

Histochemical and phytochemical study of drug Indian Sarsaparilla *Hemidesmus indicus* (L.) R. Br. ex Schult

Kharat TD¹, DN Mokate^{2*}

^{1,2} Department of Botany, Savitribai Phule Pune University, Pune, Maharashtra, India

Abstract

Hemidesmus indicus (L.) R. Br. ex Schult. Belongs to family Apocynaceae. It is a perennial, slender, twinning undershrub with woody fragrant roots and majorly contains phyto-constituents such as hexatriacontane, lupeol, α -amyirin, β -amyirin and sitosterol. The roots of the drug used as coolant and a blood-purifier in traditional systems of medicines having properties like emetic, diuretic, anti-inflammatory, etc. Due to its over exploitation, limitations for natural regeneration the said drug is in the line of scarcity in nature. For fulfilling the demand of industry it is essential to take study on propagation, cultivation and conservation.

The morphological, anatomical and phytochemical characteristics are essential for determining the taxa at taxonomic level and drug authentication. In the present study an attempt has been made to identify alkaloids, protein, starch, glucoside and tannin by using histochemical tests of different reagents in root, stem and leaf. All the histochemicals tests indicated presence of above mention constituents in the studied plant parts except fats and glucoside. Phytochemical study was carried out for detection of active constituents like alkaloids, flavonoids, glycosides, anthroquinones, amino acids, protein, saponins and tannins. Among all five solvents viz. ethanol, methanol, n-hexane, dichloromethane and aqueous extract, ethanol showed positive tests in all plant parts. The present result can be used for the standardization of drug at the time of utilization by the pharmaceutical industry.

Keywords: *Hemidesmus indicus*, Histochemistry, phytochemical, anatomical

Introduction

India is having richest biodiversity in the world (Arisdason and Lakshminarasimhan, 2016^[1]; Kharat and Mokate, 2018)^[14]. As per FRLHT (2018)^[5] India has 6,198 number of medicinal plant species used in different traditional systems and locally used by ethnic people of the country. Traditional medicine like Ayurveda, Unani and Siddha utilizes the therapeutic potential of plants. About 80% of the third world population still depends entirely on traditional medicine for primary healthcare (Gayathri and Kannabiran, 2008)^[6]. WHO (World Health Organization) has proposed guiding principle for standardizing of the herbal medicines (Kotnis *et al.*, 2004)^[16].

Medicinally important and reputed drug *Hemidesmus indicus* (L.) R. Br. ex Schult. Belonging to family Apocynaceae is once again receiving some consideration in the world of ayurvedic medicine. A climbing plant that grows in central, western and southern parts of India, *Hemidesmus indicus* was used by the native therapists in India for nephritic complaints, syphilis, and sore mouth (in children) (Kharat and Mokate, 2020)^[15]. In 1831, *Hemidesmus indicus* was introduced into European medicine (Remington *et al.*, 2014)^[21]. Majorly phyto-constituents of hexatriacontane, lupeol, α -amyirin, β -amyirin, sitosterol, etc. Today, *Hemidesmus indicus* is receiving renewed attention as an Ayurvedic medicinal an herbal product that may have health benefits for blood purification, kidney and urinary disorders, and skin infections (Greenish, 1899; Karnick, 1998)^[8, 12].

H. indicus is an official drug in Indian and British pharmacopoeia. The plant has wide ranging medicinal

Benefits. The main medicinal properties of the plant are attributed to its roots even though leaf and stem extracts also have medicinal values (Saha *et al.*, 2003)^[22]. 2-hydroxy-4-methoxy benzaldehyde is a high value flavor product and is expensive. It is often used as a substitute for vanilla in ice creams. It is used as an ingredient in herbal tea preparations (Khanna and Kannabiran, 2008)^[13]. The plant is often adulterated with *Ichnocarpus frutescens* or *Decalepis hamiltonii* (Giridhar *et al.*, 2004)^[7].

Due to its over exploitation, limitations for natural regeneration the said drug of *H. indicus* is in the vulnerable in the forests (Saha *et al.*, 2003)^[22]. *Hemidesmus indicus* reveals that the biomass of said drug extensively used in Ayurvedic and other medicinal preparations. To complete the gap it is necessary to develop propagation and agro technique for the *Hemidesmus indicus*. Most of the industries are approaching to farmers/tribes for getting the quality raw material but it is not obtained as the source is forest only. To get quality material its propagation is highly essential.

