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COMPARATIVE PHARMACOGNOSTIC AND PHYTOCHEMICAL INVESTIGATION OF TWO ALPINIA SPECIES FROM ZINGIBERACEAE FAMILY

Ajay G. Namdeo* and Vijaykumar M. Kale

Department of Pharmacognosy, Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Pune, Maharashtra, India.

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***Correspondence for Author**
Dr. Ajay G. Namdeo
Department of Pharmacognosy, Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Pune, Maharashtra, India.

ABSTRACT

The rhizomes of two *Alpinia* Species, namely *Alpinia galanga* and *Alpinia officinarum* belonging to family *Zingiberaceae* are well known for their traditional uses. The present research work was conducted for detailed comparative pharmacognostic and phytochemical investigation of the two species with the aim to establish the diagnostic keys for these important drugs based on the macroscopic, microscopic (histological distinction), qualitative tests, physico-chemical constants (ash and extractive values) and phytochemical analysis. All the parameters were studied according to the WHO and pharmacopoeial guidelines. The macroscopic characters of rhizomes were determined organoleptically and for microscopically. The measurement of different tissues and cell components were performed with the help of ocular, stage micrometer and camera lucida. The present study will provide the information with respect to detailed diagnostic and distinctive characteristics for the differentiation of the two *Alpinia Species* which can be also used as quality control parameters of these species and may provide the standard parameters to establish the authenticity of commercialized varieties and can probably help to differentiate the drug from the other species. Results obtained from this study will be helpful to generate information concerning relevant pharmacognostical, phytochemical and physicochemical data needed for proper identification and authentification of *Alpinia galanga* and *Alpinia officinarum*. It will serve as standard reference for identification and distinguishing rhizomes of two *Alpinia Species* from its substitutes and adulterants.

KEYWORDS: Zingiberaceae, *Alpinia*, Pharmacognostic, Phytochemical investigation, Physicochemical.

INTRODUCTION

Risks associated with herbal medicine products were first reported for medicinal plants of the Asteraceae family, *Hypericin* and *Aristolochia* genus, and kava-kava. A number of cases of inadvertent or deliberate substitution of the constituents of Chinese herbal preparations are cited in the literature. For example, Siberian ginseng (*Eleutherococcus senticosus*), American ginseng (*Panax quinquefolium*), and Japanese ginseng (*Panax pseudo-ginseng*) have been substituted for Korean or Chinese ginseng (*Panax ginseng*). Development of authentic analytical methods which can reliably profile the phytochemical composition, including quantitative analyses of marker/bioactive compounds and other major constituents, is a major challenge to scientists (Katiyar et al., 2011; Majumder et al., 2011). In order to assure a consistent and acceptable quality herbal product, care should be taken right from the identification and authentication of herbal raw materials to the verification process of final product (Mosihuzzaman and Choudhary, 2008).

The family Zingiberaceae is well known for its immense medicinal values are distributed widely throughout the tropics, particularly in Southeast Asia. Zingiberaceae family is an important natural resource that provides many useful products for food, spices, medicines, dyes, perfume and aesthetics (Tushar et al., 2010). *Alpinia* is the most important and widespread genus in the *Zingiberaceae* with some 230 species occurring from Sri Lanka and the Western Ghats of India to China, Japan, all of southeast Asia, the Pacific as far as Fiji, Samoa, and the Caroline Islands, and Australia as far south as northern New South Wales (Joy et al., 1998). Rhizomes of certain gingers like *Alpinia galanga* and *Alpinia officinarum* have high medicinal values (Indrayan et al., 2009).

