ISSN : 0974 - 7427 Volume 5 Issue 2

Trade Science Inc.

BIOCHEMISTRY
An Indian Journal

Regular Paper

BCAIJ, 5(2), 2011 [109-114]

Morphological, histo-anatomical and biochemical studies in *Alpinia spp.*

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Received: 28th September, 2010 ; Accepted: 8th October, 2010

ABSTRACT

The genus Alpinia (zingiberaceae) comprises about 230 species. *Alpinia*, a traditional herbal plant, has been used as an aromatic stomachic, analgesic, and antiemetic in Asia. It contains many types of flavonoids, such as *galangin* and 3-omethylgalangin. The varieties of galangal vary in their hotness and flavor. Three varieties of galangal were investigated for their morphological, anatomical and biochemical characters. Different varieties of *galanga* exhibited significant degree of variation for the morphological traits observed. A significant variation in the constituents was observed among the plants studied. The quantity of the biochemical constituents varied with different variety of *galanga*. The accumulation of starch in the rhizome was confirmed by staining the cross sectioned rhizomes with iodine.

KEYWORDS

Alpinia;
Galangal;
Morphology;
Biochemical;
Staining.

INTRODUCTION

Plants are an integral part of nature. For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade[1]. *Alpinia Roxb*, is the largest and most widespread genus in the *Zingiberaceae* family with some 230 species occuring from Srilanka and Western Ghats of India to China, Japan, Fiji, Samoa and Australia[2]. These plants grow up to 10 feet high with 3-foot spread. Many species of *Alpinia* are appreciated for their medicinal properties, they have a long history of use in traditional medicine in China and India[3,4]. The most commonly found varieties of *galanga* are- greater *galanga*, lesser *galanga* and *kaempferia galanga*. Different galangal varieties vary in their hotness and flavor. Biological diversity has become one of the most popular topics recently for discussion both in scientific and political forum at local, national, regional and global level. It has been well documented that geographical conditions affect the active constituents of the medicinal plant and hence their activity profile[5]. In the present study morphological, histo-anatomical and biochemical characteristics of varieties of galangal were investigated.

EXPERIMENTAL

Collection of samples

Three species of *Alpinia* and one genus of *galanga* were collected from the center for medicinalplant heritage, kangikode. The collected samples were grown in

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the green house of Avinashilingsm University for Women,Coimbatore.

Morphological characterization

Four varieties of *galanga* collected from kangikode were planted in sand. The plants of same age were taken and the data was recorded for nine morphometric traits at intervals of two months: plant height, number of leaves/ plant, leaf area, number of roots/rhizome, fresh rhizome weight, rhizome color, number of buds, number of nodes and inter nodes. An average of ten plants was measured each time.

Histoanatomical studies of rhizome

The histoanatomical investigations were performed on the rhizomes of three species of Alpinia and one genus of galanga. The cross sections of the rhizomes were taken and they were analysed using the stain Touledine blue. The pattern of starch accumulation was also checked by staining the sectioned rhizome with Iodine.

Biochemical analysis of samples

The crushed rhizome samples of *Alpinia* were analyzed for their total carbohydrate, reducing sugars, starch, proteins, total amino acid content, flavones, flavonol and total phenolic content.

Estimation of total carbohydrates

The extraction procedure of Hedge and Hofreiter[6] was followed for the estimation of total carbohydrates present in fresh rhizome samples of *Alpinia.* Total carbohydrate present in 100mg of samples was hydrolyzed with 5 ml of 2.5N HCl and cooled to room temperature. Then neutralized the hydrolysate with sodium carbonate until the effervescence ceased and made up the volume to 100 ml. Centrifuged at 2500rpm for 2 minutes and the supernatant was taken for the estimation of carbohydrates by Anthrone method. The total carbohydrate present in 1 g of the sample was calculated.

Estimation of reducing sugars

The extraction procedure of Krishnaveni et al.[7], was followed for the estimation of reducing sugars present in the rhizome samples of *Alpinia.* Reducing sugars present in 100mg the crushed fresh samples were extracted twice with 5 ml of hot 80% ethanol.

