GENETIC BARCODING AND BOTANICAL STUDIES ON *POTHOS SCANDENS* L. LEAVES-AN EXTRA-PHARMACOPOEIAL DRUG OF AYURVEDA

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**ABSTRACT**

**Objective:** This study was undertaken to carry out genetic barcoding, pharmacognostical and phytochemical studies on leaves of *Pothos scandens* L. belonging to the family Araceae.

**Methods:** *P. scandens,* was collected from Cochin, Kerala, during the month of August 2019. Its leaves were examined for pharmacognostical characters and physicochemical, qualitative analysis and HPTLC studies was carried out following standard protocols recommended by the Ayurvedic Pharmacopoeia of India. Genetic barcoding of the fresh tender leaves samples was carried out through following standard methods.

**Results:** Leaves are simple, alternate, broadly winged petiole. Transverse section through winged petiole portion showed U-shaped, epidermal cell along with cluster crystals. Transverse section through midrib boat-shaped structure, mesophyll undifferentiated, consist parenchyma cell, epidermis with thick cuticle along with waxy coating. Leaves powder shows green in color and microscopy reveals the presence of prismatic crystals, raphides, fibres, acicular crystals and monocot type 1 stomata. The plant is identified as *Pothos scandens* based on sequence homology and phylogenetic analysis. Qualitative results show the presence of carbohydrates, protein, alkaloids and tannins. HPTLC profile shows 7 spots at both short UV and long UV and ferulic acid and kaempferol were identified through quantitative HPTLC.

**Conclusion:** *Pothos scandens* is a climber with simple, alternate leaves. Acicular crystals and raphides, mucilaginous cavities, undifferentiated mesophyll are key characters of the identification of its leaf. DNA Barcoding results helps in correct identification of *Pothos scandens*. The results obtained from the phytochemical study will be helpful in the determination of the strength of purity and also for further standardization of the plant.

**Keywords:** Analytical, *Anukta drouya*, Genetic barcoding, *Pothos scandens*, Wound healing

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**INTRODUCTION**

Plant resources are a natural treasure of traditional system of medicines, widely used for treatments of a variety of ailments and the correct identification, and quality assurance of the starting materials is a prerequisite to ensure the reproductive quality of herbal medicine which will ultimately contribute to its safety and efficacy [1]. *Pothos scandens* L. (family- Araceae), is an epiphytic climber with rooting branches and is one of the frequently used medicinal plants by the traditional healers of Kerala, West Bengal and Karnataka states of India. In Srilanka the leaves are used to reduce swelling in trauma area, whereas in China it used as blood coagulant. Its leaves are consumed as tea by the Dai ethnic minority. The powdered leaves are traditionally used to cure smallpox, increases milk in cows, stem and leaves in snake bites [2]. The species is native to Himalayas, Madagascar and Indo-Burma region, and is widely available on the moist tree trunk, wet forests, and wall and on the rocks in northeast India. It is also frequently available in Bihar, Goa, Maharashtra, Tamil Nadu, Andaman and Nicobar Islands of India.

The leaves of *Pothos scandens* are simple, alternate, obovate or lanceolate, acute or acuminate, bright green, rounded at the base, petiole winged. Flowers are regular, bisexual, embedded in a globalup spadix with a cymbiform spathe. Spadix is yellow, ovoid or oblong as long as stipe. Fruit is berry, scarlet and oblong [3, 4]. The species has shown its therapeutic potency in various ailments i.e. asthma, snake bite, diarrhea, cancer, smallpox, sprains, epilepsy, skin diseases, boils, swellings, wounds, ulcers, dropsy, vomiting, flatulence, strangury, burning sensation, intermittent fever, skin disorder, reduce swelling, malaria, convulsions and wounds etc [5]. It is also useful in the management of bone fracture, diarrhoea, cuts, wounds, sores, abscesses etc [6]. Various studies on phytochemical analysis of *Pothos scandens* reports phytochemicals like alkaloids, flavonoids, tannins, terpenoids, saponin, catechin, coumarin, phenol, sugar, glycocide and xanthoprotein steroids, proteins, reducing sugars, carbohydrates and cardiac glycosides, phytosterols, fixed oils and fats, gums and mucilage, etc. The preliminary pharmacognostical and phytochemical studies were conducted on the leaves and stem of the species [7]. Although looking at the medicinal relevance, the species needs to be evaluated in a more scientific way. Hence, the present study was carried out to analyze the pharmacognostical parameters of *Pothos scandens* L. leaves covering its morphology, micrometric, genetic barcoding, physicochemical parameters and HPTLC quantification of phenolic acids. The study will lay the QC standards of raw material, ensuring its quality and efficacy of finished products containing the targeted species. The study will also aid in the identification of authentic raw material for the industry and which will ensure the both consistency/uniformity in *Pothos* containing herbal formulations.

