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PHARMACOGNOSTIC AND PHYTOCHEMICAL EVALUATION OF *CURCUMA AMADA* ROXB

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ABSTRACT

Curcuma amada, (Zingiberaceae) is commonly known as '*ambahaldi'*. Its rhizomes are made in to paste and used for bruises and skin diseases. A rhizome possesses bioactive components like curcuminoids which are responsible for anti-inflammatory properties; wound healing, hypoglycemia, anticoagulant and antimicrobial activities. The pharmacognostic study shown that leaves have trichome with oil cavities, rhizome also has starch grains and spiral, pitted and scalariform xylary elements. Phytoconstituents present in rhizome are mainly mucilage, lignin, starch, alkaloids, tannins, calcium oxalate crystals and saponins. Pharmacognostic studies of curcuma species confirm the traditional uses as medicine and biologically validate them, besides offering evidences to its transparency to reduce adulteration of drugs. The present study will be distinguish adulterants. Such study establishes standards on recognition, purity, quality and classification of plants, which are gaining significance in plant drug research.

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

INTRODUCTION

Curcuma amada, commonly known as mango ginger (*amba haldi*) has wide distribution in India Thailand, Indochina, Malaysia, Indonesia and Northern Australia.[1] Rhizomes of *C. amada* are buff colored outside with a light yellow or white inner epidermal layer. They are used in folklore medicines, in culinary preparation such as preserves, candy and for manufacture of oleoresin, essential oil, etc.[2] It is reported that possesses anti-inflammatory, antioxidant and antitumor properties.[3] It is used topically in poultices to relieve pain and

inflammation.[4] The rhizome of the plant has been used for centuries in traditional medicine and known to have cancer preventive or therapeutic capabilities.[5] It has been shown to suppress multiple signaling pathway and cell proliferation invasion, metastasis, and angiogenesis.[6] Medicinal plant materials are being adulterated commercially as they share similar morphological features, same name as written in classical text, presence of similar active principles in the adulterants that may badly affect the therapeutic activity of the finished products. The pharmacognostic study acts as the major and reliable criteria for identification of plant drug. The pharmacognostic parameters are necessary for confirmation of the identity and determination of quality and purity of the crude drug. The detailed and systematic pharmacognostic and phytochemical evaluations of *Curcuma amada* give valuable information in order to produce standardized finished herbal products.

MATERIALS AND METHODS

Collection of Plant Sample

Curcuma amada was collected from Kottayam and Poonjar (Kerala, India) during the month of April-July, 2014. 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 specimen (RHT 65181) was deposited at Rapinat Herbarium.

Microscopic – Anatomical Studies

The 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 section. The sectioned samples were observed in digital microscope attached to computer system for the diagnostic features of the anatomical details of leaf, rhizome and roots. For the anatomical studies fresh plants and tissue culturally produced plants were used.

Histochemistry Studies

Hand sections of fresh leaf, rhizome, root and tissue cultured leaf and root were stained with a series of histochemiacal reagents: a) safranine (1%) (safranine in 50% alcohol) and lignin, b) iodine solution and cellulose, c) ruthenium red and mucilage, d) iodine and starch, e) Wagner's reagent and alkaloids, f) dilute Ferric chloride solution and tannins, g) Million's reagent and protein, h) Conc.H_2SO_4 and saponin, i) sudan III and fat/oil globules, j) 20% NaOH and sugars, k) Con. HCl and calcium oxalate crystals and l) Con.H_2SO_4 and saponin.[7]

Powder Drug Microscopy

Rhizome and leaves of *C.amada* were collected and cleaned well to remove dust and adhering materials and then shade dried for a few days and coarsely powdered separately for powder drug detection. All powders were evaluated for microscopic structure and each of them was separately stained with the reagent such as phloroglucinol 1% and con. HCl(lignified structure), H_2SO_4 (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 Epiflorescence microscope at 10X followed by 40X magnification and important identifying characters were photographed with the help of camera.[8,9]

Extractive Values

Rhizomes of both *Curcuma* spices collected and cleaned thoroughly and then dried. Coarsely powdered and 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 weighed. The content of extractable matter in percentage of air dried material was calculated using the standard method by Kokate.[10]

