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ORIGINAL ARTICLE

Cytochemical localization of aromatic volatile oils in the rhizome of *Cyperus rotundus* Linn. (Cyperaceae)

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Abstract

Objectives: Anatomical and cytochemical studies on the rhizome of *Cyperus rotundus* were conducted to explore the relationship between aroma production and secretory structures. **Method:** Rotary microtome- prepared sections were subjected to light microscopic examination, and oil cells were localized using appropriate histochemical staining methods. **Finding:** The aromatic underground organ (rhizome) containing secretory cells was confirmed to be the primary unit responsible for aroma release. **Novelty:** Aroma-secreting cells represent the simplest secretory structures; they are larger than adjacent cells and possess comparatively thicker walls. This study employed a direct yet simple technique to prove the localization of the active agent.

Keywords: *Cyperus rotundus*; Nut grass; cytochemical and aromatic volatile oils studies

1 Introduction

Cyperus rotundus Linn., commonly called as nut grass, is a member of the Cyperaceae. The genus Cyperus is the second largest in this family and comprises approximately 5,500 species that are distributed across the world [(1)], [(2)]. It is a highly persistent and problematic weed in agricultural fields of throughout the world, owing to its robust underground tubers and rhizomes, which enable rapid vegetative propagation and survival under adverse environmental conditions [(3)] In traditional culinary practices, the powered rhizome was used as flavouring agent in cooked meat, and starch extracted from the tubers was utilized in the preparation of noodles [(4)]. *C.rotundus* rhizome is medicinally used as antifungal, antimicrobial and mosquito repelling properties. Numerous pharmacological characteristics, including anti-diabetic, anti-inflammatory, anti-obesity and neuro protective action [(5)]. The cytotoxicity of methyl triporiate B as a novel anticancer agent; further research, including molecular dynamic stimulates and in vitro assay, must be undertaken to justify methyl triporiate B is potential as an anticancer candidate [(6)]. The contribution of offers an extensive and well-documented examination of the rhizome of *C. rotundus* with respect to morphological, microscopical and physico-chemical aspects. The study encompasses structural features, microscopic

parameters, powder characteristics, and physico-chemical analysis, and is supported by photo micrographs [7].

In aromatic plants, the aroma originate from three categories of chemicals: phenolic compounds, fatty acid derivatives and isoprenoids [8]. The tuber of *C. rotundus* containing rich compounds like flavonoid, alkaloids and sequiterpenes. Some researchers have reported that secretory cells can be present as solitary cells, clusters of cells, or well-organized tissues; structurally, they are typically larger than neighboring cells and frequently resemble enlarged parenchyma cells with intense staining properties [9].

A comparative anatomical analysis of the foliage and bract leaves of six *Cyperus* species revealed important structural characters useful for taxonomic classification. A through investigation of leaf anatomy was carried out following the approach of [10]. Comparative studies on leaf and stem of *Cyperus rotundus* and *Cyperus difformis*. The anatomical study of the leaf and stem revealed several important features. Leaf blade structure, stem ground tissue, and air spaces were also observed . These combined anatomical characters are helpful for taxonomic identification [11]

Earlier anatomical investigations of *Cyperus rotundus* have mainly concentrated on the leaf and stem, the rhizome with only limited attention given to their secretory structures. The current study examines the internal organization of the rhizome and the localization of aroma-secreting compounds, thereby addressing an important knowledge gap. Histochemical analyses confirmed the presence of volatile oils within secretory cells, indicating their role in aroma synthesis.

2 Methodology

The present study aimed to investigate the secretory structures involved in aroma biosynthesis in the rhizomes of *Cyperus rotundus* Linn. Rhizome samples were collected from paddy field in Melamengelam, Tamil Nadu, India, and were taxonomically authenticated using standard local flora [12]. The authenticated specimens were preserved in the herbarium of the P.G. Research Department of Botany, Arignar Anna Government Arts college, Villupuram, Tamil Nadu, India. Accurate anatomical examination requires appropriate pre-sectioning preparation of plant material. For anatomical studies, freshly collected samples were promptly fixed in FAA solution and stored in sealed plastic bags. A separate batch of rhizome material was maintained in distilled water and utilized for cytochemical analysis.

