**Original Research Article****DOI - 10.26479/2018.0401.16****ANATOMY, MICROMORPHOLOGY AND HISTOCHEMICAL
LOCALIZATION OF DIFFERENT PHYTOCHEMICALS OF TWO
MEDICINALLY IMPORTANT TAXA OF THE FAMILY ZINGIBERACEAE****Alokesh Das¹, Krishna Kanta Pal² and Sudipa Nag^{3*}**

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ABSTRACT: The study was focused on two important plant genera of Zingiberaceae i.e. *Zingiber zerumbet* (L) Smith (Bitter Ginger) and *Amomum compactum* Soland ex. Maton. (Round Cardamom). This study provided a detailed summary of pharmacognostical, anatomical, micromorphological and phytochemical characters of these two genera. The study revealed that the presence of oil cells and olea-resin in the cortex and central cylinder region and globose, ovoid or irregularly rounded starch grains were the distinguishing features and these could be used as anatomical markers for *Zingiber zerumbet*. Preliminary phytochemical analysis of the rhizomes of *Zingiber zerumbet* and seeds of *Amomum compactum* revealed the presence of alkaloids, reducing sugar, starch, flavonoids, tannin, saponin, aminoacids, tannin and protein in different restricted localized areas. The presence of these phytochemicals were determined through colourization using different reagents like Wagners' reagents, Benedict's reagents, iodine solution, 10% NaOH, 10% lead acetate, 1% lead acetate, 5% Ninhydrine, 5% FeCl₃ and Millon's reagent. This method resolved the quality of the compound in herbal formation.

KEYWORDS: Pharmacognostical, Micromorphology, Histochemicals, Zingiberaceae, Bitter Ginger, Round Cardamom.

***Corresponding Author: Dr. Sudipa Nag**Associate, Professor, Department of Botany, Rampurhat College, Rampurhat, Birbhum,
West Bengal-731224, India.* Email Address: botanydeptprph@gmail.com**1.INTRODUCTION**

There are numerous ginger species (Zingiberaceae) in most of the country in Southeast Asia. They are not only used for their unique flavor but also their medicinal properties. Some of the dietary

ingredients have been identified and their biological activities elucidated [1, 2]. In the present investigation an attempt was made by pharmacognostical and phytochemical tests such macroscopic, microscopic and histochemical localization of these (alkaloids, saponin, reducing sugars, flavonoids, proteins, amino acids, tannin and starch) phytochemicals of the family Zingiberaceae. These two important plants are *Zingiber zerumbet* (L) Smith (bitter ginger) and *Amomum compactum* Soland ex. Maton. (Round Cardamom). *Zingiber zerumbet* is a perennial herb with leafy stems growing to about 1.2m in height that is widely cultivated throughout the tropics including Southeast Asia, Korea, India and Bangladesh for its medicinal properties [3] Rhizomes are employed against cough, stomachache, asthma and also as vermifuge. It is used in leprosy and other skin diseases. The rhizomes yield an essential oil which is used as perfume in soap and other toilet articles (4). It is also used as an anti-inflammatory agent in traditional medicine [3]. A monocyclic sesquiterpene, zerumbone (2E, 6E, 10E-humulatrien-1-one), which was found as a major component of the essential oil of *Z. zerumbet*, has been studied insively for potential use in anti-inflammatory, chemopreventive and chemotherapeutic strategies [3]. It is also used in fish poisoning, cough remedies, against bacterial diseases, thrush and diabetes. The rhizomes also have medicinally important as antihypertensive, carminative and flavouring agent. It is also used in peptic ulcers stomach problems and many infections. The extract of rhizomes have also anti-inflammatory, chemo preventive and anti HIV properties. It is noticed that the importance of rhizomes not only used in Ayurvedic medicine but also in modern medicine. The microscopic and macroscopic study of rhizome reveals many distinct characters [4]. *Amomum* is a profitable for flavor species, perfume, medicinal stuff, etc [5, 6]. Their fruits contain high amount of volatile oil. This robust, perennial aromatic herb grows to 1-1.5m high much branch underground, hard subterete rhizome where give rise to leafy stem and separate inflorescence stalk. *A. compactum* seed was reported to contain 2-5% essential oil comprising mainly 1,8 cineol and β -pinene, α -pinnene, α -terpineol and humulene were also found [7]. The current study has been undertaken the detailed anatomy, micromorphology, pharmacognostic and histochemical localization of different phytochemicals for these two genera of the family Zingiberaceae.