Alpinia galanga Linn is a perennial aromatic rhizomatous herb and an important plant from Zingiberaceae family, which is cultivated in India, China, Thailand, Malaysia and Indonesia. Many *Alpinia* species are appreciated for their medicinal properties and are also used in traditional medicines as a spasmolytic, hypotensive, anti-emetic, anti-oxidant, anti-inflammatory, bacteriastatic, fungistatic effects in India, China and other regions (Parida et al., 2011). *Alpinia galanga* is commonly used in cooking as an aromatic stimulant or flavoring agent and as folklore medicine in bronchial catarrh, rheumatism and respiratory ailment like asthma (Chudiwal et al., 2010). *Alpinia galanga* is one of the many ingredients

in polyherbal preparations for relieving pain of different etiologies such as rheumatoid arthritis, back pain and pain in individuals who had Chikungunya fever in South India (Acharya et al., 2011). Rhizomes of *Alpinia galanga* find varying medicinal uses for treatment of diseases like neuroprotective, antileishmanial, antiallergic, immunostimulating, hypoglycemic, antimicrobial, antitumor, antifungal, inhibitors of nitric oxide production, antioxidant, inhibition of human immunodeficiency virus type 1 replication and the major active constituent of this plant is 1'S-1'-acetoxychavicol, dihydrogalangal, acetoxy-1, 8-cineoles, essential oils, galangoflavonoid, 1'-Acetoxychavicol acetate, 1'S-1'-Acetoxyeugenol acetate (Ghosh et al., 2011). *Alpinia galanga* contains various secondary metabolites like, terpenoids, flavonoids, phenylpropanoids (Namdeo et al., 2014).

Alpinia officinarum Hance is also widely cultivated in southern China. There are about 46 species in this genus in China. *Alpinia officinarum*, a pungent and aromatic rhizome was used as a traditional Chinese medicine with anti-inflammatory, antioxidant, anti-proliferative, anticancer and antiemetic activities. *Alpinia officinarum* has also found medicinal importance due to presence of diarylheptanoides which possess anti-inflammatory, anticancer, antifungal, Pancreatic lipase inhibitor, antibacterial, antitubercular activity, antiplatelet and number of chemical constituent of *Alpinia officinarum* have been reported, specifically, officinin A, 5-ethoxyl-7-(4-hydroxy-3-methoxy-phenyl)-1-phenyl-3-heptanone, galangin, 3-O-methyl galangin, kaempferide, alpinin, galangol and some diarylheptanoids (Zhang et al., 2010).

MATERIALS AND METHODS

Collection of Plant Material

Plant material of *Alpinia galanga* (L) Willd and *Alpinia officinarum* Hance was collected from the Jawaharlal Nehru Tropical Botanic & Research Institute, Palode, Thiruvananthapuram.

Identification and authentication of plant materials

The rhizomes of *Alpinia galanga* (L) Willd and *Alpinia officinarum* Hance were identified and authenticated by Dr. A.S. Upadhye, Department of Botany, Agharkar Research Institute, Pune,India. *Alpinia galanga* (L) Willd: voucher specimen sample no. R-167; *Alpinia officinarum* Hance: voucher specimen sample no. R-168.

Chemicals and reagents

Petroleum ether (Merck, India) and ethanol (ChangshuYangyuan Chemicals, China) were purchased from respective vendors. The reagents for phytochemical identification were obtained from the freshly prepared stock used in pharmacognosy and pharmaceutical chemistry laboratory of the college.

Macroscopical and microscopical Studies

The macroscopic characters of rhizomes were determined organoleptically (Anonymous et al., 2003; Evans, 2004). Observations of shape, size, colour, odour, taste and external markings were done in macroscopical studies. Microscopic studies were done according to the method of (Brain and Turner., 1975). For microscopical studies; a few drops of chloral hydrate solution were added to a sample of powdered plant material on a slide and heated gently over a micro Bunsen. The slide was covered with a glass cover slip for examination under the microscope and different cell components were observed. Free hand section of drug material was taken and stained with safranin and phloroglucinol followed by concentrated hydrochloric acid (Johansen, 1940). The measurement of different tissues and cell components were performed with the help of ocular, stage micrometer and camera lucida (Babu et al., 2010).

Preparation of Extracts

The rhizomes of *Alpinia galanga* and *Alpinia officinarum* were shade dried and powdered in hand mixer. About 100 g of powdered material were taken and extracted with cold percolation using different solvents (petroleum ether, carbon tetrachloride, chloroform, ethyl acetate, acetone, ethanol, methanol and water). Each process was repeated thrice for complete extraction after extraction; extracts were combined and evaporated to dryness *in vacuo* at 40^0C.