Transverse section of the fresh rhizome were trimmed into optimum dimensions and fixed in FAA fixative. The FAA solution consisted of Formalin (5 ml), Acetic acid (5 ml), and Ethyl alcohol (90 ml), and the materials were preserved in it for 24 hours. The rhizome samples were subsequently dehydrated and infiltrated with paraffin following standard procedures [13]. Serial sections measuring 10-12μm thickness were obtained using a rotary microtome and stained with 0.1% Toludine-blue [14]. To localize aromatic substances, fresh hand-cut sections were prepared using a sharp blade and stained with Neutral red, which serves as a rapid ans specific stain for intact tissues containing aroma-storing cell [15]. Photomicrographs at various magnifications were captured using a Nikon coolpix digital camera**.** All experimental analyses were conducted at the Plant Anatomy Research Centre, West Thambaram, Chennai-45, Tamil Nadu, India.

3 Results and Discussion

3.1 Anatomical Observations

1. Macroscopic characters (Figure 1)-The rhizome of *Cyperus rotundus* have elongate, slender stolons and bearing long hard, ovoid, tunicate, black with fragrant tubers
2. Microscopic characters (Figure 2- 8) -The cross section of the rhizome is characterized by four regions i. Epidermal ii. Cortical iii. Endodermoid and iv. Stelar
3. Epidermal region-It has a thin epidermis followed by one or two layers of sub-epidermal parenchymatous cell. Inner to the sub-epidermal layer is continuous cylinder of sclereids; the sclereids are polyhedral or spherical and closely arranged ; they are two to three layered and posses dark olive green contents.

   Cortical region (Figure 2)-The cortical cells are circular, delicate -wall, parenchymatous and they are exhibit in some what larger, some layers are thick under the hypodermis and towards the endodermis. They form typical aerenchymatous cellular structure with large inter-cellular slots. Cortex is also characterized by the presence of special cell containing oleo-resinous and numerous starch grains. There are no cortical vascular bundle.
4. Endodermoid region-The endodermoid layer divides the cortical region and stelar region (Figure 3). It is fairly distinct; it is thin and consists of small squarish cells.

Stelar region (Figure 4)-The stelar vascular bundle are diffusely spread out in the pith, interior to the endodermis. These vascular bundles are numerous, similar in shape, size and diffusely spread through out the stelar region. They are circular and elliptic.



Fig 1. Habit profile of the rhizome

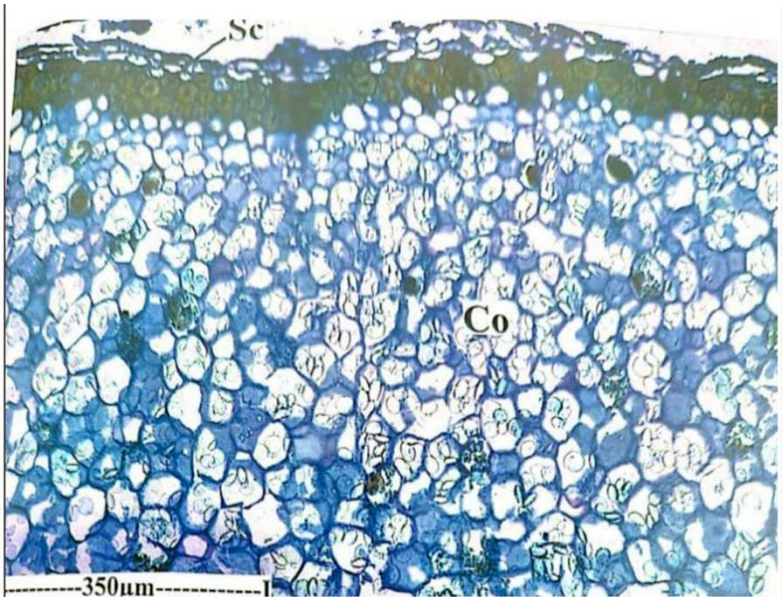


Fig 2. T.S of rhizome through cortical zone (Stained with Toluidine blue)