2. MATERIALS AND METHODS

Plant materials for the present study, were collected from the medicinal plant garden of Rampurhat College, Rampurhat, Birbhum, located in the lateritic belt of West Bengal, India. The two plants species taken under investigation were *Zingiber zerumbet* (L) Smith and *Amomum compactum* Soland ex. Maton. of the family Zingiberaceae. The selected plant species have been carefully identified with the help of different floras [8, 9, 10, 11]. These plants were compared and identified with the help of anatomical study. Plant materials (leaf, petiole, stem, root and rhizome) collecting from the garden , hand sections (transverse) were made and stained it diluted with saffranin (50%) and fast green solutions (1%) for anatomical study [12] and observed under 10X x 10X and 10X x

40X microscopic lens (OPTILAB-MX). Photo-micrograph were obtained by observing under light microscope with the help of camera (Sony DSC W830 Cybershot 20.1 MP Point and Shoot). Histochemical localization and characterization of secondary metabolites from the fresh materials were performed using few reagents such as Wagners' reagents, 1% lead acetate, 10% lead acetate, 5% Ninhydrine, 5% FeCl₃, Bendict's reagents, 10% NaOH, iodine solution and Millon's reagent according to the method of Choudhury *et al* [13]. Observing the coloration of these materials with reagents the characterization of the secondary metabolites were made and listed out. For pharmacognostic study, powdered drug sample were prepared from the rhizome of *Zingiber zerumbet* (L) Smith and *Amomum compactum* Soland ex. Maton. according to the method of Jana *et al* [14]. By drying the fresh materials at 35 °C in a hot air oven for 48 hours, powdery drugs were boiled with saturated chloral hydrate solution, kept for 24 hours and studied under microscope. For micromorphological study peeling were made from the fresh leaf materials of both the plants and mounted with 10% glycerine, then observed under microscope.

3. RESULTS AND DISCUSSION

Morphology, Anatomy and pharmacognosy of rhizome, leaf, stem and root of *Zingiber zerumbet* (L) Smith

External Morphology:

Rhizomes were irregularly branched with nodes and internodes. Scale leaves were present; it was 6-12 cm long and 1.5-2.5 cm broad. The outer surface of the rhizome was grey but inner yellow colour with bitter odour and fragrant aromatic odour (Figure.1).



Fig.1: Rhizome of *Zingiber zerumbet* (L) Smith.



Fig 2: Plant of *Amomum compactum* Soland ex. Maton.

Microscopic Characters of rhizome:

Transverse section of the rhizomes was circular in outline with epidermis and cortex. There were closed, collateral vascular bundles. Epidermal cells were irregular followed by cork cells which were 6-10 layers. Just beneath the cork layers, there was parenchymatous cortex with intercellular air space and starch grains. Many oil cells were present in the cortex. Centre cells were with yellow to orange coloured oleoresin. Endodermis was single layer and stele consisted of central parenchymatous zone. The tracheids had reticulate spiral or scalariform thickening on the walls (Figure.3E).

Micromorphological characters:

Leaf: Upper and lower epidermal cells were irregular in shape. The cell wall outline was wavy. Stomata were mainly paracytic type with subsidiary cells (Figure.3F).

Stem: The vascular bundles (Vbs) were many, collateral and closed. The endodermis was not found. Each Vb remained surrounded by a well developed sclerenchymatous sheath. Hypodermis was also sclerenchymatous (Figure.3G).