Physico –Chemical Analysis

For the determination of ash values of both species, rhizome powder was treated through sieve no. 20 and the following tests were performed as per the methods of Kandelwal *et al*.[11, 12]

Total Ash Analysis

About 3 g of each 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. sulphuricacid, 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.[10]

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.[13, 14]

RESULT AND DISCUSSION

Macroscopic study of the Plant Morphology

Rhizome large, 4-5.3 x 3-4 cm; light yellow inside and white towards the periphery with smell of green mango; sessile tubers thick, 5-10 x 2-3 cm, cylindric or ellipsoid, branched, horizontal. *Roots* fleshy, root tubers absent. Pseudo stem 30-35 cm tall. *Leaves* 4-6; petiole 5-10 cm long; lamina of lower leaves much smaller, c.18 x 8 cm, upper larger, 45-60x 14-15

cm, oblong, lanceolate, upper glabrous, tip hairy. *Inflorescence* lateral or central; peduncle 20-22 cm long, covered by 5-6 sheaths; *Spike* 12-19cm long; *Coma bracts* 14-19.c.4 x 3.5cm, orbicular – obtuse, slightly recurved, lower 2/3fused to form a pouch, green; *Bracteoles* small; *Flowers* large, c.5cm long, 3-5 in each bract, longer than the bracts. *Calyx* trunicate, 1 cm long, deeply cleft on one side, 3-lobed at the tip, minutely pubescent. *Corolla* tube funnel shaped, 3 cm long, pale yellow, minutely pubescent; lobes unequal, white; dorsal lobe larger, 1.5 x 1.2 cm. hoode at the apex; lateralsc.1.4 x 1 cm long, tip rounded, glabrous. *Labellum* somewhat elliptic, 1.8 x 1.5 cm 3 lobed, mid lobe emarginated, recurved, pale yellow with a median dark yellow band, glabrous. *Lateral staminods* 1.5 x 0.9 cm. apex slightly incurved, glabrous, pale yellow. *Stamens* white, thecae parallel, 4 mm long, basal spur 1 mm long, slightly convergent, glabrous. *Epigynous glands* two, linear, 6mm long, tip acute. *Ovary* trigonous, 3 mm long, tricarpellary, syncarpous with many ovules, densely hairy. *Style* long, filiform; stigma closely appressed within the anther lobes. *Fruits* setting not seen (Table 1).

Microscopic study

Leaf: leaf isobilateral and consists of upper and lower identical, single layered, oval, rectangular epidermal cells covered with cuticle perforated by paracytic stomata. *Trichomes* and stomata are present on both sides. *Mesophyll*: palisade and spongy parenchyma are not demarcated. They are compactly arranged, thin walled, isodimetrical, chlorophyllous. *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 the thin walled cork cells. *Cortex*: The inner cortical region consists of three rings of collateral closed vascular bundles. Numerous oil cells scattered in the cortex. Cortex cells filled with starch grains. *Endodermis*: single layer with compact collenchymatous filled with starch grains. *Pericycle*: single layer. Vascular bundles: conjoint and collateral, xylem exarch, protoxylem towards endodermis, metaxylem towards pith. *Phloem* composed of sieve tubes and phloem parenchyma. *Pith*: occupied major portion with parechymatous filled with starch grains.

Root: single thick cells. *Pericycle*: single layered. *Vascular tissue*: conjoint, collateral layered epidermis covered by cuticle. Epidermal hairs are also present. *Cortex*: large parenchymatous cells. *Endodermis*: stele surrounded by endodermis with very and radially arranged. *Xylem*

consists of vessels and xylem parenchyma. *Phloem* composed of sieve tubes and phloem parenchyma. *Pith* is parechymatous.

Histochemistry

Histochemically all parts were tested with various reagents for their accumulation of phytoconstituents in the tissue system (Table 2).

Extractive values

Physico-chemical constant is an important parameter in detecting adulteration on improper handling of drug. Extractive values of *C. amada* were studied. In *C. amada* aqueous, alcohol and chloroform extracts ranged from 7.34, 5.9 and 2.1 mg respectively (Table 3).

