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COMPARATIVE PHARMACOGNOSTIC AND PHYTOCHEMICAL EVALUATION OF *CURCUMA AMADA* AND *CURCUMA AERUGINOSA*

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ABSTRACT: *Curcuma amada* and *Curcuma aeruginosa* are economically and medicinally very important species in India. Both of the species look similar in morphological nature. In the present study, an effort has been made to work out the identifying characters and detailed comparative account of both species for ready identification of the entire plant, live or in the herbarium and also the discrimination of drugs made from the rhizome of both species. The studies of both species revealed the presence of entire major compounds like alkaloids, flavonoids, glycosides, phenols, and tannins, saponins *etc.* The macroscopic and microscopic studies of both species expressed difference. The activities of the powder with different chemicals and fluorescent studies showed characteristic colorations.

INTRODUCTION: Nature has been a source of medicinal agents for thousands of years. Plants still comprise one of the major sources of drugs in modern as well as traditional medicine throughout the world¹. A study of the World Health Organization (WHO) depicts that over 80% of world's population directly depends on the natural diversity and its associated traditional system of medicine for their primary healthcare demands². Though, traditional medical practices are empirical, it has been estimated that over 200 million people in India with limited access to the organized Primary Healthcare Service Centers, depending on varietal aspects of the traditional system of medicine to cater to their health care needs³.

Zingiberaceae are the largest family of Zingiberals and comprises nearly 50 genera and 1000 species with a large group of rhizomatous and aromatic plants characterized by the presence of volatile, oils and oleoresins. *Curcuma* is a tropical genus comprising of 120 species of rhizomatous herbs, and they are used both as spices and medicines in traditional Indian and Chinese medicines. *Curcuma amada* Roxb. commonly known as "Ama Haldi" is found all over India.

Curcuma amada is an herbaceous perennial with erect to semi-erect plant stature. *Curcuma aeruginosa* is known in Thai region as Waan- Ma-Haa-Mek. The rhizome of the plant is used medicinally to treat asthma and cough, scurvy and mental derangements⁴. It is also added to a beverage given to women in confinement to accelerate the lochia and decrease pain and inflammation of uterus⁵. The present work, 'Comparative pharmacognostic and phytochemical evaluation of *Curcuma amada* and *Curcuma aeruginosa*' is there to establish a simple and

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efficient protocol to determine the drug adulteration.

MATERIALS AND METHODS:

Collection of the Plant Material: *Curcuma aeruginosa* was collected from Pathampuzha in Kottayam (Kerala, India) during the month of July-October, 2013. *Curcuma amada* was collected from Kottayam and Poonjar (Kerala, India) during the month of April-July, 2014. They were identified and authenticated by Dr. S. John Britto, The Director and Head, Rapinat Herbarium, The Centre for Molecular Systematics, St. Joseph's College (Autonomous), Tiruchirappalli, Tamil Nadu, India. The voucher specimens were deposited at the center (RHT 65179 and RHT 65181).

Macroscopic and Morphological Studies: Morphological characters were documented together in the macroscopic features.

Pharmacognostic Studies:

Extractive Values: Rhizomes of both species were collected and cleaned thoroughly and then dried. Coarsely powdered and air dried material of 20 gm has placed in a glass stopper conical flask with 200 ml of solvent shaking frequently and then following it to stand for 18 h. It was filtered rapidly through Whatman 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 h, cooled in desiccators for 30 min and weighed immediately. The content of extractable matter in % of air-dried material was calculated using the standard method by Kokate ⁶.

Physicochemical 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 as the methods of Khandelwal, Evans and Trease ^{7,8}.

Total Ash Analysis: About 3 g in of each sample powder was accurately weighed and taken separately in silica crucible, which was previously ignited and weighed. The powder was spread like a fine layer on the bottom of the 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 concerning the air-dried powder.

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

Water Soluble Ash Analysis: The ash obtained as described for the total ash was boiled for 5 min with 25 ml of water. The insoluble matter was collected on ashless filter paper and washed with hot water. The insoluble ash was transferred into silica crucible, ignited for 15 min 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 in weight was considered as water soluble ash. The percentage of water-soluble ash was calculated concerning air-dried part respectively.

Fluorescence Analysis: A small quantity of dried and finely powdered leaf, rhizome and root were placed on a grease-free clean microscopic slide and to it was added 1-2 drops of the con. sulphuric acid, 50% sulphuric acid, concentrated 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 daylight, short (245 nm) and long (360 nm). UV radiations were recorded as per the method of Kokate ⁹.

