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Microscopic Study of the Aroma-Secreting Structure of *Syzygium aromaticum* (L.) Merr. & L.M. Perry (Myrtaceae)

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Abstract

Objectives: To study the microscopic features of the organs that contain the aroma-producing components in clove. **Methods:** Microtoming was used to elucidate microscopical information and aroma secreting structure of the leaf, flower bud and fruit of *Syzygium aromaticum*. **Finding:** The anatomical study confirmed that the aroma-secretion mechanisms are located mostly in aerial parts of flower bud, mother fruit and leaf as they exhibit the secretory cavities. **Novelty:** This study presents a simple, yet direct evidence, of determining the accumulation of bio-active components in the various parts of the clove which traditionally known as spice and condiments.

Keywords: Microscopic; Aroma; Secretory Cavity; Myrtaceae; *Syzygium aromaticum*

1 Introduction

Syzygium aromaticum (Synonym-*Eugenia caryophyllata* Thunb.) is an evergreen tree with simple leaf, aromatic terminal flower buds and fleshy mother fruit. Clove buds are widely recognized as a spice and are extensively used in culinary applications for their strong aromatic and pungent properties. Moreover, cloves exhibit anti-inflammatory, antioxidant properties armed with flavonoid and phenolic, secondary metabolites accounting for their therapeutic potential [(1)]. The pharmacologically active phytochemicals present of *S. aromaticum* flower buds include eugenol as the dominant essential oil, commonly utilized in Ayurvedic medicine [(2)]. The bio-actives of *S. aromaticum* essential oil-include antibacterial, antifungal, antioxidant and insecticidal properties-are largely linked to its high level of eugenol [(3)]. A comprehensive review article of *S.aromaticum* deals with recent advances in nanotechnology have opened new possibilities for edible film and coating formulations, where the addition of active ingredients may result in nanocapsule formation [(4)]. Cloves as a medicinal agent for the prevention and treatment of COVID-19, with the treatment being implemented at the early stages of the illness [(5)].

Most plants are able to secrete chemical compounds, such as salt, nectar, volatile oils, gums, latex and resins, from the cytoplasm to the cell exterior. Secreted compounds that are beneficial to plants are commonly termed secretions [6]. Secretions are of special significance for plants to adapt to environmental stresses and to attract pollinators or defend against herbivores and pathogens [7]. Secretory cavities and canals are structurally more complex as they are inter-cellular spaces surrounded by secretory epithelium and non-secretory cells forming a sheath [8].

Early leaf development was characterized by rapid cell division and expansion, which initiated secondary metabolite accumulation. At full maturity, biosynthesis processes stabilized, whereas during senescence essential oils and other metabolites were remobilized or degraded, consequently reducing yield [9]. The observed seasonal differences in leaf secretory cavities reveal the species anatomical plasticity in response to light and water availability. As the cavity area in *Myrica splendens* changes seasonally, this must be considered in crop management for essential oil and alkaloid yield [10]. Integration of cytological, analytical-chemical, and chemometric approaches enabled a detailed analysis of secretory cavity ontogeny and temporal essential oil accumulation in *Zanthoxylom nitidum* leaves. Cytological evidence indicated that marginal tooth-slit secretory cavities consist exclusively of a spherical cavity originating through domain a schizogenous mechanism [11].

Earlier investigations have reported to the leaf morphology and anatomy of secretory structures in two aromatic plants species using light microscopy. Their study revealed that secretory cavities are the primary sites of aroma production in *Syzygium aromaticum* and *Clausena exavata*, and the nature of the accumulated substances within these structures was confirmed through histochemical analysis [12].

Previous anatomical studies on *S. aromaticum* have primarily focused on the flower bud and leaves, providing limited information on their secretory structures, while the mother fruit has remained largely unexplored. This lack of anatomical data on the mother fruit highlights a significant knowledge gap. Therefore, the present study was undertaken to investigate and compare the secretory structures of the flower bud, mother fruit, and leaf of *S. aromaticum*, with special emphasis on their role in aroma production. Histochemical analyses confirmed the presence of essential oils within the secretory cavities, highlighting their functional role in aroma biosynthesis.