The microscopic studies are also important to ascertain evolutionary line of development as well as relationship between the closely related taxa. This information will also help in authenticating the herb during drug preparation.

Material and Method

Collection of plant material

The plant material was collected in the month of September 2018 from Mulshi Latitude-18° 30' 18.0252" N and Longitude-73° 31' 5.1780" E (Maharashtra). The fresh parts of plant were used for microscopic studies.

Microscopic studies

Microscopic studies (Wallis, 1950) [27] were carried out by preparing thin section of root, stem, and leaf. Safranin stain was used for staining and mounted in glycerin for observation. Thin sections were observed under binocular microscope (leica-DF450). Photographs at different magnification level were taken by using 12 megapixels Nikon digital camera.

Histochemical Tests

The sections of root, stem, leaf and petiole were tested with the respective reagents. The detection and localization of alkaloids, proteins, starch, fats, glucoside and tannins were determined using methods given by Johanson, (1940) [11]; Trease & Evans, 1983 [25], Fransworth (1966) [4], and Krishnamurthy (1988) [17].

Phytochemical tests

Preliminary phytochemical test for the presence of Alkaloids (by using Wagner's, Mayer's, Dragendorff's and Hager's tests), anthroquinone, amino acids, carbohydrates, flavonoids, flavanols, glycosides, phenols, protein, saponins, steroids, starch and tannins were carried out by standard methods of Horborne, (1973) [9] and Trease & Evans, (2002) [26].

Result and Discussion

Root anatomy

The T. S. through the root showed main tissue systems i.e. the phellem, cortex, vascular tissue. The root was phellem thin walled with reddish brown coloured rectangular cells. Starch grains all cortical cells are fully loaded with large sized starch grains Laticiferous ducts Scattered throughout cortex. Xylem vessels vary in size and phloem alternate with uni-seriate medullary rays. Cambium 1-2 layered compressed cell and medullary rays large number of uniseriate medullary rays, sometimes biseriate.

Sariga and Shajahan (2017) [23] studied the anatomy and powder microscopic characters of Sweta Sariva which showed resembles with present studies of the root. Rajan *et al.* (2011) [19] investigated pharmacognostical and phytochemical studies on *Hemidesmus indicus* root. They found three major components namely phellem, inner cortex and wood regions. Phellem cells were brownish pink, cork cells were radially flattened and rectangular which filled with brownish granules. Several layers of cortical cells were specific characteristic. Phloem consists of parenchyma, sieve elements, ray cells along with several laticiferous ducts. Xylem was narrow medullary rays and woody tissue occupied in central region (fig. 2, A & B).

Stem anatomy

The T. S. through the stem showed the three tissues such as epidermis, cortex and vascular tissue systems. The epidermis layer consists of multiseriate cells with irregular shape and thin walled. The innermost layer of the cortex consists of xylem and phloem. The vascular bundles were relatively different in size and number. The pith is large and consists of hexagonal parenchymatous cells which small triangular intracellular spaces were visible.

The revealed study corroborated with Dina *et al.*, (2015) [3] anatomical studies of the stem of *Pachypodium lamerei* Drake, (Apocynaceae). The cortex constitutes a smaller region than the middle part. It contains starch granules and

prisms of calcium oxalate. The pith was relatively narrower than the upper region of the stem. It was formed of nearly rounded, large parenchyma cells filled with starch grains and showed vascular bundle (fig. 2C).

T.S. of Leaf

Anatomy of leaf studied in lamina and well developed midrib region. Lamina of the leaf exhibits dorsi-ventral nature and showed the upper and lower epidermis with angular polygonal tubular cells covered with smooth cuticle. Mesophyll consists of single layer of palisade parenchyma which has radially elongated cells. Upper epidermis has unicellular covering trichomes. Midrib showed convex shape at the lower side and elevated shape at upper side. Well-developed, collateral vascular bundle was seen at the center of the midrib. The xylem and phloem were collateral (fig. 2D).

