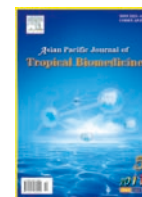




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Microscopic evaluation and physiochemical analysis of *Dillenia indica* leaf

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ABSTRACT

Objective: To study detail microscopic evaluation and physiochemical analysis of *Dillenia indica* (*D. indica*) leaf. **Methods:** Fresh leaf sample and dried powder of the leaf were studied macroscopically and microscopically. Preliminary phytochemical investigation of plant material was done. Other WHO recommended parameters for standardizations were also performed. **Results:** The detail microscopy revealed the presence of anomocytic stomata, unicellular trichome, xylem fibres, calcium oxalate crystals, vascular bundles, etc. Leaf constants such as stomatal number, stomatal index, vein-islet number and veinlet termination numbers were also measured. Physiochemical parameters such as ash values, loss on drying, extractive values, percentage of foreign matters, swelling index, etc. were also determined. Preliminary phytochemical screening showed the presence of steroids, terpenoids, glycosides, fatty acids, flavonoids, phenolic compounds and carbohydrates. **Conclusions:** The microscopic and physiochemical analysis of the *D. indica* leaf is useful in standardization for quality, purity and sample identification.

1. Introduction

Dillenia indica (*D. indica*) Linn. (Family: Dilleniaceae) grows in moist and evergreen forests of India. It has been grown in gardens for its handsome foliage and attractive flower as an ornamental plant. The fruit is said to possess tonic laxative properties and used for relieving abdominal pain. The bark and leaves are astringent^[1,2]. The mixed juices of leaves bark and fruits are given orally for the treatment of cancer and diarrhea^[3]. The alcoholic extract of *D. indica* leaves is reported to possess central nervous system (CNS) depressant activity^[4]. The methanolic leaf extract shows anti-inflammatory activity in carrageenan induced paw edema and acetic acid-induced capillary permeability methods^[5]. Phytochemical studies have shown the presence of the lupeol group of triperpene like betulinic acid and betulin and flavonol such as myricetin. Flavonoids such as kaempferol, quercetin, isorhamnetin, naringenin, and phenolic materials are also present^[6,7]. Four compounds namely, lupeol, betulinol, betulinic acid and stigmaterol can be isolated from the stem extract of the plant^[8]. The crude methanol extract of the roots

shows analgesic, antidiarrhoeal activities and reduced GI motility in animal models^[9]. For standardization and quality assurance purposes, the following three attributes must be verified: authenticity, purity and assay^[10]. Hence, in this work we report an attempt for the standardization of *D. indica* leaf by microscopic evaluation and physiochemical analysis.

2. Materials and methods

2.1. Chemicals

Phloroglucinol, glycerin, hydrochloric acid, chloral hydrate, potassium hydroxide and all other chemicals used in the study were of analytical grade.

2.2. Plant material

D. indica leaves were collected from the campus of Kurukshetra University, Kurukshetra, India and identified by Dr. HB Singh, Scientist F & Head, Raw Material Herbarium & Museum, NISCAIR, New Delhi, India. A voucher specimen of the plant was preserved in the herbarium for reference (NISCAIR/RHMD/Consult/-2009-10/1381/182/1).

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2.3. Macroscopic and microscopic analysis

The macroscopy and microscopy of the plant were studied according to the method of Brain *et al*[11]. For the microscopic studies, cross transverse sections of fresh leaves were mounted in glycerin as well as stained with phloroglucinol–HCl and studied per standard procedures[12,13]. Coarse powder was used to study microscopical characters of leaf powder.

2.4. Physiochemical analysis

Physiochemical parameters such as ash and extractive values were performed according to the official method prescribed and the WHO guidelines on quality control methods for medical plants material[14–16].

2.5. Preliminary phytochemical screening

Preliminary screening was carried out using the standard

procedure Kokate^[10].

3. Result

3.1. Macroscopic characteristics

D. indica is a handsome evergreen tree, 30–80 feet. in height and 6 feet. in girth, with a dense round crown (Figure 1a). The leaf is oblong–lanceolate 8–14 inch long and 2–4 inch broad with pointed end and toothed. The upper part of the leaf as well as vein beneath is covered with hairs (Figure 1b).

