A pharmacognostical report on the rhizome of
Alpinia galanga Linn. (Willd).

M. Chitra and J.E. Thoppil
Genetics and Plant Breeding Division, Department of Botany
University of Calicut, Kerala - 673 635, India

Received : 30.10.2007
Accepted: 12.02.2008

Abstract:

Alpinia galanga Linn. (Willd) is an important raw drug used in large quantities in various indigenous formulations. In this paper the major diagnostic characters and the qualitative chemical and physical tests responsible for the pharmacognostic identity of the rhizomes have been reported.

Introduction

Alpinia galanga Linn. (Willd) belonging to Zingiberaceae family is known as

| S.No. | Language | Name |
|-------|----------|------|
| 1     | English  | Java galangal, great or greater galanga |
| 2     | Hindi    | Kulanjan |
| 3     | Malayalam| Aratta, Peraratta |
| 4     | Tamil    | Anandam, Arattai, Arbudam, Attumam, Kandanaguliyan, Ormarundu, Perattai, Sattiradji, Sugandam, Tittiram, Tumarattagam |
| 5     | Sanskrit. 1,7,8,14 | Aruna, Dhumala, Dhumparastma, Elaparni, Gandhamula, Gandhavaruni, Kapidruma, Koraja, Kulanja, Kemooka, Kulantana, Kulanjana, Kulin-jana, Kulinjana, Kushtam, Mahabaravach, Mahabharavacha, Mahabharivaca, Malayavaca, Nakuli, Patala, Purusha, Raktarenu, Raktapupshpa, Rasna, Sthulagranthi, Sugandhavacha, Sugandha, Sugadhamula, Sugandhayoga, Tikshnamula |

The plant is distributed throughout the Western ghats and is also cultivated.1,2 It is used in more than sixty two ayurvedic formulations like Dasanoolarishtam, Rasnadi kashayam (Small), Rasnadi ghritam, Rasnadi choornam, Chandanasavam, Gulguluthikthakam kashayam, Aswagandharishtam, Balarishtam etc.10 The plant is used in rheumatism, dyspepsia, cough and in kidney stones.7,8,10,14 Pharmacognostical studies were carried out on the rhizomes which includes detailed microscopy, ash values, extractive values and preliminary phytochemical studies.

Materials and Methods

The rhizomes of A. galanga were collected locally from mature plants during the month of October-November, 2006. The plant materials were identified and authenticated. The voucher specimens were deposited in the Herbarium, Department of Botany, University of Calicut, for future reference. The collected specimens were washed with tap water to remove adhering dirt and dust. Some of the rhizomes were
fixed in FAA, while the remaining were air dried for powder analysis.

Macroscopic characters of the rhizomes were observed. To study the anatomical details of the rhizome sections were cut at 10-15 μm thickness using Sledge microtome and customary methods were followed for dehydration and maceration studies. All the stained micro slides were observed under Olympus B x 50 research microscope and photo micrographs of important parts were taken using SLR camera for image repository for future reference. Measurements of cells/tissues were made with the help of micrometers under Olympus B x 50 research microscope. Histochemical tests such as calcium oxalate crystals, tannins, starch and lignins were carried out. Standard procedure was followed for estimation of ash values, extractive values, thin layer chromatography and fluorescence analysis. Preliminary phytochemical tests of different extracts were performed by using specific reagents.

Results
Macroscopic Characters of A. galanga Rhizome

| Character                  | Description                                                                 |
|----------------------------|----------------------------------------------------------------------------|
| Length                     | 2 – 8 cms                                                                  |
| Width                      | 2-3 cms                                                                    |
| Shape                      | Cylindrical                                                                |
| Branching                  | Cylindrical branches short, base of the aerial shoot swelling and becoming subglobose |
| Rootlets Present or absent | Present                                                                    |
| Kind                       | Numerous                                                                   |
| Direction of growth        | Horizontal                                                                 |
| Surface characters         | It is marked with wavy annulations of the leaf bases, which possess a lighter colour than the remaining surface (Fig. 1). |

Histological Studies:
The transverse sections of the rhizome of Alpinia galanga show the following characters.

