

PHARMACOGNOSTIC AND PHYTOCHEMICAL EVALUATION OF *CURCUMA AERUGINOSA* ROXB

*Mariat George, S. John Britto and Thamacin Arulappan

The Rapinat Herbarium and Centre for Molecular systematics St. Joseph's College
(Autonomous), Triruchirappalli, Tamil Nadu.

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*Correspondence for Author

Mariat George

The Rapinat Herbarium
and Centre for Molecular
systematics St. Joseph's
College (Autonomous),
Triruchirappalli, Tamil
Nadu.

ABSTRACT

Cucurma is a tropical genus comprising of 120 species of rhizomatous herbs. They are used both as spices and medicines. *Curcuma aeruginosa*, is commonly known as 'kali haldi'. The rhizome of plant is used medicinally to treat asthma and cough, scurvy, mental derangements, and dysentery. It also possesses bioactive components like curcuminoids which are responsible for anti-inflammatory properties, wound healing, hypoglycemia, anticoagulant and antimicrobial activities. In the pharmacognostic study, leaves are seen with paracytic stomata and trichome with oil cavities. Rhizome also has starch grains and spiral, pitted and scalariform xylary elements. In rhizomes, phytoconstituents like mucilage, lignin, starch, alkaloids, tannins, calcium oxalate crystals and saponins are identified. The

present study will be useful towards establishing pharmacognostic standards on identification, purity, quality and classification of plants, which are gaining relevance in plant drug research.

KEYWORDS: pharmacognostic, histochemical, physico-chemical, ash values.

INTRODUCTION

Zingiberaceae are the largest family of zingiberals and comprise nearly 50 genera and 1000 species with a large group of rhizomatous and aromatic plants characterized by the presence of volatile, oils and oleoresins. Zingiberaceae are usually aromatic in all or most parts or at least one of the plant parts and many species are known to be rich in terpenoids. There are being used in traditional medicine in treatment of several diseases. The medicinal properties of the rhizome have been widely discussed and accepted worldwide. The rhizome of

zingiberaceae possesses diverse biological activities like antimicrobial ^[1], antiulcer ^[2], anti-inflammatory, antioxidant, cytotoxic and antitumor activities. They are rich in substances having therapeutic value, such as flavanoids, which have been detected in several species and considered chemosystematic markers of the order ^[3]. *Curcuma aeruginosa*, a rhizomatous herbaceous plant is commonly known as 'kali haldi'. Fresh rhizomes are aromatic and deep blue or bluish black coloured cortex with pungent odor ^[4]. In India, it grows in West Bengal, Madhya Pradesh, Orissa, Bihar and Utter Pradesh and is used by the tribals to cure various ailments ^[5]. Rhizomes are applied for sprains and bruises and also employed as cosmetics ^[6,7] records that the rhizome of *C. aeruginosa* is used in treating leucoderma, asthma, tumor, piles, bronchitis and its paste is applied on contusions and rheumatic pains.

MATERIALS AND METHODS

Collection of Plant Material

Curcuma aeruginosa was collected from Pathampuzha in Kottayam (Kerala, India) during the month of July-October, 2013. They were identified and authenticated by S. John Britto, The Director and Head, Rapinat Herbarium, The Centre for Molecular Systematics, St. Joseph's College (*Autonomous*), Tiruchirappalli, Tamilnadu, India. The voucher specimens were deposited at the centre (RHT 65179).

Macroscopic and Morphological Studies

Morphological characters were documented together with macroscopic features.

Microscopic - Anatomical Studies

The fully matured rhizome and stems were preserved in fixative solution FAA (Formalin-5ml +Acetic acid -5ml +70% ethyl alcohol-90ml) for more than 48 hours. The preserved specimens were cut into thin transverse section and cross section using sharp blade. The sectioned samples were observed in digital microscope attached with computer system for the distinguishing characters of the tissue system of leaf, rhizome and roots. For the anatomical studies fresh plants and tissue culturally produced plants were used.

Histochemistry Studies

Hand section of fresh leaf, rhizome, root and tissue cultured leaf and root were stained with a series of histochemical reagents: a) safranin (1% safranin in 50% alcohol) and lignin, b) iodine solution and cellulose, c) ruthenium red and mucilage, d) iodine and starch, e) Wagners reagent and alkaloids, f) dilute Ferric chloride solution and tannins, g) Millons

reagent and protein, h) conc.H₂SO₄ and saponin, i) sudan iii and fat/oil globules, j) 20% NaOH and sugars, k) con. HCl and calcium oxalate crystals and l) con.H₂SO₄ and saponin ^[8].

