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Histochemical studies of the rhizome of *Alpinia galanga* L. willd.

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ABSTRACT

The rhizome of *Alpinia galanga* L. Willd (Family: Zingiberaceae), were collected from three different regions, i.e., Udupi, Bisle ghat and Shringeri, shade dried under normal room temperature conditions and were subjected to histochemical studies for the presence of chemical components like calcium, starch, callose, chitin, lignin, phenolics, tannins and total proteins. The analyses revealed different colouration that indicated the presence either partially/completely or absence which were due to the growth of *Alpinia galanga* L. Willd in different regions had experienced change in various climatic factors like light, temperature etc.

Keywords: *Alpinia galanga* L. Willd, calcium, starch, callose, chitin, lignin, phenolics, tannins, total proteins.

INTRODUCTION

***Alpinia galanga* L. Willd. : (Family: Zingiberaceae)**
Synonyms: ***Amomum galanga* (Linn.) Lour.**
Vernacular Name: Eng: Greater galangal, Siamese ginger, Java galangal, Siamese galangal; Beng, Hin and San: Barakulanjar, kulanjan; Kan: Dumparasmi; Mal: Arattha, Kolinji, Peratta; Tam: Peraratthei; Tel: Peddadumparashtram (Prajapathi *et al.,* 2003).

A moderate shrub mostly referred as galangale or galanga, is a very popular spice in whole South East Asia and especially typical for the cuisine of Thailand. It is also known and used in Malaysia, Indonesia, Cambodia, Vietnam and Southern China. Chinese *five spice powder* is sometimes enhanced with galangale. In Western countries, however, galanga is not well known, at least in recent days; it has, however, been a valued spice in the early Middle-ages. Galangale is sometimes confused with other spices of the ginger family. Its taste and appearance are, however, characteristic; it cannot be substituted by any other spice (Prajapathi *et al.,* 2003). It is a rhizomatous herb which grows in dry deciduous forests, it almost resembles

that of ginger plant but it can be differentiated by the presence of minor hairs on leaf blades, broad leaves than ginger, inflorescence is in a cluster, flowers are pinkish white in color (Prajapathi *et al.,* 2003).

In India it is grown in south Kerala upto Wynad, Karnataka (Udupi, Sirsi, Shringeri, Bisle Ghat, Hassan) and parts of Tamilnadu. It is cultivated in december 2009 in Kerala.

The rhizome contains essential oils (1,8 cineol, α-pinene, eugenol, camphor, methyl cinnamate and sesqui terpenes). In dried galanga, the essential oil has quantitatively different composition than in fresh one. Whereas α-pinene, 1,8-cineol, α-bergamotene, *trans*-β-farnesene and β-bisabolene seem to contribute to the taste of fresh galanga equally, the dried rhizome shows lesser variety in aroma components (cineol and farnesene, mostly). The resin causing the pungent taste (formerly called galangol or alpinol) consists of several diaryl-sheptanoids and phenylalkanones (the latter are also found in ginger and grains of paradise). Furthermore, the rhizome is high in starch Zhao *et al*. (1992).

The fruits of *Alpinia galanga* are used as a traditional Chinese medicine; but the dry fruits of *A. conchigera*, *A. suishaensis*, *A. maclurei* and *A. polyantha* are also used as the medicine in local areas. Because dry fruits of these related plants are similar to those of *Alpinia galanga* L. Willd. in odor, morphological characters and chemical components, and even anatomical characters, it is difficult to identify the medicine. Nuclear ribosomal DNA internal transcribed spacer (ITS) regions of the five taxa were directly sequenced using an automated sequencer (Zhao *et al.,*1992).

The rhizome of *Alpinia galanga* L. Willd is extensively used in the preparation of the antibiotic, analgesic drugs which are useful in releiving the body pain (Zhao *et al.,*1992).

MATERIAL AND METHODS

The rhizomes of the *Alpinia galanga* L.Willd were collected from three locations i.e., Udupi, Bisle ghat and Shringeri. These rhizomes were subjected to shade dry under normal room temperature i.e., 36°C (Celsius) and atmospheric pressure i.e., 1013 mb (millibars). Later the rhizomes were powdered and subjected to the histochemical studies.

HISTOCHEMICAL STUDIES:

1. CALCIUM: Alkaline-Pyrogallol method:(Lison, 1936, Krishnamurthy, 1988) Fresh plant materials were taken and sectioned by free hand sectioning. The sections were treated in the Alkaline Pyrogallol reagent for 5 minutes and washed thoroughly until the sections become destained. Further, the sections were allowed to rest in water for several hours so that the colour developed slowly. Calcium stains to yellowish brown in colour.

2. STARCH: Iodine-Potassium iodide method: (Johansen, 1940, Krishnamurthy, 1988):The fresh plant materials were sectioned and mounted in Iodine-Potassium iodide solution. Starch appeared blue to balck in colour after few minutes. Newly formed starch appeared red to purple in colour.

3. CALLOSE: Soda method: (Chamberlain, 1924, Krishnamurthy, 1988):The fresh plant materials were sectioned and placed in a 4% aqueous solution of soda for 10 minutes. Later the sections were transfered to glycerin and mounted. Callose changes to a bright red.

