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## COMPARATIVE POWDER MICROSCOPICAL SCREENING OF THE RHIZOME AND LEAF OF *ALPINIA CALCARATA* AND *ALPINIA GALANGA*

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**ABSTRACT:** The therapeutic use of herbal medicine has gained considerable momentum in the world during the past decade. Hence, Quality control standards for various medicinal plants used in indigenous systems of medicine are becoming more necessary. Adulteration and misidentification of medicinal plants can cause serious health problems to consumers and pose legal problems for pharmaceutical firms. So, the aim of this investigation was to establish a simple and efficient protocol to determine possible drug adulteration by use of similar plants. Powder microscopy can help the correct identification and establish standardization of plant drugs. The present study has revealed an easy way to identify *Alpinia calcarata* and *Alpinia galanga* microscopically and thus it can also be employed to detect the degree of adulteration in powdered raw medicinal plant materials as well. Likewise, microscopical detection is easy, reliable and cost effective tool for detection of adulteration in medicinal plants. *Alpinia calcarata* Rosc. and *Alpinia galanga* Linn. of Zingiberaceae popularly known also as Lesser galangal and greater galangal, have a widespread occurrence in India, Malaysia, Bangladesh and China. Drug prepared by using rhizomes of *Alpinia calcarata* are used in the treatment of rheumatism, bronchial catarrh and asthma. It is used against infection of the skin and also possesses antibacterial activity. It is also used to stimulate digestion, purify blood, prevent bad breath, improve voice and also to treat inflammation and arthritis. Rhizomes of *Alpinia galanga* are used to treat various kinds of diseases including diabetes, cancer, kidney diseases, vata, bronchitis and diseases of heart.

**INTRODUCTION:** Medicinal plants since ancient times have been a source of relief in the control of different types of diseases. If we pool the knowledge from diverse traditions, we have a cure for every illness known to man. Since time immemorial man has used various parts of plants in the treatment and prevention of many ailments.

Plant derived drug research has become more promising in recent years and also a better alternative for synthetic medicine and therapeutics inspite of many challenges<sup>1</sup>. According to WHO, almost 80% of the population of developing countries have utmost faith in phytomedicines, of which plants have an important role<sup>2</sup>. They are used in diseases such as dyspepsia, fever, urinary disorder, antiperiodic, and anti-inflammatory<sup>3</sup>.

Medicinal plants are therapeutically effective and culturally acceptable. The increase in the use of synthetic chemicals has led to many side effects and undesirable hazards.

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It has been estimated about 10% of the plants or around 30,000 plant species are used for medicinal purposes throughout the world<sup>4</sup> and of which 6500 species are found in Asia<sup>5</sup>. Pharmacognostic characters of herbs play an important role since particular macro and microscopic features are unique for each plant<sup>6</sup>. Herbal therapeutics has become more popular in developing and developed countries owing to its natural origin and lesser side effects. As the most important source of medicines herbals play a key role in world health<sup>7</sup>. It is no wonder that the world today seems to consider it as famous chemical factory for biosynthesis of a huge array of secondary metabolites<sup>8</sup>. Most of the traditional systems of medicines are effective but the need is just to validate them to assess the quality, quantity and purity of the drugs.

The pharmacological activity of members of the family zingiberaceae which exhibit antimicrobial<sup>9</sup>, anti-inflammatory, anti-hyperglycemic, hepato-protective, immunomodulator, cytotoxic activities<sup>10</sup> and have the presence of flavonoids<sup>11</sup>. The ethnomedical practices of the tribal communities of North East India were critically studied and documented for the Zingiberaceae family towards their future pharmacological diagnostics<sup>12</sup>.

In the present study, the rhizomes and leaf powder of *Alpinia calcarata* and *Alpinia galanga* (Zingiberaceae) were studied in detail to check the adulteration. *Alpinia calcarata* is a widely distributed aromatic medicinal plant native to India<sup>13</sup>. It is extensively grown in gardens for its showy flowers and aromatic leaves.

Drug prepared by using rhizomes are used in the treatment of rheumatism, bronchial catarrh and asthma<sup>14</sup>. It also contain  $\alpha$ -fenchyl acetate (51.4%) and 1, 8-cineole (15.1%) as major constituents. *Alpinia calcarata* is a perennial herb with non-tuberous pungent rootstock. It is also used to stimulate digestion, treating colds and reducing swelling<sup>15</sup>.

The ethanolic extract of *Alpinia calcarata* exhibit significant anticancer property by inhibiting tumour cells proliferation, reducing tumour burden enhancing survival of tumour bearing mice<sup>16</sup> and also shows antioxidant property<sup>17</sup>. The hot water extract and hot ethanolic extract of *Alpinia calcarata* shows the antidiabetic activity<sup>18</sup> and essential oil possesses antibacterial activity<sup>19</sup>.