**MATERIALS AND METHODS**

**Collection of plant material**

The *Pothos scandens* was identified with the help of different flora and authenticated by the Pharmacognosy expert and its fresh leaf sample were collected from its natural habitat (Longitude: 76 ° 27' 23.9058" and Latitude: 10 ° 10' 39.23328"), Cochin, Kerala. Samples were collected in mature condition during August 2019 and the
herbarium specimen was deposited at the institute museum with the reference number (Phm/6283/2019-20). The material was also preserved in FAA-Formaldehyde Alcohol Glacial acetic Acid (90:5:5) for botanical studies (fig. 1A-D). The collected leaves were washed with potable water and dried under shade, powdered through a mechanical grinder and sieved (60 mesh). The coarsely powdered sample was kept in an airtight glass container for its pharmacognostical and analytical evaluation.

**rbcl amplifications**

Amplification of ribulose-1, 5-bisphosphate carboxylase/oxygenase large subunit (rbcl) region was performed in Veriti Thermal Cycler (Applied Biosystems). The 50μl reaction mixture containing 50ng genomic DNA, 10μM primer, 2.5μl of Taq Master Mix Red with 1.5 mmol MgCl2 (AMPLIQON). To enhance and facilitate the PCR amplification, DMSO (1%) was used in each assay [15]. Polymerase chain reaction (PCR) was performed using rbcl forward Primer ATGTGACCACAAGAGACTAAAGC and rbcl reverse Primer GTAAAATCAAGTCCACCRG [16, 17]. The PCR was set according to the thermal profile as mentioned (table 1). PCR products were sequenced after confirming amplification in 1% agarose gel electrophoresis, and purified using Mini Elute PCR Purification Kit (Qagen).

| Temperature | Time   | No. of cycle |
|-------------|--------|--------------|
| 98 °C       | 45 seconds | 35           |
| 98 °C       | 10 seconds  |              |
| 55 °C       | 30 seconds  |              |
| 72 °C       | 40 seconds  |              |
| 72 °C       | 10 min     | 1            |

**Data analysis**

The initial raw sequences were viewed and trimmed using Bioedit Sequence Alignment Editor Version 7.2.5 [18]. MEGA version 7.0.14 was used for nucleotide Basic Local Alignment Search (BLAST) and multiple sequence alignments to compare the highly similar sequences [19].

**Physico-chemical analysis and preliminary phytochemical screening**

Pharmacognostical parameters of the powdered drug such as moisture content, ash value, acid-insoluble ash, water-soluble extractive value, alcohol-soluble extractive value and pH were carried out as per standard procedure recommended by the Ayurvedic Pharmacopoeia of India (API) [20]. Readings were recorded in triplicate and the mean value was taken into consideration along with the standard deviation. For a qualitative analysis under preliminary phytochemical screening, the methanolic and water extract of leaf were screened for the presence or absence of the major class of compound [21].

**HPTLC study**

**Extraction (samples) protocols**

The coarsely powdered sample was macerated with methanol for 24 h at room temperature (25 °C±2), filtered and the residue was again extracted with fresh solvent. This process was repeated thrice and pooled filtrate was dried in the rotatory evaporator (Buchi, USA) under standard conditions of temperature (55 °C±2) and, pressure (40 mbar), lyophilized (Labconco, USA) and stored at 4 °C for analytical work.