Preliminary phytochemical screening

Preliminary phytochemical screening was carried out for alkaloids, glycosides, resins, tannins, saponins, carbohydrates, amino acids, phenols, terpenoids, coumarins and gums using standard procedures described to identify the constituents (Khandelwal, 2010; Kokate, 1994; Kokate, 2006).

Quantitative Evaluation

Physico-chemical analysis

Physico-chemical analysis i.e. percentage of ash values and extractive values was performed according to the official methods prescribed by WHO guidelines on quality control methods for medicinal plant materials (WHO/QCMMPM guidelines, 1992). Fluorescence analysis was carried out according to the method prescribed (Chase and Pratt, 1949; Kokoski et al., 1958). The color and consistency of the extracts were also recorded.

RESULTS

Pharmacognostic studies

***Alpinia galanga* (L) Willd**

Macroscopic studies

Alpinia galanga are cylindrical, branched, 2 to 8 cm in diameter, longitudinally ridged with prominent rounded warts marked with fine annulations; scaly leaves arranged circularly. Externally reddish- brown, internally orange yellow in color, fracture hard and fibrous, fractured surface rough; odour pleasant and aromatic, taste spicy and sweet.

Fig 1 Macroscopic characteristics of *Alpinia galanga* (L) Willd

Microscopic studies

TS of rhizome

Transverse section of rhizome in *Alpinia galanga* shows an outer cortical region and an inner stelar region. The outer cortex is 1 to 1.1 cm wide and the central stele is 1.2 cm in diameter. Cortex consists of an epidermis, which is composed of a single row of tangentially elongated cells. Below the epidermis, in the remaining part of the outer zone consists of numerous vascular bundles in scattered manner and each bundle is surrounded by lignified layer. Vascular bundles are nearly circular in shape and consist of groups of xylem elements and

small patch of phloem. Xylem consists of parenchyma, vessels, tracheids & fibers. Phloem is seen above the xylem and consists of sieve elements, companion cells. The cells of the cortex are thin walled, polygonal and arranged with small intercellular spaces. Cortical and stellar region demarcated by a continuous ring of endodermoidal layer. The stellar region is composed of thin walled parenchymatous cells with numerous vascular bundles. Small vascular bundles are radially arranged xylem elements are present beneath this layer. These parenchymatous cells are slightly smaller than that of the cortical region and polygonal to circular in shape and compactly arranged with small intercellular spaces. Smaller bundles are towards the peripheral region and larger ones occupy the central part. Oleoresin cells are present near to each bundle. Sclerenchymatous sheath partially encircling the bundle is less thickened. Starch grains are simple, elongated and are more in number. Numerous oleoresin cells are scattered throughout the rhizome.