4. CHITIN: Potassium hydroxide- Iodine-Potassium iodide method: (Johansen, 1940; Roelofsen and Hoette, 1951, Krishnamurthy, 1988):Fresh plant materials were treated in 120°C 23M Potassium hydroxide. Later it was autoclaved at 15 Psi, washed and stained in Iodine- Potassium iodide solution in 1% Sulphuric acid. A voilet or red-voilet colour indicated the presence of Chitin.

5. LIGNIN: Maule's reaction: (Johansen, 1940; Gibbs, 1958, Krishnamurthy, 1988)Fresh plant material were sectioned and treated in 1% neutral potassium permanganate solution for about 5 to 20 minutes. Later it was washed with distilled water and decolourized with 2% dilute hydrochloric acid. Further it was repeatedly washed thoroughly with water and treated with few drops of ammonium hydroxide or sodium bicarbonate solution. Lignin stained red, indicated its presence.

6. PHENOLICS: Nitroso reaction: (Reeve, 1951, Krishnamurthy, 1988):Fresh plant material were sectioned and 10% sodium nitrite, 10-20% urea and 10% acetic acid were added in equal volumes. Later after 3 - 4 minutes and to this 2 volumes of 2N sodium hydroxide were added.
Cherry-red colour indicated the presence of phenolics.

7. TANNINS: Ferric chloride method: (Reeve,1951, 1959; Svendsen, 1951, Jensen, 1962; Mace, 1963, Krishnamurthy, 1988): Fresh plant materials were sectioned and placed in 10% formalin solution containing 2% ferric chloride. Blue or blue green precipitate indicated the presence of tannins.

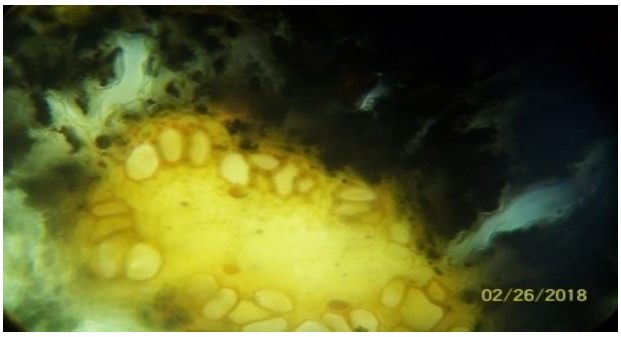
8. TOTAL PROTEINS: Fast green method: (Ruthmann, 1970, Krishnamurthy, 1988)
Fresh plant materials were sectioned and stained with fast green solution, gently heated by bunsen burner until the stain was evaporated (30-60 seconds). The stain was removed using distil water. Proteins appeared bright green.

RESULTS AND DISCUSSION

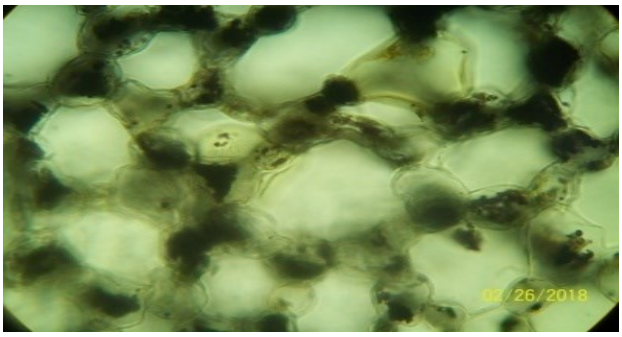
Dhale *et al.,* (2011) in their work stated that temporary macerated materials were involved for the analyses of histochemical of *Adhatoda zeylanica* Medik., *Ruta graveolens* L. and *Vitex negundo* L., 0.5% of $FeCl_3$ was used for the analysis of tannins but in the present study,

Table- 1: HISTOCHEMICAL TEST FOR IDENTIFICATION OF COMPOUNDS:

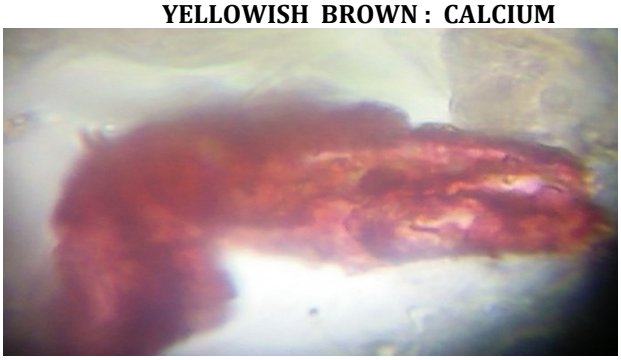
PLANT NAMES	LOCATIONS	CALCIUM	STARCH	CALLOSE	CHITIN	LIGNIN	PHENOLICS	TANNINS	TOTAL PROTEINS
***Alpinia galanga.* L.Willd.**	Bisle ghat-Dakshin Kannada dist.	Yellowish-brown Present	Blue-black Completely formed	Bright red-Present	Reddish appearence-Partially Present	Red-Present	Cherry Red-Present	Blue-blue green Present	Bright green Present
	Gonikoppa-Coorg	Yellowish-brown Present	Blue-black Completely formed	Bright red-Present	Voilet Partially Present	Red-Present	Cherry Red-Present	Blue-blue green Present	Bright green Present
	Udupi Shankaranarayana temple-Kodavoor	Yellowish-brown Present	Blue-black Completely formed	Bright red-Present	Reddish appearence-Partially Present	No red colour-Absent	Cherry Red-Present	Blue-blue green Present	Bright green Present