**Chromatographic conditions**

Chemical profiling and method optimization for quantification of metabolites was carried out on 20x10 cm TLC aluminum pre-coated plates with 0.2 mm layer thickness of silica gel 60 F254 (Merck, Germany). Tracks (standard and sample) were applied as 8 mm band with an automatic TLC sampler (CAMAG ATS4, Switzerland) under a flow of Nitrogen gas. The linear ascending development was carried out in an automated developing chamber (CAMAG ADC-2). The saturation time of chamber was conditioned and optimized to 5 min at room temperature (25 °C±2) and relative humidity (55±2%) for better resolution with mobile phase vapors. The plate was allowed to develop up to the height of 85 mm under the tertiary solvent system of toluene ethyl acetate formic acid (5:4:1) from the point of application and the total run time was standardized to 20 min. After development, the plates were air-dried for 30 min and scanning was performed using the CAMAG TLC scanner at...
absorption maxima in reflectance-absorbance mode. Metabolites were quantified based on regression equation of standard area vs. concentration of standard marker and results are expressed on % dry wt. basis of powder sample.

RESULTS

Macroscopic study

Leaves are simple, alternate, obovate to lanceolate, entire, acute apex, coriaceous, bright green above while light green beneath and measure about 7.9-9.8 × 2-2.6 cm, broadly winged petiole with amplexicaul base, veins inconspicuous with midrib and lateral veins almost parallel to the margin, intercostal, reticulate venules (fig. 2A).

Microscopic study

T. S. of winged petiole

Diagrammatic section passing through the winged petiole is 'U' shape and consist of the epidermis. Ground tissue, undifferentiated mesophyll and many vascular bundles. Detailed transverse section shows upper and lower epidermis made up of compactly arranged square shaped epidermal cell covered with thick cuticle. Both the epidermis are interrupted by stomatal openings. Epidermal cells are heavily filled with cluster crystals of CaO. Lamina is few undifferentiated consisting parenchyma cells filled with green chlorophyll pigment. Most of the cell enriched by acicular crystals (1.1 µm, 40x), prismatic crystals (0.5 µm, 40x), cluster and rosette crystals (0.5 µm, 40x). Randomly larger parenchyma cells are filled with mucilage forming mucilaginous cavities. Closed vascular bundle are distributed throughout lamina. Large vascular bundle alternates with small sized vascular bundle. Vascular bundle covered by bundle sheath and it is consists of 5-6 xylem elements and large amount of phloem (fig. 2B-F).

T. S. of lamina passing through midrib

Diagrammatic section pass through midrib is boat shaped. Ground tissue and mesophyll are undifferentiated, vascular bundles are distributed throughout the transverse section. Detailed transverse section shows upper and lower epidermis made up of compactly arranged square shaped epidermal cell with thick cuticle. Both the epidermal cell interrupted by stomata openings. Lamina is made up of undifferentiated parenchyma cell filled with chlorophyll pigments. Most of the cells are enriched by acicular, prismatic, cluster and rosette crystals. Large numbers of mucilage cavities are distributed through-out the lamina. Vascular bundle: large vascular bundle is located at the centre of the undifferentiated lamina and smaller meristems are located towards the epidermal region. Vascular bundle closed type made up of 1-2 xylem vessels and phloem made up of phloem fiber and its elements forming a cap like structure. Some of the isolated fibro vascular bundles are situated at both the epidermal region, consisting only of sclerenchyma cells without any vascular tissue (fig. 3A-I).

Fig. 2: A) Macro measurement of leaf; B) T. S. of winged petiole; C) Upper epidermis; D) Bundle sheath with vascular bundle; E) Parenchyma cells along with prismatic crystals; F) Epidermal cell with cluster crystals

Fig. 3: A) Leaf and winged petiole measurement; B) T. S. of leaf passing through midrib; C) Upper and lower epidermis; D) Epidermis along with thick waxy cuticle; E) Vascular bundle with sheath and micro measurement of vascular bundle; F) Undifferentiated parenchyma cells; G) T. S. of leaf passing through midrib (stain); H) Pericyclic fibres, phloem and xylem; I) Lignified pericyclic fibres, phloem and xylem; J) Monocot type 1 stomata in lower surface; K and L) Micro measurement of stomata (µm)
Surface study of lower epidermis was carried out to determine the type and distribution of stomata and epidermal cell. Quantitative microscopy of leaves was carried out to determine the epidermal cell number, stomatal number, stomatal index and size of the stomata (fig. 3J-L). The stomatal number, stomatal index, stomatal size, epidermal cell size were calculated by trial and error method (by taking 3–5 successive readings. Mean value was taken into consideration). Results are tabulated in table 2.