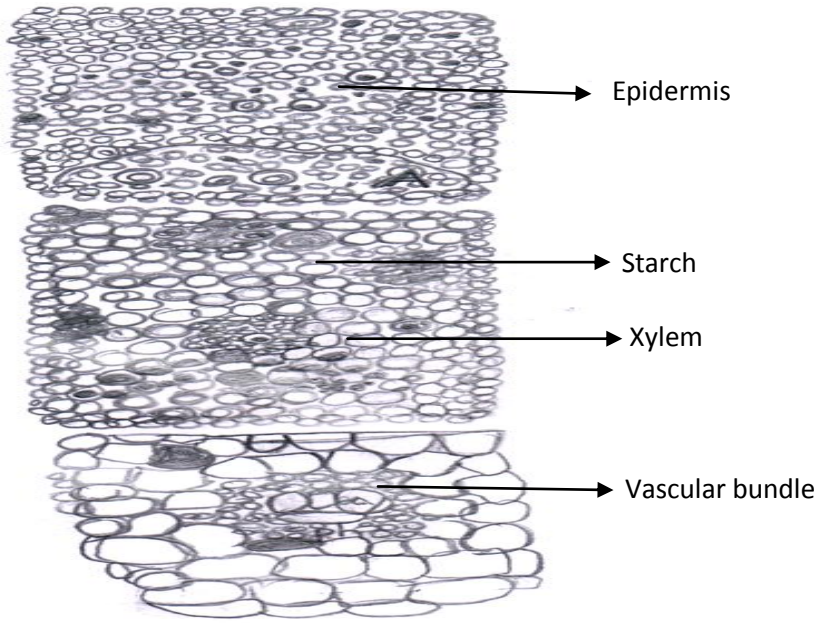


Fig 2 T. S. of rhizome of *Alpinia galanga* (L) Willd

Powder microscopy

Powdered microscopy revealed the presence of fragments of epidermal cells in surface view, parenchymatous cells with yellow colouring matter and starch grains. Vessels are thick walled, alongated having scalariform or reticulate thickening with simple pitting. Starch grains show variation in size and shape, few are circular. The majority are long rod shaped with rounded broad end. Tracheids are present.

