

DRUG RESEARCH

Pharmacognostic & Physico-chemical evaluation of *Alpinia galanga* Sw.in

*P. Padma Rao**, *P. Subramanian*** and *M. Prabhakar****

Abstract

Alpinia galanga Sw. belonging to family Zingiberaceae is an important plant drug in Ayurveda and Unani Systems. It forms the basic source of Mahabharivacha and Rasna in Ayurveda and as Kulanjan-e-kabir in Unani. It is used for maintaining youthful vigour and strength. It is a popular remedy for rheumatism, fevers, digestion, cough, bronchitis, asthma and other respiratory ailments. Some indigenous plants are being tried and adopted into homoeopathic system of medicine. *Alpinia galanga* as well is being introduced in homoeopathy. The reddish pink tuberous rhizomes of *Alpinia galanga* are the officinal part. They are 2 to 4.5 cm thick, rounded with horizontal sheathy marking over surface. The cortex is often interrupted by oil canals. Starch grains densely fill in the cells of ground cortex. Fibro-vascular bundles are scattered in the cortex and more densely in the vicinity of endodermis. Sclerenchymatous fibres enclose xylem and phloem. Powder microscopic characters along with organoleptic characters are presented. Physico-chemical standards viz., ash values, moisture content, extractive values of raw drugs, constants such as weight per ml. total solids alcohol content besides TLC, HPTLC and HPLC profiles of mother tincture are given.

Introduction

The rhizomes of *Alpinia galanga* belonging to family Zingiberaceae is commonly known as greater galangal in English, Kulanjan in Hindi; aruni in Sanskrit; pera-rattai in Tamil; pedda dumpharashtrakamu in Telgu. *Alpinia galanga* is a perennial rhizomatous herb. It is a native of Java and Sumatra, cultivated throughout India, including Bengal and Western Ghats. It is reported to contain 40 compounds comprising of monoterpenes, sesquiterpenes and diterpenes viz., A- and B-pinene, limonene, cineol, eugenol acetate, chavicol acetate, myricene and galangal A and galangal B as major constituents.

It is a popular drug used in indigenous systems of medicine. For instance in Ayurveda, the rhizome is used as stomachic, in heart ailments, vata, bronchitis and for the improvement of appetite. In Unani as an aphrodisiac, diuretic, rheumatic and chest pains, diabetes and kidney troubles. Ethnomedicinally, it is employed as hot and stimulating; has a sweet scent, and also put into bazaar sprits to make it more intoxicating. It is also used as domestic medicine for bronchial catarrh.

Though medicinally it is widely known in all indigenous systems except homoeopathy, there is a

strong urge for introducing some of the well known indigenous drugs into homoeopathic system. In view of this, the rhizomes of *A. galanga* is also being tried.

The review of literature reveals that there is no work done on this drug in homoeopathy. Hence the present work is undertaken which involves pharmacognosy and physico-chemical parameters, along with UV, HPLC and HPTLC profiles of the mother tinctures.

Material and Methods

Pharmacognosy

Dried rhizomes were collected locally and were authenticated at the Department of Botany, Osmania University, Hyderabad. The boiled rhizomes were cut at 13-15 μ m thick (in TS and LS) on Spencers sliding microtome. The sections were double stained with saffranin and light green and mounted in Canada balsam. The rhizomes were macerated to study the nature of tracheary elements following Jeffreys maceration method (Johansen, 1940). The fresh sections were also studied for histochemistry following Youngken (1951) and Johansen (1940). The dried powder (1 gm) was boiled with 2 ml water and the suspension after thorough shaking was laced

*A.R.O.(Chem.), **A.R.O.(Chem.), *** Project Officer, Drug Standardization Unit (H), O.U.B. 32, Road No. 4, Habsiguda, Hyderabad-500 007.