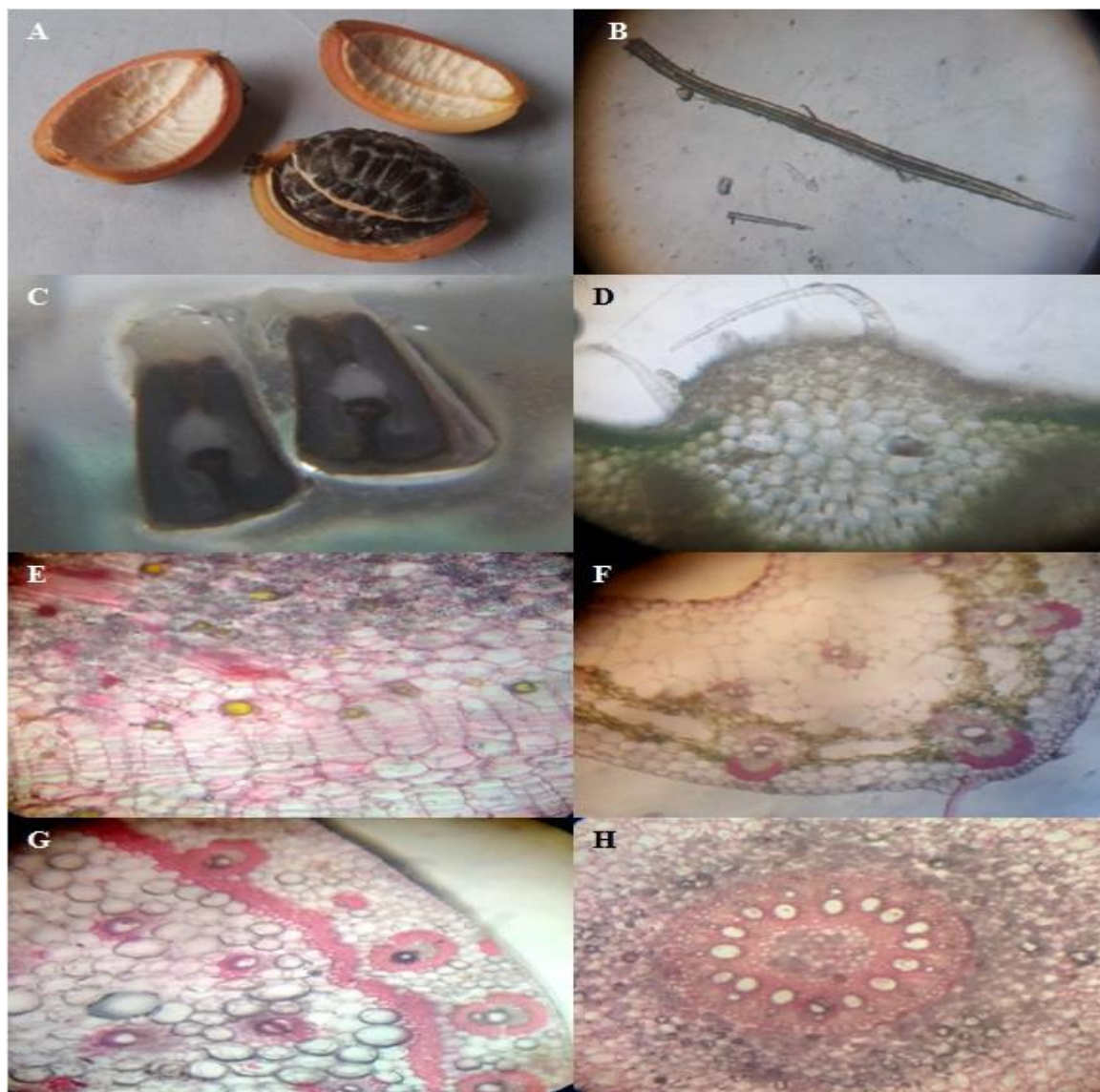


Figure.3: Light microscopic photographs of anatomy of different parts of *Amomum compactum* Soland ex. Maton. and *Zingiber zerumbet* (L) Smith.

- A. Fruit of *Amomum compactum*.
- B. Trichome of *Amomum compactum* leaf
- C. Aril of *Amomum compactum* seed.
- D. Transverse section of the leaf of *Amomum compactum*.
- E. Transverse section of the rhizome of *Zingiber zerumbet*.
- F. Transverse section of the leaf of *Zingiber zerumbet*.

G. Transverse section of the stem of *Zingiber zerumbet*.

H. Transverse section of the root of *Zingiber zerumbet*.

Root: The xylem groups were polyarch condition and 16-18 in number. The pith was large and parenchymatous (Figure.3H).

Chemical constituent of rhizomes of *Zingiber zerumbet* (L) Smith:

The methanolic extracts of rhizomes of *Zingiber zerumbet* (L) Smith. gave positive tests for alkaloids, saponin, tannin, amino acid, proteins, starch, flavonoids and reducing sugar. In the study of histochemical localization, it was found that alkaloids were present in few cortical cells, xylem and periderm. Reducing sugar was present in the parenchymatous cells and also in xylem. Protein and amino acids were also present in parenchymatous cortex and tracheids. Tannin was present in only parenchymatous cells. Starch, flavonoids and saponins were distributed throughout the rhizome except periderm (Table No. 1).

