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PHARMACOGNOSTIC STANDARDISATION OF THE LEAVES OF *PIMENTA DIOICA* LINN.

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ABSTRACT

Pimenta dioica commonly known as Allspice was used by early Central American civilizations as a flavoring for chocolate and found to have good anti-microbial action. The current study was therefore carried out to provide requisite pharmacognostic details about the plant. The results of the study could be useful in setting some diagnostic indices for the identification and preparation of a monograph of the plant.

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INTRODUCTION: Standardization means adjusting the herbal drug preparation to a defined content of the active constituent. The concept of standardized extracts definitely provides a solid platform for scientific validation of herbals. Medicinal plant materials are characterized according sensory microscopic and macroscopic characteristics. Organoleptic evaluations can be done by means of organs of sense, which provides the simplest and quickest means to establish the identity and purity and thereby ensure quality of a particular sample. A number of different bases are used for morphological studies and natural variations in these characteristics play an important role for preliminary evaluation of crude drugs. The basis of analysis by evaluation of microscopic characters is that there are always sufficient differences in the same type or different types of plants as far as the cell characteristics are concerned. Standardization profiles of herbal drugs are not available for most drugs¹. This study is an attempt to study the complete pharmacognostical profile of *Pimenta dioica* leaf, which belongs to the family Myrtaceae.

Myrtaceae is a family of about 100 genera and 3,000 species of evergreen shrubs and trees; well represented in Australia, the East Indies and tropical America. The family is divided into two sub-families, the Myrtoideae [fruit, berry or drupe] and the Leptospermoideae [fruit, or loculicidal capsule]. Genera of the Myrtoideae include *Myrtus* [100 spp.], *Psidium* [140 spp.], *Pimenta* [18 spp.], *Eugenia* [1000spp.], *Pseudocaryophyllus* and *Syzigium* [Jambosa]. Many of the genera provide important volatile oils and spices- for example, Cloves and its oil, Eucalyptus oil, Cajuput oil and Pimento. *P. dioica* is indigenous to West Indies and tropical America and grown in gardens in India. The plant is reported to be cultivated in gardens, especially in Bengal, Bihar and Orissa. It is said to grow well in

Bangalore and fruit heavily. It has been recommended for growing in the hilly districts of Mysore and along the river valleys. It is also found to grow in poor but well drained soil, both in high ranges up to 1065 m, above sea-level and in the plains of Kerala. *Pimenta dioica*, commonly known as Allspice was used by early Central American civilizations as a flavoring for chocolate. As a medicine, Allspice has much the same use as Cloves and their oils are similar. It works well as a digestive and has an antiseptic and slightly anaesthetic action².

Allspice berries owe their characteristic odor to the presence of an essential oil [3.3-4.5%] which contains eugenol [65-85%] as the principal constituent, together with eugenol methyl ether [9.6%], phellandrene, cineole, caryophyllene and a terpene alcohol. The dried leaves on steam distillation yield 0.7-2.9 % of an essential oil, which like berry oil contains, eugenol as its main component³. According to Pruthi⁴, allspice berry owes its characteristic odor to the presence of an essential oil [3.3-4.5%], concentrated mainly in the pericarp.

The oil of the pimenta berries has a yellow to yellowish-red color, darkening with age, and possesses the characteristic color and flavour of allspice. It contains eugenol [65-80%] as the principal constituent. As per the work of Purseglove and Brown⁵, *P. dioica* leaf oil of Jamaican origin contains more eugenol [95-98.5%], than the other Caribbean leaf oils along with 1, 8-cineole, caryophyllene and alpha pinene. Two oils produced by steam distillation of the leaves of *P. dioica* of Jamaican origin were examined by GC/MS, by Tucker and Maciarelo⁶, wherein the oils were found to be rich in eugenol [66.38-79.24%]. The leaf oil of *P. dioica* of Cuban origin has been analyzed by a combination of capillary gas chromatography and GC/MS. The main constituent was found to be eugenol -

54.26%⁷. In an earlier study one of the authors, Rao *et al*⁸ reported the *in vitro* antimicrobial activity of the essential oil of the leaves of allspice obtained from India. As the leaves of *Pimenta dioica*, has not been subjected to any pharmacognostical investigations hitherto, it is felt that this important spice has to be studied in this direction.

