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Valuable assessment of the Foliar Micromorphology and phytochemical physicochemical profile of *Myristica fragrans* Houtt.(Myrsticaceae)

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ABSTRACT

To study in detail the micromorphology and physicochemical analysis of the leaves of *Myristicafragrans* Houtt family Myrstecaceae. Methods: Macroscopy, microscopy, physicochemical analysis, preliminary phytochemical screening and other WHO recommended parameters for standardizations were performed. Results: Leaves are elliptical or oblong lanceolate, pointed, alternate 5-15cm long and 2-7 cm broad. Dark green with entire margin, acuminate base. `Microscopic evaluation revealed the presence of paracytic or rubiaceous stomata in lower epidermis and apostomatic upper epidermis, two layered palisade cells run along the midrib also, tanniniferous cells, vascular bundles capped with sclerenchyma fibres xylem vessels, phloem, fibres. Vein islet numbers, vein termination numbers, stomatal number, stomatal index and other physico chemical tests like ash values, loss on drying, extractive values were determined. Preliminary phytochemical screening showed the presence of alkaloids,steroids, tannins, proteins and aminoacids, flavonoids, terpenoids, mucilage, volatile oil, saponin , carbohydrates and absence of mucilage, fixed oil. Conclusions: The microscopic using histological identification , microscopic constants and other physico chemical examinations of the leaves of *M.fragrance* can be used as a rapid, inexpensive botanical identification technique and is useful in standardization, hence would be of immense value in authentication of the leaf.

Keywords: *Myristica fragrans; Myrstecaceae; Micro morphology; Macroscopy.*

1. INTRODUCTION

Plants have been the basis for medical treatments through much of human history, and such traditional medicine is still widely practiced today. In many developing countries, a large proportion of the population relies on traditional practitioners and their armamentarium of medicinal plants in order to meet health care needs.The ancient scholars only believed that herbs are only solutions to cure a number of health-related problems and diseases. Most of the drugs, thus formulated, are free of side effects or reactions. This is the reason why herbal treatment is growing in popularity across the globe. These herbs that have medicinal quality provide rational means for the treatment of many internal diseases, which are otherwise considered difficult to cure. [1]

In this study we selected a widely available plant *Myristica fragrans* Houtt belonging to family Myristicaceae is known for its medicinal properties in traditional medicinal practices for various diseases. The Myristicaceae family belongs to magnoliids and is the members of flowering plant. They commonly originate from their native of Asia, America, Pacific islands and Africa. The trees do not give flowers until around 9 years old, but once start flowering they continue to do so for further 75 years. The trees bear 2 to 3 crops a year. [2] It is being now cultivated in tropical regions, especially Grenada in the West Indies, Sri Lanka and India. The plant grows at an altitude of 700-4500m with a temperature 25-30ºC. It requires a rainfall of 2000-3500mm.Nutmeg can grow on any kind of soil provided there is sufficient water but without any risk of water logging. [3] Traditionally the infusion of *M.fragrans* leaf were used to relieve flatulence, intestinal spasm and hypertension. [4] The leaf essential oil has herbicidal properties and is used for preparing soaps and chewing gum. The fruit essential oil of *M.fragrans*possess Anti angiogenic activity, Antimicrobial activity, Anti-cancer activity. [5]

2. MATERIALS AND METHODS

Formalin, acetic acid, ethyl alcohol, chloral hydrate, toluene blue, phloroglucinol, glycerine, hydrochloric acid, and all other chemicals used in this study were of analytical grade.