3.2. Microscopical characteristics

3.2.1. Leaf microscopy

D. indica leaf surface shows the anomocytic types of stomata which is characteristics of family Dilleniaceae (Figure 2a). Leaf surface also shows the presence of veins, vein–



Figure 1. Macroscopic characteristic of *D. indica*.
a: Uniform look of *D. indica*; b: Oblong–lanceolate leaves of *D. indica*.

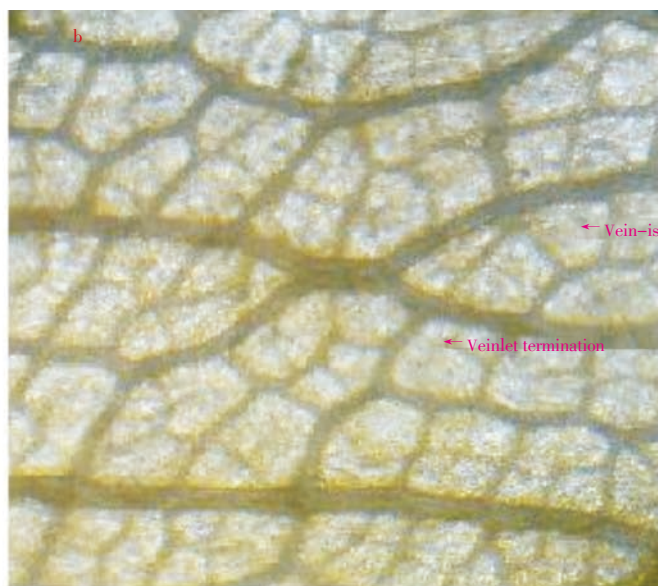
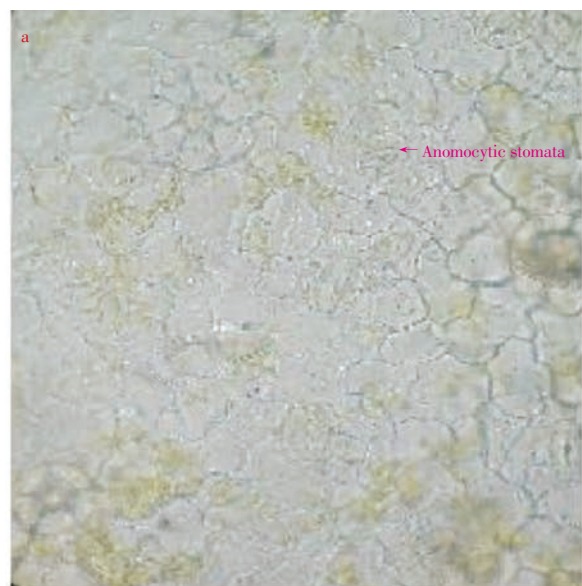


Figure 2. Leaf surface of *D. indica*.
a: Stomata; b: Veins, veinlet termination & vein–islet.

islets and vein terminations (Figure 2b). Transverse section of leaf (Figure 3a) shows the epidermis layer, and patches vascular bundles (xylem and phloem), collenchymas, etc. The vascular bundles were stained pink with phloroglucinol and conc. HCl (Figure 3b) Trichomes are unicellular and lignified. Strips of collenchyma are present below and upper layer of epidermis (Figure 3c). Leaf constants such as stomatal number, stomatal index, veinlet terminations and vein-islet number were measured. The results were shown in Table 1.

Table 1

Leaf constants.