*Alpinia galanga* Wild. (Zingiberaceae) is a perennial herb found in countries like Thailand, Indonesia, China and Malaysia. Most of the South Indian Physicians of traditional Ayurveda and Siddha medicine use *Alpinia galanga* to treat various kinds of disease including Diabetes mellitus<sup>20</sup>. It is also used for the treatment of some inflammatory conditions as a digestive, spleen and liver tonic, and in dyspepsia, gastralgia, sea sickness. Rhizomes of *Alpinia galanga* (Linn.) are cooked as spice in many food preparations in Southeast Asia and it is also reported to have anti-HIV agents<sup>21</sup> and contain more bioactive compounds compared to other species in the genera<sup>22-24</sup>. *Alpinia galanga* also have compounds like galangoisoflavanoid<sup>25</sup>,  $\beta$ -sitosterol diglucosyl caprate and galanganol<sup>26</sup>. The rhizomes of *Alpinia galanga* possess Antileishmanial<sup>27</sup>, Insecticidal<sup>28</sup> properties.

## MATERIALS AND METHODS:

**Location and mode of Plant collection:** For powder microscopic studies the mature rhizomes and leaves of *Alpinia* species were collected from botanical garden of St. Joseph's Training College, Mannanam, Kottayam (Dt), Kerala during December 2012. Confirmation was further done with the help of floras and other reputed literature and annotated with the date of collection, the locality and medicinal uses. The voucher specimens were deposited in the Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College (Autonomous), Tiruchirappalli.

## Botanical description of plant materials:

### *Alpinia calcarata*:



FIG. 1: *ALPINIA CALCARATA* – HABIT, INSET DEPICTS A SINGLE INFLORESCENCE

Herbaceous plants nearly 2.5 m tall. Leaves distichous, a linear-lanceolate or linear, margin entire, apex long-acuminate, panicles terminal, to 10 x 4cm, narrow, rachis pubescent, bracts small, ovate, 1 or 2 flowered, calyx-tube funnel shaped, to 1cm, corolla white, labellum oblong or obovate, staminodes teeth-like, anther to 0.7 mm, ovary to 3.5 mm, stigma subglobose<sup>29</sup> (Fig. 1).

### *Alpinia galanga*:



FIG. 2: *ALPINIA GALANGA* – HABIT, INSET SHOWS A SINGLE FLOWER

An herbaceous plant up to 2 meter in height leaves long, narrow, green above, pale beneath, whitish on margins, median nerve very strong. Flowers about 3cm long, greenish white, in compound dense bunches; lip of corolla white, streaked with red. Fruit orange - red, size like that of a small cherry. Rhizome slightly aromatic Flowers greenish-white, streaked with red, in panicles<sup>29</sup> (Fig. 2).

### Microscopic study of powdered plant material:

The collected plant was washed with distilled water to remove the dust and adhering materials and then was dried under shade. The shade-dried material was powdered by means of mechanical grinder and the powder was used for powder microscopy. The macroscopic characters of both drug and powder were observed. Pinch of *Alpinia calcarata*'s leaf and rhizome powder were placed on the grease-free microscopic slide along with the help of glycerine and water (1:1), observed under Epifluorescence microscope at 10x followed by 40x magnification and important identifying characters were photographed. The same procedure was done for *Alpinia galanga*'s rhizome and leaf powder as well.

**RESULTS AND DISCUSSION:** The present study includes rhizomes and leaves of *Alpinia calcarata* Roscoe and *A. galanga* (Linn.) Willd. whose microscopical build up is different from each other and assessed by standard Pharmacognostical methods.

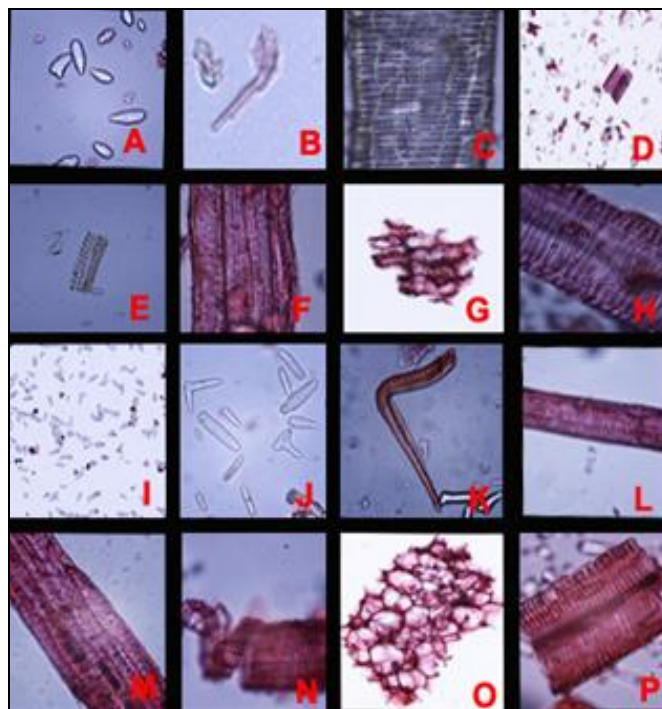


FIG. 3: RHIZOME POWDER STUDIES – A - H: *Alpinia calcarata*: A. Simple starch grains (oval shaped), B. Simple trichome, C. Pitted tracheids, D. 4x view of rhizome powder, E. Spiral tracheid, F. Presence of fibres, G. Parenchyma cells at places with aggregation of small silica crystalline matter, H. Scalariform tracheid. I – P: *Alpinia galanga* : I. 4x view of rhizome powder, J. Simple starch grains (oval shaped & Muller shaped), K. Simple trichome, L. Pitted tracheid, M. Presence of fibres, N. Spiral tracheid, O. Parenchyma cells at places studded with prismatic Crystals of Calcium oxalate, P. Scalariform tracheid.

