

Development of pharmacognostic profile of *Alpinia galanga*, Willd. (Zingiberaceae)

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Abstract

Alpinia galanga, Rosc. (Zingiberaceae), commonly known as Kulanjan, Greater galangal, well known Ayurvedic herb and found throughout India. It is an Indian folkloric Ayurveda medicine primarily used as a medicine due to its anti-bronchitis, anti-inflammatory, intermittent fever, anti-cough, gastric disorder anti-bacterial, properties; The aim of present study was to gather information for the systematic identification and authentication of this particular species and pharmacognostic standardization of aerial part (leaves) and underground part (Rhizome) of this plant as per WHO guidelines. The result obtained in the present investigation might be useful in the drug industry for the identification, authentication & quality of the commercial samples supplied by suppliers. The present study may also be used for making monographs on this plant for different pharmacopoeias & official books.

Keywords: *Alpinia galanga*, Willd. (Zingiberaceae) Pharmacognostic evaluation, standardization, WHO guidelines.

1. Introduction

It is well known fact that plants are the major resource of food and medicine for mankind. Since human civilization to till date, plants are primary resources of medicines[1]. Millions of the people in the third world use herbal medicines because they believe in them and regard them as their own system of medicine 1.80% of the world's population has faith in traditional medicine, particularly plant drug for their primary health care[2]. The use of herbal medicine, the dominant form of medical treatment in developing countries, has been increasing in developed countries in recent years[2].

According to World Health Organization (WHO) about 25% of modern medicines are descended from plants first used traditionally. Many others are synthetic analogues built on prototype compounds isolated from plants[4]. In ancient time, vaidyas used to treat patients on individual basis, and prepare drug according to the requirement of the patients. But the scenario has changed now; herbal medicines are being manufactured on the large scale in pharmaceutical units, where manufacturers come across many problems such as availability of good quality raw material, authentication of raw material, availability of standards, proper standardization methodology of plant etc[5].

Therefore it has become extremely important to make an effort towards quality control and standardization of the plant material to be used as medicine. The quality control of phyto-pharmaceuticals may be defined as the status of a drug, which is determined either by identity, purity, content, and other chemical, physical or biological properties, or by the manufacturing process. The process of standardization can be achieved by step wise pharmacognostic studies. WHO has given certain guidelines for the quality control and standardization of medicinal plants. The objective of WHO guidelines is to define basic criteria for the evaluation of quality, safety and efficacy of drugs herbal medicines[6].

Alpinia galanga, Willd. (Zingiberaceae), commonly known as Sugandhamula in Sanskrit and Kulanjan in Hindi, it is evergreen plant up to about 2.0m high bearing perennial rhizome, growing in eastern Himalayas and southwest India. The essential oil containing alpha-pinene, beta-pinene, limonene, cineole, terpinene-4-ol and alpha-terpineol are reported in the leaves and rhizome of this plant. The plant is used in Indian folkloric medicine (Ayurveda) for svasa (asthma), pratisayaya (coryza), udararoga (abdomen disorder), visama javara (intermittent fever), and sula (colic pain) properties[7].

The rhizome of the plant is well studied for its medicinal properties but the aerial part (leaves) is nearly virgin. The aim of present study is to gather information for the systematic identification and authentication of this particular species and pharmacognostic standardization of underground part (rhizome) and aerial part (leaves) of this plant, as per WHO guidelines. No scientific specifications regarding its pharmacognostical profile has been yet published by any author & agencies.

2. Materials and Methods

2.1 Collection of plant material and authentication

The fresh rhizome and leaves of *Alpinia galanga*, Willd were collected from the Government Garden, Saharanpur and Arosol Pharmaceutical Pvt. Ltd. Saharanpur in the month of April and authenticated by Taxonomist Dr. S. K. Upadhyaya (Ex.HOD and Reader of Department of Botany, Maharaj Sing P.G. College Saharanpur and Dr. M.R. Uniyal Ex. Director Bhartiya Kalyan Chikitsa Sansthan, Patiyala.). The collected plant material was dried in shade for about four weeks and then in oven at 60⁰ C for 12 hours. The material was then crushed to powder and stored in air tight container for further analysis. Macroscopic and microscopic analysis to categorize medicinal plant materials sensory, macroscopic and microscopic characteristics are considered to determine the identity and the purity of plant materials.

Firstly, we have examine macroscopic and microscopic characteristics. Morphological studies of leaf (such as shape, apex, surface, base, margin, venation, taste and odor) and rhizome (such as color, odor and taste) were carried out. Free hand transverse section of fresh rhizome, and leaf were taken, cleaned in chloral hydrate solution with warming, stained with phloroglucinol and concentrated hydrochloric acid. They were mounted on slide in glycerin and studied under microscope. Microphotographs of sections were made using 'Nikon coolpix L21' digital camera.

Physico-chemical studies like ash values, acid insoluble ash value, extractive values and loss on drying were performed according to the officinal methods as prescribed in WHO guidelines and Ayurvedic Pharmacopoeia of India on quality control methods for medicinal plants materials.