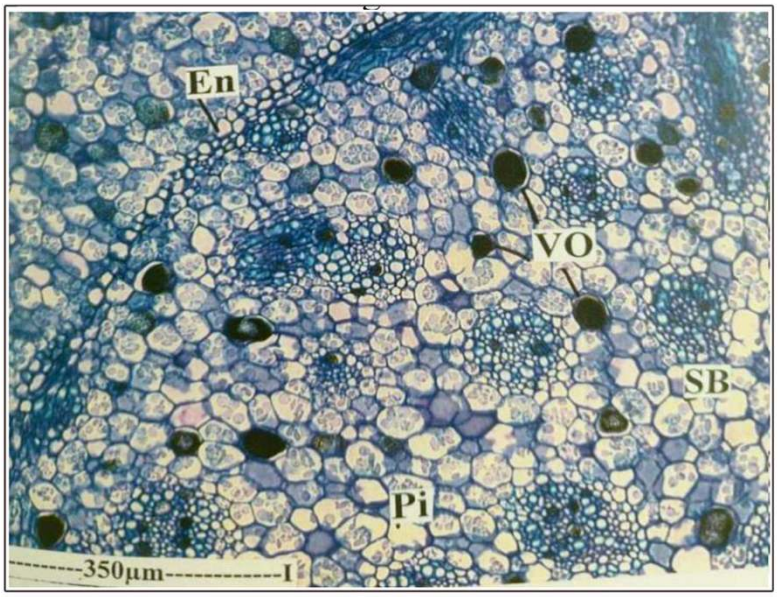


Fig 3. T.S of rhizome through stellar zone (Stained with Toluidine blue)

Each vascular bundle consists of scattered xylem and phloem elements surrounded by a sclerenchymatous sheath of one to three layers of cells [*Amphivasal* type of vascular bundle]. Water conducting tissues are polygonal, wide and compact walled. The larger, late-developing xylem cells are up to 20μm wide. Each vascular bundles are surrounded by sclerenchymatous bundle sheath cells. The bundle sheath cells are either incomplete or complete around the vascular bundles, when the bundles are smaller. Parenchymatous cells of the pith also contain oleo-resin and starch grains.

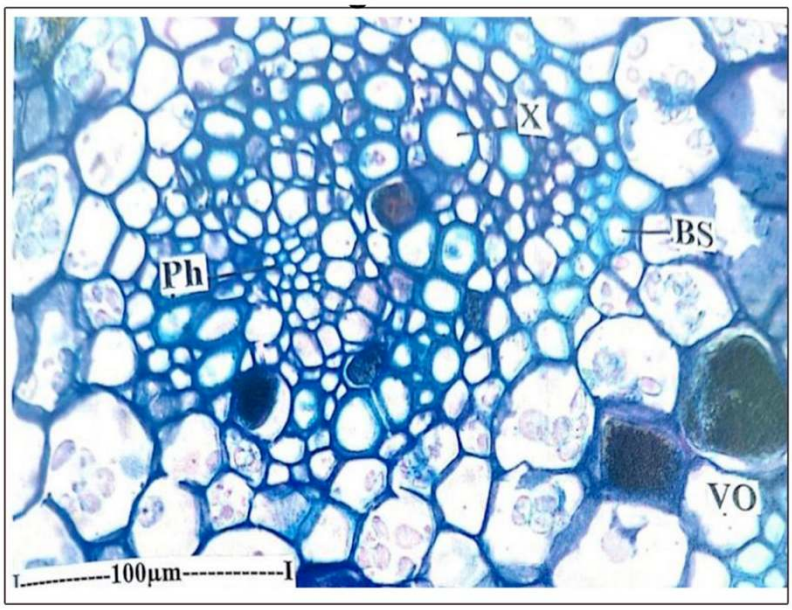


Fig 4. A Vascular bundle enlarged with volatile oil deposition in the parenchyma cells

Starch grains distribution (Figure 5)- Sections stained with IKI, exhibit the granular inclusions small starch densely crowded to within the cortical cells and stelar portion of the ground cells. The starch grains are large, ovoid or narrowly oblong; they are

10-20μm long and 10-12μm wide. Some of the cortical cells have large, solitary, spherical bodies stained orange color which are probably the aromatic compound.