MATERIALS AND METHODS:

Procurement of leaves: The leaves used for the investigation were collected from a private garden situated at Puttur, Karnataka, India and also from a private farm in Kasaragod, Kerala, India during the months of March/April and May/June. The leaves were identified by two local taxonomists, viz., Prof. Rajagopal, Mahatma Gandhi Memorial College, Udupi and Prof. K. Gopalakrishna Bhat, Poorna Prajna College, Udupi, Karnataka. The leaves were shade-dried and powdered and stored in air-tight containers to prevent the loss of volatile oil till further use. A herbarium was prepared and the voucher specimen PD08-01 is stored with us. Collected fresh leaves were washed and used for evaluation of microscopic and organoleptic characteristics. The shade-dried powder was used for physicochemical analysis and preliminary phytoprofilng.

Microscopy: Fresh leaves of *Pimenta dioica* were selected for the microscopical studies. Microscopical sections were taken by free hand sectioning. Histochemical detection of the cell contents were carried out. Photomicrographs of the microscopical sections were taken. Microscopic examination of the powder was carried out using different chemical reagents.

Quantitative Microscopy: The leaf constants for *Pimenta dioica* were calculated by standard methods⁹.

Physico-chemical parameters: The physico-chemical parameters such as total ash, acid insoluble ash, water soluble ash, extractive values were carried out by the method described in the Indian Pharmacopoeia¹⁰.

Preliminary Phytochemical Profiling: For the preliminary phytochemical profiling, 50 g of the air-dried powdered drug was successively extracted with the following solvents of increasing polarity in a soxhlet apparatus, viz., petroleum ether [60°C - 80°C], toluene, chloroform, acetone, methanol and water. All the extracts were concentrated by distilling the solvents and the extracts were dried in an oven at 50°C. Each time before extracting with the next solvent, the marc was dried in an air oven below 50°C. The marc was finally macerated with water for 24 hours to obtain the aqueous extract. The completion of the extraction was confirmed by evaporating a few drops of extract from the thimble on a watch glass to observe that no residue remained after evaporation of the solvent. The liquid extracts obtained with different solvents were collected. The consistency, odour, colour, appearance of the extracts and their percentage yield were noted. The extracts were then subjected to various qualitative tests using standard methods, to determine the presence or absence of various phytoconstituents such as alkaloids, glycosides, flavanoids, carbohydrates, amino acids, saponins, sterols and terpenoids, anthraquinone glycosides, coumarins, carotenoids, tannins, phenolic compounds, fixed oils and fats by usual methods¹¹.

RESULTS:

Organoleptic Characters: *Pimenta dioica* is a bushy evergreen tree, 6.6-10 m tall, occasionally attaining a height of 13.3 m. It has a slender upright trunk, much branched at the top and

covered with a smooth grey bark. The leaves are polished green, opposite, oblong-lanceolate, tapering to the petiole, blunt and somewhat emarginate at the top; entire dark green above, paler and gland dotted beneath, and a prominent midrib 4-6 inches long. Leaves are 11-11.5 cm X 5-6 cm in size. The leaves are very aromatic when fresh, abounding in essential oil.

Microscopy:

Transverse section of the leaf: The transverse section of the leaf shows a dorsi-ventral condition as shown in **Fig. 1 [a]-[e]**. The tissues represented in the lamina and the midrib regions are as follows:

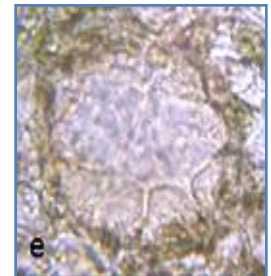
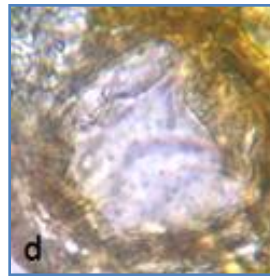
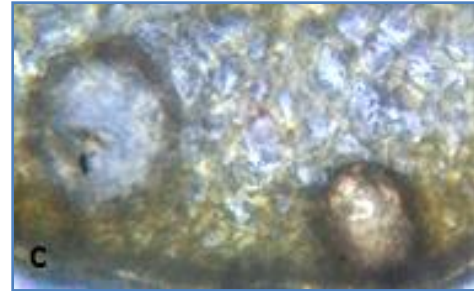
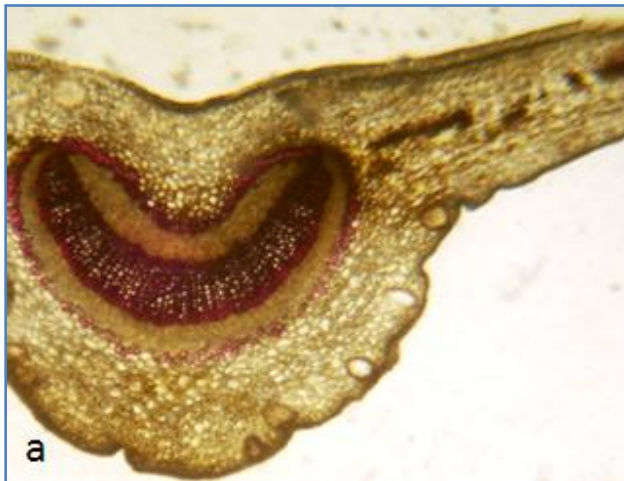