2.2 Preliminary phytochemical screening

The dried plant material (Rhizome and leaves) were extracted with methanol and chloroform. The behaviour of powder with various chemical reagent and preliminary chemical tests for various extracts were also carried out.

2.3 Macroscopic characters

The leaves are green in color, 30-50 cm. in length alternate, oblong lanceolate, upper surface is glabrous and shining. The leaves are simple, dorsi-ventral, petiolate, stipulate, venation is linear. *Alpinia galanga* is a perennial herb. The roots are adventitious, in groups, fibrous, persistent in dried rhizomes, The rhizome is about 08 to 10 cm long and 06 to 12 cm in diameter and yellowish-brown in colour, cylindrical, branched, stout, aromatic, longitudinally ridged with prominent rounded warts (remains of roots) marked with fine annulations; scally leaves arranged circularly; externally reddish-brown, internally orange yellow in colour; fracture, hard and fibrous; fracture, surface rough; odour, pleasant and aromatic; spicy and sweet in taste.

(b) Microscopic characters

Root: T.S. of root circular in outline, single layered epidermis with barrel shaped cells having unicellular root hairs, hypodermis 3 or 4 cells deep and sclerenchymatous cortex, many cells deep, with well developed intercellular spaces; endodermis showing prominent casparian strips and 'v' shaped thickening, followed by many celled sclerenchymatous pericycle; xylem and phloem in separate radial strands; centre occupied with a parenchymatous pith.

Rhizome: T.S. of young rhizome circular in outline; epidermal cells small and angular, thick cuticle present, rhizome differentiated into a wide cortex and a central cylinder, both regions having irregularly scattered vascular bundles, each vascular bundle with a prominent fibrous sheath; inner limit of cortex marked by rectangular parenchymatous cells; stele with irregular, closely placed vascular bundles towards periphery, root traces present, schizogenous canals and oil cells with suberized walls found in cortex and in central region; most of the parenchymatous cells filled with starch grains which are ellipsoidal to ovoid, sometimes beaked, simple, 10 to 64 µm, xylem eccentric, circular or crescent shaped at the broad end, the narrow beak-like end become black when stained with dil. iodine water and chlor-zinc iodide but the remaining part become light blue or brown. Macerated preparation shows vessels 95 to 710 µm long and 19 to 190 µm broad, tracheidal fibres 68 to 920 µm long and 19 to 30 µm broad.

Leaves of *Alpinia galanga*, Willd.



Rhizome of *Alpinia galanga*, Willd.



Powder: Powder is orange brown in colour, spicy and pungent in taste, shows parenchymatous cells containing starch (as described under microscopy of rhizome), oil cells, schizogenous canals, vessels with scalariform and reticulate thickenings and tracheidal fibres.

2.4 Physicochemical parameters

Physicochemical parameters like foreign matter, loss on drying, total ash, water soluble ash, acid insoluble ash, water soluble extractive, alcohol soluble extractive, are summarized in **table 1**.

2.5 Extractive values

Extractive values of powder of aerial part and underground part (Rhizome) in various solvents are summarized in table 2.

2.6 Preliminary phytochemical screening

Various tests were applied on the methanolic extract of the plant material using specific reagents. The results obtained are summarized in the **Table 3**. The tests showed the presence of alkaloids, carbohydrates, glycosides, flavanoids and tannins. Gibbs[8] suggested some simple tests for studies of phytochemicals. Which can be performed directly on fresh or dried plant material. Some qualitative tests were also described by other authors[9][16].

(1) Test for alkaloids: To the extract dilute hydrochloric acid will be added and filtered. The filtrate will be treated with various alkaloid reagents.

A) Mayer's test: The filtrate will be treated with Mayer's reagent: appearance of cream colour indicates the presence of alkaloids.

B) Dragon draff's test: The filtrate will be treated with dragon draff's reagent: appearance of reddish brown colour precipitate indicates the presence of alkaloids

C) Hager's test: The filtrate will be treated with Hager's reagent: appearance of yellow colour indicates the presence of alkaloids.

(2) Test for glycosides: When a pinch of the extract was treated with glacial acetic acid and few drops of ferric chloride solution followed by addition of concentrated H_2SO_4 , formation of a ring at junction of two liquids take place.

(3) Test for tannins: The extract will be treated with 10% lead acetate solution. Formation of a white colour indicates the presence of tannins

(4) Test for steroids

Libbermann bur chard's test: The extract will be treated with 3ml of acetic anhydride, few drops of glacial acetic acid followed by a drop of concentrated H_2SO_4 .

(5) Test for flavonoids

A) 5ml of extract will be hydrolyzed with 10% of H_2SO_4 and cooled. then it will be extracting with diethyl ether and divided into three portions in 3 separate test tubes, 1ml of diluted sodium carbonate, 1ml of 0.1 N sodium hydroxide and 1ml of strong ammonia solution will be added to the first, second and third test tube respectively in each test tube. Development of yellow colour demonstrates the presence of flavonoids.

B) Shinoda's test

The extract will be dissolved in alcohol. To which few magnesium turnings will be added followed by concentration HCl drop wise and heated and appearance of magenta colour indicates the presence of flavonoids.