Plate 1 : Varieties of *galangal*: (1) Alpinia officinarum; (2) Alpinia galangal; (3) Kaempferia galangal; (4) Alpinia calcaratta

The supernatant was collected and evaporated by keeping it on a water bath maintained at 80°C. Dissolved the sugars with 10 ml of water and aliquots of 0.1 and 0.2 ml were used for estimation. The amount of reducing sugars present in 1 g of the sample was calculated.

Estimation of starch

The extraction procedure of Thayumanavan and Sadasivam[8] was followed for the estimation of starch present in 1g of fresh rhizome samples of *Alpinia.* Crushed sample (100mg) was extracted with hot 80% ethanol to remove the sugars. To the dried residues added 5.0 ml of water and 6.5 ml of 52% perchloric acid and extracted the starch in ice for 20 minutes. Centrifuged and saved the supernatant. Repeated the extraction using fresh 52% perchloric acid. Centrifuged, pooled the supernatants and made up volume to 100 ml. The supernatant (0.1ml) was used for estimation. The starch content in 1g of the rhizome samples was calculated.

Estimation of proteins

The extraction procedure of Lowry[9] was followed for the estimation of proteins. The proteins present in 100 mg of the samples was extracted with 15 ml of the phosphate buffer (pH 6.8) with a mortar and pestle. Centrifuged and used 0.1 ml of the supernatant for estimation. The protein content in 1g of the sample was calculated.

Estimation of total amino acids

The extraction procedure of Moore and Stein[10] was followed for the estimation of total aminoacids. The total amino acids present in 0.1 g of sample was extracted with 10ml of hot 80% ethanol. Filtered and centrifuged. Repeated the extraction twice with the residue

and pooled the supernatants. 0.1 ml of the supernatant was used for estimation. The total amino acid present in 1 g of the sample was calculated.

Estimation of flavone and flavonol

About 500mg of fresh rhizomes of *Alpinia* are crushed with 5ml of 70% ethanol and extrated 24 h at room temperature. It was then filtered, and the procedure was repeated twice. The extracts were filtered, combined and diluted to 10 ml with 70% ethanol. 1.0 ml of the solution was used for estimation. The flavone present in 1g of the sample was calculated.

Estimation of flavanone and dihydroflavonol content

About 0.5g of fresh rhizomes of *Alpinia* are crushed with 5ml of 70% ethanol and extracted for 24 h at room temperature. It was then filtered, and the procedure was repeated twice. The extracts were filtered, combined and diluted to 10 ml with 70% ethanol. 1.0 ml of the solution was used for estimations. The flavanone and dihydroflavonol present in 1g of the sample was calculated.

Estimation of total phenolics

About 0.5g of fresh rhizomes of *Alpinia* are crushed with 5ml of 70% ethanol and extracted for 24 h at room temperature. It was then filtered, and the procedure was repeated once again. The extracts were filtered, combined and diluted to 10 ml with 70% ethanol. 1.0 ml of the solution was used for estimation. The total phenolics present in 1g of the sample were calculated.

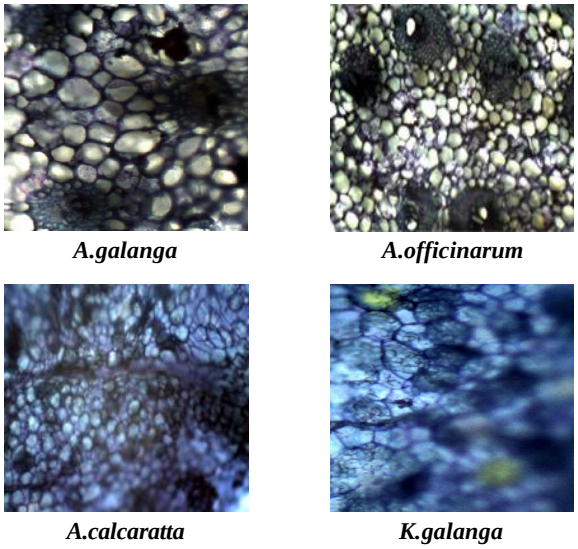
RESULTS AND DISCUSSION

Morphology of collected samples

Morphological variability

The mean values of 9 morphological traits for *Alpinia* are presented in TABLE 1. There exists a large variation in the morphology among the varieties of galanga.