No	Parameters	Value (in 1mm ² area)
1	Average stomatal number in 25 different fields (400×)	130
2	Stomatal index (4000×) lower surface	15.6-18.5
3	Vein-islet number (50×)	16-18-20
4	Vein-termination number (50×)	10-12-14

3.2.2. Powder microscopy

The fine powder was mounted in glycerin as well as

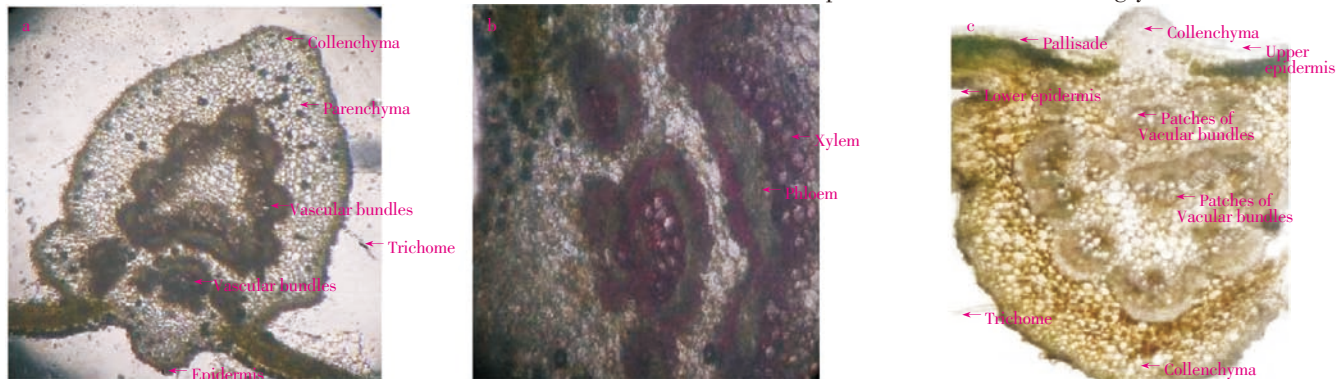


Figure 3. Transverse section of *D. indica* leaf.

a: T.S. (stained) of *D. indica* leaf (100 ×); b: Vascular bundles (400 ×); c: T.S. (unstained) of *D. indica* leaf (100 ×).