Powder Drug Microscopy

Rhizome and leaf of both species were collected and cleaned well to remove dust and adhering materials and then dried under shade for few days and coarsely powdered separately for powder drug detection. All powders were evaluated for microscopic structure; each of them was separately stained with the reagent such as phloroglucinol 1% and con. HCl (lignified structure), H₂SO₄ (350g/L) (calcium oxalate crystals), iodine solution (starch granules), sudan red G (cuticular cell walls). A small quantity of rhizome and leaf powder of both plants were placed on grease free microscopic slide along with the help of glycerine and water (1:1), observed under Epifluorescence microscope at 10x followed by 40x magnification and important identifying characters were photographed with the help of camera ^[9,10].

Extractive Values

Rhizomes of curcuma spices collected and cleaned thoroughly and then dried. Coarsely powdered air dried material of 20g was placed in a glass stopper conical flask with 200 ml of solvent shaking frequently, and then following it to stand for 18 hours. It was filtered rapidly through Whatmann No.1 filter paper, taking care not to lose any solvent. 25 ml of filtrate was transferred to flat- bottom dish and evaporated on a water bath, then dried at 105°C for 6 hours, cooled in desiccators for 30 minutes and weighted. The content of extractable matter in percentage of air dried material was calculated using the standard method by Kokate ^[11].

Physico-Chemical Analysis

For the determination of ash values of both species, rhizome powder was treated through sieve no. 20 and the method of Kandelwal *et al.*, ^[12,13] was used in the following tests.

Total Ash Analysis

About 3 g of sample powder was accurately weighed and taken separately in silica crucible, which was previously ignited and weighed. The powder was spread as a fine layer on the bottom of crucible. The powder was incinerated gradually by increasing temperature to make it dull red until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get a constant weight. The percentage of total ash was calculated with reference to the air dried powder.

Acid Insoluble Ash Analysis

The ash obtained as described above was boiled with 25 ml of 2N HCl for 5 minutes. The insoluble ash was collected on the filter paper and washed in hot water. The insoluble ash was transferred into crucible, ignited and weighed. The procedure was repeated to get a constant weight. The acid insoluble ash was calculated with reference to the air dried drug.

Water Soluble Ash Analysis

The ash obtained as described for the total ash, was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on ash less filter paper and washed with hot water. The insoluble ash was transferred into silica crucible, ignited for 15 minutes and weighed. The procedure was repeated to get a constant weight. The weight of insoluble matter was subtracted from the weight of total ash. The difference of weight was considered as water soluble ash. The percentage of water soluble ash was calculated with reference to air dried part respectively

Fluorescence Analysis

A small quantity of dried and finely powdered leaf, rhizome and root was placed on a grease free clean microscopic slide and on it was added 1-2 drop of con. sulphuric acid, 50% sulphuric acid, con. Hydrochloric acid, 50% hydrochloric acid, con. Nitric acid, 50% nitric acid, 10% sodium hydroxide, 5% ferric chloride, 5% potassium hydroxide, water and acetic acid, gently tilting the slide and waited for 1-2 min. Then the slide was placed inside the UV and viewed in day light, short (245nm) and long (360nm). UV radiations were recorded as per the method of Kokate ^[11].

Preliminary Phytochemical Analysis

The ethanol, chloroform and water extract subjected to preliminary qualitative chemical analysis. Standard methods were used for preliminary phytochemical screening of the extract [14,15].

RESULTS AND DISCUSSION

Macroscopic study of the Plant Morphology

Rhizome large, 5-7 x 9-10 cm, blue in the centre, verging towards grey, the blue color highly variable; strongly aromatic. Sessile tubers branched, condensed. *Root* fleshy; root tubers many, ovate-oblong, pale. *Plants* large, 70-100 cm tall, pseudo stem 31-36 cm tall, sheaths green. *Leaves* distichous, 79-100 cm; petiole as long as leaf; leaf 35-40 x 10-13 cm, oblong