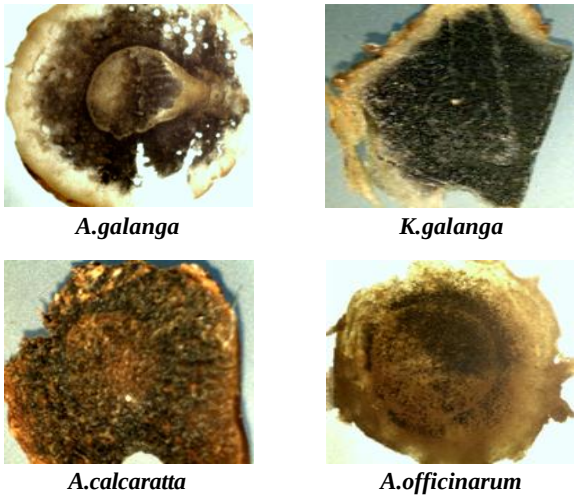
The shape and appearance of leaves were assessed, *Alpinia galanga* and *Kaempferia galanga* had short-broad leaves and *Alpinia calcaratta* had long-thin leaves whereas long-broad leaves were observed in *Alpinia officinarum* (Plate 2). *Alpinia calcaratta* had a thin rhizome whereas all the others had a thick rhizome. Different varieties of galanga exhibited significant degree of variation for the traits observed. In case of rhizome color each variety had a unique color (TABLE 1).



A.galanga *A.officinarum*

A.calcaratta *K.galanga*

Plate 2 : C.S of Rhizomes stained with toluedine blue



A.galanga *K.galanga*

A.calcaratta *A.officinarum*

Plate 3 : C.S of Rhizomes

Shoot length

On comparing the plant height among different varieties of galanga, *Alpinia officinarum* has the highest shoot length (19±0.52) and the lowest shoot length was observed in *Kaempferia galanga* (7±0.81cm) (TABLE 1).

Number of roots

The number of roots varied among the samples. The highest number of roots was observed in *Alpinia*

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TABLE 1 : Morphology of different vatrieties of galanga

Morphological traits	*Alpinia officinarum*	*Alpinia calcaratta*	*Alpini galanga*	*Kaempferia galanga*
Color of the rhizome	Pale yellow	Brown	Pale brown	white
Nature of rhizome	Thick	Thin	thick	thick
Nature of the leaves	Long-broad	Long-thin	Short-broad	Short-broad
Color of the rhizome after cross sec.	Pale yellow	Brown	Pale brown	white
Number of leaves per plant	4±0.67	6±0.67	5±0.82	3±0.47
Shoot height	19±0.52	15.5±0.47	16.5±0.82	7±0.82
Number of roots	12±0.48	18±0.82	6±0.87	5±0.87
Number of nodes per 3cm	10±0.49	5±0.47	4±0.47	4±0.47
Number of internodes per 3cm	8±0.47	4±0.67	3±0.67	3±0.82
Weight of rhizome(g)	28.87	24.54	14.89	8.6

TABLE 2 : Biochemical composition of ethanol extracts of galangal

Constituents(mg/g)	*Alpinia officinarum*	*Alpinia calcaratta*	*Alpinia galanga*	*Kaempferia galanga*
Total carbohydrates	37.2±0.46	122.8±0.33	146.3±0.23	80.8±0.54
Starch	11. 5±0.26	38.2±0.65	93.1±0.11	60.3±0.12
Reducing sugars	3.4±0.08	7.9±0.07	6.9±0.08	9.5±0.24
Non reducing sugars	22.4±0.26	76.7±0.17	46.4±0.56	11.3±0.34
Proteins	156.5±0.39	246.2±0.8	380.9±0.56	193.2±0.07
Amino acids	11.7±0.339	5.7±0.56	12.1±0.40	9.2±0.76
Flavone & Flavanol	80.8±0.41	128.8±0.29	148.8±0.32	98.6±0.62
Flavonone & dihydroflavanol	194.9±0.77	207.5±0.22	267.7±0.11	74.5±0.55
Total phenols	4.1±0.07	13.42±0.18	3.2±0.46	2.5±0.47

calcaratta (18±0.82) followed by *Alpinia officinarum* (12±0.48). The lowest number of roots was seen *Kaempferia galanga* (5±0.87).