Epidermis:
Composed of a single row of narrow tangentially elongated cells (Fig. 2). Outer tangential walls are thicker than inner tangential and radial walls. In fresh material cells are found filled with same dark contents (Fig. 3).

Hypodermis:

Cells are smaller and arranged without inter cellular spaces and are devoid of starch grains (Fig. 3).

Cortex:
Fairly broad and parenchymatous. Outer cortical cells are polygonal thin walled and arranged compactly with small triangular spaces. Numerous oleoresin cells are seen in this area (Fig. 2). Inner cortical cell is composed of thin walled polygonal cells with numerous vascular bundles in them. The cells are filled with long simple rod shaped starch grains.

Endodermis:
Is composed of a row of thin walled cells appearing barrel shaped in cross section. Casparian thickening is not conspicuous. Starch grains are absent in this area (Fig. 7).

**Pericycle :**

Is composed of single row of thin walled cells (Fig. 7).

**Vascular bundles :**

Fibrovascular bundles are scattered through out the cortex and stele without any definite orientation and are collateral endarch and closed type. Peripheral bundles are small with a group of central xylem and with a small patch of phloem surrounded by the bundle sheath (Fig. 4) and the inner bundles are either similar to peripheral bundles or seen as two or three separate strands of xylem with a patch of phloem and surrounded by the bundle sheath. Stelar bundles have irregular orientation. Bundles towards the interior are larger and better developed than the outer bundles (Fig. 6).

**Bundle Sheath :**

Is sclerenchymatous which is broader towards the xylem side. In the small bundles the sheath is composed of one or two rows. In the larger bundles it is composed of 3, 5 or 6 rows (Fig. 6).

**Cambium :**

Meristematic layers of cells present (Fig. 7).

**Xylem :**

Vessels are with scalariform or reticulate thickening with paratracheal xylem parenchyma (Fig. 6).

**Phloem :**

Sieve tube, 1-2 companion cells and phloem parenchyma are present (Fig. 6).

**Ground parenchyma :**

Cells are smaller than the cortical cells and are filled with starch grains but their quantity is less than in the cortical cells. Oleoresin cells are also present.

**Starch grains :**

Show variation in size and shape. Few are circular, some muller shaped but the majority are long rod shaped with one rounded broad end and one pointed end. Some starch grains have small protrusions in them (Fig. 7).

**Diagnostic characters :**

In the stelar bundles, the bundle sheath does not completely encircle the vascular bundle as seen in the cortical bundle (Fig. 7). Bundle sheath is well developed in the larger bundles and has an arch like appearance. The occurrence of two bundles together is a common feature of the cortical region (Fig. 5). The peripheral portion of ground tissue is translucent, interior opaque, whitish and central cylinder is translucent. In older rhizomes the peripheral translucent zone turns opaque due to starch deposition.

**Powder Characteristics**

The powder is light orangish brown in colour with light aromatic agreeable odour and pungent taste. It shows the following characters.

1. Fragments of epidermal cells are present.
2. Parenchymatous cells, oleoresin cells with yellow coloring matter are present.
3. Fibres with elongated, lignified and tapering ends (Fig. 9).

4. Vessels are thick walled, elongated having scalariform or spiral thickening and with blunt ends (Fig. 8).

5. Starch grains show variation in size and shape. Few are circular some muller shaped but the majority are long rod shaped with one rounded broad end and one pointed end (Fig. 7).