Alpinia Galanga



Flower of *Alpinia Galanga*

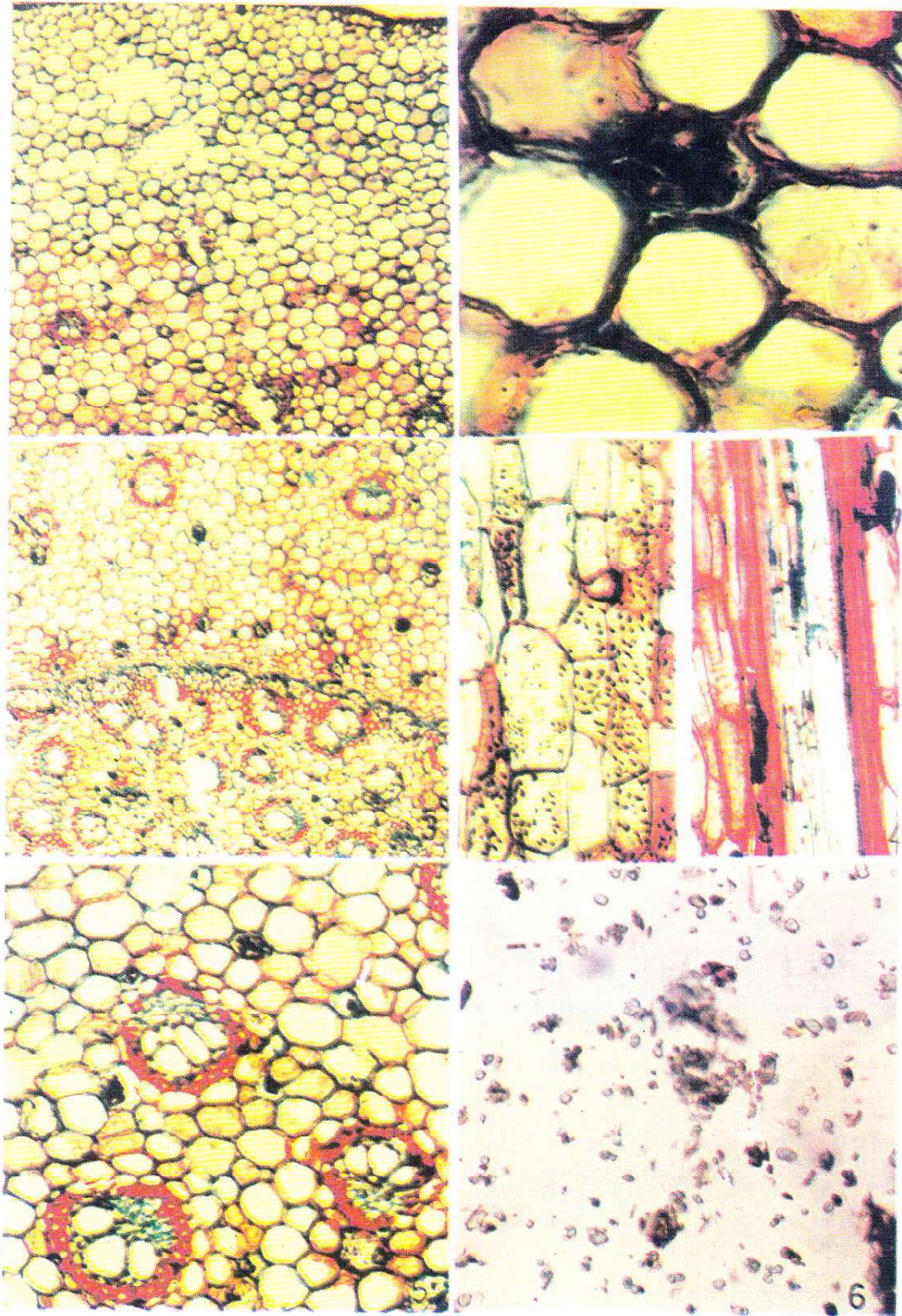


FIG. 1

Microscopy:

1. Starch grains abundant, cylindrical to clavate and pyriform and few irregular in shape; 14-55 μm in length and 7-19 μm in breadth.
2. Fragments of sclerenchymatous fibres.
3. Pieces of cortical parenchyma tissue with dense contents including starch grains.
4. Fragments of tracheary tissue of fibrovascular bundle with helical thickenings and fibers.
5. Fragments of parenchyma tissue with brownish tanniniferous content.
6. Pieces of cortical tissue with oil canals.