2 Methodology

The present investigation was carried out to examine the secretory structures associated with aroma biosynthesis in the flower bud, mother fruit and leaf of *S.aromaticum* (L.) Merr.&L.M.Perry. Plant materials were collected from Courtallum Hills of the Western Ghats, Tamil Nadu, India. The collected specimens were preserved and deposited as herbarium vouchers in the Department of Botany, Arignar Anna Government Arts College, Villupuram, Tamil Nadu, India. Species identification was performed using relevant local Floras [13], [14]. Proper specimen preparation prior to sectioning is critical for obtaining precise anatomical observations. For anatomical analysis, freshly collected samples were immediately placed in re-sealable plastic bags containing FAA for fixation. In contrast, a separate set of leaf samples was immersed in distilled-water and processed for histochemical investigations.

Fresh samples were trimmed to appropriate sizes and properly oriented according to the desired planes of sectioning. The materials were fixed in FAA (Formalin 5ml; Acetic acid 5ml and Ethyl alcohol 90ml 70%) for 24 h. Dehydration was carried out using a graded tertiary butyl alcohol (TBA) series following the protocol described by [15]. After dehydration, the specimens were infiltrated with paraffin wax and embedded to obtain wax-blocks. Longitudinal sections (LS) of the flower buds and transverse sections of the mother fruit and leaf were prepared at a thickness of 10-12μm using a rotary microtome. The sections were stained with Toludine-blue as described earlier [16]. For the localization of aroma substance, fresh hand sections were obtained using a razor blade stained with Neutral red as described before [17]. Micro-photographs were captured using a Nikon Trilocular microscope equipped with a Nikon digital camera. All laboratory investigations were carried out at the Plant Anatomy Research Centre, West Thambaram, Chennai-45. Tamil Nadu, India.

3 Result and Discussion

3.1 Anatomical Observation

3.1.1. Leaves Organoleptic character

Leaves are smooth, shining, coriaceous and gland dotted; when crushed the leaf emits strong pleasant aroma; taste is slightly hot and acrid.

Fig 1. A shoot with flower shoot

3.1.2. Microscopic features

The leaf has thick and planoconvex midrib and even surfaced lamina. The midrib has a flat adaxial side and convex abaxial side. It is 700μm thick. It has narrow epidermal layer of semicircular cells with thick cuticle. The ground tissue consists of angular, thin walled compact parenchyma cells. The vascular strand is wide and prominent; it is omega shaped in sectional view and has several parallel lines of xylem elements with phloem both on the inner and outer parts of the xylem; thin sheath of sclerenchyma occurs all around the vascular strand.

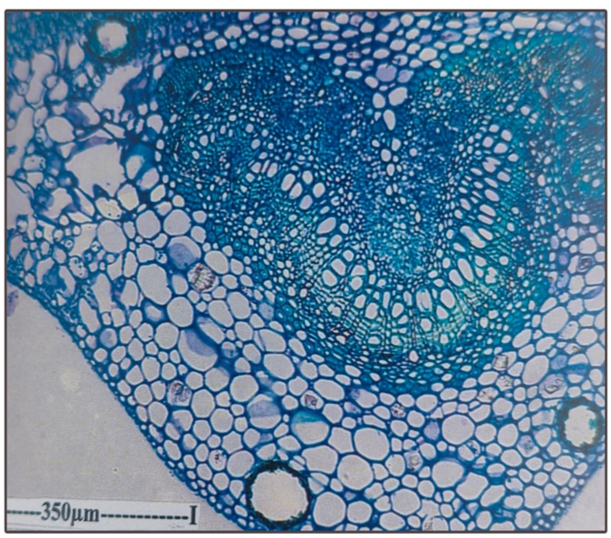

Fig 2. Microtomy *of Syzygium aromaticum* through midrib region

3.1.3. Localization of aroma-secreting structure

The secretory cavities occur in upper and lower part of the lamina and in the outer zone of the ground tissue of the midrib and petiole. The cavities are mostly situated near the epidermal layer. They have intact epithelial cells, which break on maturity to liberate the aroma compounds.