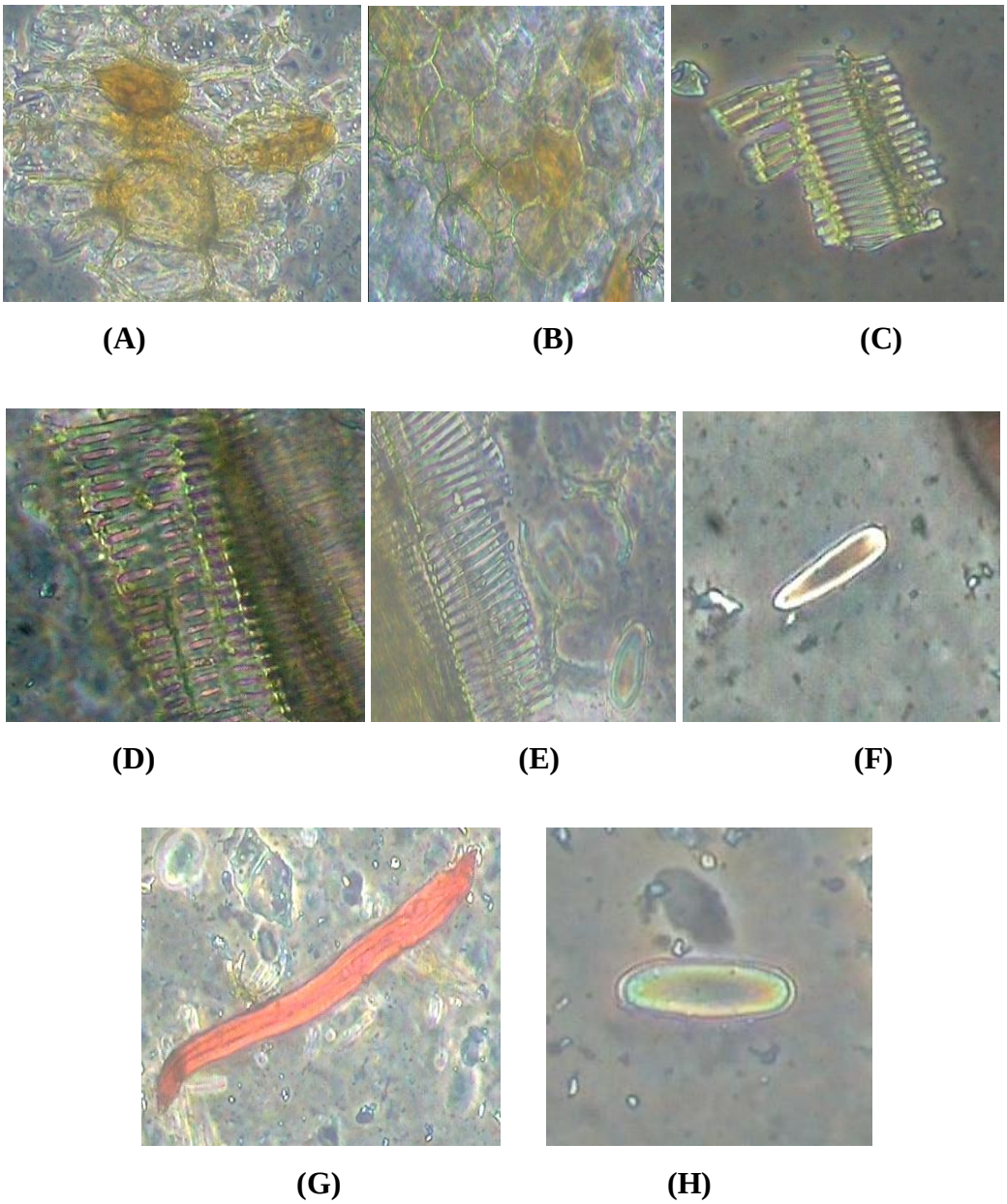


Fig 3 *Alpinia galanga*: (A) Epidermal cells, (B) Parenchyma cells with starch grains, (C and D) Vessels with scaiariform thickening, (E) Vessels, (F) Starch grains, (G) Trachied, (H) Starch grains.

Alpinia officinarum

Macroscopic studies

Alpinia officinarum rhizome is a slightly curved and cylindrical rhizome, sometimes branched; 2.8 cm in length, 6. 15 mm in diameter; externally red-brown to dark brown with fine striped lines, grayish white nodes and several traces of rootlet; hard to break; fracture surface, light brown in color and thickness of cortex is approximately the same as that of stele. Odor is characteristic, taste is extremely pungent.

Fig 4 Macroscopic characteristics of *Alpinia officinarum* Hance

Microscopic studies

T.S of rhizome

Under a microscope, transverse section reveals epidermal cells often containing resin-like substances; cortex, endodermis and stele present beneath the epidermis; cortex and stele divided by endodermis; vascular bundles surrounded by fibers, scattered throughout the cortex and stele, cortex and stele composed of parenchyma interspersed with oil cells; parenchymatous cells containing solitary crystals of calcium oxalate and starch grains, starch grains generally simple (sometimes 2 to 8 compound), ovate, oblong or narrowly ovate, 10 to 40 mm in diameter and with an eccentric navel.

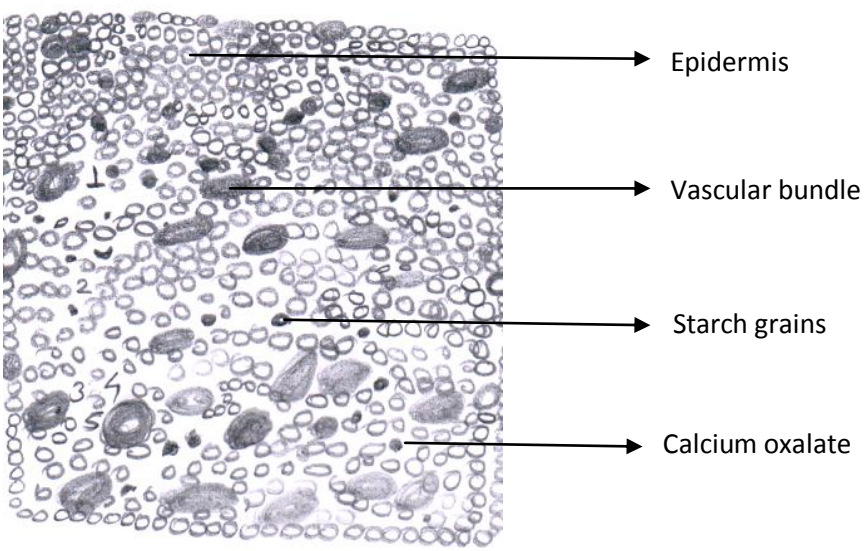
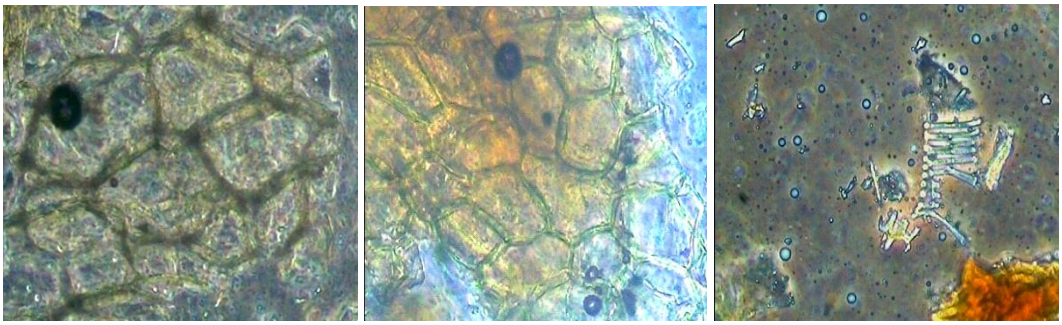


Fig 5 T. S. of rhizome of *Alpinia officinarum* Hance.