Powder microscopy

Organoleptic characters of leaf powder show green in colour with slight pungent odour, astringent taste and fibrous touch (fig. 4A).

The microscopy of leaf powder shows the presence of prismatic crystals, raphides, fibres, acicular crystals, rod shaped crystals, brown content, fragment of spongy parenchyma cells, fragment of stomata with epidermal cell, crystal fibre, simple fibre, rhomboidal crystal, oil globule, fragment of epidermal cells, starch grain, fragment of annular vessels, parenchyma cells with chlorophyll pigment, group of lignified fibres (fig. 4B-L).

Table 2: Quantitative microscopy of Pothos scandens leaf

| S. No. | Parameter                      | Result            |
|-------|--------------------------------|-------------------|
| 1     | Type of the stomata            | Monocot type-1    |
| 2     | Length of the stomata (n=5)    | 0.8±0.054μm       |
| 3     | Width of the stomata (n=5)     | 0.7±0.044μm       |
| 4     | Stomatal index                  | 11.1 mm²         |

Table 3: Sequences producing significant alignments through BLAST analysis

| Description                                                                 | Max score | Total score | Query cover | E value | Per. ident | Accession     |
|-----------------------------------------------------------------------------|-----------|-------------|-------------|---------|------------|---------------|
| Pothos scandens voucher Chase, 9989, (K) ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcl) gene, partial cds; plastid | 728       | 728         | 77%         | 0.0     | 92.40%     | JQ933452.1    |
| Pothos scandens plastid partial rbcl gene for ribulose bisphosphate carboxylase | 728       | 728         | 77%         | 0.0     | 92.40%     | AM905732.1    |
| Pothos longipes isolate OF6 ribulose-1, 5-bisphosphate carboxylase/oxygenase large subunit (rbcl) gene, partial cds; Chloroplast | 693       | 693         | 73%         | 0.0     | 92.39%     | KF496718.1    |
| Pothos longipes voucher 129308471 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcl) gene, partial cds; chloroplast | 689       | 689         | 72%         | 0.0     | 92.36%     | KM895540.1    |

DNA barcoding

The taxonomically identified Pothos scandens L. DNA sequences were aligned and was queried for highly similar sequences in NCBI GenBank using nucleotide BLAST Tool from NCBI. It showed that the queried sample sequences were best matching with Pothos scandens voucher 9989 (GenBank accession JQ933452.1 with 0.0 E value and 92.40% ident. It showed 73% query cover for other Pothos longipes species. Therefore the sequence derived from this study confirms its identification as Pothos scandens and the final sequence derived from this study was deposited in NCBI GenBank with accession number MW323301. The sequence derived producing significant alignment through BLAST analysis are given in table 3 and phylogenetic tree of P.scandens is depicted in fig. 5.
Fig. 5: Phylogenetic tree of *P. scandens*

Physicochemical parameters

Leaves powder was found to be devoid of any foreign matter. Detailed results of physicochemical parameters are given in table 4 along with calculated standard deviation.

**Table 4: Physicochemical parameters of *P. scandens* leaves**

| S. No. | Parameter                          | Result       |
|--------|-----------------------------------|--------------|
| 1      | Loss on drying(% w/w)             | 4.60±0.34    |
| 2      | Ash value(% w/w)                  | 4.83±0.85    |
| 3      | Acid insoluble ash (% w/w)        | 0.26±0.39    |
| 4      | Water soluble extractive value(% w/w) | 6.70±0.32 |
| 5      | Alcohol soluble extractive value(% w/w) | 12.48±0.61 |
| 6      | pH(5% aqueous)                    | 6.4          |

Values are expressed in mean±SD (n=3).

Preliminary phytochemical screening

Qualitative test revealed presence of carbohydrate, alkaloids and tannin in aqueous as well as methanolic extract of leaf. The results of tests performed are depicted in table 5.