Discussion

The macroscopic as well as microscopic studies of A. galanga Linn.(Willd) revealed that using these diagnostic features one can identify this plant very easily for further investigation. Bundle sheath is well developed in the larger bundles and has an arch like appearance. The occurrence of two bundles together is a common feature of the cortical region. The peripheral portion of ground tissue is translucent, interior opaque, whitish and central cylinder is translucent. In older rhizomes the peripheral translucent zone turns opaque due to starch deposition. These are useful identifying features obtained after studying histology of the rhizomes of A. galanga. The information obtained from ash values and extractive values are useful during the time of collection of rhizomes and also during the extraction process. The colour and consistency of the various polar and non-polar extracts of rhizomes, the behaviour of rhizome powder on treating with various chemical reagents and the histochemical changes observed on the T.S. of rhizomes due to the action of various reagents are useful phytochemical parameters which can be of great help in identifying samples of genuine drug. TLC provides semi quantitative information on the chief constituents of the plant drug and enables the assessment of drug quality. Using these standards especially histological and chemical studies the plant can be authenticated, identified and differentiated from other related species. Also these pharmacognostic parameters help in the detection of adulteration in commercial samples.

Table - 1. Micrometrical measurement of cells/tissues of A. galanga rhizome in micron (µ)

| Type of Cells/Tissues | Measurement in micron(µ ) |
|-----------------------|--------------------------|
|                       | Range               | Mean ± S.D. |
| Epidermal Cells       | Length 12 – 24      | 18.0 ± 4.32 |
|                       | Breadth 9 – 15       | 12.0 ± 1.91 |
| Hypodermal cells      | Length 25 – 52       | 41.28 ± 10.12 |
|                       | Breadth 24 – 37      | 30.28 ± 4.49 |
| Outer zone            | Length 38 – 196      | 103.14 ± 55.56 |
|                       | Breadth 45 – 74      | 57.85 ± 11.93 |
| Inner zone            | Length 46 – 82       | 64.28 ± 12.31 |
|                       | Breadth 32 – 60      | 47.14 ± 10.23 |
| Endodermis            | Length 18 – 46       | 34.71 ± 9.98 |
|                       | Breadth 14 – 23      | 18.71 ± 3.04 |
| Pericycle             | Length 15 – 26       | 20.71 ± 3.95 |
|                       | Breadth 10 – 15      | 13.14 ± 1.77 |
| Vessels               | Length 35 – 74       | 55.57 ± 13.96 |
| Nature of Ash               | % of (W/W) ash |
|----------------------------|----------------|
| Total ash                  | Not more than 5 % |
| Acid insoluble ash         | Not more than 2 % |
| Water soluble ash          | Not less than 13 % |
| Loss in weight on drying at 110°C | Not more than 2 % |

**Table - 3. Extractive values of different solvents, percentage of extractability, colour and consistency of extract of A. galanga rhizome**

| S.No. | Solvent used           | Percentage of extractability | Colour of extract | Consistency of extract |
|-------|------------------------|-------------------------------|-------------------|------------------------|
| 1     | Petroleum ether (60-80°C) | 2.33 + 1.06                   | Brown             | Oily                   |
| 2     | Benzene                | 0.62 + 0.45                   | Pale Brown        | Sticky                |
| 3     | Chloroform             | 1.37 + 0.15                   | Pale Brown        | Powdery               |
| 4     | Acetone                | 3.08 + 0.18                   | Yellowish Brown   | Oily & Sticky         |
| 5     | Methanol (90%)         | 3.26 + 1.10                   | Brownish Yellow   | Semi solid            |
| 6     | Distilled water        | 10.8 + 1.67                   | Brown             | Powdery               |

