Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(4): 1421-1429
Received: 23-05-2017
Accepted: 24-06-2017

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Pharmacognostic, physicochemical and phytochemical evaluation of *Indigofera cordifolia* L

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Abstract
The pharmacognostic and phytochemical investigations on the whole plant of *Indigofera cordifolia* (Linn.) were carried out. Macroscopic study of leaf and stem *I. cordifolia* revealed some of the characteristic features like size, shape, colour, odour and taste of the crude drug and in microscopic study internal characteristic features like epidermis, cortex, xylem, phloem, trichomes, and stomata were noted. In physicochemical analysis, total ash, acid insoluble ash, water soluble ash and sulphated ash were estimated which were within limits. Extractive value was maximum in methanol as compared to other solvents. Fluorescence analysis with various reagents showed characteristic colouration at day (visible light) and under UV light. The qualitative phytochemical analysis revealed the presence of many bioactive constituents like flavonoids, steroids, saponins, tannins, cardiac glycosides and anthocyanins. This study will be helpful in the future pharmacognostic standardization of this important plant. The parameters laid down will be useful and suitable for compilation of a monograph and help in dentifying this plant in its crude form and prevent it from adulteration and ensure its thereapeutic efficacy.

Keywords: *Indigofera cordifolia* (Linn.), pharmacognosy, phytochemical, physicochemical, macroscopic, microscopic

Introduction
Indigofera cordifolia (Linn.) belongs to the family Fabaceae (Papilionaceae). It is commonly known as 'Heart leaf indigo'. It is a wild herb, commonly plants have nitrogen fixing bacterial nodules in their lateral roots. It is found in Central and Western India. It is an annual plant and grows prostrate on the ground. It is dark green in colour with little pink flowers in clusters which are used commercially to produce indigo dye. *Indigofera* sp. are extensively used in Ayurveda, Unani, Homeopathy and Traditional systems of medicine. The plant is used to treat various diseases like indigestion, rheumatoid arthritis, toxicities, fever, etc. It consists of various Chemical Constituents and Components like 1,2-Benzene dicarboxylic acid, bis(2-methylpropyl) ester, fatty acids like n-hexadecanoic acid (Palmitic acid), 9,12-octadecadienoic acid (Linoleic acid), aliphatic hydrocarbons like Hexadecane, Hexadecene, 3-octadecane, 5-octadecane, terpenoids, steroidal compounds like Cholestane, 3,5-dichloro-6-nitro (3β, 5α, 6β), Pregnan-18-oic acid, 20-hydroxy-(5α), Butanedial, Coumaran (2, 3-dihydro Benzofuran), 7-ethyl, 2, 4-dimethyl, 4H-thiazolo [5,4-b] Indole, 1- (1-oxo, 7, 10-hexadecadienyl) pyrrolidine and 2, 3- dihydro-3, 5-dihydroxy-6-methyl 4H-pyran-4-one in the HA and CH extracts etc, (Anjaria *et al*., 2002) [1]. The plant shows antioxidant activity (Khatri *et al*., 2013) [2].
Each plant drug possesses unique properties in terms of its herbals in botany, chemical constituents and therapeutic potency. So it is important to study pharmacognostic characters of each medicinal plant to differentiate the authentic plant sample from an adulterated one. Adulteration and substitution intentionally or accidentally has to be prevented and the best method is to lay down standardization parameters for each plant which will help in exact identification of the plant in intact form or powdered form. Some of the plants whose pharmacognostic studies have been done are reported in Table 1.
In the present work, an attempt has been done to lay down pharmacognostic, physicochemical and phytochemical characters of *Indigofera cordifolia* L. These parameters will be useful in authentification and standardization of the drug, which can guarantee the quality and purity of the drug and maintain its therapeutic efficacy.