lanceolate, tip acute, base acuminate, glabrous, purple or reddish –brown patch along the sides on the distal half of the mid rib on upper side only, fading at maturity, groove of the mid-rib is green. *Inflorescence* lateral, 24-30 cm long, peduncle 14-18 cm; spike 12-15 x 5 cm; coma bracts large, pink to violet, lower one streaked green. *Fertile bracts* 18-20.4.5-5x 4.4-5 cm, each subtending a cincinnus of 8-10 flowers. *Bracteoles* large, 3.5 x 25 cm, white with median light green patch. *Flowers* 4.4-5 cm, equal to or slightly shorter than the bracts. *Calyx* 1 cm, truncate, 3-lobed at apex, slit on one side. *Corolla* tube 3-3.3 cm long, pink, lobes unequal; dorsal lobes 1.5x1.2 cm, concave, hooded; lateral lobes 1.5x 1 cm, tip rounded, pink. *Labellum* 1.5-1x.71.8 cm, tip emarginate, yellow with a deep yellow median band. *Lateralstaminoids* 1.5x1 cm, yellow. *Anther* 7 mm long, without crest, squarred at the base, spurs 3 mm long, divergent. Epigynous glands 2, 5 mm long, linear yellowish green. *Ovary* 5 mm, trilocular, with many ovules. Style long, filiform; stigma bilipped, slightly exerted above the anther lobes. *Fruting* not common (Table 1) (plate I).

Microscopic study of the Plant Anatomy

Leaf: Leaf isobilateral and consists of upper and lower layers, covered with cuticle, single layered stomata. Trichomes and stomata are present on both sides. *Mesophyll*: palisade and spongy parenchyma are compactly arranged, thin walled, isodimetrical, chlorophyllous and scattered oil cavities present. *Vascular bundles* alternating with oil cavities; collateral and closed. *Xylem* towards upper side and *phloem* towards lower side surrounded by bundle sheath; xylem consists of vessels and *phloem* with sieve tubes and companion cells. *Sclerenchyma* cells in patches on both sides of mid rib region to give mechanical support of the vascular bundles. *Rhizome*: Periderm consists of many layered thin walled cork cells. *Cortex*: The inner cortical region consists of many rings of collateral and closed vascular bundles. Numerous oil cells scattered in the cortex, filled with starch grains. *Endodermis*: single layered with compact collenchymatous filled with starch grains. *Pericycle*: single layered. *Vascular bundles*: conjoint and collateral, xylem exarch, protoxylem towards endodermis, metaxylem towards pith. *Phloem* composed of sieve tubes and *phloem* parenchyma. *Pith*: parenchymatous filled with starch grains. *Root*: Single layered with thick cells. *Pericycle*: single layered. *Vascular tissue*: conjoint, collateral, epidermis covered by cuticle. Epidermal hairs are also present. *Cortex*: large parenchymatous cells. *Endodermis*: stele surrounded by endodermis and radially arranged. *Xylem* consists of vessels and xylem parenchyma. *Phloem* composed of sieve tubes and *phloem* parenchyma. *Pith* is parenchymatous.

Histochemistry

Histochemically all parts were tested with various reagents for their accumulation of phytoconstituents in the tissue system (Table 2)(plate II,III,IV,V,VI,VII).

Extractive Values

Physico-chemical constant is an important parameter in detecting adulteration on improper handling of drug. Extractive values of *C. aeruginosa* were studied. In *C. aeruginosa* extractive values of aqueous, alcohol and chloroform extract ranged from 8.5, 6.2 and 2.5 mg (Table 3)

Physico-Chemical Contents

The physical content evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs. Equally important in the evaluation of crude drugs is the ash value and acid insoluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, i.e., the presence or absence of foreign organic matter such as metallic salt and / or silica (Table 4). Total ash 5.8%, acid insoluble ash 0.56%, water soluble ash 3.96%.

Plate I: Macroscopic-morphological studies



Habit

Rhizome

Table 1: Macroscopic characters of *C.aeruginosa*.