IMAGES OF ALPINIA GALANGA

BEFORE DERVATIZATION

IMAGE AT 254 nm

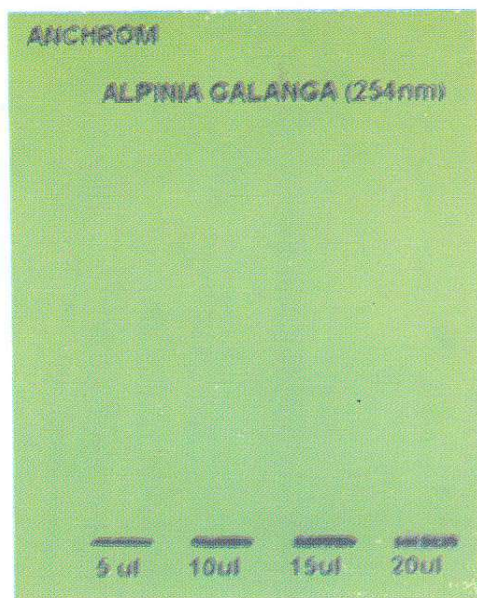
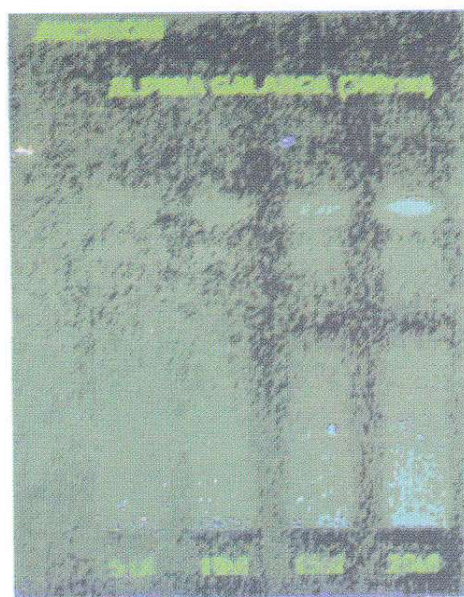


IMAGE AT 366 nm

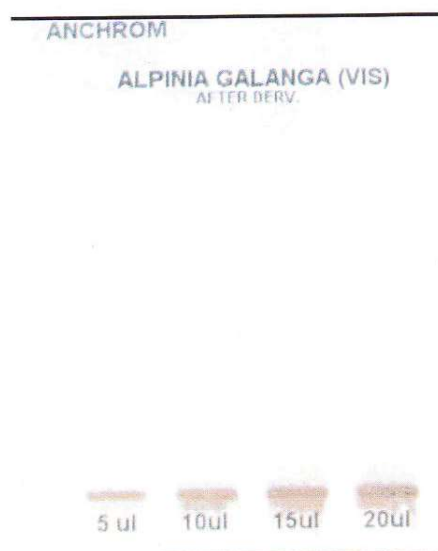


AFTER DERVATIZATION

IMAGE AT 366 nm



IMAGE AT VISIBLE



Derivatization Reagent: 5% Methanolic Sulphuric Acid

HPTLC FINGER PRINTING OF *ALPINIA GALANGA* SCANNED AT 300 nm

on a slide with a drop of saffranin added to it. After washing with alcohol and mounted in glycerine and observed for broken elements and recorded. Photomicrographs of anatomical details of sections of rhizomes and powder were obtained through Olympus automatic photomicrographic microscope.