FIG. 1: PHOTOMICROGRAPH OF TRANSVERSE SECTION THROUGH THE MIDRIB REGION OF *PIMENTA DIOICIA* LINN. LEAF- (A) MIDRIB AND LAMINA REGIONS; (B) VASCULAR BUNDLE IN MID RIB REGION; (C) OIL GLANDS; (D) LYSIGENOUS OIL GLAND- ENLARGED VIEW; (E) SCHIZOGENOUS OIL GLAND- ENLARGED VIEW

Lamina: The lamina of the leaf shows the upper epidermis, mesophyll and lower epidermis. The upper epidermis is single-layered with a thick cuticle, rectangular cells and no trichomes of any kind. The Mesophyll being a dorsiventral leaf is differentiated into palisade and spongy parenchyma. Lysigenous and Schizogenous oil glands, brown-colored cork tumors, sphaeraphides [cluster crystals] and obliquely cut vascular strands are seen very frequently in the mesophyll.

Palisade cells are single layered, cells elongated and compact. Spongy Parenchyma is 5 to 8 layered, loosely arranged with intercellular spaces. Big cluster crystals [sphaeraphides] are seen frequently. Vascular strands are seen at times. Lysigenous and Schizogenous oil glands are seen towards the upper and lower epidermis. The lower epidermis resembles the upper epidermis.

Midrib: The lower and upper epidermal layers are continuous over the midrib. The midrib is occupied by the cortical parenchyma with the vascular bundle embedded in the middle. Vascular bundle is arc shaped, collateral with xylem towards upper epidermis and phloem towards the lower epidermis. Pericyclic fibers are seen as a ring around the vascular bundles. Surface preparation shows beaded epidermal cells and Cruciferous stomata.

Powder characteristics: The powdered leaves of Allspice have an aromatic aroma and pungent taste. The powder sample was mounted in chloral hydrate and in phloroglucinol: concentrated hydrochloric acid respectively, the following diagnostic characters were observed

Epidermal cells: With irregularly beaded walls as seen in surface view.

Stomata: Cruciferous stomata or anisocytic type, meaning thereby that the stomatal pore is surrounded by three epidermal cells of which one is invariably smaller than the other two.

Oil Glands: Large secretory oil glands, either entire or in fragments.

Calcium Oxalate Crystals: Fragments of mesophyll tissue, containing big cluster crystals.

Cuticle: Thick, shining, hyaline and curved fragments.

Fibers: Lignified fibers, seen along with vascular strands.

Quantitative Microscopy: Different leaf constants are tabulated in **Table 1**.

TABLE 1: QUANTITATIVE MICROSCOPY OF ALLSPICE LEAF

Leaf Constants	Mean Values
Stomatal Index	2.947
Palisade Ratio	4
Vein Islet Number	6

Physico-chemical parameters: The percentage of total ash, acid insoluble ash, water soluble ash and extractive values are tabulated in **Table 2** and **3**.

TABLE 2: PHYSICO-CHEMICAL ANALYSIS OF LEAVES OF ALLSPICE

Nature of the Ash	Percentage w/w of ash [Mean Values]
Total Ash	‡ 9.7
Acid insoluble Ash	‡ 1.4
Water soluble Ash	‡ 2.7

TABLE 3: EXTRACTIVE VALUES OF ALLSPICE LEAF

Nature of the Extractive	Percentage w/v of Extractive [Mean Values]
Water soluble extractive	‡ 4.612
Alcohol soluble extractive	‡ 7.453
Petroleum ether extractive	‡ 0.213

Preliminary Phytochemical Screening: Preliminary phytochemical screening indicated the presence of only tannins in the aqueous and methanolic extracts [**Table 4**].

TABLE 4: PHYTOCHEMICAL PROFILE OF ALLSPICE LEAF

TYPE OF CONSTITUENT	PETROLEUM ETHER	TOLUENE	CHLOROFORM	ACETONE	METHANOL	WATER
Tannins	–	–	–	□–	+	+
Glycosides	–	–	–	–	–	–
Steroids	–	–	–	–	–	–
Carbohydrates	–	–	–	–	–	–
Alkaloids	–	–	–	–	–	–
Proteins	–	–	–	–	–	–
Amino acids	–	–	–	–	–	–
Flavonoids	–	–	–	–	–	–

-: Absence; +: Presence

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