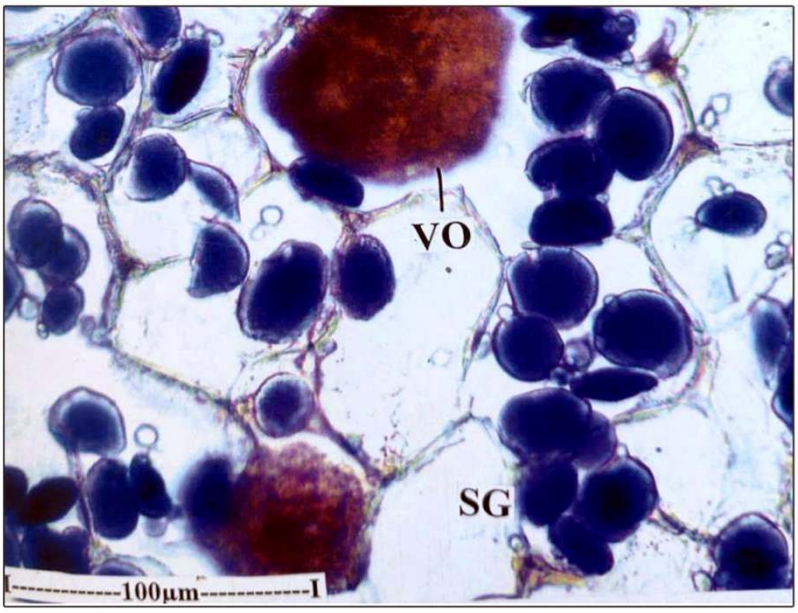


Fig 5. Distribution of the starch grains and volatile oil substance in the parenchyma cells (Stained with IKI)

3.2 Distribution of aromatic compounds

Both manual sections with cutting blade and serial sections with rotary microtome were prepared for the study. Dilute Neutral red [1:100] was employed to localize the aromatic compounds in the rhizome cells. In the paraffin embedded microtome sections, the cortical cells of ground parenchyma and stelar portion darkly stained parenchyma cells were seen diffusely distributed. The fragrance substance is stained dark red (Figure 6). These cells containing dark red contents are ensheathed by five to seven parenchyma cells which radiate from the central cells containing dark contents. The encircling cells are the rosette cells and the central cells is the fragrance substance containing cell. The fragrance containing cells are similar to other non-fragrance cells in shape, size and wall thickness.

In the hand sections of the fresh samples, the distribution pattern and localization of fragrance substances are similar to those in the paraffin sections. The colour of the fragrance substance in the fresh materials is purplish in contrast to dark red in the fixed materials (Figure 7).

The internal structure of the *Cyperus rotundus* rhizome showing a single layer of epidermal cells and followed by two or three layers of thick walled, lignified Sclerenchymatous hypodermis. However, the structure of the fibro vascular strand, starch filled ground parenchyma cells and sclerotic hypodermal cells seen to be specific for *Cyperus rotundus.* Evidence suggest that the distribution of lignified sclerenchyma fibers along the epidermis and the overall lignification of the tubers play a key role in protecting the mature tubers from flood stress (Figure 8). The result concurs with previous literature suggesting that lignified peripheral tissues may facilitate the adaptation of *C.rotundus* to in-undulated conditions[16]. According, the authors report several anatomical and histochemical attributes that likely promote the successful adaptation of *C.rotundus* to amphibious habit.

4 Legend for the Figures

(BS-Bundle Sheath; Co- Cortex; Ph-Phloem; En- Endodermis; Pi-Pith; SB- Stellar Bundles; Sc-Sclereids; SG-Starch Grains; SB-Stellar Bundles; VO- Volatile oil substances)

Cyperus rotundus is commonly known as nut grass, has been widely utilized and valued for centuries for its aromatic and medicinal properties. The production of odoriferous secondary metabolites is predominantly associated with its rhizome. However the structural organization of the aroma-producing regions and the specific cellular components involved in the