Table 1: Review of some medicinal plants

No.	Plant name	Family	Part used	References
1	*Acacia auriculiformis* A. Cunn. ex. Benth	Mimosaceae	Stem	Sharma *et al.,* 2017 [3]
2	*Baccharis milleflora* DC.	Asteraceae	Stem, leaf	Pereira *et al.,* 2014 [4]
3	*Brachystelma edulis* Coll. and Helmsl.	Asclepiadaceae	Leaf	More and Jadhav, 2015 [5]
4	*Callistemon citrinus* L.	Myrtaceae	Bark	Netala *et al.,* 2015 [6]
5	*Cjanus cajan* L.	Fabaceae	Seed	Pratima and Mathad, 2017 [7]
6	*Emblica officinalis* L.	Euphorbiaceae	Fruit	Raja *et al.,* 2014 [8]
7	*Gmelina arborea* Roxb.	Verbenaceae	Root	Panda *et al.,* 2016 [9]
8	*Hydrolea zeylanica* Vahl.	Hydrophyllaceae	leaf	Qureshi *et al.,* 2017 [10]
9	*Limonia acidissima* L.	Rutaceae	Leaf, stem	Pandavadara and Chanda, 2014 [11]
10	*Mimusops elengi* Linn.	Sapotaceae	Whole part	Srivastava *et al.,* 2017 [12]
11	*Naravelia zeylanica* Linn. DC.	Ranunculaceae	Leaf	Sreeshma *et al.,* 2016 [13]
12	*Nardostachys jatamansi* DC.	Valerianaceae	Rhizomes	Purnima and Kothiyal, 2015 [14]
13	*Operculina turpethum* L.	Convolvulaceae	Leaf	Jalaj and Radhamany, 2014 [15]
14	*Pongamia pinnata* L.	Fabaceae	Leaf	Menpara and Chanda, 2014 [16]
15	*Psidium guajava* L.	Myrtaceae	Leaf	Kaneria and Chanda, 2011 [17]
16	*Pterocarpus santalinus* Linn	Fabaceae	Leaf, stem, bark	Donga *et al.,* 2017 [18]
17	*Quercus infectoria* Olivier.	Fabaceae	Insect gall on leaf	Shrestha *et al.,* 2014 [19]
18	*Tephrosia purpurea* (Linn.) Pers.	Fabaceae	Root	Shah *et al.,* 2011 [20]
19	*Terminalia arjuna* Roxb.	Combretaceae	Bark	Dambal *et al.,* 2014 [21]
20	*Zanthoxylum armatum* DC.	Rutaceae	Fruit, bark leaf	Zafar *et al.,* 2017 [22]

Materials and methods

Plant collection and extraction

The leaf, stem and whole plant was collected in August, 2016 from Rajkot, Gujarat, India. The plant parts were separated, washed thoroughly with tap water, shade dried and homogenized to fine powder and stored in closed container for further studies. For physicochemical studies, 10 g of dried powder of plant was extracted by using solvents (petroleum ether, toluene, ethyl acetate, methanol and water) of different polarities by cold percolation method (Rakholiya *et al*., 2014) [29]. The solvent was evaporated to dryness and dried crude extracts were stored in air tight bottles at 4 ºC. Macroscopic and microscopic characters were studied as described in quality control method (Khandelwal, 2008) [24]. Photographs at different magnifications were taken by using digital camera.

Pharmacognostic studies

Macroscopical studies

Macroscopic studies were carried out by using organoleptic evaluation method. The shape, size, colour, odour, taste, base, texture, margin, apex of leaves and stem of plants were observed (Tyler *et al.,* 1977) [25].

Microscopic studies

Microscopic studies were carried out by preparing thin sections of leaf and stem. The thin sections were further washed with water, stained with congo red, malachite green and mounted in glycerine for observation and confirm its lignifications (10x, 40x). The powder microscopic studies were also carried out and the specific diagnostic characteristic features were recorded (Khandelwal, 2008) [24].

Qualitative phytochemical analysis

The detection of alkaloids, flavonoids, tannins, phlobatanins, saponins, steroids, cardiac glycosides, triterpenes and anthocyanins were carried out following the procedure of Harborne, (1998) [26]. The details of the procedure followed is as described earlier (Pande and Chanda, 2017) [27]

Physicochemical analysis

The physicochemical parameters like loss on drying, total ash, acid-insoluble ash, water-soluble ash, sulphated ash and extractive values were determined as per WHO guidelines (WHO, 2002) [28] in dried powder of different plants. The details of the procedure followed is as described earlier (Rakholiya *et al.,* 2012) [23].

