Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
https://www.phytojournal.com
JPP 2023; 12(1): 668-671
Received: 11-10-2022
Accepted: 16-12-2022

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Studies on anatomy, physico-chemical and thin-layer chromatography of rhizome, root and leaf of *Dracaena trifasciata* (Prain) Mabb

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DOI: https://dx.doi.org/10.22271/phyto.2023.v12.i1f.14611

Abstract
Dracaena trifasciata (Prain) Mabb., commonly known as mother-in-law's tongue, used in the treatment of various ailments such as ulcer, jaundice, skin itches, urinary diseases, asthma, cough, snake and insect bites in folklore medicine. In the present investigation, anatomical, physico-chemical and thin-layer chromatographic identification of rhizome, root and leaf has been studied in detail and provided diagnostic key to identify the original drug from the adulterant(s).

Keywords: *Dracaena trifasciata*, rhizome, root, leaf, anatomy, thin-layer chromatography

Introduction
Dracaena trifasciata (Prain) Mabb. (Syn: *Sansevieria trifasciata* Prain; Family - Asparagaceae), commonly known as snake plant, mother-in-law's tongue in English, a perennial, erect, herbaceous evergreen succulent plant, native to tropical West Africa, found in wild tropical and subtropical regions, also grown as an ornamental plant in many places of the world. In folklore medicine, it is used for the treatment of different ailments such as ear-ache, ulcer, jaundice, pharyngitis, skin itches, urinary diseases, analgesic and antipyretic is well known [1]. In Bangladesh, it was used as a whole plant for treatment of alopecia, malaria, tonic and snake bite [2]. Leaves and rhizomes were used for treating bronchitis, asthma, cough, snake and insect bites [3]. It is used in traditional medicine for influenza, cough and respiratory inflammation. Roots and leaves have secondary metabolites such as saponins that exhibit remedy for cough, snake bite, sprain, bruise, boil, abscess, respiratory inflammation and hair tonic [4]. The plant also possess such as antidiabetic [1], anti-allergic, anti-anaphylactic [3], and thrombolytic activities [5]. The leaves extract possess antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* bacteria [6]. Recent study proved that the leaf has anti-alopecia activity [7]. The plant contains phyto-constituents such as flavonoids, steroidal sapogenins namely 25S-ruscogenin and sansevierigenin, pregnane glycosides, and steroidal saponins [8-11], Methyl pyrophaeophorbide A, Oliveramine, (2S)-3′,4′-Methylenedioxy-5,7-dimethoxyflavane, 1-Acetyl-β-carboline, Digiprolactone, Trichosanic acid and Methyl gallate [7].

Several pharmacological and toxicological studies have been carried out in *D. trifasciata*. However, there is lack in detailed pharmacognostical study to identify the crude drug. Hence, the present study has been carried out to standardize the anatomical features of leaf, rhizome, root, physico-chemical analysis and thin-layer chromatographic finger-print profile to serve as a possible tool for proper identification.

Materials and Methods
Anatomical studies
Fresh plants (5 year old) were collected from Cholayil Medicinal Plant Conservation Park, near Uthukkottai, Thiruvallur district, Tamil Nadu. The fresh rhizome, root and leaf were cut in to small pieces and fixed immediately in FAA for 24 hr, embedded in paraffin wax after dehydration and infiltration. Sections were taken using rotary microtome to the thickness of 8-12 µm [12], stained with toluidine blue [13] and photographed.

Physico-chemical evaluation
For the analysis of water soluble extractives, alcohol soluble extractives, Total ash and Acid insoluble ash methods adopted from the Ayurvedic Pharmacopoeia of India [14].

Thin-Layer Chromatographic (TLC) Analysis

For the TLC analysis, rhizomes and leaves were shade dried separately for a week and powdered. 2 g of powdered samples was extracted under reflux with methanol (3 times) in a water-bath, then concentrated and dried. The residue was re-dissolved in methanol and used for the TLC spotting. For stationary phase precoated Silica Gel F^{254} (Merck) plate and for mobile phase Toluene: ethyl acetate (3:1) was used. After the plate development, dried and was dipped in 1% Vanillin H_2SO_4 and heated at 105 °C for colour development and the spots Rf value are recorded.

Observations

Macroscopic characters of rhizome (Figure- 1:1-2)

Fresh rhizomes are cylindrical, 10 to 20 mm thickness, light brown in colour, surface smooth, shiny, brittle, mucilage present in cut surface and fibrous up to 10 cm long with prominent leaf scars. Dried rhizomes are wrinkled longitudinally, outer surface papery, thin, light brown in colour, cut surface shows white in colour and fibrous; odour not specific and taste bitter.