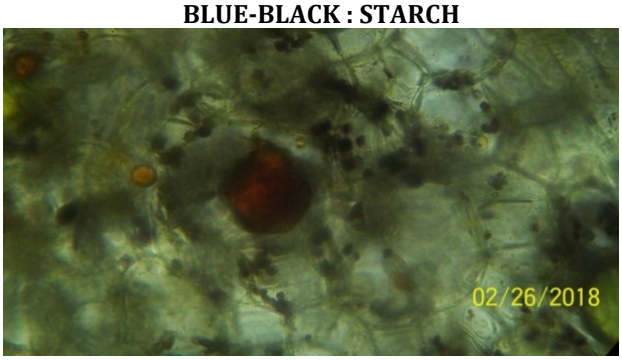
YELLOWISH BROWN : CALCIUM



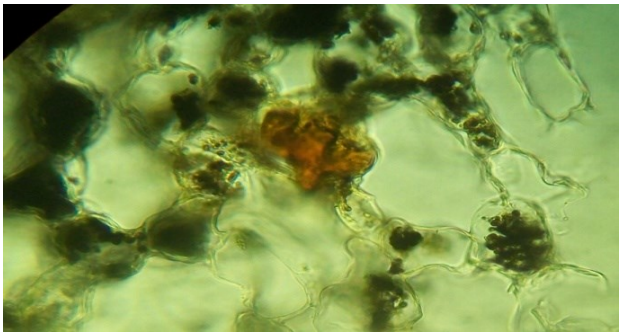
BLUE-BLACK : STARCH



BRIGHT RED : CALLOSE

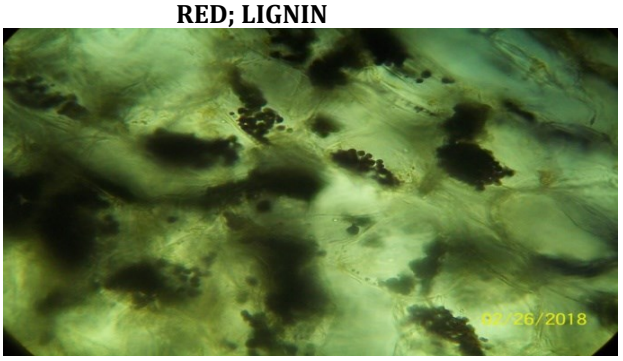


REDDISH APPEARENCE: CHITIN

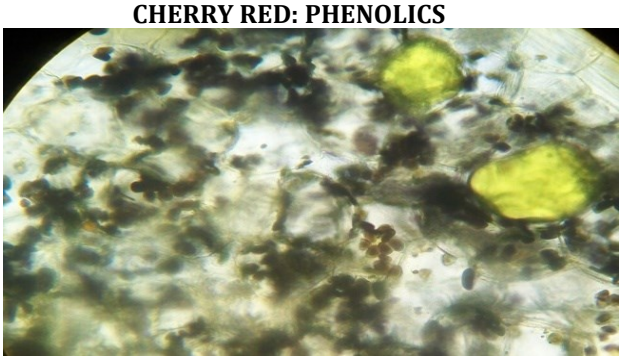


RED; LIGNIN

CHERRY RED: PHENOLICS



BLUE-GREEN : TANNIN



BRIGHT GREEN : PROTEINS

2% of $FeCl_3$ was utilized for the same with fresh rhizome of *Alpinia galanga.* L. Willd. along with formalin. Fernando and Vale (2016) in their studies employed a parallel method for the analysis of tannins., viz., Vanilin –hydrochloric acid method and Lignin, a compound organic polymer was detected through phloroglucinol method whereas it indicated its presence through potassium permanganate, hydrochloric acid and sodium bi-carbonate in case of rhizome of *Alpinia galanga.* L. Willd.

From the present studies on histochemical analysis, it was evident that the chemical compounds present in the root, rhizome and leaves vary in its extent of ratio like partial presence, complete presence etc.

In case of *Alpinia galanga.* L. Willd. the rhizome collected from Gonikoppa accession possessed all the copounds analyzed, except for chitin which was partially present. Lignin remained absent in case of Kodavoor accession. Bisle ghat and Kodavoor both exhibited the same features in common for calcium, starch, tannins and total proteins.

Bisle ghat proved to be the best among the three for the growth of *Alpinia galanga* L. Willd. which possessed all the histochemicals that were analyzed and their presence in the matured plant in full-fledged form irrespective of the seasonal and climatic changes.

Conflicts of interest: Not declared

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