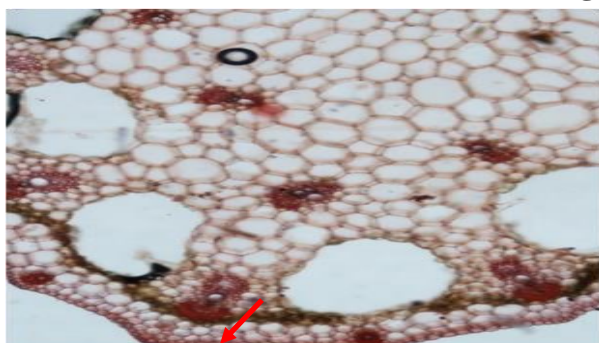
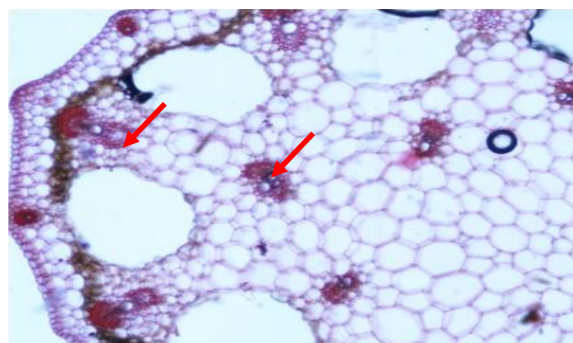
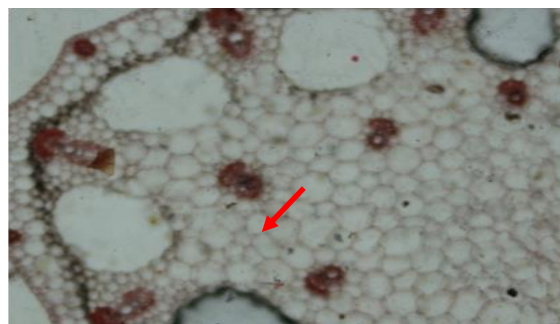
S. No	Characters	Leaf	Rhizome	Root	Flower
1	Color	Thick green	Brown	Light brown	Yellow
2	Shape	Lanceolate	Oblong –irregular	Long, cylindrical	Funnel
3	Texture	Smooth	Rough	Smooth	Smooth
4	Size	79-100cm	9-10 cm	11-12 cm	5-6 cm
5	Taste	Bitter	Slightly bitter	Slightly bitter	Bitter
6	Odour	Aromatic	Aromatic	Slightly aromatic	Slightly aromatic

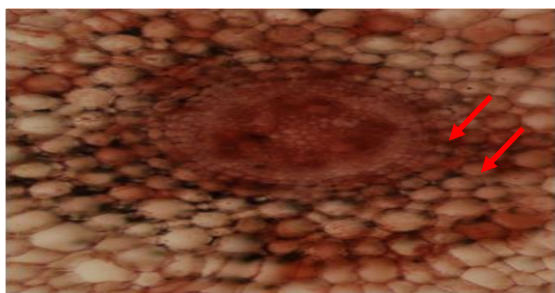
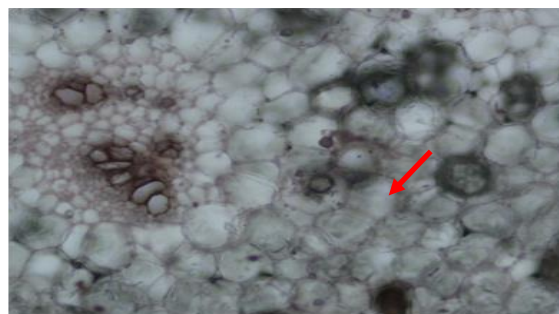
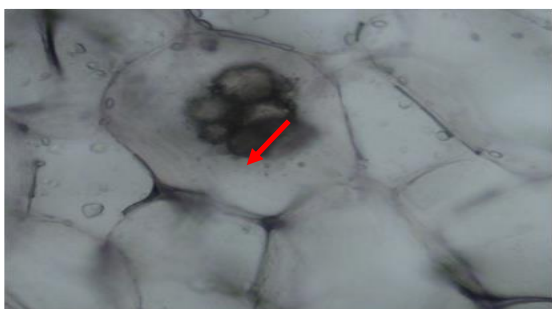
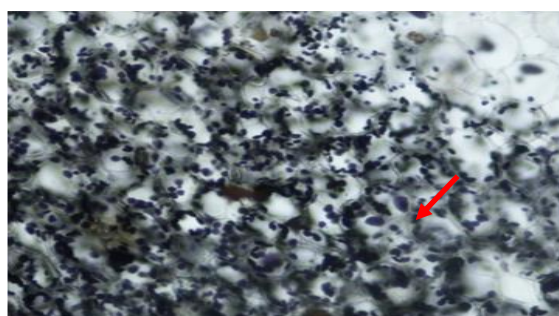
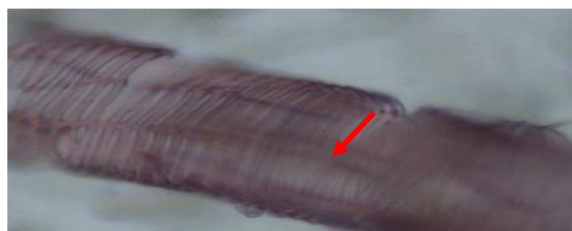
Table 2: Histochemistry of *C. aeruginosa*.