The revealed study asserted with Allam *et al.* (2016) [2] the leaf, stem and root of *Carissa macrocarpa*, (Apocynaceae) and observed the transverse section of root, different region *viz.*, outer cork, narrow cortex, the pericycle. The vascular system consists of outer zone phloem and inner zone xylem. Cambium was present in between phloem and xylem. The cortex region showed starch grains which were simple or compound. T. S. of stem showed epidermis enclosing a moderately cortex. Vascular tissues pass through medullary rays enclosing extensive parenchymatous pith in the center region. T. S. of leaf showed that convex with dorsiventral mesophyll cell. Vascular tissues were collateral forming an open curve of xylem, perimedullary phloem and phloem. The parenchymatous pericycle was present.

Histochemical tests

H. indicus is source of alkaloids, protein, starch, glucoside and tannin so in the present study histochemical test of different plant parts was conducted. To analyze histochemistry of *H. indicus* the free hand sections of root, stem and leaf were treated with different reagents. Presence of alkaloids was detected by using different two reagents such as Wagner's reagent that displayed reddish brown colour. Although Hager's reagent gave yellow precipitate indicating presence of alkaloids. All the above stated tests were positive for alkaloids in the parts investigated (Fig. 3, 4, 5, A and B). For ratifying presence of proteins, another set of sections was treated with potassium ferrocyanide which indicated characteristic sequence of colour reactions beginning with instant occurrence greenish blue. This was detected in all the sections which confirmed that protein were present in the plant parts (Fig. 3, 4, 5, C). For confirmation of starch, section was treated with iodine and Potassium iodide indicated colour reactions beginning with instant occurrence dark blue (Fig. 3, 4, 5, D). Set of sections were kept in the Sudan stain for 20 min which not indicated precipitate there for absent of fats ((Fig. 3, 4, 5, E). Section treated with KOH, 90 % alcohol, 2.5% FeSO₄, 20% FeCl₃, 20% HCl, and 1% picric acid. There were not indicated deep blue colure so glucoside absent (Fig. 3, 4, 5, F). For confirming presence of tannin, another set of sections was treated with dilute acidic ferric chloride solution which gave greenish colour (Fig. 3, 4, 5, G). The present study verified with Rajan *et al.*, (2011) [19] and Jeewandara *et al.*, (2017) [10] Pharmacognostical and Phytochemical Studies on *H. Indicus* Root and comparative pharmacognostical

investigation of three different members of Sariva used in Ayurveda.

Phytochemical analysis

Presence of crucial metabolites in crude drug was confirmed by pharmacological action. In the present study, the qualitative screening of alkaloids and glycosides were detected in alcoholic extracts of root, stem and leaf using different reagents. Alkaloids in all studied plant parts were detected using Wagner's, Dragendorff, Mayer's and Hager's reagents. It was found that they were found to be positive in ethanol, methanol and dichloromethane whereas negative in n-hexane and aqueous. Ratha *et al.*, (2012) [20] reported that preliminary phytochemical analysis such as alkaloids, flavonoids, amino acid, tannins and saponins from *H. indicus* and *Vetiveria zizanoides* plants there were observed the same results. Carbohydrate was present in all plant parts with positive reaction to Molisch's reagent which were indicated occurrence of red cum violet ring. The presence of flavonoids and flavanols was confirmed by ammonia test. This test in ethanol and methanol whereas it was negative in n-hexane, dichloromethane and aqueous extracts. Glycosides were also present in all parts those were subjected to Fehling test and Ferric chloride test that gave characteristic white precipitation. The test showed positive results in all solvents except for water extracts. Khanna and Kanabiran, (2008) [13] investigated similar result of phytochemical analysis of *H. indicus* plant presence of different chemical compound *viz*; saponins, flavanoids, terpenoids, tannins, glycosides, saponins, phytosterols, carbohydrates, proteins and phenolic compounds. Anthroquinones were detected in root, stem and leaf extracts, which gave pink to red colour in Borntrager's test.