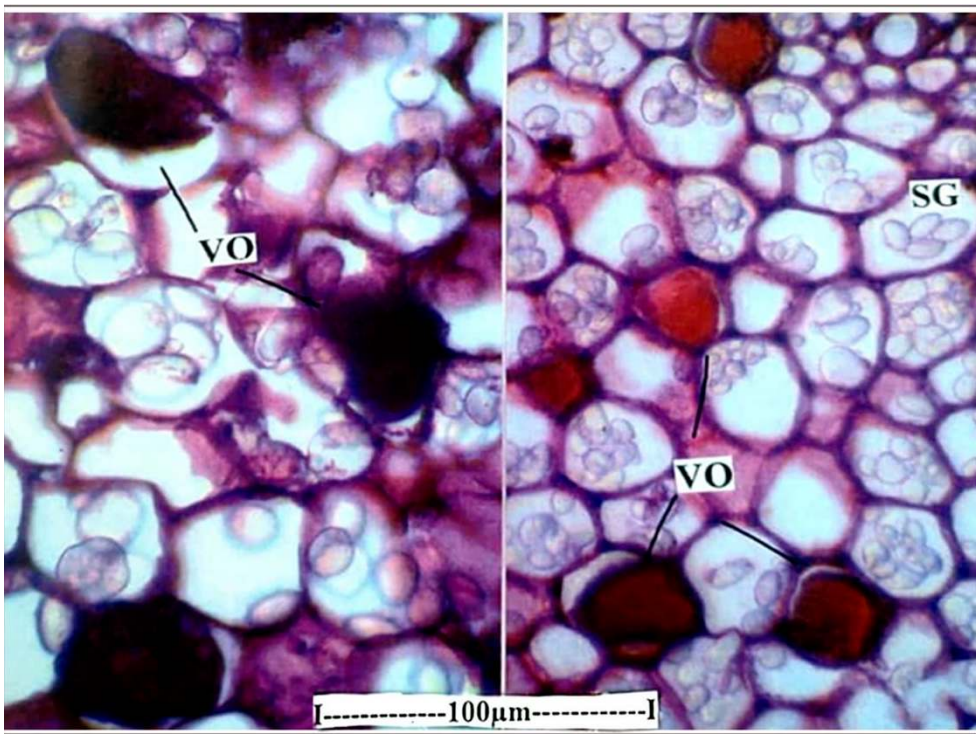


Fig 6. T.S of rhizome stained with Neutral Red - Cortical and Stellar parenchyma cells containing volatile oil substances

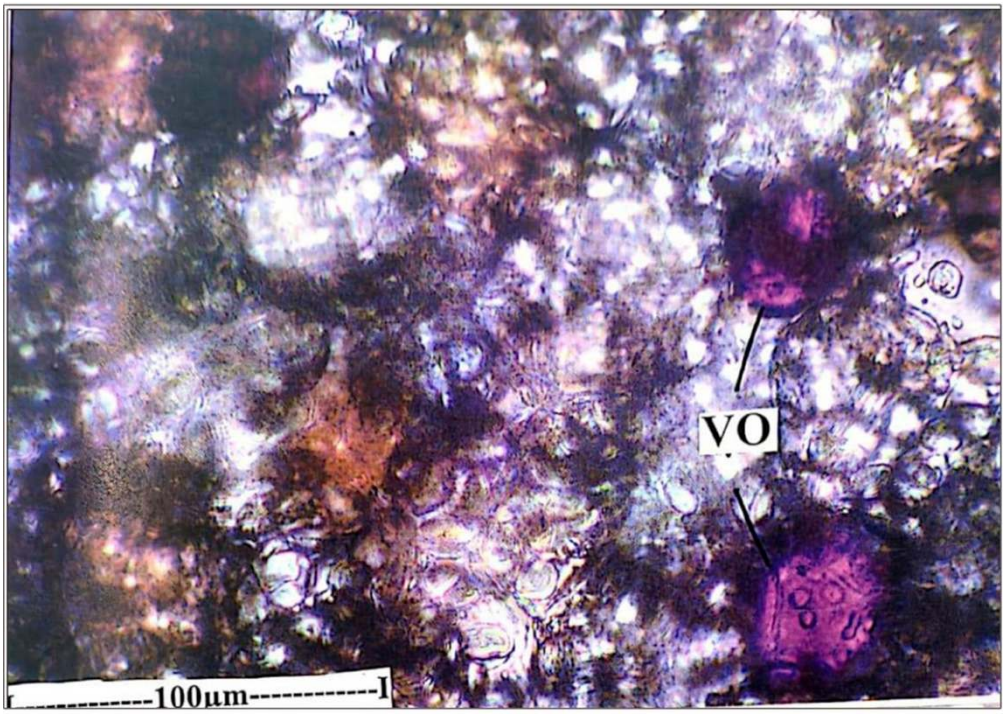


Fig 7. Hand sectioning of the rhizome showing volatile oil substances in the cortical region