Both Toludine blue and Neutral red located the aroma substances in the epithelial cells of the cavities. Toludine blue stained the substance bluish green while the Neutral red stained in dark red colour. In paradermal sections, the mesophyll cells radiate

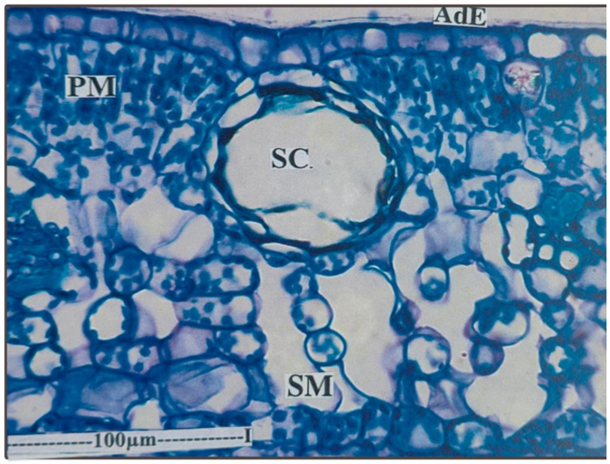

Fig 3. Transverse Section of lamina showing adaxial secretory cavity

from the cavities and spread all around the cavities. Calcium oxalate druses occur in the mesophyll cells, especially in the radiating cells around the cavities. The cavities are 60μm wide in the leaf, 80μm in the midrib and 100μm in the petiole.

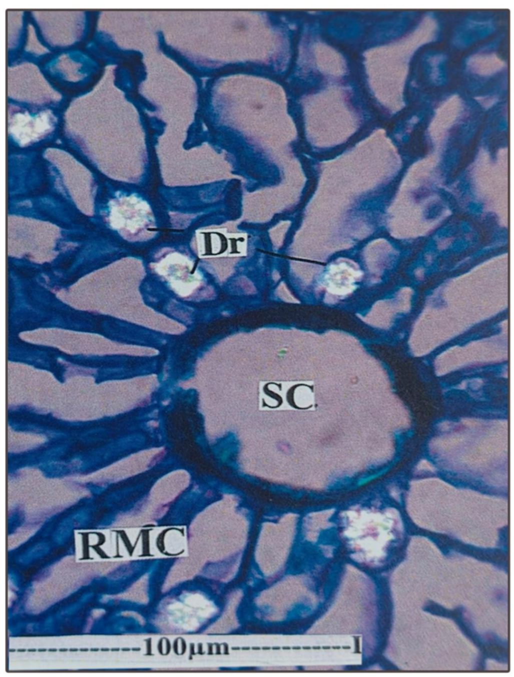

Fig 4. Paradermal section showing secretory cavity with rosette cells and crystal distribution (under polarized light microscope)

3.2 Macroscopic characters of flower bud (Clove)

Flower buds are cylindrical with globose head. The buds are yellowish green when fresh, become dark brown when dried. The flower buds have four lobed, turbinate persistent sepals, four calyptrate caducous petals and many free incurved stamens. Flower buds have strong fragrant and spicy odour and pungent aromatic taste. When the dried buds are pressed with nails, they exude oil; both fresh and dried flower buds sink in water.