Fluorescence analysis

Fluorescence study of different plants powder was performed as per. A small quantity of the plants powder was placed on a grease free clean microscopic slide and 1-2 drops of freshly prepared various reagent solutions were added, mixed by gentle tilting of the slide and waited for few minutes. Then the slide was placed inside the UV chamber and observed in visible light, short (254 nm) and long (365nm) ultra violet radiations. The colours observed by application of different reagents in different radiations were recorded.

Results

Organoleptic and macroscopic characteristics of *Indigofera cordifolia*

Organoleptic and macroscopic characteristics of *Indigofera Cordifolia* leaf is given in Table 2 and Fig. 1.

Leaves

The leaf was simple and small, green coloured, phyllotaxy opposite decussate, shape lanceolate to ovate, marginentire, apex sub-acute, leaf base symmetrical, small and smooth appearance, veinationreticulate, odour was characteristic and taste was acrid. The average leaf size was 1-1.5 cm in length and 1.1.5 cm in width (Fig. 1).

Stem

The stem was non-woody, green, procumbent, less hairy when growing among dense grasses, cylindrical, up to 10-15 cm height bearing numerous branches and 1 mm thickness, outer surface was rough at base and soft at apex. The odour was characteristic and taste was acrid.

Table 2: Organoleptic features of *Indigofera cordifolia* L.

Parts	Observation
Part	Leaves
Arrangement	Opposite
Size	1-1.5 cm long, 1-1.5 cm wide
Shape	Lanceolate to ovate
Colour	Green
Odour	Characteristics
Taste	Acrid
Appearance	Scabrous
Margin	Entire
Apex	Sub-acute
Base	Symmetrical
Petiole	Short
Texture	Short smooth
Veination	Reticulate veination

Microscopic characteristics

Petiole

The transverse section of *I. cordifolia* is shown in Fig. 2. The petiole was found to be semi-cylindrical shaped towards the distal end of laminal side. The upper epidermis and lower epidermis was single layered. Hypodermis was 2-3 celled with collenchymataous tissue. Ground tissue was parenchymatous, vascular bundles were conjoint collateral open type, the vascular bundles were 'Arc" shape and the size varied from large too small. These were centripetal arranged i.e. xylem surrounded by the phloem (Fig. 2 a).

Leaf

The transverse section of *I. cordifolia* leaf is shown in Fig. 2. The leaf lamina was dorsiventral in nature. The upper epidermis and lower epidermis was single layered (Fig. 2 b). The palisade tissue was single or double layer, elongated and very loosely arranged on both the surfacesof leaf pinna (Fig. 2 c). The mesophyll layer was small 3-5 celled. T.S. passing through the mid rib region showed vascular bundles present towards the ventral surface. Ground tissue was parenchymatous, centrally located collateral vascular bundles surrounded by some parenchymatous cells filled with dark content (Fig. 2 d). The paracytic stomata were present on the lower epidermis. The two subsidiary cells were surrounded by small stomata (Fig. 2 e).

Stem

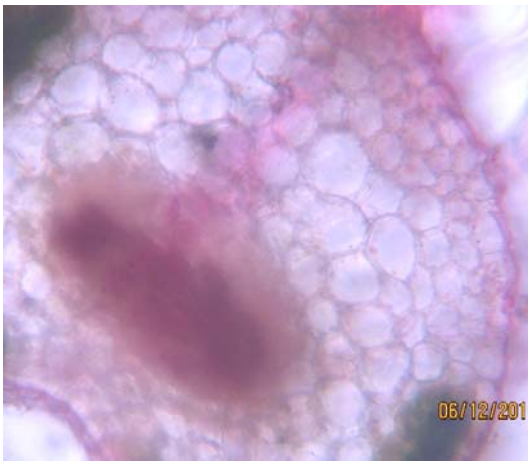
The transverse section of *I. cordifolia* stem is shown in Fig. 3. The epidermis was singlelayered and was covered by thick walled cuticle followed by 2-3 layers of cork cells (Fig. 3 a). The cortex was 6-7 layered; vascular bundles were surrounded by polygonal lignified parenchymatouss and sclerenchymatous cells (Fig. 3 b). The central parenchymatous pith was very small and the vascular bundles were arranged in a ring (Fig. 3 c). The vascular bundles consisted of secondary phloem and secondery xylem; the secondary phloem consisted of sieve tubes, companion cells and phloem parenchyma. The secondery xylem consisted of lignified trachea, tracheids, fibres and vessels. The xylem fibres were pitted elongated and moderately thickened (Fig. 3 d).