2.1. Plant collection and authentication

Leaves of the plant *M.fragrans* selected for our study was collected from ellappara , Idukki District, Kerala, India during the month of July 2018 and was authenticated by Dr.Stephen, Department of Botany, American college, Madurai

2.2. Macroscopic analysis

Macroscopic observation of the plant was done. The shape, size, surface characters, texture, colour, odour, taste was noted. [6]

2.3. Microscopic analysis

Transverse section midrib region of fresh leaf pieces were cut and fixed in FAA and then dehydrated by employing graded series of ethyl alcohol and tertiary butyl alcohol [7], sections were taken using microtome, permanent mount was prepared using saffranin fast green double staining technique[8]. In order to supplement the descriptive part, the photomicrographs in different magnifications of all necessary cells and tissues were taken with NIKON coolfix 84(x) digital camera and Lab photo 2 microscopic unit.

2.4. Powder microscopy

The coarse powder of leaf was used to study the microscopial characters of the leaf powder [9,10]. Physiochemical analysis Total ash, acid insoluble ash, water soluble ash, sulphated ash, loss on drying, extractive values and leaf constants such as vein islet numbers, vein terminal number, stomatal number and stomatal index, were determined [9,10].

2.5. Preliminary phytochemical screening

Preliminary phytochemical screening was carried out to find out the presence of various phytoconstituents using standard procedure[11].

3. RESULTS

3.1. Macroscopy

M.fragrans is a spreading aromatic evergreen tree growing up to 5 to 13m high, occasionally 20m belonging to the family Myrsticaceae. The leaves are pointed, Elliptical, or oblong lanceolate, glabrous, obtuse and leathery in shape Arranged alternate and are borne on stems about 1 cm long with a length of 5-15cm × 2-7 cm and dark Green in colour and glossy above with entire Margin and acuminate Base(Fig 1, 1A, 1B).

Figure - 1: Habit and habitate of *M.fragrans*

Figure - 1A- Dorsal view of *M.fragrans* leaf.

Figure - 1B- Ventral view of *M.fragrans* leaf.

3.2. Microscopy of the leaf

The leaf is dorsiventral with prominent midrib. Transverse section (T.S) of the leaves through the midrib showed the following tissue systems (Fig 2). Shape: Adaxial side (Fig:4) is slightly raised flat whereas the abaxial side(Fig:5) is convex. Epidermis is composed of single layered and have a strongly thickened cuticle on the top of the papillose in adaxial side. Smaller in size than the abaxial epidermal cells. Polygonal with straight walls, apostomatic. Palisade tissues runs along the midrib region.(Fig 3) The abaxial epidermis has undulating anticlinal walls and contains clustered crystals.

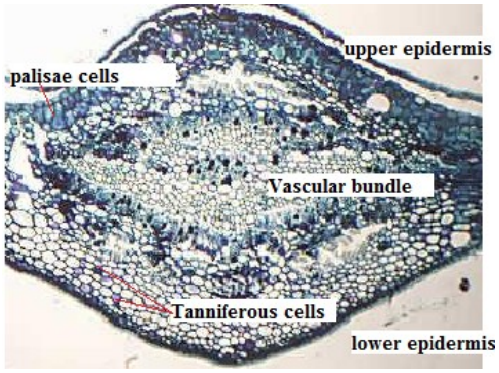

Figure - 2: T.S through midrib.

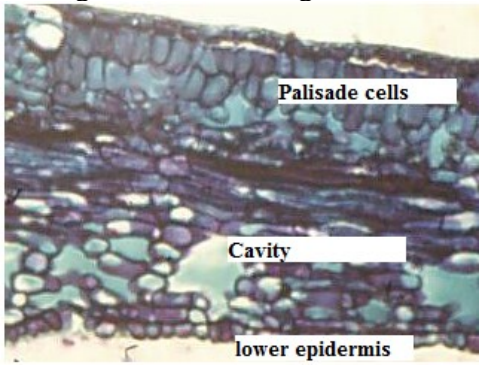

Figure - 3: T.S of Lamina.

Paracytic or rubiaceous type of stomata are present confined to lower epidermis.