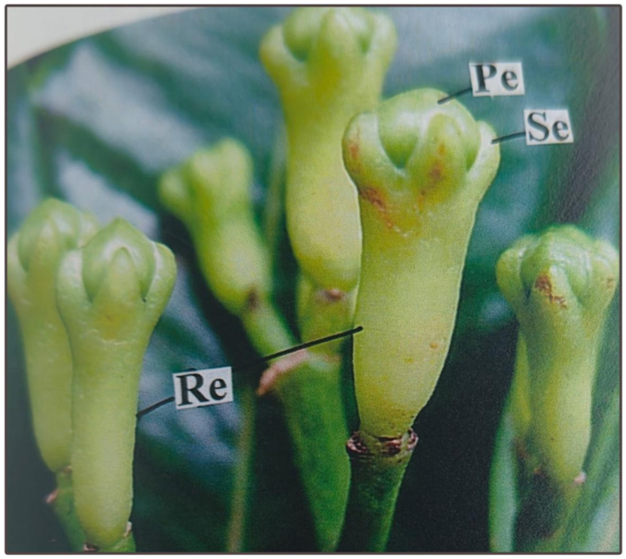

Fig 5. Fresh flower buds

3.2.1. Microscopic features of flower bud (Clove)

The sepals have thick midrib with adaxial sloping hump; the margins become thin and pointed. The sepal is 400μm thick in the middle and 15μm thick along the margin. The petals are thick in the middle, without a hump, and gradually thinning along the margins. They are 200μm in middle part and 30μm thick along the margins. The sepals and petals have thick epidermal layers of rectangular cells and thick walled compact ground parenchyma cells. Wide circular schizogenous oil cavities are abundant both in the sepals and petals. They are distributed in the inner part of the epidermis. The cells bordering the oil cavities are spindle shaped; they become disintegrated liberating their contents into the cavity. The anthers have four pollen-chambers and a wide, circular secretory cavities in the middle. The cavities are surrounded by darkly staining disintegrated cells. Calcium oxalate crystals are diffusely distributed in the ground parenchyma of the anther and the receptacle.

3.2.2. Floral Receptacle (Hypanthium)

is thick and circular in cross-sectional view. It has thick epidermal layer of radially oblong cells and prominent cuticle. The outer ground tissue consists of radially elongated thick walled compact parenchyma cells. The cells in the interior of the receptacle assume circular or polyhedral outline. In the peripheral zone of the receptacle are closely arranged numerous, wide secretory cavities. The cavities are either circular or radially ovate and are schizogenous in origin. They have amorphous content formed by the disintegrated bordering epithelial cells of the cavity.

3.2.3. Localization of aroma-secreting structure

In the sections of the receptacle stained with Neutral red have dark red contents filling the entire lumen of the cavity. The epidermal cells as well as the ground parenchyma cells exhibit mild affinity for the Neutral red.

3.3 Organoleptic characters of fruit

The mother cloves are thick, hard with smooth and shinning surface. When broken, they contain one or two mature seed. The seeds have less aroma than the flower buds, they have spicy odour, pungent aromatic taste.

3.3.1. Microscopic features of the fruit (Mother clove)

The fruit has wide pericarp measuring 1.5mm thick. Within the cavity of the fruit occurs a pair of large cotyledons. The pericarp has thin epidermal layer and prominent cuticle, inner to the epidermis is a broad zone of radially elongated parenchymatous tissue in which occur wide, elliptical schizogenous secretory cavities. The cavities have dark lining layer, the ground parenchyma around the cavities are radially elongated and rosette-like. The middle portion or the mesocarp has small polygonal, thin walled compact parenchyma cells. The endocarp has densely crowded, large circular vascular strands. The strands have collateral xylem

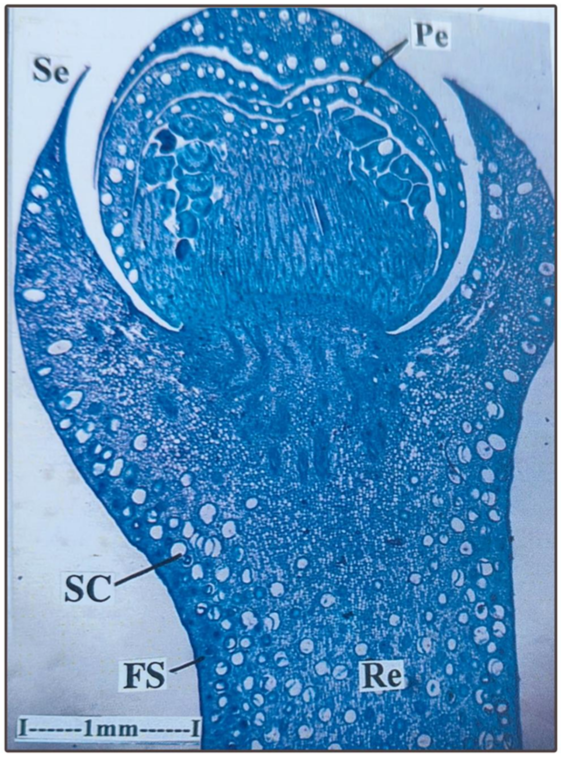

Fig 6. Longitudinal section of flower bud showing sepals, petals and stamens having secretory cavities