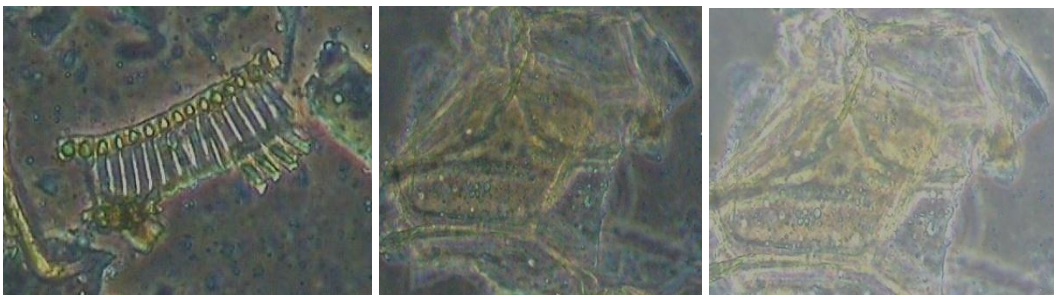
Powder microscopy

Powdered microscopy revealed the presence of parenchymatous cells in surface view with fragments of epidermal cells. Fragments of vessels having scalariform thickening are

presents. Many circular starch grains are presents. Many fragments of parenchymatous cells containing starch grains are present.



(A) **(B)** **(C)**



(D) **(E)** **(F)**

Fig 6 *Alpinia officinarum*: (A) Parenchymatous cells, (B) Epidermal cells, (C) Fragments of vessels, (D) Vessels with scalariform thicknings, (E and F) Parenchyma cells containing starch grains.

Table 1 Comparative pharmacognostic screening of *Alpinia galanga and Alpinia officinarum*.

Parameter	**Characters**	***Alpinia galanga***	***Alpinia officinarum***
Macroscopical study	**Colour**	Externally Reddish- Brown	Externally Red-Brown To Dark Brown
	Odour	Pleasant and Aromatic	Characteristic
	Taste	Spicy and Sweet	Extremely Pungent
	Shape	Cylindrical and Branched,	Slightly Curved and Cylindrical
	Size	2 To 8 Cm In Diameter	6-15 Mm In Diameter
Microscopic Study		Cortex	Epidermal Cells
		Vascular Bundles	Cortex
		Xylem	Endodermis
		Parenchyma	Vascular Bundles
		Tracheids	Parenchymatous Cells
		Fibers	Calcium Oxalate
		Starch Grains	Starch Grains
Powder Microscopy		Epidermal Cells	Parenchymatous Cells
		Starch Grains	Epidermal Cells
		Vessels With Scaiariform	Fragments Of Vessels

	Starch Grains	Vessels With Scalariform
	Starch Grains	Starch Grains
	Trachied	-

Physicochemical analysis

Ash value

The percentage of active chemical constituents in crude drugs is mentioned on air-dried basis. Therefore, the loss on drying of plant materials should be determined and the water content should also be controlled. This is especially important for materials that absorb moisture easily or deteriorate quickly in presence of water. The test for loss on drying determines both water and volatile matter. The acid insoluble ash consist mainly silica and indicate contaminate on with earthy material. The water soluble ash is used to estimate the amount of inorganic elements present in drugs.

Extractive values

The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particulars solvent.