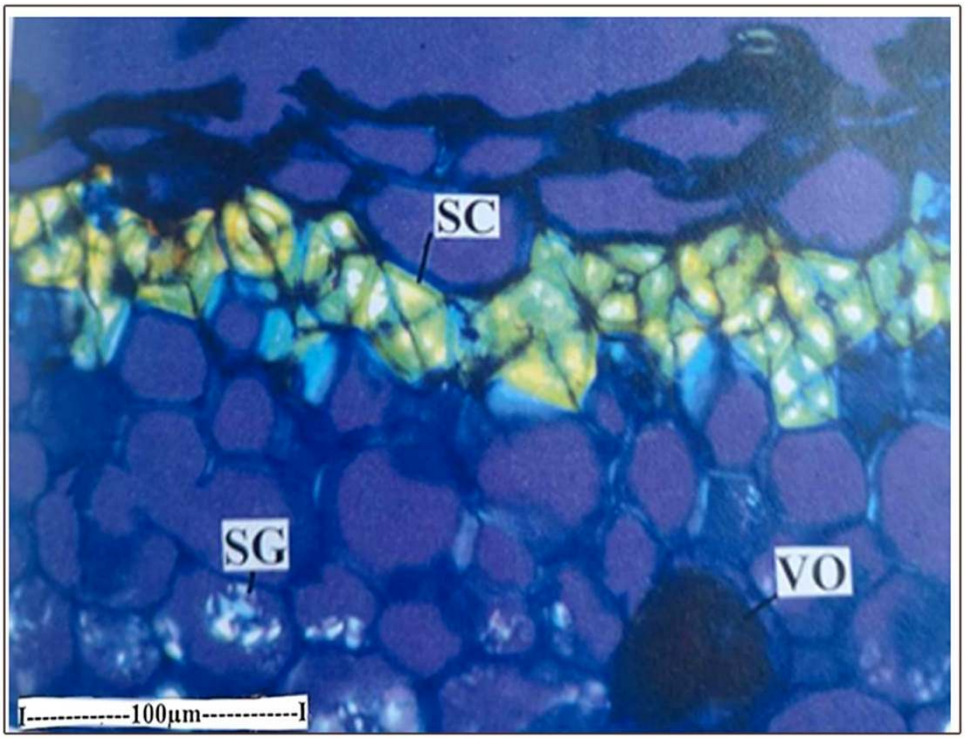


Fig 8. Sclereid band in the outer cortical zone of the rhizome(under polarized light microscope)

synthesis and storage of these compounds remain insufficiently documented. Therefore, a detailed anatomical and cytochemical investigation of *C. rotundus* is of considerable scientific importance. The secretory cells are the simplest type of structure; these cells can be distinguished from the neighbouring non-secretory cells only by the actual contents, which are stained by specific vital stain. However, the secretory cells may be larger in size than the surrounding cells with thicker walls. It becomes more obvious that barring that a fore mentioned reference, there is no study the cytochemical of the aromatic compounds in the rhizome.

The present investigation had certain limitation. Technical challenges faced during microtome sectioning reduced the number of suitable sections available for observation, which prevented a more thorough and detailed examination of the secretory structures. Future research using refined sectioning procedures and advanced imaging techniques could help address these issues and provided clearer evidence regarding the role of simple secretory cells in aroma secretion.

5 Conclusion

The present study provides clear anatomical and histochemical evidence regarding the structure and functional significance of the rhizome. Detailed morphological and anatomical investigations revealed distinct secretory cells distributed within the rhizome tissues. Cytochemical staining confirmed that these secretory cells are the principal sites of essential oil accumulation and aroma production. The dense amorphous contents observed within these cells correspond to volatile aromatic compounds characteristic of the rhizome. These finding highlight the value of anatomical and histochemical parameters in accurate identification of rhizome-based crude drugs and help reduce adulteration in herbal markets. Overall, the study strengthens the diagnostic importance of secretory structures in authentication, quality control, and pharmacognostic evaluation of *Cyperus rotundus* rhizomes.

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