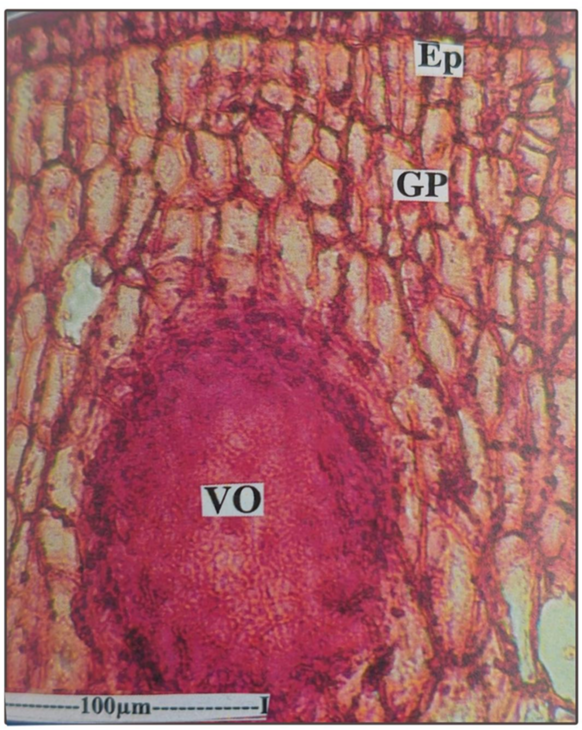

Fig 7. A secretory Cavity stained with Neutral red

Fig 8. Two mature Mother fruits

and phloem with one or two layers of large bundle sheath sclereids. The sclereids are elongated or polyhedral with thick walls and wide lumen. The cotyledons have thin, less conspicuous epidermal layer and homogenous parenchymatous ground tissue. Small secretory cavities filled with dense content are seen in the inner to the epidermis. Starch grains are abundant in the cells of the cotyledons.

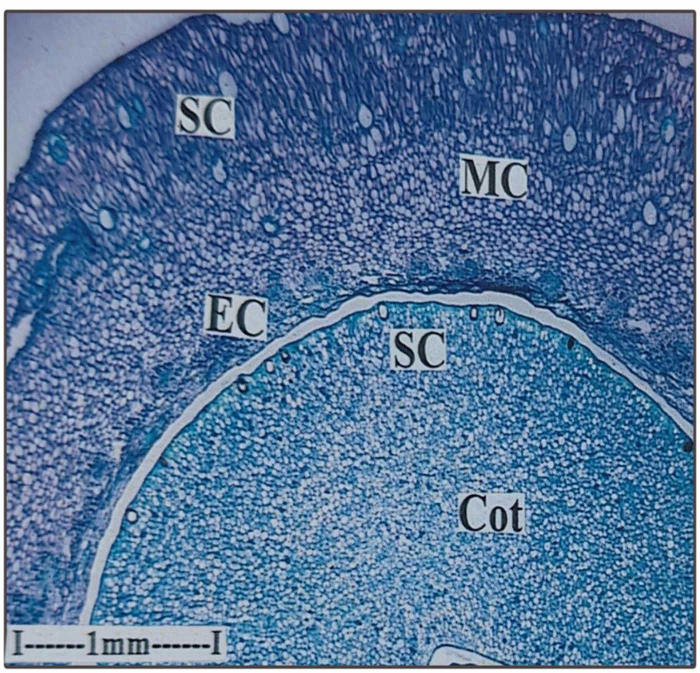

Fig 9. Cross section of Mother fruit showing pericarp and cotyledons

3.3.2. Localization of aroma-secreting structure

Section stained with Neutral red exhibited localization of aroma substances in the secretory cavities of the pericarp and in the cotyledons. The cavities and the cells of the cotyledons have darkly stained cells. The cotyledons have dense accumulation of starch grains.