Physico-chemical

The dried rhizomes were powdered to obtain coarse powder 10/44 (sieve size). It was subjected to determination of moisture content (L.O.D. at 105 C). Ash values and extractive values. The above parameters were determined in accordance with the procedures recommended in Homoeopathic Pharmacopoeia of India (HPI). The mother tincture was prepared as per HPUS (1993). Percolation method was employed for preparing the mother tincture.

The alcoholic extract (mother tincture) was studied for a) Physico-chemical constants, b) Chromatography (TLC/HPLC/HPTC) and c) U.V. absorbance.

All chemicals and solvents used were of analytical grade. Silica gel G (E Merck, India) was used for thin layer chromatography. U.V. spectra was recorded using U.V. spectrophotometer (Elico make).

Physico-chemical constants viz., organoleptic characters, weight per ml, total solids, alcohol content and pH value, was determined as per the procedures laid down in the HPI(1971).

Chromatography (TLC)

For thin layer chromatography, 25ml alcoholic extract was evaporated on water bath to remove alcohol. The remaining aqueous part was extracted with 25 ml. chloroform (three times). All the three fractions were combined and concentrated to 2 ml. 15 µl was applied on activated silica gel G coated TLC plate. It was developed using Acetic acid – Chloroform (1:9 v/v) as mobile phase and 10% FeCl₃ solution was sprayed for visualization.

UV absorbance

For UV absorbance, the mother tincture as such, as well as diluted were scanned for UV visible spectra in the range of 202-897 nm. The peaks of maximum absorption are given in table-5.

HPLC study

This method is adoptable for routine analysis in quality control laboratories. Owing to the relative

simplicity of this method and because of high precision of the results it provides.

In this study, the retention time and %age peak area for major compounds of 65% alcoholic extract of *Alpinia galangal* Q was summarized in table-2 besides overlaid spectra.

HPTLC study

Precoated high performance TLC plated (silica gel 60 F 254 as stationary phase) were used. For sample application CAMAG linostat 1 V sample applicator was employed. The speed of application was maintained at 4 mm/sec. The R_f values of the various components (before and after) derivatisation are provided in table-4.

The solvent system used for development was toluene: ethyl acetate: methanol (9:1:1) 5% methanolic sulphuric acid was employed for derivatisation.

Results

Pharmacognosy

Observations

Morphology: Rhizomes are 2-4.5 cm in thickness, tuberous, rounded, thickened at nodes, with horizontal sheathy markings, surface smooth to striated with fine wiry rootlets.

Microscopy: Epidermis is 1-layered, cells barrel shaped and isodiametric, barrel shaped ones 17-27 µm (19) long and 10-15 µm (13) wide; and isodiametric ones 7-15 µm (11) in diameter; contents slightly dense with tannins in few. Hypodermis is 3-6 layered, parenchymatous, cells closely packed, polygonal, 12-37 µm (24) in diameter, walls slightly thick, contents dense, in few with tannins and starch grains. The outer cortex is 8-10 layered, cells polygonal to spherical, 43-130 µm (83) in diameter, walls slightly thick, contents dense with tannins and starch grains and interspersed with oil canals, few fibrovascular bundles are present dispersed in the cortex.

The inner cortex is abundant inside the endodermis. The fibrovascular bundles are found closely arranged along the endodermal region and also dispersed throughout cortex. The cells of the cortex are spherical to polygonal, 43 -127 µm (88) in diameter, contents dense with tannins, resinous matter and with oil canals interspersed in between.

Fibrovascular bundles are distributed evenly in cortex but more frequent in the inner cortex. Vascular

bundles of outer cortex are larger, oval to spherical and elliptic, 254-566 μm (424) in diameter while those of inner cortex diffuse, numerous, smaller, 141-376 μm (252) in diameter, endarch, conjoint with xylem at center and phloem concentric at the poles. A layer of fibrous tissue encloses the xylem and phloem. Secondary walls of xylary elements of vascular bundles helical, few scalariform or reticulate; fibres are medium sized, thick walled, septate and non-septate, non-libriform, pits simple.