Powder microscopy of plant

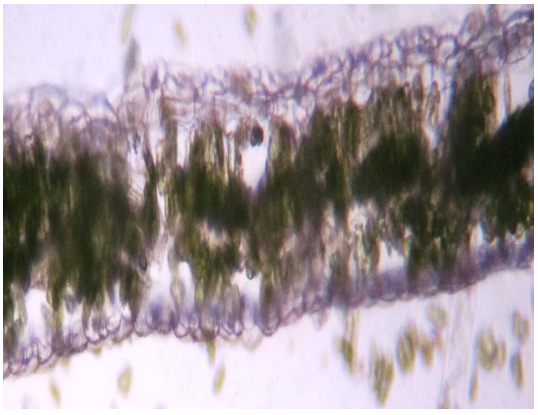
The crude powder of *I. cordifolia* plant was light green in colour, fine, odour was characteristic and taste was slight acrid. The powder microscopy characteristics are shown in Fig. 4. The specific characteristics determined from the powder study under microscopic investigation showed annular vessels, spiral vessels, pitted vessels, unicellular trichomes, paracytic stomata, etc.

Fig. 1: Macroscopic characteristics of *I. cordifolia* leaf

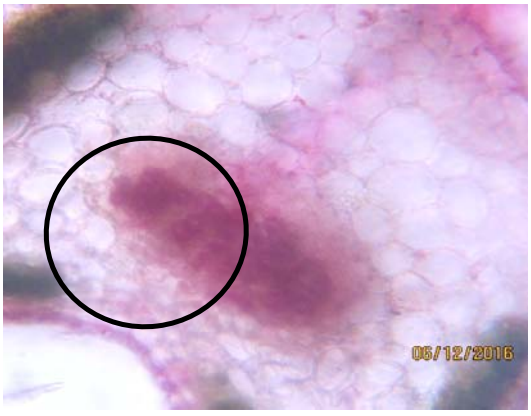
a) T.S of petiole with trichomes



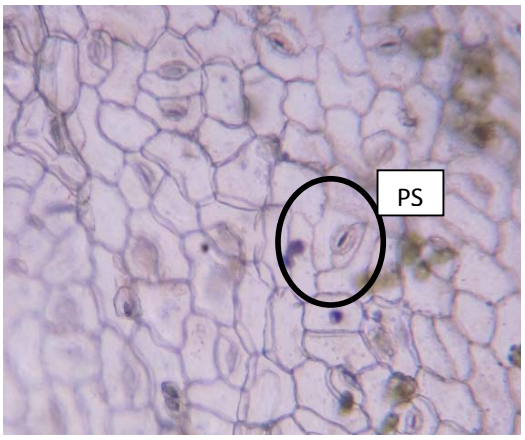
b) T.S of leaf with upper and lower epidermis