The vascular bundles are Collateral and have sclerenchyma caps at the adaxial and abaxial side of the bundle. It is surrounded by groups of sclerenchyma fibres often associated with peripheral phloem groups. Fibre groups also occur in the centre of midrib, associated with and often even in the centre of the phloem bundles. An arc shaped three large VB at the abaxial part and one more or less straight bundle on the adaxial side. A few strands of intraxylary phloem in the midrib. The phloem is arranged in separate strands often in 2 layers. Numerous phloem bundles are interspersed in the ground tissued between the main bundles often accompanied by xylem elements. In the vein region elongated tanniniferous sacs present.

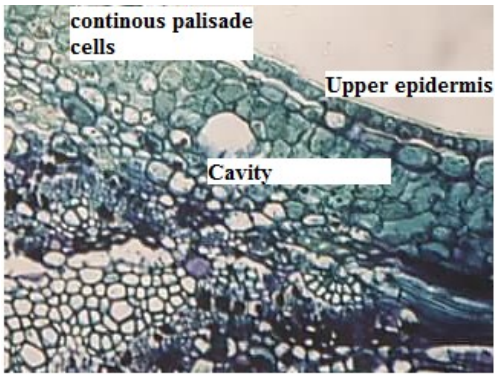

Figure - 4: Adaxial side enlarged

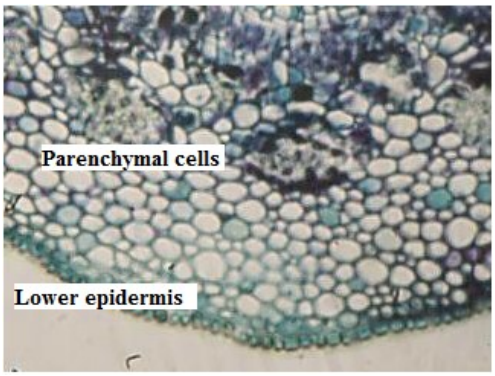

Figure - 5: Abaxial side enlarged.

The larger more or less spherical oil cells are seen in the parenchymatous ground tissue of the midrib. Tanniniferous sacs present in the ground tissue.

Lamina is Dorsiventral. Epidermis is single layered with cuticle. Two layered columnar closely packed palisade cells. Spongy tissue is interspersed to variable extents with bifringent walls. Spherical secretory cells with aromatic contents present in the mesophyll tissue mostly in the spongy tissue.

Distinct vein islets are formed by the secondary and tertiary veins.Vein islets are small and clearly seen,some of the vein terminations are forked.

Trichomes are Uniseriate hairs, the cells of which have arms.

3.3. Petiole

Shape:

Nearly planoconvex in outline with flat surface on the adaxial side with two short lateral wings on both the ends (Fig 6).

The epidermis are single layer made up of small rectangular cells covered by thick cuticle.

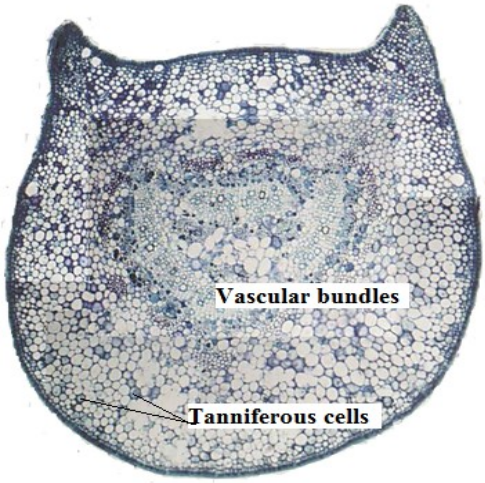

Figure - 6: T.S of Petiole

The vascular system of the basal end of the petiole consists of 3, more or less arc shaped collateral bundles, also with free phloem bundles adaxially, giving the appearance of bicollateral bundles, the sclerenchyma is usually confined for the greater part to the abaxial sides. Vascular system of the distal end of the petiole is just like that of the vascular system of the midrib. Abaxial side consists of an arc shaped 3 vascular bundles and one bundle on the adaxial side. The phloem is arranged in separate strands. A few strands of intraxylary phloem is seen. Tanniniferous cells and spherical secretory cells are scattered in the parenchymatous ground tissue. Secretory cells are mostly occur in the peripheral cortical region.