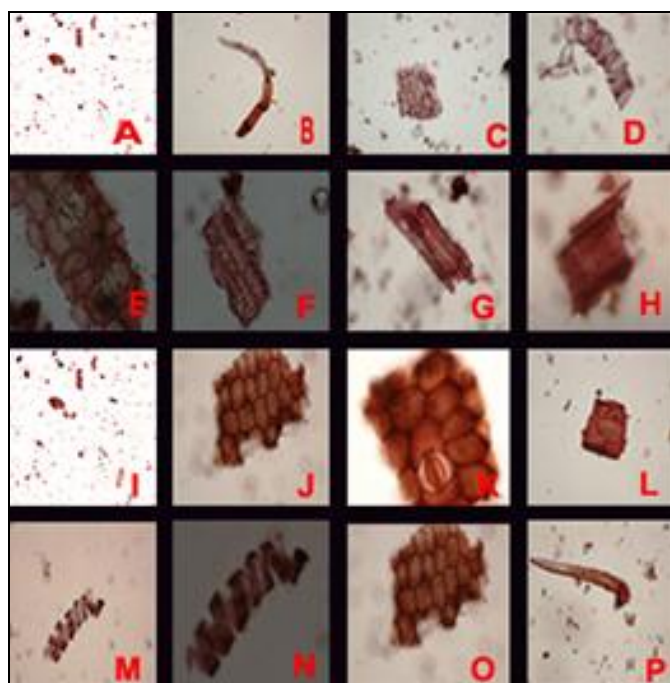
Present work as well as earlier reports reveals the presence of simple starch grains, simple trichome, vessels, fibres and pitted parenchyma in rhizome powder studies of both species<sup>30</sup>.

The rhizome powder is light brown colour with ginger like odour, and it shows some specific characters. The results show that *Alpinia galanga* consists of plenty of starch grains.

Some of the starch grains were muller shaped, some were triangular, pear shaped and most of them were round and oval shaped (Fig. 3). In the case of *Alpinia calcarata* Roscoe, simple starch grains mostly round and oval shaped were also found.

Parenchyma cells at places studded with aggregation of small silica crystalline matter were found only on *Alpinia calcarata* Roscoe and a few silica crystals were found in the parenchyma cells of *Alpinia galanga* (Linn.) Willd (Fig. 3).

Besides, other identification characters are vessels, fibers and trichomes that are bigger in *Alpinia galanga* (Linn.) Willd. with the diameter between 200 to 300 $\mu$ . Pitted fibres are more prominent in *Alpinia galanga* (Linn.) Willd. and parenchyma cells near vessels are at places studded with prismatic crystals of calcium oxalate. But in the case of leaf powder it is greenish colour with specific odour and shows the isolated reticulated vessel and open stomata (Fig. 4).



**FIG. 4: LEAF POWDER STUDIES - A- H: *Alpinia calcarata*:** A. 4x view leaf powder, B. Simple Trichome, C. Cortical parenchyma cells, D. Spiral tracheids, E. Open Stomata, F. Pitted tracheid, G. Presence of Fibres, H. Annular tracheid. **I- P: *Alpinia galanga*:** I. 4x view leaf powder, J. Stomata (20x view), K. Open Stomata (40x view), L. Pitted tracheids, M - N. Spiral tracheids (20 & 40x view), O. Cortical large parenchyma cells, P. Trichome.

Thus, Present study reveals an easy technique to identify two similar medicinal plant materials microscopically and this method can also be employed to detect the degree of adulteration in powdered raw medicinal plant materials as well.

**CONCLUSION:** 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. The observation of cellular level morphology or anatomy is a major aid for the authentication of drugs. From the above work it may be concluded that *Alpinia calcarata* and *Alpinia galanga* are important medicinal plants of great deal and are useful for its various properties by a number of pharmaceutical companies and general public. Still a lot of scope is there for research on these plants to explore it further for the well-being of humans.

A number of pharmaceutical preparations make it clear that these herbs are contributing a lot not only in the field of Ayurveda but also in modern system of medicine. These pharmacognostic characters can be used as a diagnostic tool for the correct identification of *Alpinia galanga* and *Alpinia calcarata*, which will be very useful in designing the monograph on these drugs in the Indian Pharmacopoeia.

The aim of the present work was to evaluate the microscopic features of the powdered form of both plants to support the pharmacological effects well. Although numerous studies have shown that the medicinal values of these plants, there still remains ample scope for further research.

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