Fig 1: *Dracaena trifasciata* plant

1. Habit
2. Dried rhizome
3. Dried leaf

Microscopic characters of rhizome (Figure - 2:1-2)

The cross section of rhizome about 10 mm in diameter shows circular in outline with prominent outer multi-layered epidermis. Epidermis consists of 10-12 rows of rectangular, tangentially elongated, thin-walled suberized cells. Ground tissue is undifferentiated, composed of parenchymatous cells which contain mucilage. Vascular bundles are numerous, scattered throughout the ground tissue and are collateral, endarch and closed. The outer bundles are smaller and become gradually larger towards inner. (Figure – 2:1). Each vascular bundle is surrounded by a conspicuous sclerenchymatous bundle cap (fibre sheath). Xylem consists of 6-8 vessels and metaxylem vessels situated towards outside and protoxylem vessels at the base. The phloem present just above the metaxylem, consists of sieve tubes and companion cells. No calcium oxalate crystals are found (Figure – 2:2).

Macroscopic characters of root (Figure - 1:1)

Roots are thin, wiry, surface rough, brown in colour, 1.5 to 2.0 mm thickness and up to 15 cm long. No characteristic odour and taste.

Microscopic characters of root (Figure - 2:3-4)

The root about 2 mm thickness shows circular in outline with distinct portions of outer epidermis, middle cortex and vascular region and inner pith (Figure – 2:3). Epidermis consists of 4 rows of thick walled suberized cells which are cubical or triangle in shape. Cortex composed of multi-layered thin walled isodiametric parenchymatous cells with intercellular spaces. Endodermis and pericyle markedly separate the vascular region from the cortex (Figure – 2:4). Vascular cylinder consists of xylem and phloem which are arranged alternatively. Xylem exarch and phloem circular or oval in shape surrounded by xylem. Pith occupies centre region made up of parenchymatous cells. No calcium oxalate crystals are found in cortex and pith regions.

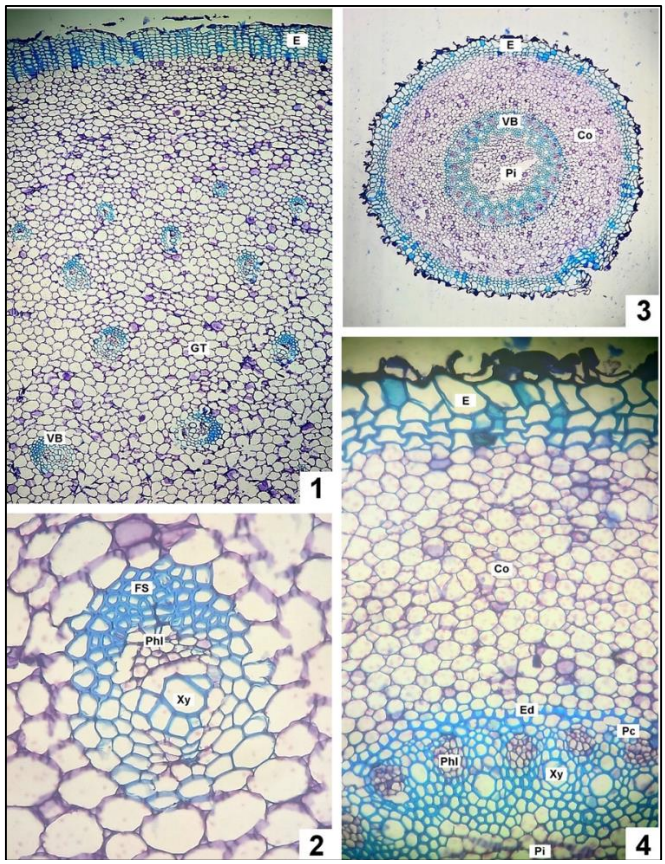

Fig 2: Anatomy of rhizome & root

1. T.S. of rhizome
2. Enlarged rhizome vascular bundle
3. T.S. of root
4. Enlarged root portion

Macroscopic characters of leaf (Figure - 1:1 & 3)

Leaves erect, clusters, up to 1 m or more tall, leathery, linear-lanceolate, sword –shaped, succulent, deep green with light green to grey-white waxy cross-striped, margin entire, tip acute. Dried leaves pale green to light brown in colour, longitudinally wrinkled, curved, cut surface fibrous; no characteristic odour, taste bitter.

Microscopic characters of leaf (Figure - 3:1-5)

The transverse section of leaf shows single layer of epidermis with thick cuticle in both upper and lower surface and cells are square to oval shape in outline (Figure – 3:1-2). In upper view, the epidermal cells are short or long, rectangular or polygonal, elongated and parallel to the leaf axis. Cuticular ornamentation is smooth without trichomes or any other

appendages on both surfaces (Figure – 3:1-2). The leaf is amphistomatic and tetracytic stomata (Figure – 3:5). The cross section of lamina midrib region is usually more or less D-shaped with the thickness ranging from 1.0 mm to 5.0 mm. The mesophyll cells are isobilateral, divided into an outer region of chlorenchyma and inner water-storage tissue, which is clearly distinct from the peripheral mesophyll (Figure – 3:1). Vascular bundles are oval shape in outline, collateral, closed, endarch and consist of a well-developed sclerenchyma cap above the phloem and oriented towards centre region (Figure – 3:3). Calcium oxalate crystals are represented by raphides, and they are present in the chlorenchyma and the central mesophyll with varying frequency (Figure – 3:4).