Physicochemical 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).

Powder Drug Microscopy

Leaf, rhizome and root of both plant were analyzed which contained epidermal cells, cortical tissue, scalariform, spiral and pitted vessels. Rhizome consisted of starch grains, oil gland and epidermal. 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 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 light produced fluorescence in many natural products which did not visibly fluorescence in day light. The main color of the powder in natural light *C. amada* leaf was blackish green, rhizome pale yellow; in short wave black and brown; where as in long wave black and blackish yellow (Table 6).

Table 1: Macroscopic characters of *C.amada*

S. No	Characters	Leaf	Rhizome	Root	Flower
1	Color	Dark green	Slightly brown	Light brown	Yellow
2	Shape	Lanceolate	Oblong –palmate	Long cylindrical	Funnel
3	Texture	Smooth	Rough	Smooth	Smooth
4	Size	30-45 cms	4-5.3 cms	9-10 cms	5 cms
5	Taste	Slightly bitter	Sour	Slightly sour	bitter
6	Odour	Aromatic	Aromatic	Slightly aromatic	Slightly aromatic

Table 2: Histochemistry of *C.amada*

Test for	Reagents used	Nature of change	Leaf	Rhizome	Root
Lignins	Safranine (1%)	Red	Upper and lower epidermis hypodermis, vascular bundle		Cortex, endodermis pericycle and vascular tissue.
Mucilage	Ruthenium Red	pink	Upper and lower epidermis hypodermis, mesophyll, vascular bundle	Hypodermis, cortex, endodermis, pericycle,pith	Cortex, endodermis, pericycle, vascular bundles,pith
Starch	Iodine	Blue		Cortex and pith	Cortex ,pith
Alkaloids	Wagner's reagent	Orange		Cortex and pith	Cortex
Tannins	Dil .$FeCl_3$	Blackish blue	Upper epidermis, lower epidermis, sclernchymatous tissue	Cortex, Endodermis,	Vascular tissue
Saponins	Conc .H_2SO_4	Light yellow	Upper epidermis, lower epidermis	Endodermis, pericycle,vasculartisse	-
Calcium oxalate crystals	Conc .HCl	Bright effervesces	-	-	Cortex, pith

Table 3: Extractive value (mg)

Parameter	*mg*
Water	7.34
Ethanol	5.9
Chloroform	2.1

Table 4: Ash value and Percentage

Ash value	Percentage
Total ash	5.4%
Water soluble ash	3.3%
Acid insoluble ash	0.43%

Table 5: Phytochemical constituents of *C. amada*

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

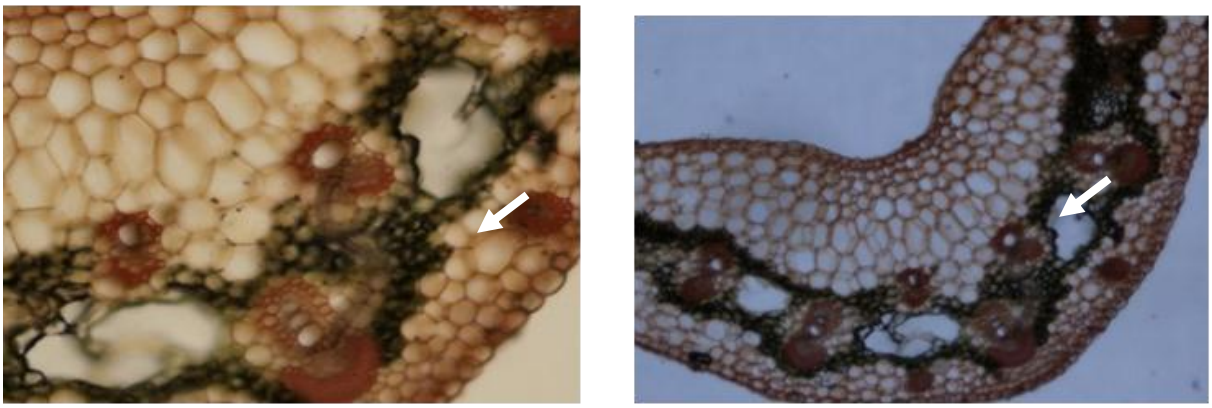
Table 6: Fluorescence Analysis of *C.amada*