Tanniniferous cells and spherical secretory cells are scattered in the parenchymatous ground tissue. Secretory cells are mostly occurred in the peripheral cortical region

3.4. Powder microscopy

The analysis of the dried powder microscopy of the leaf showed the presence of Xylem, phloem vessels Stomata, Fibers and Epidermal cells(Fig 7).

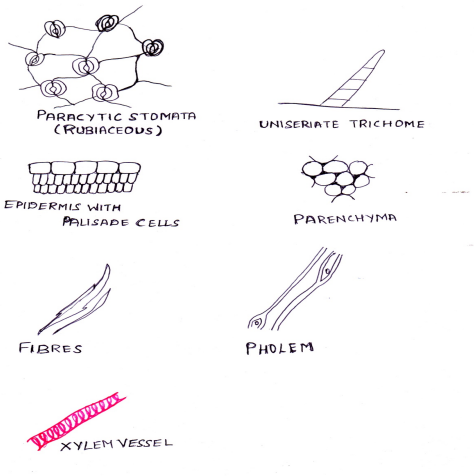

Figure - 7: Powder microscopy.

3.5. Physiochemical analysis

Physiochemical parameters were found as follows: total ash 7.64 w/w, acid insoluble ash 1.06 %w/w, water soluble ash 2.22 %w/w, ethanol soluble extractive value 2.8 %w/w, water soluble extractive value 4.6 %w/w, loss on drying 1.77 %w/w and foreign organic matter was nil. Leaf constants were as follows vein islet number 7, vein termination number 8, stomatal number (lower epidermis)9, stomatal index (lower epidermis) 12.

3.6. Preliminary phytochemical screening

Preliminary phytochemical screening showed the presence of alkaloids, carbohydrates, phytosterol, tannins, flavonoids, terpenoids, volatile oil and absence of mucilage, fixed oil and glycoside.

4. Discussion

Adulteration and misidentification of medicinal plants can cause serious health problems to consumers and legal problems for the pharmaceutical industries. The past decade has witnessed the introduction and implementation of new Good Manufacturing Practices (GMP) in quality control of raw materials, intermediates and finished products of botanical origin. The initial step in quality control of medicinal plants is ensuring the authenticity of the desired species for the intended use. It can be conducted via a variety of techniques, namely macro and microscopic identification and chemical analysis especially description of microscopic botanical aspects to determine definitively the proper species of plant material while it is still in its non-extracted form. The observation of cellular level morphology or anatomy is a major aid for the authentication of drugs. These characters are especially important for identification of powdered drugs, because in these cases most of

the morphological diagnostic features are lost.[12]Microscopic evaluation is one of the simplest and cheapest methods for the correct identification of the source of the materials.

In our present work we selected a plant *M.fragrans* Houtt. (Myristicacae). The microscopic and organoleptic characters, presence of anomocytic stomata in the lower epidermis, prismatic crystals, and druses of the leaf can serve as diagnostic parameters. The physical constant evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs, the ash values is particularly important to find out the presence or absence of foreign inorganic matter such as metallic salts and or silica (earthy matter). The extractive values are primarily useful for the determination of exhausted or adultered drug. Preliminary phytochemical screening will reveal the useful information about the chemical nature of the drug. Preliminary phytochemical screening showed the presence of alkaloids, carbohydrates, proteins, phytosterol, tannins, flavonoids, terpenoids, glycosides and absence of volatile oil, fixed oil, mucilage

5. CONCLUSION

The present work concluded the microscopic using histological identification, microscopic constants and other physico chemical examinations of the leaves of *Myristica fragrance* can be used as a rapid, inexpensive botanical identification technique and is useful in standardization, hence would be of immense value in authentication of the leaf.

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