**Table - 4. Fluorescence analysis of the rhizome powder of A. galanga under Ultra Violet light**

| S.No. | Treatment                   | Fluorescence                      |
|-------|------------------------------|-----------------------------------|
|       |                              | In daylight | In UV light (365nm) |
| 1     | Rhizome powder as such       | Yellowish Brown                     | Off white          |
| 2     | Powder + 1 N NaOH            | Yellowish Brown                     | No fluorescence    |
| 3     | Powder + 1 N KOH             | Yellowish Brown                     | No fluorescence    |
| 4     | Powder + 50% H₂SO₄           | Dark Brown                          | Black              |
| 5     | Powder + 1 N HCl             | Yellow                             | Pale Yellow        |
| 6     | Powder + 50% HNO₃            | Chocolate Brown                     | Black              |
| 7     | Powder + 5% FeCl₃            | Dark Brown                          | No fluorescence    |
| 8     | Powder + 5% Iodine Solution  | Bluish Black                        | No fluorescence    |
| 9     | Powder + Picric acid         | Pale yellow                         | Pale Brown         |
| 10    | Powder + Acetic acid         | Yellowish Brown                     | Pale yellow        |
| Tests                      | Mayer’s Reagent | Dragendorf’s Reagent | Wagner’s Reagent | Hager’s Reagent | Molisch Test | Fehling’s Reagent | Leagal’s Test | Millions Test | Ninhydrin Test | Lieberman Burchard’s |
|---------------------------|----------------|----------------------|------------------|----------------|--------------|------------------|-------------|-------------|--------------|---------------------|
| Alkaloids                 | -ve            | +ve                  | +ve              | -ve            | -ve          | -ve              | -ve         | -ve         | -ve          | -ve                  |
| Carbohydrates and Glycosides | -ve            | -ve                  | -ve              | -ve            | -ve          | +ve              | -ve         | -ve         | +ve          | -ve                  |
| Proteins and Amino acids  | -ve            | -ve                  | -ve              | -ve            | +ve          | -ve              | -ve         | +ve         | -ve          | -ve                  |
| Steroids/Terpenids        | +ve            | -ve                  | -ve              | -ve            | -ve          | -ve              | -ve         | -ve         | -ve          | -ve                  |

Table - 5. Preliminary phytochemical screening of various extracts (obtained by successive solvent extraction of plant material) of rhizome of A.galanga

| Tests       | Petroleu m Ether (60-80°C) | Benzene | Chloroform | Acetone | Methanol | Water |
|-------------|----------------------------|---------|------------|---------|----------|-------|
| Alkaloids   | Mayer’s Reagent            | -ve     | +ve        | +ve     | -ve      | -ve   |
|             | Dragendorf’s Reagent       | -ve     | +ve        | +ve     | -ve      | -ve   |
|             | Wagner’s Reagent           | -ve     | +ve        | +ve     | -ve      | -ve   |
|             | Hager’s Reagent            | -ve     | +ve        | +ve     | -ve      | -ve   |
| Carbohydrates and Glycosides | Molisch Test | -ve | -ve | -ve | -ve | -ve |
|              | Fehling’s Reagent          | -ve     | -ve        | -ve     | -ve      | +ve   |
|              | Leagal’s Test              | -ve     | -ve        | -ve     | -ve      | +ve   |
| Proteins and Amino acids  | Millions Test              | -ve     | -ve        | -ve     | -ve      | +ve   |
|              | Ninhydrin Test             | -ve     | -ve        | -ve     | -ve      | +ve   |
| Steroids/Terpenids        | Lieberman Burchard’s       | +ve     | -ve        | -ve     | -ve      | -ve   |
| Test     | Saponin | Foam Test | -ve | -ve | -ve | -ve | -ve | -ve |
|----------|---------|-----------|-----|-----|-----|-----|-----|-----|
| Tannins  | Braemers Test | -ve | -ve | -ve | +ve | -ve | -ve | -ve |
| Resins   | Resin Test   | -ve | -ve | -ve | +ve | -ve | -ve | -ve |

Table - 6. Histochemical Analysis of the T.S. of rhizomes of A.galanga

| S.No. | Reagents           | Test for   | Nature of Change | Histological zone | Result |
|-------|--------------------|------------|------------------|-------------------|--------|
| 1     | Phloroglucinol + Conc. HCl + alcohol | Lignin     | Pink             | Xylem vessel      | +ve    |
| 2     | Iodine solution    | Starch     | Black            | Cortex & Stele   | +ve    |
| 3     | Sudan III          | Oil        | Pink             | Cortex & Stele   | +ve    |
| 4     | H₂SO₄ (20%)        | Calcium Oxalate | No change    | Whole Section | -ve    |
| 5     | Methylene Blue     | Mucilage   | No Change        | Whole Section    | -ve    |