Table 2 Comparative physicochemical screening of *Alpinia galanga and Alpinia officinarum*

Parameter	***Alpinia galanga***	***Alpinia officinarum***
Ash value (%)		
Total ash	10.2	7.5
Acid in soluble ash	4.1	1.5
Water soluble ash	5.3	1.6
Loss on drying at110°C	11	1.5
Extractive value(%)		
Pet-ether	3.47	0.60
chloroform	1.48	1.8
Ethanol	9.04	14
Methanol	4.7	2.7
Water	12.50	1.6

Preliminary phytochemical screening

Preliminary phytochemical screening was helpful in prediction of nature of drugs and also useful for the detection of different constituents present in different polarity solvent. So it could be helpful to extract out particular constituents by solvent. The phytochemical study of *Alpinia galanga* revealed the presence of alkaloids, tannins, terpenoids and phenolics, alkaloids, carbohydrates, tannins, aminoacids, and saponins, while the phytochemical study

of *Alpinia officinarum* revealed the presence of alkaloids, tannins, coumarins, terpenoids and phenolics, carbohydrates, tannins, glycosides, aminoacids, phenols, gums and saponins.

Table 3 Comparative Preliminary phytochemical screening of *Alpinia galanga and Alpinia officinarum*

Chemical compounds	*Alpinia galanga*	*Alpinia officinarum*
Carbohydrates	-	-
Tests for Proteins	+	+
Tests for Steroids	-	-
Volatile oils	-	-
Glycosides	-	+
Saponins	-	-
Tannins	+	+
Flavonoids	+	+
Alkaloids	+	+

(+Present;-Absent)

Morphological characteristics of *Alpinia galanga* and*Alpinia officinarum*

All the extracts of *Alpinia galanga* are brown to brownish yellow in color and showed semisolid consistency while aqueous extract is brown in color and showed solid consistency. All the extracts of *Alpinia officinarum* are brown to brownish yellow in color and showed semisolid consistency while aqueous extract is brown in color and showed powder consistency.

Table 4 Table shows morphological characterization of rhizome extracts of *Alpinia galangal* and *Alpinia officinarum*.

		Alpinia galanga		*Alpinia officinarum*	
Sr. No.	**Extracts**	**Colour**	**Consistency**	**Colour**	**Consistency**
1.	Hexane	Brown	Oily	Yellowish Brown	Sticky Oily
2.	Pet.ether	Brownish yellow	Semisolid	Brownish yellow	Semisolid
3.	Chloroform	Dark brown	Semisolid	Dark brown	Semisolid
4.	Acetone	Brownish yellow	Semisolid	Brownish yellow	Semisolid
5.	Ethyl acetate	Brown	Solid	Brown	Solid
6.	Ethanol	Dark brown	Semisolid	Dark brown	Semisolid
7.	Methanol	Brownish yellow	Semisolid	Brownish yellow	Semisolid
8.	Water	Brown	Solid	Brown	powder

Fluorescence analysis of rhizome extracts of *Alpinia galanga*.

Table 5 Fluorescence analysis of rhizome extracts of *Alpinia galanga*.

Sr. No.	Extracts	Visible light	Short UV light	Long UV light
1.	Hexane	Pale yellow	Yellowish green	Brown black
2.	Pet.ether	Yellowish Brown	Reddish Yellow	Brown
3.	Chloroform	Brownish yellow	Emerald Green	Reddish Brown
4.	Acetone	Yellowish Brown	Yellowish green	Pale Green
5.	Ethyl acetate	Brown	Pale Green	Reddish Orange
6.	Ethanol	Dark brown	Greenish Brown	Emerald Green
7.	Methanol	Brownish yellow	Reddish Orange	Pale Green
8.	Water	Brown	Greenish Brown	Yellowish Brown

Table 6 Study of fluorescence analysis from the extracts of *Alpinia officinarum*

Sr. No.	Extracts	Visible light	Short UV light	Long UV light
1	Hexane	Pale yellow	Yellowish green	Brown black
2	Pet.ether	Yellowish Brown	Emerald Green	Brown
3	Chloroform	Yellowish Brown	Reddish Yellow	Reddish Brown
4	Acetone	Brownish yellow	Yellowish green	Pale Green
5	Ethyl acetate	Brown	Greenish Brown	Reddish Orange
6	Ethanol	Dark brown	Greenish Brown	Emerald Green
7	Methanol	Emerald Green	Yellowish Brown	Pale Green
8	Water	Brown	Pale Green	Reddish Orange

DISCUSSION

Standardization is an important tool for herbal drugs in order to establish their identity, purity, safety and quality (Mukherjee et al., 2002). In order to standardize a drug, various macroscopic, physicochemical analyses, phytochemical analysis, and fluorescence analysis were done. The quantitative determination of some pharmacognostical parameters is useful for setting standards for crude drugs. (Ravichandra et al., 2011).