It was positive in n-hexane and aqueous extracts but negative in ethanol and methanol. To confirm presence of proteins and amino acids in plant parts extracts when added to Ninhydrin and Million's reagent gave blue colours and greenish black respectively. Proteins and amino acids were present in all the solvents subjected to investigation. Saponins were absent in all the plant parts. Steroids were confirmed in all plant parts by Salkowski test indicated by occurrence of lower layer that turned to cherry red colour. Steroids were found to be positive in n-hexane and ethanol whereas negative in methanol and aqueous extracts respectively. I₂KI reagent gave bluish black precipitate to the extract which confirmed presence of starch in root, stem and absent in leaf extracts. Root, stem and leaf extract when added with Trease and Evans and acidic FeCl₃ test gave blue green and greenish colour respectively that confirmed presence of phenols and tannins. It was positive in all solvents except aqueous extract. Fats absent in all plant part of root, stem and leaf. These results were in agreement with Rajan *et al.*, (2011) [19] who performed phytochemical study of *H. indicus* root. Similar results were reported for alkaloids, steroids, tannin, flavonoids and phenols, etc. in root extracts. Najafi and Deokule (2010) [18] investigated the same result of phytochemical study in stem and leaves of *Tylophora dalzellii* Hook.f. (Apocynaceae). Allam *et al.*, (2016) [2] studied a variety of secondary metabolites with important biological and economic values were detected in Apocynaceae as; triterpenes and sterols, cardenolides and alkaloids. Saryam (2012) [24] investigated the Physico-chemical and preliminary phytochemical screening of *H. indicus* root and present of alkaloids, carbohydrates, glycosides, steroids, saponins, terpenoids and polyphenol.

Table 1: Phytochemical analysis of extracts of *H. indicus*

Compounds	Name of Test	Extracts														
		Root					Stem					Leaf				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Alkaloids	Wagner's test	+	+	-	+	-	+	+	-	+	-	+	+	-	+	-
	Mayer's test	+	+	-	+	-	+	+	-	+	-	+	+	-	+	-
	Dragendorff's test	+	+	-	+	-	+	+	-	+	-	+	+	-	+	-
	Hager's test	+	+	-	+	-	+	+	-	+	-	+	+	-	+	-
Anthroquinone	Borntrager's test	-	-	+	-	+	-	-	+	-	+	-	-	+	-	+
Amino acids	Ninhydrin test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carbohydrates	Molisch's test	+	-	+	-	+	+	-	+	-	+	+	-	+	-	+
Flavanoids	Ammonia test	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-
Flavanols	Ammonia test	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-
Phenols	Trease and Evans test	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-
Glycosides	Fehling test	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-
	Ferric chloride test	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-
Protein	Million's test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fats	Copper sulphate tests	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Saponins	Foam test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Steroids	Salkowski test	+	-	+	-	-	+	-	+	-	-	+	-	+	-	-
Starch	Iodine test	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
Tannins	Ferric chloride test	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-

1: Ethanol; 2: Methanol; 3: n-Hexane; 4: Dichloromethane; 5: Aqueous; +: Present; -: Absent



Fig 1: *Hemidesmus indicus*

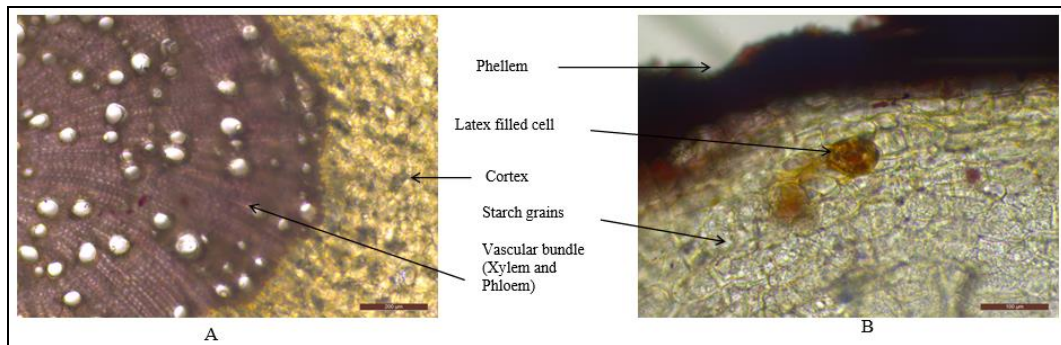


Fig 2: A - T. S. of Root and B - T. S. of Root with starch grains and latex filled cell