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	L.Br	L.Br	G	P.G	P.G	T.A	L.G	L.G
2	50%H_2SO_4	R.B	B	B	R.B	Br	Br	B	B.Br	B
3	Con.HCl	L.Br	B	B	B	B	B	B	B	B
4	50%HCl	Br	B	B	G	B.G	B.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_2SO_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	L.Br	P.G	G	G	G.Y	Y.G	Y.G
11	Methanol	P.G	L.Br.Y	L.Br	B	P.Y	P.Y	B.G	B.Br	B.Br
12	Acetic acid	Br	L.Br	L.Br	L.G	Y.G	Y.G	B	Y	Y

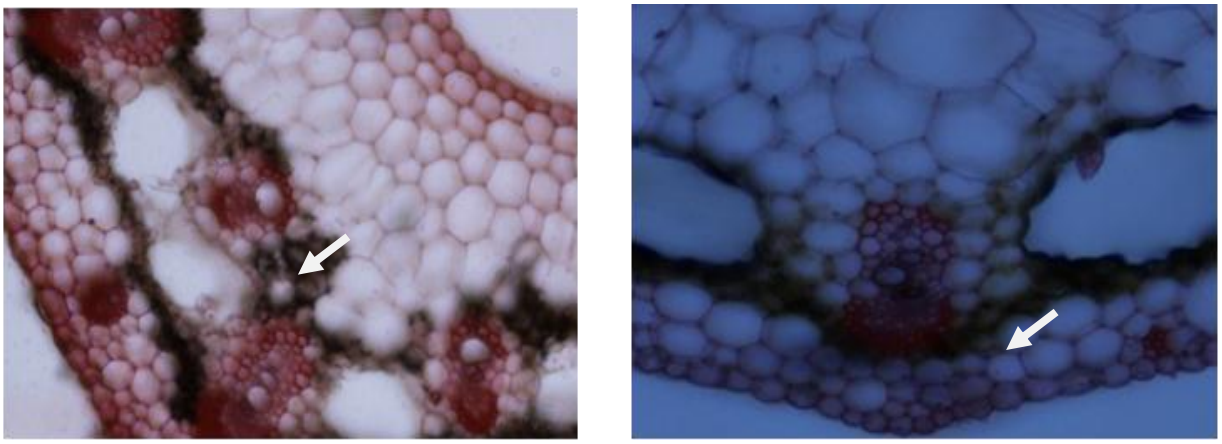
L:Leaf; Rh:Rhizome; Rt:Root

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:Thick Yellow, R.G:Reddish Green, L.Br.Y:Light Brownish Yellow, Y.G:Yellowish Green, L.B.Y:Light Blackish Yellow,B.R:Blackish Red.