c) T.S of leaf pinna

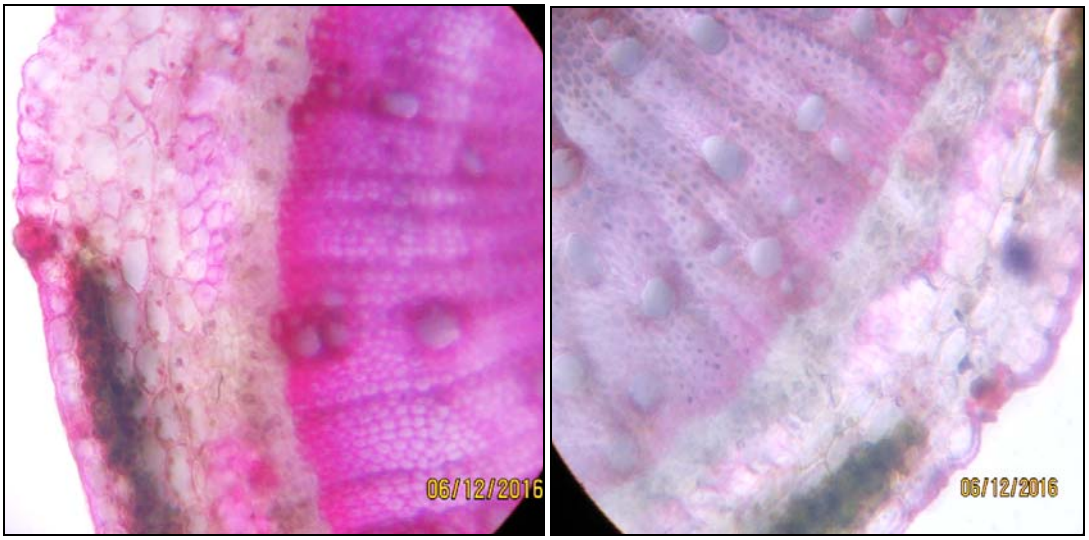


d) T. S of leaf with vascular bundle



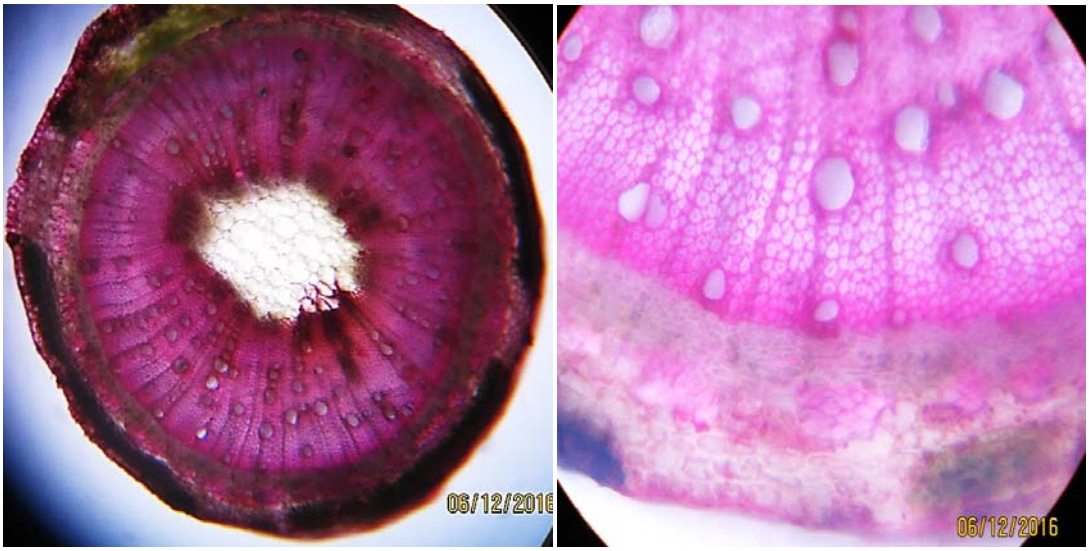
f) Paracytic stomata

Fig 2: Microscopic characteristic of *I. cordifolia* leaf



a) T. S of stem single layer epidermis

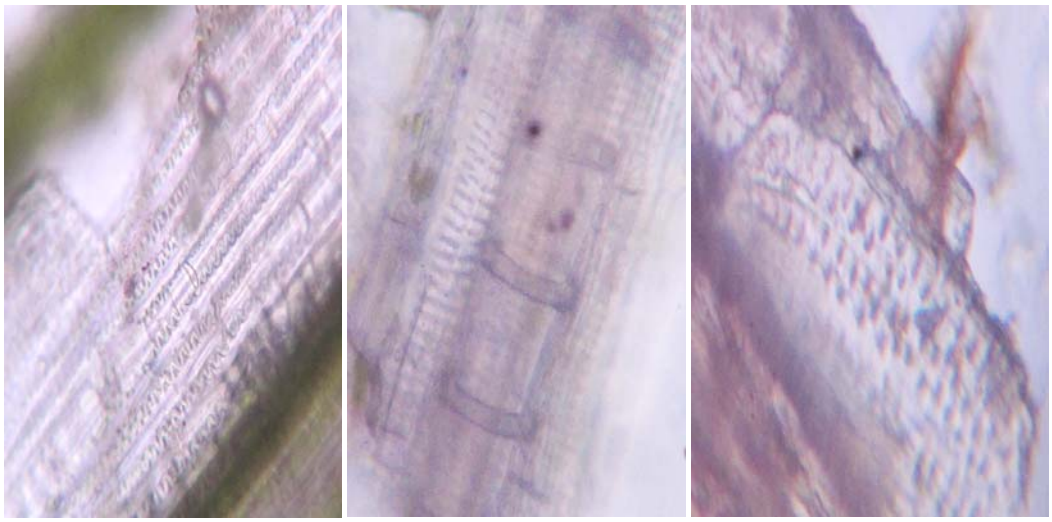
b) T. S of stem with cortex



c) T. S of stem with small pith

d) T. S of stem with xylem vessels, medullary ray

Fig 3: Microscopic characteristic of *I. cordifolia* stem



a) Annular vessels b) Spiral vessels c) Pitted vessels

d) Unicellular trichome e) Paracytic stomata

Fig 4: Microscopic powder study of *I. cordifolia* plant

Qualitative phytochemical analysis

The qualitative phytochemical screening of the crude powder of *I. cordifolia* plant is shown in Table 3. In this plant, flavonoids were present in maximum amount followed by alkaloids (Table 3). Other phytoconstituent were present in trace amount and anthocyanins and phlobatanins, tannins and saponins were absent.

Table 3: Qualitative phytochemical analysis of *I. cordifolia*

Sr. no.	Phytochemicals	Observation
1	Alkaloids	
	(1) Mayer's reagent	+++
	(2) Dragondroff's reagent	+
	(3) Wagner's reagent	+
2	Flavonoids	++++
3	Tannins	-
4	Phlobatanins	-
5	Saponins	-
6	Steroids	+
7	Cardiac glycosides	+
8	Triterpenes	+
9	Anthocyanins	-

Note – (++++) vary more amount, (+++) more amount, (++) less amount, (+) vary less amount, (-) absent.

Physiochemical analysis