**Table 5: Results of preliminary phytochemical screening of *P. scandens* leaves**

| S. No. | Parameter   | Test                  | Water extract | Alcohol extract |
|--------|-------------|-----------------------|---------------|-----------------|
| 1      | Carbohydrates | Molisch’s test       | +             | +               |
| 2      | Protein     | Biuret test           | +             | +               |
| 3      | Amino acids | Ninhydrin test        | -             | -               |
| 4      | Steroids    | Salkowski reaction    | -             | +               |
| 5      | Glycosides  | Keller Kiliani test   | -             | +               |
| 6      | Saponins    | Foam test             | -             | -               |
| 7      | Flavonoids  | Lead acetate          | +             | -               |
| 8      | Alkaloids   | Dragendorff’s test    | +             | +               |

[‘+’ = Present, ‘-’ = Absent]

HPTLC study

The HPTLC study reveals that the presence of 7 spots at short UV (254 nm) and long UV(366 nm) in methanol extract of leaf. The *Rf* values obtained are depicted in table 6. The HPTLC quantification of identified phenolics was carried out on methanol extract (extractive yield 12.48%) under the binary solvent system of toluene: ethyl acetate (9:1)[22]. The sample was screened for identification of several poly-phenolics, among which ferulic acid and kaempferol were identified, separated and quantified. The markers were quantified in sample after matching the UV spectra and *Rf* (fig. 6 and 7). The ferulic acid (Rf 0.54) was scanned at 520 nm and kaempferol (Rf 0.58) at 300 nm. Ferulic acid and kaempferol were identified in sample and content are 0.068% and 0.146% on dry wt. respectively (table 7). The method was linearly calibrated with statistically significant correlation coefficient of 0.999 and 0.996 for ferulic acid and kaempferol respectively (table 8, fig. 8). The residual plot curve of targeted standards reveals a decent fit of data (fig. 9) and all the other statistical parameters are within the ICH guidelines. The method was also validated as per ICH guidelines and the values were found to be within the limits (table 9 and table 10).

**Table 6: *Rf* values obtained at UV range of *P. scandens* leaves**

| Sample name | 254 nm | 366 nm |
|-------------|--------|--------|
|              | No. of spot | Max. *Rf* value | No. of spot | Max. *Rf* value |
| Leaf        | 7      | 0.37, 0.44, 0.52, 0.63, 0.79, 0.85, 0.94 | 7      | 0.15, 0.34, 0.44, 0.46, 0.55, 0.62, 0.79 |

Fig. 6: Overlay spectra of standard ferulic acid (1) and *P. scandens* leaf (2)
Fig. 7: Overlay spectra of standard Kaempferol (1) and P. scandens leaf (2)

Table 7: HPTLC quantification of poly-phenolics in Pothos samples

| S. No. | Compound name | % dry weight in samples |
|--------|---------------|-------------------------|
|        |               | P. scandens leaf        |
| 1.     | Ferulic acid  | 0.068%                  |
| 2.     | Kaempferol    | 0.146%                  |

[*- = Absent]

Fig. 8: Calibration curve for standard ferulic acid (A) and kaempferol (B) (n = 5)

Table 8: Statistical parameters for the calibration of standards

| Statistical parameter               | Values* | Ferulic acid | Kaempferol |
|-------------------------------------|---------|--------------|------------|
| Calibration range (ug/spot)         |         | 0.4–2        | 0.4–2      |
| Slope                               |         | 0.0076       | 0.009      |
| Intercept                           |         | 0.0007       | 0.0005     |
| Regression coefficient              |         | 0.999        | 0.996      |
| R²                                  |         | 0.54±0.03    | 0.58±0.05  |
| Standard Deviation                  |         | 0.0048       | 0.0062     |
| Standard Error                      |         | 0.0002       | 0.0004     |
| Average                             |         | 0.0016       | 0.012      |
| LOD (ug/spot)                       |         | 2.088        | 2.090      |
| LOQ (ug/spot)                       |         | 3.327        | 6.335      |

*n=5
DISCUSSION

The present study is an attempt to establish the diagnostic characteristics of *P. scandens.* Observed results can be employed as suitable quality control measures to ensure the quality, safety and efficacy of this herbal drug material. The parameters studied here are useful to identify and authenticate the traditionally important of medicinal plant *P. scandens.* The quality control parameters for the crude drugs, as raw materials, were established with the help of several official determinations based on morphology, microscopy and physicochemical studies [23].