Phytochemical Studies: The ethanol, chloroform and water extract were subjected to preliminary qualitative chemical analysis. Standard methods were used for preliminary phytochemical screening of the extract ^{7,8}.

RESULTS AND DISCUSSION:

Extractive Values: Extractive values of both *C. amada* and *C. aeruginosa* were studied. In *C. amada* aqueous, alcohol and chloroform extracts ranged from 7.34, 5.9 and 2.1 mg respectively. In *C. aeruginosa* extractive values of aqueous, alcohol

and chloroform extracts ranged from 8.5, 6.2 and 2.5 mg.

Physicochemical Contents: The physicochemical constant is an important parameter in detecting adulteration on improper handling of the drug. Equally important in the evaluation of crude drugs, is the ash value and acid insoluble ash value determination¹⁰. 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 silica

C. amda: Total ash 5.4%, acid insoluble ash 0.43%, water soluble ash 3.3%.

C. aeruginosa: Total ash 5.8%, acid insoluble ash 0.56%, water-soluble ash 3.96%.

TABLE 1: MACROSCOPIC STUDIES OF C. AMADA

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

TABLE 2: MACROSCOPIC STUDIES 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	79-100 cm	9-10 cm	11-12 cm	5-6 cm
5	Taste	Bitter	Slightly bitter	Slightly bitter	Bitter
6	Odor	Aromatic	Aromatic	Slightly aromatic	Slightly aromatic

TABLE 3: EXTRACTIVE VALUES

Parameter	C. amada (mg)	C. aeruginosa (mg)
Water	7.34	7.83
Ethanol	5.9	6.2
Chloroform	2.1	2.5

TABLE 4: ASH VALUE AND PERCENTAGE

Ash value	Percentage	
	C. amada	C. aeruginosa
Total ash	5.4%	5.8%
Water soluble ash	3.3%	3.69%
Acid insoluble ash	0.43%	0.56%

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	Tannis	+	–	+
9	Carbohydrates	+	–	–
10	Protein	+	–	+
11	Gum and mucilage	+	–	+

TABLE 6: 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	+	-	+

Fluorescence Analysis: Powder drugs of leaf and rhizome of both plants were treated with different acids of various concentrations observed for the color under daylight, short and long waves of ultraviolet rays. Some constituents showed

fluorescence in the visible range in daylight. The ultraviolet light produced fluorescence in many natural products which did not affect visibly fluorescence in day light⁹.

TABLE 7: FLUORESCENCE ANALYSIS OF *C. AMADA*

S. no.	Drug + reagent	Daylight			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 ₂ SO 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 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	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

TABLE 8: FLUORESCENCE ANALYSIS OF *C. AERUGINOSA*

S. no.	Drug + reagent	Daylight			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: Blackish Green, G: Green, Y: Yellow, L.G: Light Green, P.G: Pale Green, T.A: Thick Ash, Br: Brown, B:Black, T.Br: Thick Brown, R.Y: Reddish Yellow, B.Br: Blackish Brown, L. Br: Light Brown, R.Br: Reddish Brown, 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

The main color of the powder in natural light *C. amada* and *C. aeruginosa* leaf was blackish green, rhizomes of both species showed some differences.

CONCLUSION: *Curcuma* is a well-known spice of India, various *Curcuma* are conventionally used as a significant ingredient in food and traditional medicines. *C. amada* and *C. aeruginosa* are in constant use in Zingiberaceae. *C. amada* have a characteristic smell and pale yellow color of rhizome with nodes and internodes. Pharmacognostic studies reveal that the total ash is 5.4%, water-soluble ash is 3.3% and soluble acid ash is 0.43%. Extractive values of water, ethanol, and chloroform are 7.34 mg, 5.9 mg, and 2.1 mg respectively. *C. aeruginosa* identified from its large size; rhizome is pale blue and strongly aromatic. Pharmacognostic studies reveal that the total ash is 5.8%, water-soluble ash is 3.69% and soluble acid ash is 0.56% respectively. Extractive values of water, ethanol, and chloroform are 7.83 mg, 6.2 mg and 2.5 mg. Hence, each *Curcuma* species possesses specific pharmacognostic typescript enabling recognition. Thus these features by quality and quantity assist in the drug formulation, and also confirm the adulteration.

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