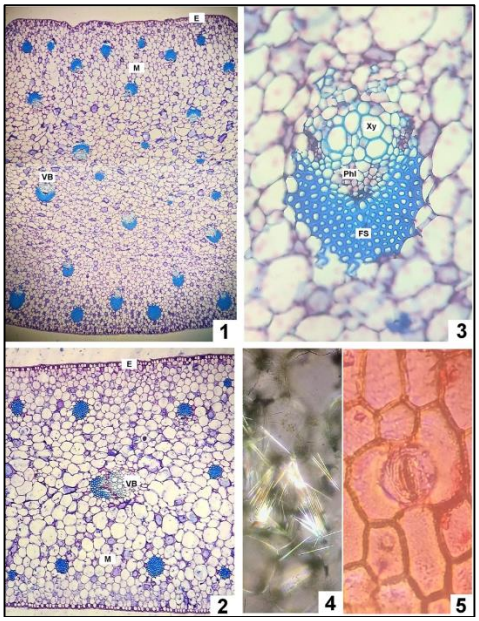

Fig 3: Anatomy of leaf

1. &
2. T.S. of leaf
3. Enlarged leaf vascular bundle
4. Raphides crystals
5. Stomata

Phytochemical analysis

The results of physico-chemical analysis and TLC fingerprint profiles are presented in Table- 1 & 2.

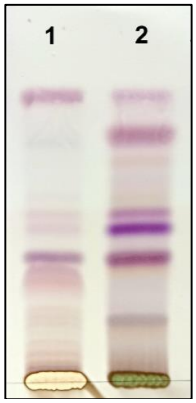

Fig 4: TLC fingerprint

Track – 1. Rhizome
Track – 2. Leaf
(Co – Cortex; E – Epidermis; Ed – Endodermis; FS – Fiber sheath; GT – Ground tissue; M – Mesophyll; Pc – Pericycle; Phl – Phloem; Pi – Pith; VB – Vascular bundle; Xy – Xylem)

Table 1: Physico-chemical analysis

Parameters	Rhizome	Leaf
Water soluble extractives	32.60 ± 0.41	18.44 ± 0.19
Alcohol soluble extractives	16.63 ± 0.44	3.06 ± 0.18
Total ash	8.75 ± 0.12	14.24 ± 0.13
Acid insoluble ash	3.88 ± 0.04	4.80 ± 0.52

Values from triplicate (Mean ± SD)

Thin-Layer Chromatographic (TLC) Analysis (Figure – 4):

Table 2: Rf values (visible light)

Rhizome	Leaf
0.33 – Pink	0.18 – Pink
0.36 – Violet	0.36 – Violet
0.45 – Light violet	0.45 – Light violet
0.49 – Light violet	0.49 – Light violet
0.70 – Light violet	0.70 – Light violet
0.83 – Light violet	0.83 – Light violet

Discussion and Conclusion

Plant drugs are extensively used in traditional systems of medicine such as Ayurveda, Siddha, Unani and Homeopathy. In traditional systems and herbal raw materials markets, different plant species are sold under the same drug name and this is one of the major problems faced. In order to avoid adulterations, standardizations of these herbal drugs are an important tool to establish their identity, purity, safety and quality. To standardize or validate a drug, various parameters such as macroscopic, microscopic and phytochemical analysis are done. Microscopic evaluation and thin layer chromatographic fingerprint is one of the simplest and inexpensive methods to establishing the correct identification of the source material and useful for setting standards for crude drugs. Hence, the present work was undertaken to study the anatomy, thin-layer chromatographic fingerprint and physicochemical evaluation of rhizome, root and leaf of *Dracaena trifasciata*.

The following anatomical features are suggested to diagnose the drug.

Rhizome

Multi-layered epidermis consist rectangular, tangentially elongated cells. Undifferentiated ground tissue contains mucilage. Vascular bundles numerous, outer bundles are smaller and gradually larger towards inner, surrounded by a conspicuous sclerenchymatous bundle cap. Xylem consists of 6-8 vessels, endarch. Phloem consists of sieve tubes and companion cells.

Root

Epidermis 4 layers thick walled, cubical or triangle shape cells. Endodermis and pericyle present. Xylem exarch and phloem circular or oval in shape surrounded by xylem. Pith occupies centre region.

Leaf

Epidermis with thick cuticle, cells are square to oval shape, in upper view, cells are short or long, rectangular or polygonal, elongated. Amphistomatic and tetracytic type stomata. Mesophyll cells are isobilateral. Vascular bundles are oval shape, collateral, closed, endarch with well-developed sclerenchyma cap, oriented towards centre region. Raphides present in mesophyll.

Thin-layer chromatographic (TLC) analysis

The Rf - values and colour of the spot shown in Table - 2 and Figure - 4 can largely distinguish the parts of the plant.

Acknowledgement

The authors are thankful to Mr. Pradeep Cholayil, Chairman & Managing Director and Mrs. Jayadevi Pradeep Cholayil, Directress - Cholayil Private Limited for their constant support and encouragement in carrying out this study.

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