Macroscopically, *Pothos scandens* is an epiphytic climber, leaves are simple, alternate, obovate to lanceolate with broadly winged petiole are distinct features. Which can be useful for plant identification [24]. Microscopy also plays an important role in establishing the authenticity and detection of adulteration/substitutes for herbal raw drugs. The importance of epidermal characters are widely recognized in taxonomic considerations and most times these are successfully used in the identification of taxa at genus and species levels [25]. Similarly, studies in stomata have a great taxonomic as well as a pharmacognostic value the proper identification of medicinal plants [26]. The salient diagnostic characteristics of leaf were monocot type 1 stomata in the lower epidermal surface, vascular bundle, acicular, rosette, prismatic and cluster type of calcium oxalate crystals. These characters can be used for standardization of drug and also used for preparation of plant monographs. The DNA barcoding results showed that the queried sample sequences were best matching with *P. scandens* isolate; therefore, the sequence derived from this study confirms its identification as *P. scandens.* The physical parameters are almost constant for a plant therefor these are helpful setting standards for a crude drug. These parameters are important for detection of drug adulteration or improper handling of raw materials [27]. The obtained value of loss on drying is (4.60±0.34 % w/w) of the leaf, which is low, showed that there is less chance for microbial degradation of the drug during storage. Ash may be derived from the plant tissue itself i.e. physiological ash or from the extraneous matter, especially sand and soil adhering to the surface of the drugs i.e. non-physiological ash, and this kind of ash are determined together, therefore it is referred to as total ash [28]. The level of contamination or adulteration by sand (siliceous earth) can be detected by the level of acid insoluble ash. Obtained values for total ash and acid insoluble ash are 4.83±0.67 and 0.26±0.39, respectively. Physiological ash may comprise mineral nutrients as well. Acid insoluble ash resembles non-physiological ash. 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. The variation in extractable matter in various solvents suggests the fact that the formation of the bioactive principle of the medicinal plants is influenced by several intrinsic and extrinsic factors. High alcohol soluble and water-soluble extractive values reveal polar substance.
like phenols, tannins and glycosides [29]. The alcohol extractive value (12.4±0.61) was observed to be higher than the water extractive value (6.7±0.32) showing that alcohol could be a better solvent for the extraction of the plant. pH value of water extraction of leaf was 6 which shows the weak acidic nature of the crude drug. The preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development [30]. Preliminary qualitative phytochemical analysis made for the leaf of P. scandens revealed the presence of carbohydrates, proteins, alkaloids, flavonoids, glycosides, steroids and tannins. HPTLC fingerprinting is a valuable tool for the analysis of phytochemical because of sensitivity and cost-effectively. The fingerprinting of a plant will help in the identification and quality control of a particular species. It can also give information that will be useful for the isolation, purification, characterization, and identification of marker compounds of the species [31].

It was discovered that Toluene: Ethyl Acetate (9:1 v/v) is the most suitable solvent system for HPTLC of methanol extract of powdered P. scandens leaves. In spectral comparison two similar spectra were found at 0.44 Rf and 0.79 Rf among the 7 peaks at 254 nm and 366 nm. The RF values obtained will be helpful in the drug's standardization. The quantification of phenolic compound, ferulic acid and kaempferol were identified in the sample at 0.54 Rf and 0.58 Rf was scanned at 320 and 300 nm, respectively.

Ferulic acid is a phenolic acid compound which arises from the metabolism of phenylalanine by shikimic acid pathway in plants. Because of its phenolic nucleus and extended conjugated side chain, ferulic acid is an important natural potent antioxidant [32]. Ferulic acid has low toxicity and possesses many physiological functions, including anti-inflammatory, antimicrobial, anticancer (for instance, lung, breast, colon and skin cancer), anti-arhythmic and antithrombotic activity [33]. Kaempferol is a natural flavonol a class of antimicrobial, antitumor, and have anticancer activities [35].

CONCLUSION

*Pothos scandens* is a climber with simple, alternate leaves with broadly winged petiole. Presence of microscopic typical feature of leaves are acicular and rosette crystals, mucilaginous cavities, undifferentiated mesophyll, closed vascular bundle are the important anatomical characters of the leaf. Stomatal index of leaf was 6 which shows the weak acidic nature of the crude drug.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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