Number of leaves per plant

Among the different species the lowest number of leaves was observed in *Kaempferia galanga* and highest in *Alpinia calcaratta* (TABLE 1).

Number of nodes and internodes per 3cm

The number nodes per 3cm of the rhizome ranged from 4±0.47 (*Kaempferia galanga*) to 10±0.49 (*Alpinia officinarum*).

The highest number of internodes was observed in *Alpinia officinarum* 13±0.67). The lowest number observed in samples *Alpinia galanga* and *Kaempferia galanga* (3±0.67).

Cross section of rhizomes

The variability in the morphological traits was also checked by cross sectioning the rhizomes of Alpinia. Cross section of the rhizomes showed three distinct concentric ring structures. The cross section is circular in outline with a yellow to brown coloured exodermis consisting layers of cells. When viewed under 10X magnification in a compound microscope vascular bundles are seen scattered in the cortex, outer layer appeared thin.

The cambium forms a continuous area, which generated, towards the inner side, a thick ring of xylem, in which the rays are either solitary or grouped in a small number on the internal side of the ring, in a rich cellulosic parenchyma, within which a few vessels are also dispersed. The middle layer showed some granules. To differentiate between the vascular bundles the rhizome sections were stained with the differential stain Toluiedine blue. The xylem vessels appeared blue-green in color whereas the phloem appeared colourless (Plate 2).

Grigore and Toma (2007) explained that the rhizomes of *Lepidium perfoliatum* have thin outer layer, followed by a continuous layer of cambium in which the xylem are scaterred either solitary or grouped. The rhizomes of galangal also have similar structure.

Alpinia officinarum and *Alpinia calcaratta* showed a similar arrangement of vascular bundles whereas in *Alpinia galanga* and *Kaempferia galanga* the whole tissue stained blue , showed some oil bodies which are green in colour and only two layers were observed (Plate 2). Similar, results were reported by Saensouk et al.[11] in three species of *Cornukaempferia*.

The cross sectioned rhizomes were also stained with iodine, noticeable differences were observed in the structure. In case of *Alpinia calcaratta* both the middle portion and inner portion stained black indicating a large accumulation of starch grains. In case of *Alpinia galanga* and *Kaempferia galanga* the whole tissue stained black. The results obtained are in accordance to he results of biochemical analysis. As observed in cross section, in biochemical analysis also the concentration of starch was more in *Alpinia calcaratta*.

The presence of bioactive metabolites can be correlated with the starch grains. *Alpinia galanga* which has showed large quantity of flavonoids exhibited a direct relationship with starch content, whereas in case *Alpinia officinarum* inverse relation was observed.

TABLE 3 : Biochemical composition of ethylacetate extracts of galangal

Constituents (mg/g)	*Alpinia officinarum*	*Alpinia calcaratta*	*Alpinia galanga*	*Kaempferia galanga*
Total carbohydrates	0.6±0.05	4.6±0.24	6.3±0.24	1.9±0.02
Starch	0.3±0.02	1.1±0.27	4.8±0.10	1.7±0.01
Reducing sugars	0.1±0.02	0.1±0.08	0.1±0.22	0.2±0.17
Non reducing sugars	0.2±0.17	3.4±0.05	1.4±0.33	0.2±0.32
Proteins	1.6±0.24	6.6±0.47	8.2±0.07	2.9±0.46
Amino acids	3.3±0.57	2.8±0.49	3.8±0.52	3.1±0.18
Flavone & Flavanol	29.6±0.03	67.8±0.75	74.3±0.68	30.8±0.73
Flavonone & dihydroflavanol	17.5±0.61	17.9±0.48	18.3±0.19	8.2±0.25
Total phenols	3.7±0.02	9.3±0.01	2.8±0.32	0.4±0.03

Biochemical analysis of samples

Khanna et al.[12] indicated that the estimation of biochemical attributes is informative for finding distinctness among the different morphotypes as these are the direct gene products. In the present study, ethanol and ethyl acetate fractions of rhizome extracts were used for quantification of proximate principles namely starch, reducing sugars, non reducing sugars, proteins amino acids, flavanoids and total phenolics present in them. All the estimations are done in 10 replicates and the results are presented in TABLE 2 and 3.