Test for	Reagents used	Nature of change	Leaf	Rhizome	Root
Lignins	Safranin (1%)	Red	Upper and lower epidermic, hypodermis, mesophyll tissue, vascular bundles	-	Cortex, endodermis, pericycle, vascular tissue
Mucilage	Ruthenium Red	Pink	Upper and lower epidermis, hypodermis, mesophyll tissue, vascular bundles	Hypodermis, cortex, pith, endodermis, pericycle	Cortex, endodermis Pericycle, vascular bundles, pith
Starch	Iodine	Blue	Hypodermis tissue	Cortex, pith	Cortex, pith
Alkaloids	Wagner's reagent	Orange	Hypodermis tissue	Cortex, pith	Cortex
Tannins	Dil .FeCl ₃	Bluish black	Upper and lower epidermal tissue sclerchymatous cell	Corex, endodermis, pericycle, vascular tissue	Vascular bundles
Saponins	Conc .H ₂ SO ₄	Light yellow	Upper and lower epidermis	Endodermis, pericycle, vascular tissue.	-
Calcium oxalate crystals	Conc .HCl	Bright effervesces	-	Cortex, pith	-

Powder Drug Microscopy: Leaf, rhizome and root were with epidermal cells, cortical tissue, scalariform, spiral and pitted vessels. Rhizome consisted of starch grains, oil gland and epidermal cells. Leaf powder contained paracytic stomata, vascular tissues and epidermal cells. Root powder also contained root hairs and starch grains.

Fluorescence Analysis: Powder drugs of leaf and rhizome of both plants were treated with different acids of various concentrations observed for the color under day light, short and long waves of ultra violet rays. Some constituents showed fluorescence in the visible range in day light. The ultraviolet produced fluorescence in many natural products which did not visibly fluoresce in day light. In *C. aeruginosa* main color in natural light in leaf was blackish green and rhizome brownish yellow; in short wave black and reddish brown; whereas in long wave black and light blackish red (Table 6).

Plate II: Histochemical studies of leaf**Lignin****Starch****Tannin****Alkaloids****Sapoins****Plate III: Histochemical studies of root****Starch****Mucilage**

**Alkaloids****Taninns****Plate IV :-Histochemical studies of rhizome****Mucilage****Saponins****Calcium oxalate crystals****Starch****Plate V:-Rhizome powder studies****Hairs****Spiral trachids**

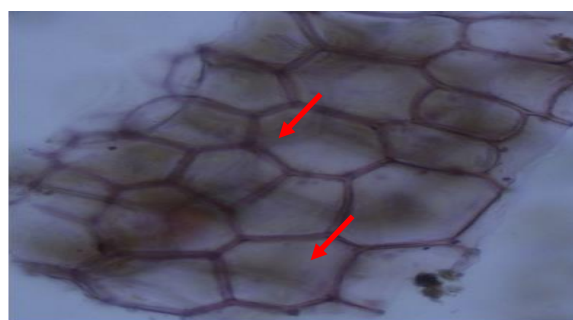
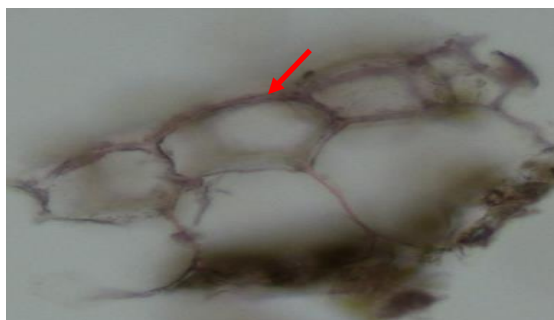
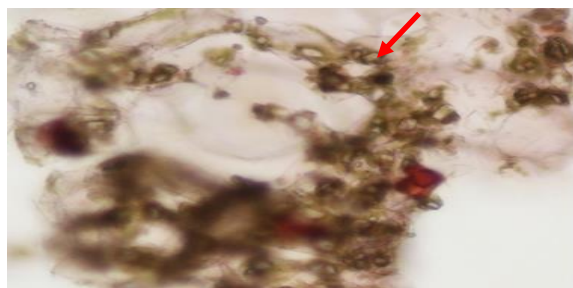
Starch grains**Presence of fibers****Plate VI:-Powder studies of leaf****Epidermal tissue****Spong tissue****Stomata****Chloroplast****Plate VII : Powder studies of root****Hypodermal tissue****Spiral tracheids****Root hairs****Vessels**

Table 3: Ash value and percentage

Ash value	Percentage
	<i>C.aeruginosa</i>
Total ash	5.8%
Water soluble ash	3.69%
Acid insoluble ash	0.56%

Table 4: Extractive value (mg)