Plate 1: Habit and Flower of *C. amada*

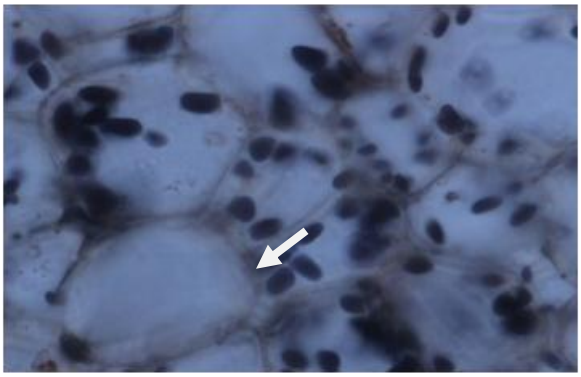


Tannin **Saponins**

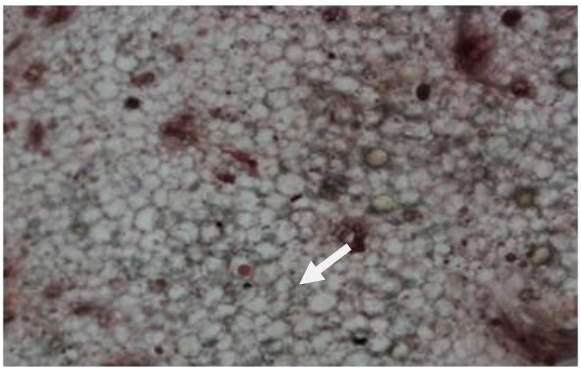


Lignin **Mucilage**

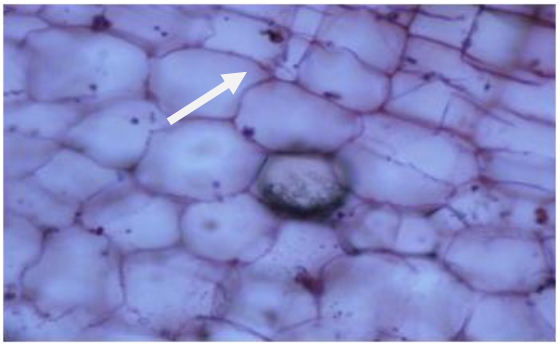
Plate 2: Histochemical Studies of leaf



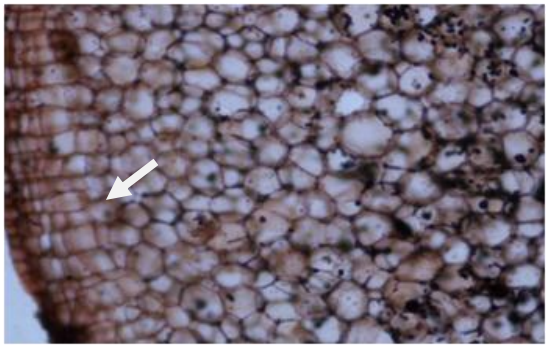
Starch



Tannins

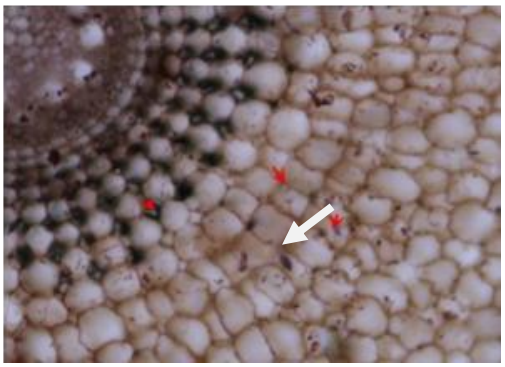


Mucilage

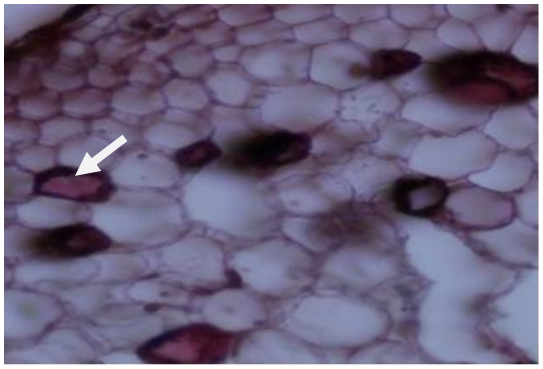


Alkaloids

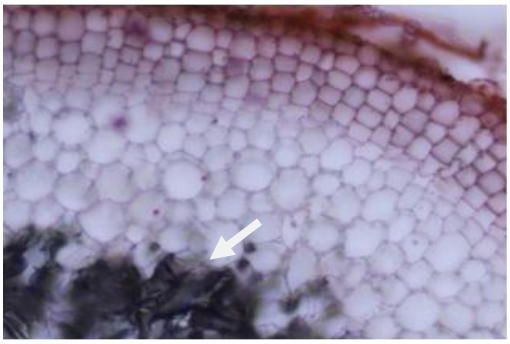
Plate 3: Histochemical studies of rhizome