Raw drugs pose problem of identification and to establish their genuineness when they lack any external diagnostic features or any organoleptic clues. During such situations, the microscopic analyses of the specimen will offer a helping hand to establish the identity of the phytodrugs (Mukherjee et al., 2008). Macroscopic studies revealed that *Alpinia galangal* are cylindrical, 2 to 8 cm in diameter. Externally reddish- brown; odour pleasant and aromatic, taste spicy and sweet, while *Alpinia officinarum* rhizome is a slightly curved and cylindrical rhizome, 2.8 cm in length, 6. 15 mm in diameter; externally red-brown to dark brown, dour is characteristic, taste is extremely pungent. Microscopic studies revealed that *Alpinia galanga* shows an outer cortical region and an inner stelar region. Cortex consists of an epidermis, Xylem consists of parenchyma, vessels, tracheids, fibers, Oleoresin cells, Starch grains.

Transverse section of *Alpinia officinarum* reveals the presence of epidermal cells, cortex, endodermis, endodermis; vascular bundles, fibers, scattered throughout the cortex and stele, parenchyma, parenchymatous cells, calcium oxalate and starch grains.

Powdered *Alpinia galanga* showed the presence of Epidermal cells, Parenchyma cells with starch grains, Vessels with scaiariform thickening, Vessels, Starch grains, Trachied, Starch grains. Powdered *Alpinia officinarum showed* the presence of Parenchymatous cells, Epidermal cells, Fragments of vessels, Vessels with scalariform thicknings, Parenchyma cells containing starch grains.

The Physio-chemical evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs. The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particulars solvent (British Pharmacopoeia 1980). The total ash, acid insoluble ash, water soluble ash, loss on drying of *Alpinia galangal* was found to be 10.2, 4.1, 5.3, 11 % respectively, and *Alpinia officinarum*7.5, 1.5, 1.6, 1.5 % respectively**.** The ether soluble extractive value, chloroform soluble extractive value, ethanol soluble extractive value, methanol soluble extractive value and water soluble extractive value of *Alpinia galangal* extract was found to be 3.47, 1.48, 9.04, 4.7and 12.50 respectively, while *Alpinia officinarum* extract 0.60, 1.8, 14, 2.7, 1.6 respectively.

Preliminary phytochemical screening was helpful in prediction of nature of drugs and also useful for the detection of different constituents present in different polarity solvent. So it could be helpful to extract out particular constituents by solvent (Harborne et al., 1998). The phytochemical study of *Alpinia galanga* revealed the presence of alkaloids, tannins, terpenoids and phenolics, alkaloids, carbohydrates, tannins, aminoacids, and saponins, while the phytochemical study of *Alpinia officinarum* revealed the presence of alkaloids, tannins, coumarins, terpenoids and phenolics, carbohydrates, tannins, glycosides, amino acids, phenols, gums and saponins. All the extracts of *Alpinia galanga* and *Alpinia officinarum* are brown to brownish yellow in colour and showed semisolid consistency while aqueous extract is brown in colour and showed solid consistency.

CONCLUSIONS

It is concluded that the above pharmacognostic and phytochemical parameters are very useful for the identification of the species. The results of the present study will also be helpful in the

preparation of monograph. The study had shown the standards which will be useful for the detection of its identity and authenticity. The other study viz. physical evaluation, preliminary phytochemical test adds to its quality control and quality assurance for proper identification. The present investigation, including comparative organoleptic, microscopic characters, physicochemical values, preliminary phytochemical screening and pharmacognostical studies, can be used as a diagnostic tool for the correct identification of the rhizomes. It will serve as standard reference for identification and distinguishing rhizomes of two *Alpinia Species* from its substitutes and adulterants.

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