Fig 10. Secretory cavities located in the epicarp stained with Neutral red

Secretory cavities are widely recognized architectural features of Myrtaceae. These secretory cavities have been typically identified in Rutaceae, Myrtaceae, and recently in Asteraceae [18]. The results revealed that *S. aromaticum* contain well developed secretory cavities responsible for oil secretion in the leaves, flower buds, and fruits. Morphologically , these cavities are glandular in nature and internally they exhibit circular to elliptical shapes, distinctly larger than the surrounding cells and encased by parenchymatous tissue. These observation are consistent with earlier reports that documented the presence of secretory cavities in *S, aromaticum* [19].

4 Legend for the figures

(AC-Aromatic Contents, AdE-Adaxial Epidermis, Cot-Cotyledons, Dr-Druses, EC-Epithelial Cells, Ep-Epidermis, FS-Flower Stalk, GP-Ground Parenchyma, MC-Mesocarp, Pe-Petals, PM-Palisade Mesophyll, Re-Receptacle, RMC-Rosette of Mesophyll Cell, SC-Secretory Cavity, SM-Spongy mesophyll, VO-Volatile Oil content)

Table 1. Distribution of secretory cavities in different organs of *S.aromaticum*

S.No	Plant Organs	No. of Secretory cavities (under low magnification)	No.of Secretory cavities (under high magnification)
1.	Leaf Midrib	03 (Figure 2)	02
2.	Lamina	06	01 (Figure 3)
3.	Petiole	08	04
4.	Petals	35 (Figure 6)	12
5.	Sepals	42(Figure 6)	15
6.	Epicarp	08 (Figure 9)	03
7.	Mesocarp	04	02

Secretory cavities were most abundantly distributed in the flower buds compared to with leaves and fruits**.** Sepals and petals contain more secretory cavities compared with leaf and Mother fruits. The aromatic nature of the bud may be linked to the persistent calyx, arising from chemical constituents localized in the secretory cavities. According to [20] studies nature of compound present in the gland may be volatile terpenes and other compounds such as flavonoid, phenyl rings, resins and carbonyl functional groups. The nature and variety of chemical classes remains to be explored with novel approaches. The present study observed that the secretory cavities functions as aroma-emitting structures.

Clove is an aromatic plant well known for its attractive fragrance and flavour. The plant synthesizes odoriferous secondary metabolites mainly in its flower bud. The clove have been widely used and valued for many centuries. Nevertheless, the structural organization of the aromatic regions and the particular cells or cavities responsible for the accumulation of aroma compounds remain inadequately explored. The mother fruit of *S. aromaticum* is worthwhile to make detailed study.

The present study was subject to certain limitations. Technical difficulties encountered during microtome sectioning restricted the number of usable sections, which in turn limited the extent of anatomical observations. Consequently, a more detailed and comprehensive analysis of the secretory structures could not be under taken. Further studies employing improved sectioning techniques and advanced imaging methods may help to overcome these constraints and deeper insight.

5 Conclusion

Syzygium aromaticum (L.) Merr. & L.M.Perry is an evergreen tree belong to Myrtaceae member. The morphology and anatomy of the flower bud, mother fruit and leaf were analyzed to identify secretory structures using light microscopy. The present investigation confirm that secretory cavities the principal sites of aroma production in *S. aromaticum*. Histochemical analyses revealed the accumulation of essential oils within these secretory structures. Essential oil deposits were consistently detected in the secretory cavities of the flower buds, fruits and leaves, indicating their widespread distribution. The presence and localization of volatile oil constituents within these cavities strongly support their functional role in conferring the characteristic aroma of different plant organs of *S. aromaticum*.

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