(6) Test for saponins

Foam test: About 1ml of the extract was diluted to 20 ml with distilled water and shaken well in a test tube; the formation of foam in upper part of test tube indicates presence of saponins.

(7) Carbohydrates Test

(A) Molish's test: To a small portion of filtrate add a little amount of molish's reagent and H_2SO_4 : formation of a violet ring indicates the presence of carbohydrates.

B) Fehling's test: To a small portion of filtrate add a little amount of Fehling's reagent A and Fehling's reagent B: formation of a reddish brown indicates the presence of carbohydrates

C) Benedict's test: To a small portion of filtrate add a little amount of benedict's reagent: formation of a reddish orange indicates the presence of carbohydrates.

2.7 Fluorescence powder drug analysis

The powdered plant material was treated with different reagents and change in color was studied in both UV (254nm and 365nm) and day light. The results obtained are summarized in table 4.

3. Result and Discussion

Alpinia galanga is widely used in Indian folkloric medicines. This plant is chiefly used for its therapeutical properties viz, antibronchitis, anti-inflammatory, intermittent fever, anticough, gastric disorder and antibacterial activities. Phytochemical analysis

Various chemical extract of leaves and Rhizome of *Alpinia galanga* have been observed reported in Tables-2, methanole +water extractive yields are greater than the other extractive yields i.e. 22.50% and 16.60% of Leaves and Rhizome respectively with total ash value of 1.73% and 4.10%, acid insoluble ash 0.89% and 125% of leaves and rhizome respectively(Table-1).

3.1 Physicochemical parameters

Table 1: Physicochemical parameters of aerial part(Leaves) and Rhizome of *Alpinia galanga*

Parameter	Result	
	Leaves	Rhizome
Foreign matter	Negligible	Negligible
Loss on Drying of shade dried drug	2.35%	4.65%
Total Ash	1.73%	4.10%
Water soluble Extractive	12.10%	15.30%
Alcohol soluble Extractive	9.10%	8.20%
Acid Insoluble Ash	0.89%	1.25%

3.2 Extractive values

Table 2: Extractive values of aerial part (Leaves) and underground part (Rhizome) of *Alpinian galanga* Willd

Solvents	Extractive value	
	Leaves	Rhizome
Acetone	3.30%	5.57%
Petroleum ether	7.50%	6.60%
Methanol	8.20%	14.40%
Water & Methanol (50:50)	22.50%	16.60%
Ethyl acetate	1.95%	2.93%
Chloroform	7.60%	5.60%
Acetic acid	1.10%	2.10%

3.3 Preliminary phytochemical screening

Table 3: Phytochemical screening of methanolic extract of aerial Part (Leaves) and Rhizome of *Alpinia galanga*

Constituents	Result	
	Leaves	Rhizome
Alkaloid	-	-
Glycoside	+	++
Tannins	-	+
Steroids	+	++
Flavanoids	+	++
Saponins	+	+
Carbohydrates	+	+

Note (-): Absent, (+): Present, (++) more intensive

2.7 Fluorescence powder drug analysis

Table 4: Fluorescence powder drug analysis of aerial part (Leaves) and Rhizome of *Alpinia galanga*

Reagent+Powder	Normal Light		UV 254nm.		UV 365nm.	
	Leaves	Rhizome	Leaves	Rhizome	Leaves	Rhizome
Dry Powder	Green	Light Brown	Green	Green	Yellowish Green	Yellowish Green
Powder +5% NaOH	Dark Brown	Dark Brown	violet	Green	Orange	Orange
Powder +5% KOH	Light Brown	Light Brown	violet	Green	Light Red	Red
Powder+ Conc.H ₂ SO ₄	Black	Black	Dark Brown	Dark Brown	Black	Black
Powder+Dil. Ammonia	Light Brown	Brown	indigo	Red	Orange	Dark Red
Powder +HCl (Conc.)	Dark Green	Dark Brown	Blue	Red	Dark Blue	Dark Red
Powder+Conc. HNO ₃	Reddish Brown	Reddish Brown	Orange	Orange	Red	Red
Powder+Dil. HNO ₃	Redish Brown	Redish Brown	Green	Green	Light Red	Light Red
Powder+Venillin H ₂ SO ₄	Blue	Dark Brown	Black	Orange	Light Black	Yellow
Powder+Iodine	Blue	Blue	Dark Blue	Dark Blue	Dark Red	Dark Red

On the basis of qualitative assessment of phytochemicals, this plant contained glycosides, sterols, flavonoids, saponins, carbohydrates, and tannins, observation of phytochemical screening says that leaf does not exhibit any positive test for tannins while rhizome show positive test for tannins. Leaves of *Alpinia galanga* impart less intensive colour test for glycosides, steroids, and flavonoids, on the other hand, rhizome exhibit more intensive colour test for glycoside, sterols, and flavonoids in the qualitative phytochemical screening. On the basis of colour intensity, rhizome of *Alpinia galanga* carries more active ingredients chiefly used for therapeutical purposes.

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