyledones, Vol. I (Ranunculaceae to Rhizophoraceae) Botanical Survey of India.

Sukhdev Swami Handa, Suman Preet Singh Khanuja, Gennaro Longo and Dev Dutt Rakesh (2008) Extraction technologies for medicinal and aromatic plants, International centre for science and high technology.

Supriya Mana, Sachin Singhal, Neeraj K. Sharma and Dharmendra Singh (2010) Hypoglycemic Effect of Holarrhena antidysenterica Seeds on Streptozotocin induced Diabetic Rats. International Journal of PharmTech Research. Vol. (2)2; pp. 1325-1329.

Thimmaiah, S.M. (1999) Standard methods of biochemical analysis. kalyani publication.