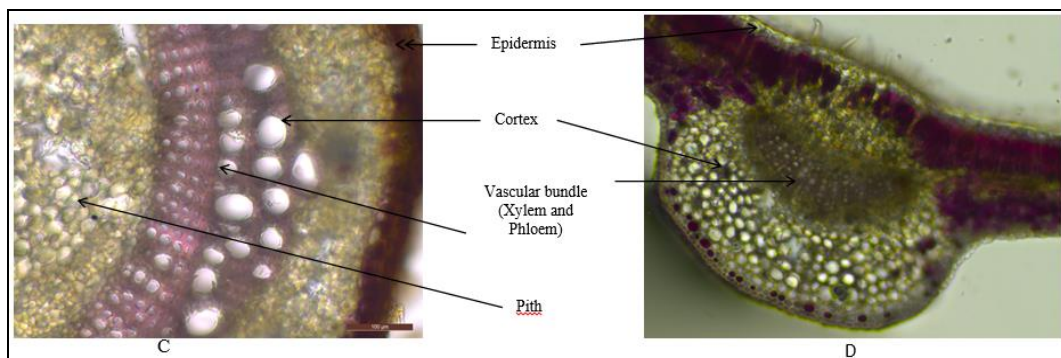


Fig 2: C - T. S. of Stem and D - T. S. of Leaf

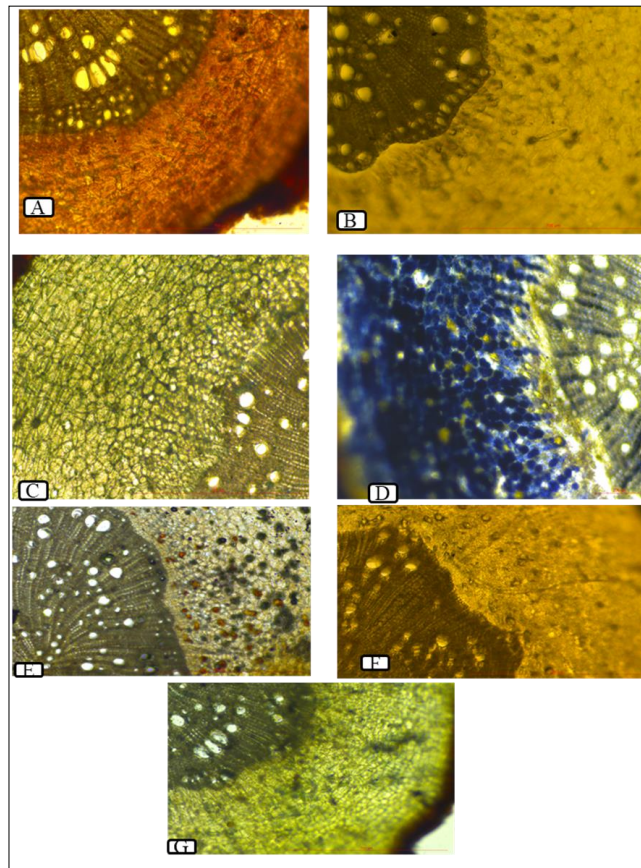


Fig 3: Histochemical tests of Root of *H. indicus* A- Wagner's (reddish brown), B- Hager's (yellow ppt), C- Protein (greenish blue) D- Starch (Dark blue), E-Fat (No colour), F-Glucoside (Absent), G-Tannin (Greenish).