Parameter	<i>C.aeruginosa</i>
Water	7.83
Ethanol	6.2
Chloroform	2.5

Table 5: Phytochemical constituents of *C.aeruginosa*

S .no	Phytochemical constituents	Extract		
		Ethanol	Chloroform	Water
1	Starch	+	—	+
2	Glycoside	+	+	—
3	Flavonoids	+	+	+
4	Steroids	+	—	—
5	Phenol	+	—	+
6	Saponins	—	—	+
7	Alkaloids	+	+	—
8	Tannins	+	-	+
9	Carbohydrates	+	—	—
10	Protein	+	—	+
11	Gum and mucilage	+	—	+

Table 6: Fluorescence Analysis of *C.aeruginosa*

Sl.no	Drug +reagent	Day light			250-270 nm			360-390 nm		
		L	Rh	Rt	L	Rh	Rt	L	Rh	Rt
1	Powder as such	B.G	Y	Y	G	P.G	P.G	T.A	L.G	L.G
2	50% H ₂ SO ₄	Br	B	B	B	Br	Br	B	B	B
3	Con.HCl	L.Br	B	B	B	B	B	B	B	B
4	50% HCl	Br	B	B	G	G	G	B	T.Br	T.Br
5	50% HNO ₃	Y	A	A	P.Y	Y	Y	Br	G.Y	G.Y
6	10% NaOH	T.Br	R.Y	R.Y	B	B.R	B.R	R.B	Br	Br
7	Con.H ₂ SO ₄	B	R.B	R.B	B	B	B	R.B	B	B
8	Con.HNO ₃	Y	A	A	P.Y	T.Y	T.Y	Br	G.Y	G.Y
9	5% FeCl ₃	G	B	B	B	G	G	G	B	B
10	With water	G.Y	L.Br	B	P.G	G	G	G.Y	Y.G	Y.G
11	Methanol	P.G	P.Br	B.G	L.G	L.Y	G.Y	B.G	L.G	L.G
12	Acetic acid	Br	L.Br	L.Br	L.G	Y.G	Y.G	B	Y	Y

L:Leaf; Rh:Rhizome; Rt:Root

B.G: BlakishGreen,G: Green,Y: Yellow,L.G: Light Green, P.G: Pale Green, T.A: Thick Ash, Br:Brown, B:Black, T.Br:ThickBrown,R.Y:Reddish Yellow, B.Br:Blackish Brown, L.Br:Light Brown, R.Br:ReddishBrown,L.Y:Light Yellow, A:Ash, P.Y:Pale Yellow, G.Y: Greenish Yellow, R.B: Reddish Brown, L.Br:Light Brown, P.Y:Pale Yellow, T.Y:ThickYellow, R.G:Reddish Green, L.Br.Y:Light Brownish Yellow, Y.G:Yellowish Green, L.B.Y:Light Blackish Yellow,B.R:Blackish Red

DISCUSSION

Adulteration and misidentification of medicinal plants can cause serious health problems to consumers and legal problems for the pharmaceutical industries. The past decade has witnessed the introduction and implementation of new Good Manufacturing Practices (GMP) in quality control of raw materials, intermediates and finished product of botanical origin. The initial step in quality control of medicinal plants is ensuring the authenticity of the desired species for the intended use. It can be conducted via a variety of techniques, namely macro and microscopic identification and chemical analysis especially description of microscopic botanical aspects to determine definitively the proper species of plant material while it is still in its non-extracted form. The observation of cellular level morphology or anatomy is a major aid for the authentication of drugs.

In *C. caesia* the rhizome is tuberous with camphoraceous sweet odor, irregularly shape, laterally flattened covered with adventitious roots and scars with longitudinal circular zones, bluish black, water soluble ash 13.6%, total ash 6%, moisture content 9%, alcohol insoluble ash 6%, acid insoluble ash 4%; extractive value highest in methanol 4.6%, followed by n-hexane 2.2%; alkaloids, carbohydrates, proteins, fixed oils, flavonoids, phenols and tannins. Cineole, camphor and α -turmeone are main volatile oils methanol extract ^[16].

In *C. neilgherrensis* leaf powder is grey, bitter in taste and slightly aromatic. Leaf covered with single layered epidermis, with cuticle and trichome, paracytic stomata present on both surfaces. Leaf moisture content 4.5%, total ash 8%, water soluble ash 6.4%, alcohol soluble ash 8%, methanol extract constitute flavonoids, alkaloids, tannins, organic acids and glycosides ^[17]. In *Zingiber zerumbet* rhizomes are irregularly branched with nodes and internodes, scales are present at nodal region, surface smooth, covered with trichome and fibers, light grayish yellow, hard and brittle, fragrant, aromatic, spicy and slightly bitter, with abundant

starch grains and oxalate crystals; water soluble ash height 24% than alcohol soluble ash 9.4% with an 11% of moisture content. Main phytoconstituents are glycosides, triterpenoids, saponins, tannins, carbohydrates, proteins, amino acids, sterols and volatiles are present only in petroleum ether extract ^[18]. Fluorescence analysis is also an important parameter for pharmacognostic evaluation of crude drugs ^[19].