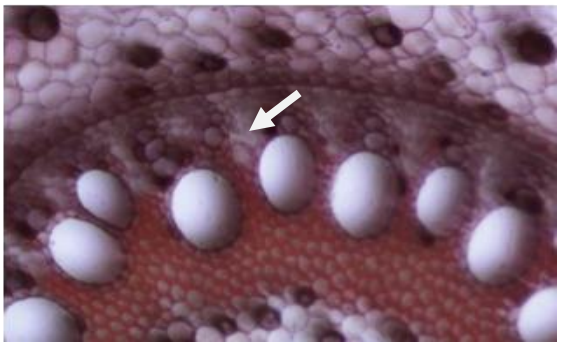
Alkaloids



Mucilage

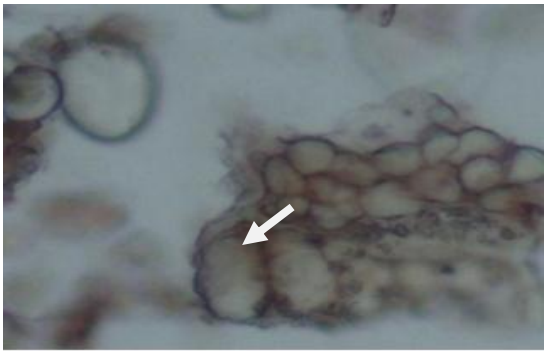


Starch

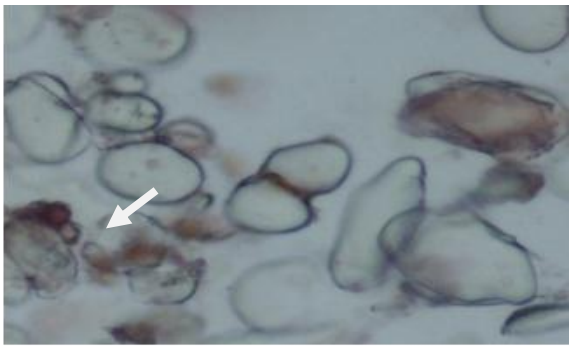


Lignins

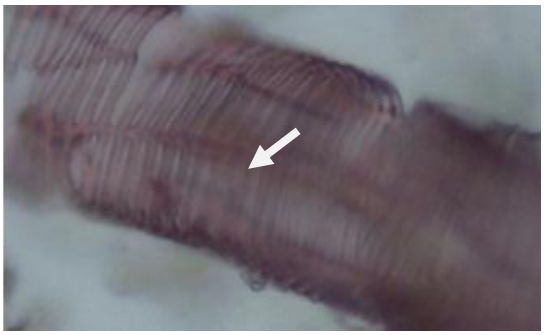
Plate 4: Histochemical studies of Root



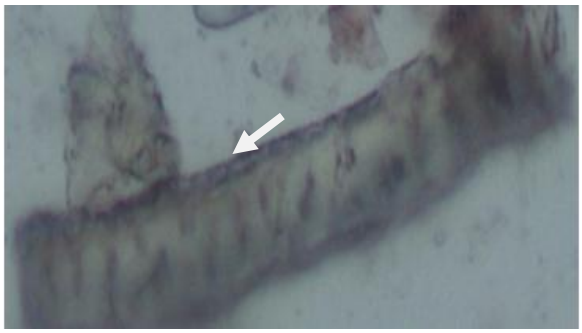
Oil globule



Starch grains

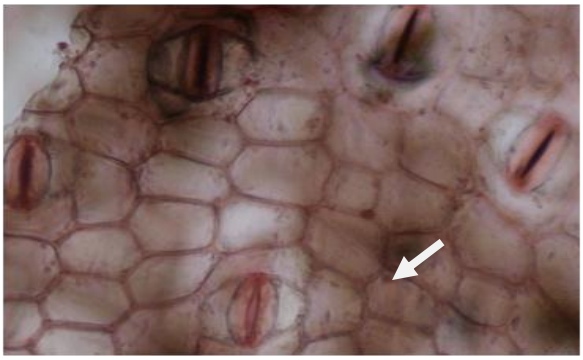


Spiral trachids

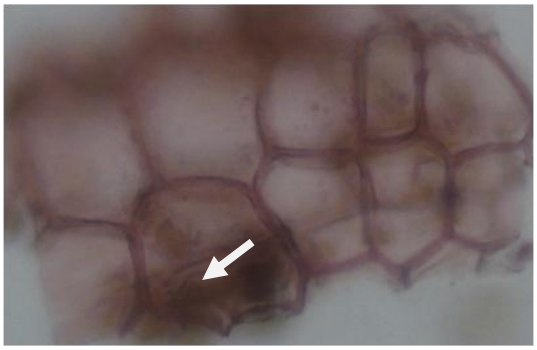


Pitted vessels

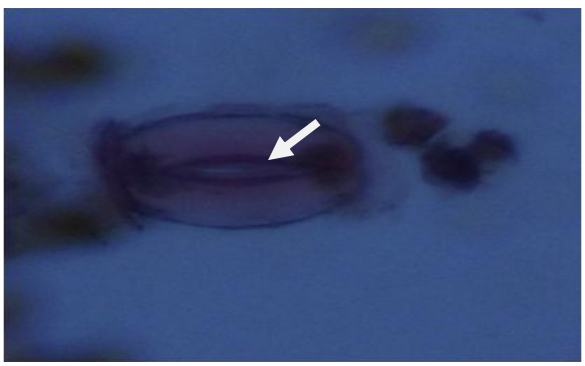
Plate V:-Rhizome powder studies



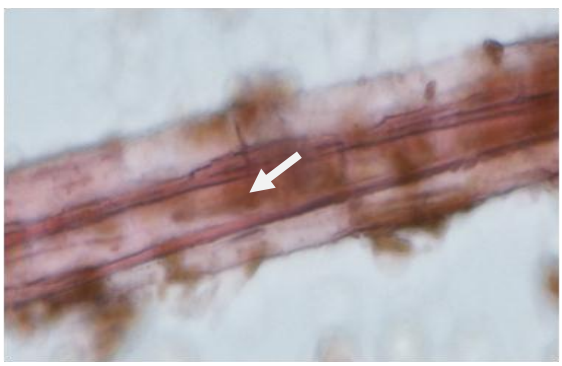
Lower epidermal tissue



Hypodermal tissue



Stomata



Xylem vessals

Plate VI:-Powder studies of Leaf

Adulteration and misidentification of medicinal plants can cause severe health troubles to clients and legal problems for the pharmaceceutical industries. The last decade has witnessed the prologue and realization of new Good Manufacturing Practices (GMP) in quality control of raw materials, intermediates and finished product of botanical origin. The preliminary step in quality control of medicinal plants is ensuring the authenticity of the desired species for the future use. It can be conducted via a series of techniques, namely macro and microscopic identification and chemical analysis especially account of microscopic botanical aspects to establish definitively the proper species of plant material while it is still in its non-extracted form.[15] The observation of cellular level morphology or anatomy is a major aid for the authentication of drugs.

CONCLUSION

C.amada is an important member of the family Zingiberaceae. Macro and microscopic studies of the specie reveal diagnostic features to identify plants and to detect degree of adulteration in powdered raw medicinal plant materials as well. The aim of the present work was to evaluate the microscopic features of the powdered form of the plant to support the pharmacological data. *C. amada* is closely related to *C.longa*, which can easily be distinguished from characteristic smell and pale yellow color of rhizome with nodes and internodes. Leaves have trichome and with oil cavities; rhizome with starch grains and spiral, pitted and scalariform xylery elements; mainly with phytoconstituents like mucilage, starch,alkaloids, tannins, calcium oxalate crystals and saponins. Physico chemical studies reveal that the total ash is 5.4%, water soluble ash is 3.3% and acid soluble ash is 0.43%. Extractive values in water, ethanol and chloroform are 7.34mg, 5.9mg and 2.1 mg respectively. Thus it is evident that each *Curcuma* species has its specific pharmacognostic characters with specific quality and quantity. There are important in the drug formulation, and also enable to check adulteration

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