The physical constant investigation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The *I. cordifolia* plant is shown in Table 4. The moisture content of dry powder of plant was 86.75 % respectively. Hence it would discourage bacteria, fungi or

yeast growth. The total ash in plant was 21.75 %, while water soluble ash and acid insoluble ash was 1.16 % and 3.66 % respectively. The sulphated ash of the plant was 33 %.The extractive value of plant is shown in Table 4. The maximum soluble extractive value was found in methanol extract (5.56 %) and minimum soluble extractive value was found in petroleum ether extract (0.61 %). The water soluble extractive value of was 15.09 %.

Table 4: Physiochemical parameter of *I. cordifolia*

Sr. No	Parameters	% value(w/w)
1	Loss on drying	86.75
2	Total ash	21.75
3	Water soluble ash	1.16
4	Acid insoluble ash	3.66
5	Sulphated ash	33
6	Petroleum ether soluble extractive value	0.61
7	Toluene soluble extractive value	0.95
8	Ethyl acetate soluble extractive value	1.23
9	Methanol soluble extractive value	5.56
10	Water soluble extractive value	15.09

Fluorescence analysis

Fluorescence study of *I. cordifolia* crude powder was treated with various reagents revealed characteristic fluorescence at 366 nm and 254 nm wavelength Table 5. Some constituents show fluorescence in the visible range in daylight. The UV light produce fluorescence in many natural products which do not visible shine in daylight. Hence crude drugs are often evaluated qualitatively in this way and it is a significant parameter for pharmacognostic evaluation of crude drugs.

Table 5: Fluorescence analysis of *Indigofera cordifolia* plant powder.