Table - 7. Thin layer Chromatographic analysis of rhizomes of A.galanga

| Visualization | Rf   | Colour                  |
|---------------|------|-------------------------|
| 254 nm        | 0.43 | Brown Black             |
|               | 0.93 |                         |
|               |      | (Fig. 10).              |
| 365 nm        | 0.53 | Off white Yellow        |
|               | 0.89 |                         |
|               |      | (Fig. 11).              |
| Vanillin Sulphuric acid | 0.18 | Black Pale brown      |
|               | 0.32 | Blue Pale violet       |
|               | 0.64 |                         |
|               | 0.93 |                         |
|               |      | (Fig. 12).              |
LEGENDS FOR THE FIGURES PROVIDED

Figs. 1-12. Macroscopic, microscopic and TLC details of Alpinia galanga rhizome

(1) Macroscopic appearance of the rhizome of Alpinia galanga
(2) Portion of outer cortex with cortical bundles x 200.
(3) Outer cortex with epidermis and oleo resin cell x 200.
(4) Outer cortex with cortical bundles x 200.
(5) Outer cortex with cortical bundles x 400.
(6) Endodermoid layer with pericycle and stelar bundles x 400.
(7) Outer cortex with endodermis, pericycle, stelar bundles and starch grains x 200.
(8) Tracheids with scalariform thickening x 200.
(9) Fibre cells x 100.
(10) TLC of Alpinia galanga at 254 nm.
(11) TLC of Alpinia galanga at 365 nm.
(12) TLC of Alpinia galanga with Vanillin Sulphuric acid.

B: Bundle sheath  CB: Cortical bundle  ED: Endodermis,  
F: Fibres  H: Hypodermal cells  OC: Outer cortex 
Ol.r.: Oleo resin cells  P: Pericycle  Ph: Phloem 
S: Spots of A. galanga  SB: Stelar bundles  t: Tracheids  
XY: Xylem Vessels
References

1. Anonymous. *The Wealth of India. Raw materials.* Vol II. CSIR. New Delhi, pp. 402-406 (1950).
2. Anonymous. Pharmacopoeia of India, Second edition, Ministry of Health and Family Welfare, Govt. of India, New Delhi, p. 947 (1990).
3. Chase, C. R. & Pratt, R. J. Fluorescence analysis of powdered vegetable drugs with particular reference to development system of identification. *J. Amer. Pharm.* pp. 38,324 (1949).
4. Johansen, D. A. *Plant Microtechnic,* 1st Ed. McGraw Hill Book Co., New York, London, pp. 182-197 (1940).
5. Kokate, C. K. *Practical Pharmacognosy,* 3rd Edn. Vallabh Prakashan, Bombay, pp. 107-111 (1991).
6. Krishnamurthy, K. V. Methods in Plant Histochemistry. Viswanathan Publishers, Madras, pp. 1-14 (1988).
7. Kirtikar, K. R. & Basu, B. D. *Indian Medicinal Plants.* Vol. IV, 3rd ed. Goyl Offset Printers, Delhi, pp. 2420-2426 (1984).
8. Nadkarni, A. K. *Indian Materia Medica,* Continental Publications, Bombay. p. 716 (1954).
9. Peach & Tracey, M. V. Modern Methods of Plant Analysis Vol. III Springer & Verlag, Berlin, pp. 321-322 (1983).
10. Sivarajan, V. V. & Balachandran, I. *Ayurvedic drugs and their plant sources.* Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, pp. 160, 167, 227, 398 (1994).
11. Stahl, E. Thin Layer Chromatography- A Laboratory Handbook, 2nd Edn.: 7-20 (1969).
12. Trease, G. E. & Evans, W. C. *Pharmacognosy,* 12th Edn., Bailliere Tindall, Eastbourne, U.K, p. 121 (1983).
13. Wallis, T. E. *Text Book of Pharmacognosy,* 3rd Edn., J & A Churchill Ltd., London, pp. 1-14,263 (1985).
14. Warrier, P. K. *Indian medicinal plants* - a compendium of 500 species. Vol I-III . p. 255 (1996).