Comparisons within the genus Curcuma

The purpose of this study is to study the pharmacognostic properties in different species of curcuma especially rhizome and leaf. The hexane extract of *C. aeruginosa* rhizomes revealed the presence of sesquiterpene and furanodiene which give strong inhibitory activity on 5- α reductase ^[20]. Rhizomes and leaves of *C. aeruginosa*, *C. brog*, *C. malabarica*, *C. rakthakanta* and *C. sylvatica* contained total phenolic content, flavonoids in them. The total phenols in methanolic extracts of rhizomes ranged from 210 to 700 mg gallic acid equivalents/100g and in leaves from 840 to 1480 mg/100g. Flavonoid content in leaves ranged from 270 to 380 mg epicatechin equivalents/100 g. The leaves of all species had higher content of phenolics, DPPH radical scavenging activity and ferric reducing power as compared to rhizomes. Chowdhury *et al.*, ^[21] also evidenced the presence of total phenol, flavonoid and curcumin contents were obtained higher in the mature part of rhizome.

Other than the pharmacognostic studies, antimicrobial and antioxidant studies have been carried out. There was good correlation between the phenol content and antioxidant activity in rhizomes, but not in leaves. The results of the study highlighted the potential of these unutilized *Curcuma* species (rhizomes and leaves) as a rich source of antioxidants for food and health ^[22]. Among the Gram positive bacteria, acetone extract of *C. caesia* showed maximum activity against *S. aureus* and hexane extract of *C. aeruginosa* exhibited maximum activity against *B. cereus*. In Gram negative bacteria, chloroform extract of *C. caesia* showed maximum inhibitory action against *S. marcescens*, whereas the methanol extract of *C. aeruginosa* showed higher inhibitory action against *S. typhi* ^[23]. Mature part mainly inner cortex of the rhizome, *C. aeruginosa* showed excellent performance of antioxidant activity which was increasing towards maturity. Total phenol, flavonoid and curcumin contents showed high quantity in mature part of rhizome. Free radical scavenging activities were significantly correlated with total phenol content. An outstanding result was observed in case of single node cutting propagation technique. The present study showed also higher amount of these phenolic compounds in *C. aeruginosa* rhizome which can explain its high radical

scavenging activity. Both methanol extractive yield and radical scavenging activity are higher in *C. aeruginosa* ^[24]. The positive correlation between antioxidant activity and phenolic compounds is also observed, which indicates that phenolic compounds are responsible for its antioxidant activity.

CONCLUSION

Curcuma is a well-known spice of India and *C. aeruginosa* is often used for medicinal purpose. Macro and microscopic studies of both species revealed an easy technique to identify and differentiate two medicinal plants and to detect degree of adulteration in powdered raw medicinal plant materials as well. Plant anatomy thus plays a positive role in the understanding of biological activities. *C. aeruginosa* is closely related to *C. caesia*, and diagnostic features are large size of plant, rhizome with pale blue colour and strongly aromatic. Leaves are with paracytic stomata trichome and with oil cavities; rhizome possesses starch grains and spiral, pitted and scalariform xylary elements; chief phytoconstituents are mucilage, lignin, starch, alkaloids, tannins, calcium oxalate crystals and saponins. Pharmacognostic studies reveal that the total ash is 5.8% water soluble ash is 3.69% and acid soluble ash is 0.56%. Extractive values are equal to value of water; ethanol and chloroform are 7.83mg, 6.2mg and 2.5mg. Thus it is evident that each *Curcuma* species is having its specific pharmacognostic characters with specific quality and quantity. There are valuable in the drug formulation, and also enable to check adulteration.