Morphology, Anatomy and pharmacognosy of fruit, seed and leaf of *Amomum compactum* Soland ex. Maton.:

External Morphology:

The plant was leafy herbaceous with stout rhizome with 4-6 ft. height (Figure.2). The inflorescence was spike with white flowers and fruit capsule. Capsule was green when immature and orange brown when mature, oblate, depressed globose, 1.5 cm across, slightly 9 grooved when dry, pilose. Seeds were irregularly polygonal, 4 mm in diameter with white aril (Figure.3A & 3C).

Micromorphological characters of leaf:

Epidermal cells were hexagonal in shape and cell wall outline was more or less straight. There were no intercellular spaces. Stomata were paracytic type. Trichomes were unicellular or multicellular with pointed tip (Figure.3B & 3D).

Chemical composition of seeds of *Amomum compactum* Soland ex. Maton.

The methanolic extract of seeds of *Amomum compactum* gave positive test for all alkaloids, tannins, amino acids, proteins, saponins, starch, flavonoids and reducing sugar. In the study of histochemical localization, it was found that alkaloids were present in the endosperm of the seed. Reducing sugar was present in testa and endosperm. Proteins present in embryo and outer seed coat. Amino acid, saponin and tannin covered the testa only. Flavonoids and starch were distributed throughout the seed (Table No. 1).

The plant *Z. zerumbet* (L) Smith. shows the characters similar to the findings of Rout *et al* (2011) [3] and *A. compactum* shows the similar type results which was found in other species of *Amomum* by Shivanand and Mahalaxmi (2010) [15]. The external morphology and anatomy of rhizome, micromorphology of *Z. zerumbet* (L) Smith. leaf, chemical constituents of seed of *Amomum compactum* Soland ex. Maton. revealed the presence of different micromorphological structure, which help for proper identification of the plants. In the study of anatomy (Figure.3) and

histochemical localization (Table No. 1), presence of the different secondary metabolites (alkaloids, flavonoids, tannins, proteins etc.) in different tissue in the rhizome of *Zingiber zerumbet* (L) Smith and seeds of *Amomum compactum* Soland ex. Maton. are clearly observed and the intensity of colouration shows the quantity of secondary metabolites present in those tissue or cells.(Table 1). Biological assays and chemical analysis are very important aspects in pharmacognostic evaluation of medicinal plants [13, 14, 15, 16].

4.CONCLUSION:

The chemotaxonomic analyses are better than the morphological and anatomical characters. It might be due to more usefulness of dried crushed materials rather than fresh materials. The character gives satisfying results and it can be placed correctly in the taxonomic classification, as long as the microbes or other materials do not contaminate. A hundred years old herbaria specimen can be examined their secondary metabolites accurately [17]. But all these parameters can act as diagnostic tool for identification and authentication of raw drug samples and play an important role in quality control and detection of adulteration [3, 6].

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Table No. 1: Histochemical localization of phytochemicals in seed of *Amomum compactum* Soland ex. Maton. and in rhizome of *Zingiber zerumbet* (L) Smith.

| Reagents | Colouration | Intensity | | Phytochemicals | Tissue Location | |
|----------------------|-----------------|-----------|-----|----------------|-------------------------|----------------------------------|
| | | A | Z | | A | Z |
| Wagner's | Orange brown | +++ | ++ | Alkaloid | Endosperm | Xylem, cortex, periderm |
| Benedicts' | Brick red | ++ | + | Reducing sugar | Testa, Endosperm | Xylem, Cortex |
| Iodine | Blue | +++ | + | Starch | Whole seed | Except periderm in whole section |
| 10% NaOH | Magenta | ++ | + | Flavonoid | Whole seed | Except periderm in whole section |
| 10% lead acetate | Yellowish White | + | ++ | Tannin | Testa | Cortex |
| 1% lead acetate | Whitish cream | ++ | +++ | Saponin | Testa | Cortex |
| 5% Ninhydrine | Purple | ++ | + | Amino acid | Testa | Cortex, Tracheid, Trachea |
| 5% FeCl ₃ | Greenish black | +++ | + | Tannin | Testa | Cortex |
| Millons' Reagent | Yellow to brown | ++ | +++ | Protein | Embryo, outer seed coat | Cortex |

(+, ++, +++) represent degree of intensity of colour change i.e. presence of phytochemical groups