Powder studies

Microscopy:

1. Starch grains abundant, cylindrical to clavate and pyriform and few irregular in shape; 14-55 μm in length and 7-19 μm in breadth.
2. Fragments of sclerenchymatous fibres.
3. Pieces of cortical parenchyma tissue with dense contents including starch grains.
4. Fragments of tracheary tissue of fibrovascular bundle with helical thickenings and fibers.
5. Fragments of parenchyma tissue with brownish tanniferous content.
6. Pieces of cortical tissue with oil canals.

Organoleptic Characters

Appearance	: Clear, non-viscous liquid
Colour	: Reddish brown
Odour	: Aromatic

Histochemistry

Fixed oils & Fats	: As faint traces in the outer cortex and parts of inner cortex.
Starches	: abundant in the cortex.
Suberin	: absent
Essential oils	: Present in patches in the cortex and also near vascular bundles.
Flavonoid glycosides	: absent
Alkaloids	: absent
Resins	: as traces in hypodermis and cortex.
Terpenoids	: as traces in hypodermis and cortex.
Carbohydrates	: present in the cortex as traces.

Discussion

The characteristic presence of a thick walled endodermis as reported by Dutta and Mukherjee, (1950) which separates the rhizome into the outer cortex from the inner containing closely scattered vascular bundles is confirmed. The fibrovascular bundle being enclosed by a layer of sclerenchyma is described as a sheath of 3-4 layers of fibers by Dutta & Mukherjee (1950). The epidermis as described as a uniform layer of parenchymatous cells and heavily thickened is the outer wall (Dutta & Mukherjee, 1950). Whereas it is 1- layered consisting of barrel shaped and isodiametric cells and not parenchymatous in nature as has been described by the former.

A 3-6 layered hypodermis is present beneath the epidermis, and the cells are often filled with dense tanniferous contents.

The inner and outer cortex is parenchymatous and almost appear uniform with cells of inner being larger in size. They are often found to contain starch grains and few with resinous matter. The oil canals are present distributed in the cortex consisting of a pore (in T.S.) in between the cortical cells as also been reported by Datta & Mukherjee (1950).

The fibrovascular bundles are numerous and found closely dispersed in the form of a ring beneath the endodermis as held by Dutta and Mukherjee (1950). Further, the bundles are evenly distributed in the cortex. In size the v. bundles are larger, 254-566 μm in diameter in the outer cortex and smaller 141-396 μm in diameter in the inner cortex.

Microscopical features of powder of rhizomes reveal the presence of abundant starch grains, cylindrical, clavate and pyriform in shape besides broken fibers, cortical parenchyma with starch grains, and tannins; tracheary elements with helical and scalariform thickenings.

Physico-chemical Standardisation

Alcoholic extract and mother tincture have shown positive test for tannins. The data pertaining to physico-chemical studies is presented in table (1). Formulation of mother tincture is given in table (2 & 3). Physico-chemical constants of mother tincture are summarized in table (4). TLC studies on chloroform extract of the mother tincture showed four prominent purple coloured spots on spraying with 10% FeCl_3 solution and heating at 110 C table (). Mother tincture a such and its dilution were scanned under UV and visible light showed three and two distinct peaks (maximum absorbance) respectively. It is inferred from the UV study that the absorbance of diluted mother tincture falls exclusively

Table-1: Standardization of raw drug

Sl.No.	Parameters	Quantitative value, % w/w
1.	Moisture content (L.O.D. at 105o C)	13.21
2.	Total ash	3.447
3.	Water soluble ash	0.946
4.	Acid insoluble ash	1.364
5.	Alcohol soluble extractive	0.5
6.	Water soluble extractive	6.98