REFERENCE

1. Yamada Y, H. Kikuzaki, N Nakatani. Identification of antimicrobial gingerols from *Zingiber officinale*, J. Antibact. Antifungal Agents, 1992; 20: 309 – 311.
2. Matsuda H, Pongpiriyadacha Y, Morkawa T, Ochi M, Yoshikawa M. Gastroprotective effects of phenylpropanoids from the rhizomes of *Alpinia galangal* in rat : Structural requirement and mode of action . Eur. J. Pharmacol, 2003; 471: 59-67.
3. Pugailli HRI., Kalpan MAC, Gottlieb OR. Chemotaxonomy of super order zingiberiflora (sensu Dahlgren) I. Flavonoids. *Acta Bot. Bras*, 1993.
4. Srivastava S, Chitranshi N, Srivastava S, Dan M, Rawat A, Pushpangathan P. Pharmacognostic evaluation of *Curcuma aeruginosa* Robx. *Nat Prod Sci*, 2006; 12: 162.
5. Pandey AK, Chowdhury AR.. Volatile constituents of the rhizome oil of *Curcuma caesia* Robx. from central India. *Flavour and Fragrance Journal*, 2003.

6. Qaisar M, SN Gilani, S Farooq, A Rauf, R Naz, Shaista, S Perveez. Preliminary Comparative Phytochemical Screening of Euphorbia Species, American-Eurasian Journal of Agricultural & Environmental Sciences, 2012; 12(8): 1056-1060.
7. Anonymous. The wealth of India, Raw materials. New Delhi: CSIR, 1962.
8. Khan SK, Karnat NM, Shankar D. India's foundation for the revitalization of local health tradition pioneering in situ conservation strategies for medicinal plants and local cultures. Herbal Gram, 2005; 68: 34-48.
9. Johansen DA. Plant micro technique. McGraw-Hill Book Co. Ltd, New York, USA, 1940.
10. Upton R, Graff A, Jolliffe G, Langer R, Williamson E. Botanical Pharmacognosy- Microscopic Characterization of Botanical Medicine. Boca Raton, CRC Press, Taylor & Francis Group, 2011.
11. WHO. Quality Control Methods for Herbal Materials, Geneva: WHO Press, 2011.
12. Khandelwal KR. Practical Pharmacognosy techniques and Experiments. Edition 16th Nirali Prakashan, Pune, 2006.
13. Evans and Trease. Edition- 12, East Bourne, U.K, 1983.
14. Kokate CK, Practical Pharmacognosy. 4th edition Vallabh Prakan, New Delhi, 1994.
15. Khandelwal KR. Practical Pharmacognosy. 12th ed. Pune Nirali Prakashan, 2004.
16. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 39th ed. Pune Nirali Prakashan, 2007; 08-109.
17. Pritesh Paliwal S, Pancholi S, Rakesh K, Patel. Pharmacognostic parameters for evaluation of rhizome of *Curcuma caesia* Journal of advanced pharmaceutical technology and research, 2011.
18. Shyam Prasad M, Anju.P, Ramachandran, Harimohanchandola, Harisha C R, Vinay J Shukla. Pharmacognostic and phytochemical studies of *Curcuma neilgerrensis* (Wight) leaf-A folklore medicine. AJU Journal, 2013; 33(2); 284-287.
19. Rout OM, Prakash, Rout Kedar Kumar, Acharya Rabinarayn, Mishra Sagar Kumar. Pharmacognostical and phytochemical studies of Zingiber zerubet (L) Rhizome. International Journal of Research in Ayurveda and Pharmacy, 2011; 2: 698-703.
20. Zhao Z, Liang Z, Guo P. Macroscopic identification of Chinese medicinal materials: Traditional experiences and modern understanding. J. Ethnopharmacology, 2011; 131: 556-561.

21. Jukkarin Srivilai, Nantaka Khorana, Neti Waranuchb, Kornkanok Ingkaninan. Anti-androgenic activity of furanodiene isolated from *Curcuma aeruginosa* Roxb. Extract. Naresuan University Journal, 2011; Special Issue.
22. Chowdhury R, Nimmanapalli R, Graham T, Reddy G. Curcumin attenuation of ipopolysaccharide induced cardiac hypertrophy in rodents. *ISR Inflamm.*, 2013; 539305.
22. Bala Nambisan, Angel G R, Vimala B. Antioxidant and antimicrobial activity of essential oils from nine starchy curcuma species. *International Journal of Current Pharmaceutical Research*, 2012; Vol 4, Issue 2
23. Sweetymol Jose, T Dennis Thomas. Comparative phytochemical and anti-bacterial studies of two indigenous medicinal plants *Curcuma caesia* Roxb. and *Curcuma aeruginosa* Roxb. *International Journal of Green Pharmacy*, Volume, 2014 : 8 ; 1 - 65-71
24. Saikat Chowdhury, K Nishteswarb. Role of volatile oil of natural source in the cosmetics and health care industries. *An International Journal of Pharmaceutical Sciences*, 2013 ; Vol – 4.