Biochemical constituents of galangal varied with different species of galanga. Among the ethanol extracts of four species, *Alpinia galanga* (146.3±0.23 mg/g) has the highest quantity of total carbohydrates followed by *Alpinia calcaratta* (122.8±0.33mg/g). The lowest quantity of carbohydrate was noticed in *Alpinia officinarum* (37.2±0.46mg/g).

The starch content in the collected rhizome samples was quantified. It was found that, the starch content varied ranging from 11. 5±0.26 mg/g to 93.1±0.11mg / g. The lowest amount of starch was recorded for the variety *Alpinia officinarum* whereas the highest amount of starch was found in *Alpinia galanga*.

So far no studies are found in Alpinia regarding the content of sugars. Reducing and non-reducing sugar content was analyzed and it was found that, reducing sugars ranged between 3.4±0.07mg/g (*Alpinia officinarum*) and 9.5±0.24 mg/g (*Kaempferia galanga*). The non-reducing sugars ranged between 11.3±0.34 mg/g (*Kaempferia galanga*) and 76.7±0.17mg/g (*Alpinia calcaratta*).

Rai et al.[13] studied the variation in the sugars and chlorophyll content in different cultivars of ginger and they also found that the carbohydrates more in the rhizomes collected from Gorubathaney.

Amino acids and protein content of the rhizome extracts were estimated and the results obtained indicate that proteins and amino acids were found in highest amounts in the variety *Alpinia galanga* (380.9 ± 0.56mg/g, 12.1 ± 0.40mg/g). The lowest amount of protein was observed in *Kampferia galanga* (193.2± 0.07mg/g) and aminoacid was lower in *Alpinia calcaratta* (5.7 ± 0.56mg/g).

Flavonoid, the major active principle in the rhizome extracts was estimated. It was observed that the concentration of flavonoids was more in *Alpinia galanga* (148.8±0.32mg/g, 267.7±0.11mg/g) less in *Alpinia officinarum* (80.8±0.4mg/g) & *Kaempferia galanga*l (74.53±0.55mg/g) (Plate 1). Nair et al., (1998) estimated the flavonoid content in common Indian foods and they reported that more than 100mg of flavonoids are present in 10g of turmeric.

Phenolics were also found, the lowest quantity was found in *Kaempferia galanga* (2.5±0.47mg/g) and highest in *Alpinia calcaratta* (13.4±0.19mg/g) (TABLE 2).

The ethyl acetate fractions of galangal varieties also showed similar results as their corresponding ethanol fractions. *Alpinia galanga* had highest amount of carbhohydrates (6.3±0.24mg/g), starch (4.8±0.1mg/ g) followed by *Alpinia calcaratta* (4.6±0.24mg/g) and *Kaempferia galanga* (1.7±0.01mg/g).

The highest quantity of protein was found in *Alpinia galanga* 8.2±0.07mg/g and lowest in *Alpinia officinarum* 1.6±0.24mg/g. Pruthi (1993)[14] studied the protein content in the rhizomes of ginger and reported they have about 8.6% of protein.

The major components flavanoids are more in *Alpinia galangal* and the phenols are more in *Alpinia calcaratta* (TABLE 3). similar results were obtained by Chan et al.[15].

ABBREVIATIONS

A.galanga-Alpinia galanga,
A.officinarum-Alpinia officinarum,
A.calcaratta-Alpinia calcaratta,

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K.galanga-Kaempferia galangal.

CONCLUSION

A wide range of variation was noticed for both quantitative and qualitative traits namely, rhizome colour, colour after cross section, nature of rhizome, leaf, weight of rhizome, number of node-internodes, number of leaves and shoot length and this indicated the existence of morphological diversity in the selected varieties. Similars results from the histoanatomical and biochemical studies also confirms the exsistance of variability among the varieties of galanga.

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