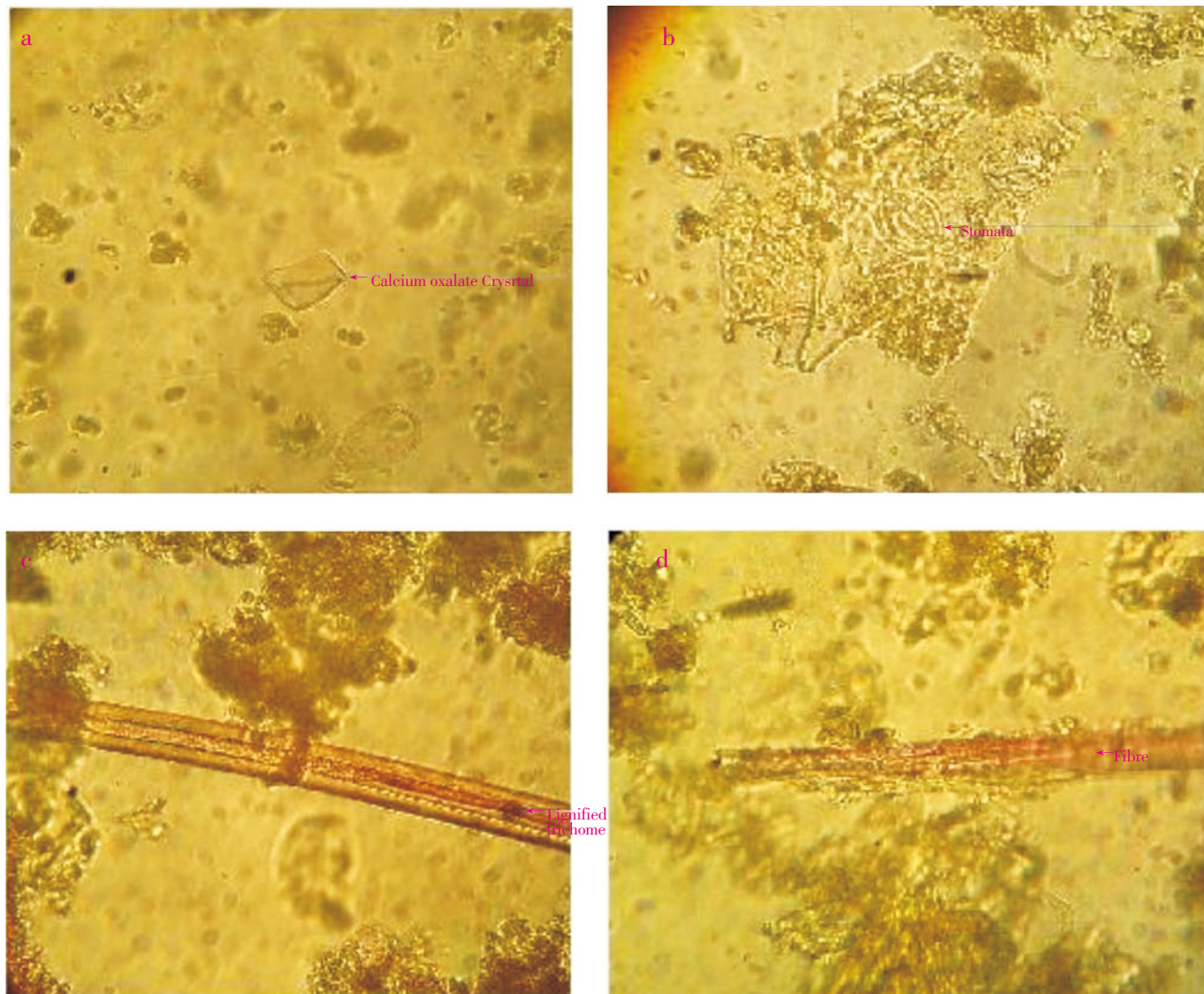


Figure 4. Powder characteristics of *D. indica* leaf (400 ×).

stained (phlorogucinol + conc. HCl). After observation under microscope, it showed presence of unicellular lignified trichomes, anomocytic stomata, calcium oxalate crystals, epidermal cells, xylem vessels, etc. (Figure 4).

3.3. Preliminary phytochemical screening

Preliminary phytochemical screening revealed the presence of steroids, terpenoids, saponins, fatty acids, flavonoids, phenolic compounds, glycosides and carbohydrates.

3.4. Physiochemical parameters

The physiochemical parameters such as ash values, losses on drying, swelling index and percentage of foreign matters were measured and shown in Table 2. The results of extractive values of different solvents were shown in Table 3.

Table 2

Physiochemical parameters.

No	Parameters	Value (%w/w)
1	Foreign matter	0.45
2	Loss on drying	3.2
3	Total ash value	11.25
4	Acid insoluble ash	8.25
5	Water soluble ash	5.5
6	Swelling index	1.75 mL

Table 3

Extractive values of crude drug.

No	Parameters	Extractive values (%w/w)
1	Pet. ether soluble extractive	9.7
2	Chloroform soluble extractive	7.2
3	Methanol soluble extractive	27.7
4	Water soluble extractive	31.51
5	Alcohol soluble extractive	26.42

4. Discussion

Today sophisticated modern research tools for evaluation of the plant drugs are available but microscopic method is still one of the simplest and cheapest methods to start for establishing the correct identity of the source materials^[17]. In the present work microscopy evaluation and physiochemical analysis of *D. indica* leaf were carried out. Morphological and histological studies of the leaf will enable to identify the crude drug. The macroscopical characters of the leaf can serve as diagnostic parameters. The microscopical studies of the transverse section showed presence of unicellular lignified or non-lignified trichomes and anomocytic stomata, which is characteristic of the family Dilleniaceae. 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^[18]. Preliminary phytochemical analysis indicated presence of steroids, terpenoids, glycosides, fatty acids, flavonoids, phenolic compounds and carbohydrates. The information obtained from preliminary phytochemical screening will be useful in finding out the genuity of the drug. Ash values of a drug give an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The percentage of total ash, acid insoluble ash and water soluble ash are carried out. Extractive values are primarily useful for the determination of exhausted or adulterated drugs^[19].

In conclusion, the present work was undertaken with a view to lay down standards which could be useful to detect

the authenticity of this medicinally useful plant. Microscopic study and physiochemical standards can be useful to substantiate and authenticate the drug.

Conflict of interest statement

We declare that we have no conflict of interest.

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