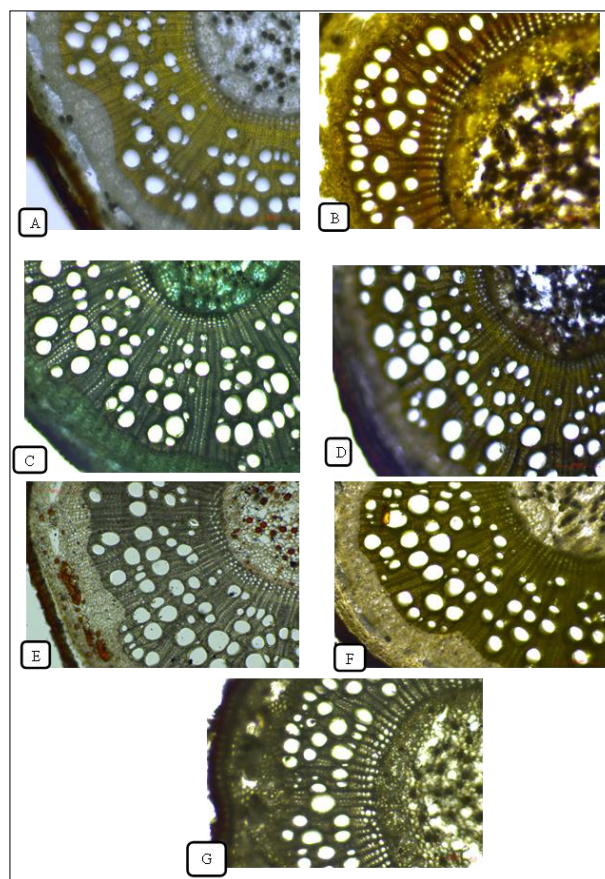


Fig 4: Histochemical tests of Stem of *H. indicus* A- Wagner's (reddish brown), B- Hager's (yellow ppt), C- Protein (greenish blue) D- Starch (Dark blue), E-Fat (No colour), F-Glucoside (brown-yellowish), G-Tannin (Greenish).

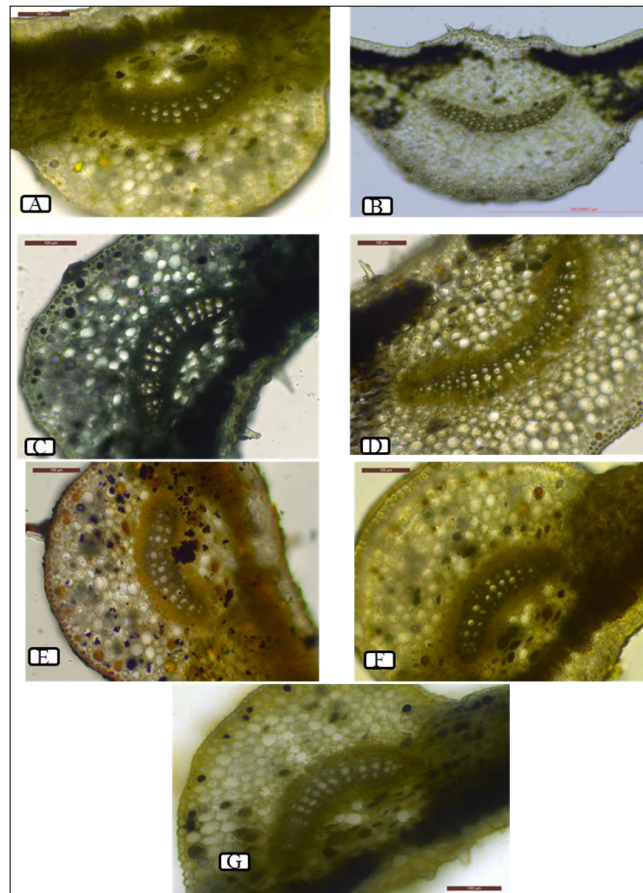


Fig 5: Histochemical tests of Leaf of *H. indicus* A- Wagner's (reddish brown), B- Hager's (yellow ppt), C- Protein (greenish blue) D- Starch (No colour), E-Fat (No colour), F-Glucoside (Absent), G-Tannin (Greenish).

Conclusions

This study is in line with the quality parameters prescribed in Ayurvedic Pharmacopeia of India and also standards set by other international agencies.

The present study has enormous practical implications to authenticate genuine material that has been used in drug trade based on family Apocynaceae in general and *H. indicus* in particular. The detailed investigation pertaining to microscopic features,

Along with biochemical investigations shall aid in confirming and further selecting appropriate plant based drug.

this study it is concluded that Pharmacognostical and phytochemical studies on *Hemidesmus indicus* roots will be highly useful in determining qualitative and quantitative standards which can ascertain the identity, quality and purity of this plant drug that in turn will result in standard herbal preparations for the betterment of human society.

The present investigation thus highlighted urgent need to undertake detailed scientific research to explore this common, endemic and least studied plant for its potential uses in herbal drug preparation.

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