Sr. no.	Treatment	Visible light	Under UV light Short Wave length (254nm)	Under UV light Long Wave length (365nm)
1	1N NaOH (aq)	Green	Black	Dark green
2	1N NaOH (alco)	Green	Black	Green
3	Ammonia	Green	Black	Dark green
4	Picric acid	Whitish green	Black	Black
5	Petroleum ether	Yellow green	Green	Green
6	50% HCL	Green	Black	Green
7	50%H2SO4	Green	Black	Brown
8	Ethyl acetate	Green	Light green	Light green
9	Ethyl alcohol	Green	Light green	Light green
10	Methanol	Black	Light green	Light green
11	50% KOH	Black	Yellowish green	Dark green
12	50%HNO3	Black	Black	Brown
13	Acetic acid	Black	Light green	Green
14	Iodine in water (1%)	Black	Dark green	Green
15	FeCl3	Black	Black	Light black

Discussion

The broad use of herbal drugs in conventional medicines, standardization becomes an important measure for ensuring quality, purity and authenticity of the crude drugs. First step in this context is authentification of plant species which can be done by morphological and anatomical analysis or pharmacognostic analysis. It is one of the simplest and cheapest method for establishing the correct identification of the source materials (Nirmal *et al*., 2012; Kumar *et al*., 2012a) [30, 31]. As there is no pharmacognostic work recorded on this medicinally potent plant, the present work was undertaken to lay down the standards which could be useful for establishing its authenticity. The present study reports the morphoanatomical, phytochemical, physicochemical and fluorescence analysis of *I. cordifolia* L. whole plant. Pharmacognostic and physicochemical studies of whole plant acts as a reliable tool for plant identification and detecting adulteration (Desai and Chanda 2014; Zhao *et al*., 2011; Raj and Radhamany, 2012) [32, 33, 34]. Studies of macroscopic and microscopic study can be valuable source of information which is usually and helpful in evaluation of purity and quality of a crude drugs. In the present study, in macroscopic investigation size, shape, odour, taste, colour and habit are identified and in microscopic study epidermis, cortex, pericycle, arregement of vascular bundles, metaxylem, phloem, and pith (small or large) are identified. The phytocemical study revealed different phytoconstituents found in maximum amount (flavonoid, alkaloid), trace amount (steroids, cardiac glycosides, and triterpenes) and absent (tannins, phlobatanins, saponins and anthocyanins). The extractive values give an idea about the chemical constitution of the drug (Kumar *et al*., 2012b) [35]. In the present study, the maximum soluble extractive value was found in methanol extract (5.56 %) as compared to other solvents. The water soluble extractive value of was 15.09 %. The ash values determine the earthy matter or inorganic composition and other impurities present along with the drug. Fluorescence characteristics are an alternative rapid method to ensure the aunthenticity of unsure specimen. When physical and chemical methods are inadequate, the plant material may be identified and differentiated from their adulterants on the basis of fluorescence characteristics (Deore and Namdeo, 2014) [36]. Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Some constituents show fluorescence in the visible range in daylight. The ultraviolet light produces fluorescence in many

natural products. Hence, some crude drugs are often assessed qualitatively in this way and it is an important parameter for pharmacognostical evaluation of crude drugs. Adulteration of the actual raw material is the main cause of degradation of desired therapeutic effect of plant species used in various traditional systems of medicine.

Conclusion

The current investigation reveals the pharmacognostic features, physicochemical and phytochemical properties of *I. cordifolia*. The present findings are associated with standardization of parameters like macroscopic and microscopic characters, phytochemical screening, fluorescent analysis and physicochemical quantification of the plant *I. cordifolia*. Ash values added more strength to crude drug standardization with prominent results indicating the involvement or non involvement of irrelevant matter. Such study on the macro and microscopic anatomy, preliminary phytoconstituent screening and physicochemical parameters are important informations which may be useful in verification and contamination for quality control of this therapeutic plant afterwards.

Acknowledgements

The authors thank UGC-CAS Department of Biosciences, Saurashtra University, Rajkot, India for providing excellent research facilities.

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