Table-2: Formulation of mother tincture

Sl.No.	Contents	Quantitative value
1.	Alcohol content	65%
2.	Drug strength	1/10
3.	Coarse powder of Alpinia galangal Sw	100g
4.	Purified water	317ml
5.	Strong alcohol	683ml

(To make one thousand milliliters of mother tincture)

Table-3: Physico-chemical Standardization of mother tincture

Sl.No.	Parameters	Observations
1.	Organoleptic properties <i>a. Appearance</i> <i>b. Colour</i> <i>c. Odour</i>	<i>Clear, non-viscous liquid</i> Reddish brown Aromatic
2.	Sediments	Absent
3.	Weight per ml	0.89 g
4.	Total solids	1.0% w/v
5.	Alcohol content	65% v/v

Table-4: TLC Results of mother tincture

Extract	:	Chloroform extract of the mother tincture.
Adsorbent	:	Silica gel-G
Layer thickness	:	0.4mm in wet condition

Solvent System	Detecting Agent	No. of Spots	Rf. Values	Colour of the Spots
Acetic acid: Chloroform (1:9 v/v)	10% FeCl ₃	4	0.68	Purple
			0.81	Violet
			0.86	Violet
			0.95	Violet

Table-5: UV Absorbance of alcoholic extract

Sl.No.	Mother Tincture	No. of Peaks	UV Absorbance, nm
1.	65% alcohol extract as such	3	305.7
			451.9
			462.2
2.	After dilution	2	236.4
			282.6

in the UV region while the mother tincture revealed only one peak in the UV and the rest two in visible range. It is evident from the raw drug studies that the value of acid insoluble ash falls within its acceptable level.

HPTLC profile shown absorbance in the longer wavelength of UV light before derivatisation whereas after derivatisation i.e. subsequent to spraying with 5% methanolic sulphuric acid reveals eith bands with Rf values 0.51, 0.26, 0.41, 0.40, 0.14, 0.35, 0.21 and 0.61.

Conclusion

The data evolved in pharmacognostic and physico-chemical studies including parameters such as UV, HPLC, and HPTLC profiles are useful as standards for both raw drug as well as finished products in Homoeopathy.

Acknowledgement

The authors thank the Director, Central Council for Research in Homoeopathy, New Delhi, for facilities and encouragement. To Anchrom HPTLC Applications Laboratory, Mumbai, for HPTLC studies and the Director, IICT, Hyderabad, for HPLC profile.

Bibliography

1. *Homoeopathic Pharmacopoeia of India, Vol. I*; Controller of Publications, Government of India, New Delhi(1971).
2. *The Wealth of India, Raw Materials, Vol. I, CSIR, Publications Directorate, New Delhi(1971).*
3. *Homoeopathic Pharmacopoeia of United States.* (1993).
4. *Second Supplement to Glossary of Indian Medicinal Plants with active principles; Part-I, (A K) Publications and Information Directorate, C.S.I.R., New Delhi(1992).*
5. Dutta, S.C., and B. Mukherjee, (1950). *Pharmacognosy of Indian Root and Rhizome Drugs*, Published by Manager, Publications, Ministry of Health, Government of India, Delhi.
6. Herman, I., et. Al. (1985). *The essential oil of Greater galangal (Alpinia galangal) from Malaysia*, Phytochemistry Vol. 24(1), P. 93-96.
7. Johansen, D.A. (1940) *Plant Microtechnique.* McGraw Hill Book Co., New York.
8. Kirtikar, K.R. and Basu, B.D. 1980. *Indian Medicinal Plants*, Bishan Singh Mahendrapal Singh, Dehradun.
9. Stahl, E. (1969). *Thin layer Chromatography. A laboratory Hand Book*, Springer Verlag, Berlin.
10. Wagner, H.S. Bladt and E.M. Zgainski (1984). *Plant Drug Analysis. A TLC Atlas*, Springer Verlag, Berlin.
11. Youngken, H.W. (1951). *Pharmaceutical Botany*, 7th Ed. The Blackiston Company, Toronto.