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Research Article

Pharmacognostic and Phytochemical Evaluation of *Curcuma angustifolia* roxb. (Rhizome) Indigenous Ethno-Medicinal Plant Used by Tribal Soliga Community of Biligirirangana Hills.

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ABSTRACT
In India there is an extensive usage of medicinal plants as an herbal drug to treat various kinds of ailments. Traditionally *Curcuma angustifolia* Roxb. (Rhizome) is used in the treatment of inflammation, cancer, wound healing, diabetes, asthma, fever and anaemia by tribal Soligas of Biligirirangana hills. Therefore the present communication deals with the pharmacognostic and preliminary phytochemical evaluation of *Curcuma angustifolia* Roxb. (Rhizome). The pharmacognostical profiles include organoleptic study, microscopy evaluation of the rhizome; powder microscopy; determination of size of fibre and starch; fluorescence analysis and physical parameters like ash value, extractive value, and moisture content of the powdered material. Preliminary Phytochemical screening to detect the presence of alkaloids, flavonoids, terpenes, carbohydrates and total phenolics has been done to know the nature of phytoconstituents present in them. The result of the present study is useful in establishing the standards for identification, authentication and evaluation of the plant material. The pharmacognostic study of *Curcuma angustifolia* Roxb. (Rhizome) has been carried out for the first time which will help in establishing the monograph of the plant.

Key words: *Curcuma angustifolia* Roxb. , fluorescence study, pharmacognosy, phytochemical studies.

INTRODUCTION

Curcuma angustifolia Roxb. is commonly called as Indian Arrow root belongs to the family Zingiberaceae. It is popularly called as Gavayodhbhava in Sanskrit and Turmeric wild arrow root in English. The rhizome of *Curcuma angustifolia* is pale pendulous tubers useful in treating leprosy, burning sensations, dyspepsia, loss of taste, bronchitis, asthma, fever, thirst, jaundice, anaemia, leucoderma, stones in the kidney and bladder, urinary discharges, ulcers and diseases of blood[1].

Curcuma angustifolia rhizome is highly valued as an article of diet. The starch obtained from the dry powdered rhizome forms the chief source of the plant and is nutritive[2].

The GC-MS analysis of the young rhizome of *Curcuma angustifolia* Roxb. showed the presence of major compound like α-amorphene, Camphor, 2,7-napthalenediol, Trans-nerolidol, Octadecanoic acid, butyl ester, Humulen-6,7-epoxide[3]. But their role in pharmacological parameters has not been evaluated.

It is very important to make an effort towards the standardisation of *Curcuma angustifolia* rhizome which can serve as an alternative medicine to cure many ailments. The standardisation process is done by pharmacognostic studies[4]. The identification and authentication of the plant material can be supported by the pharmacognostic studies done. Therefore the present study deals with the detailed pharmacognostic evaluation of *Curcuma angustifolia* Roxb. (Rhizome).

MATERIALS AND METHODS

The fresh and healthy plant material i.e. the rhizome of *Curcuma angustifolia* Roxb. (Fig 2) was collected from Biligirirangana hills, Chamrajnagar, Mysore District, Karnataka. The plant samples were authenticated by Dr. V. Rama Rao, National Ayurvedic Diabetics Research Institute (Centre Council of Research for Ayurvedic and Siddha, Department of AYUSH, Ministry of Health and F.W. govt. Of India, New Delhi Govt.), Central Pharmacy Annexe, Ashoka pillar, Jayanagar, Bangalore 560011. The authenticated samples were preserved in the herbarium with the specimen voucher number *RRCBI-MUS-0106.*

Macroscopic characters

The following characters for the fresh rhizome were noted: size, shape, colour, odour and taste [4, 5].

Microscopic characters

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Figure 1: Flower
Curcuma angustifolia Roxb

Figure 2: Rhizome, *Curcuma angustifolia* Roxb

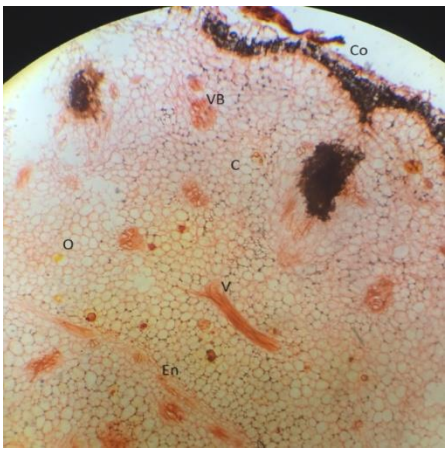

Figure 3: Transverse section of Rhizome in 10x
Co-cork, VB-vascular bundle, C-cortex, V-vesicle, O-oil cells, En-endodermis

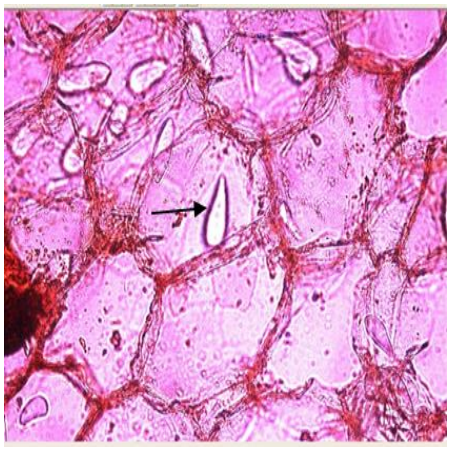

Figure 4: Presence of starch granules in 40x

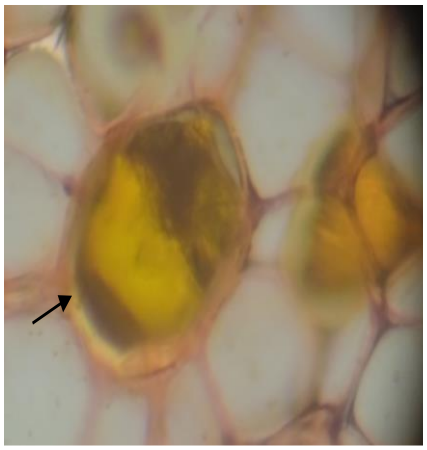

Figure 5: Presence of oil cells in 40x

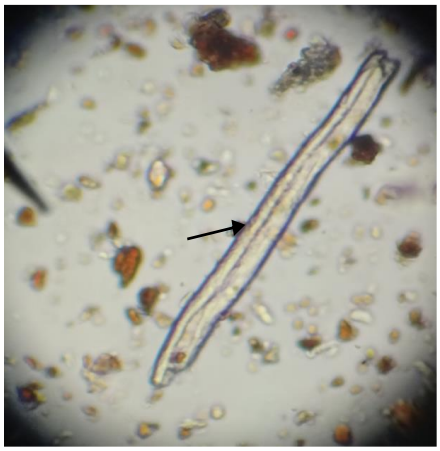

Figure 6: Presence of fibre in 40x

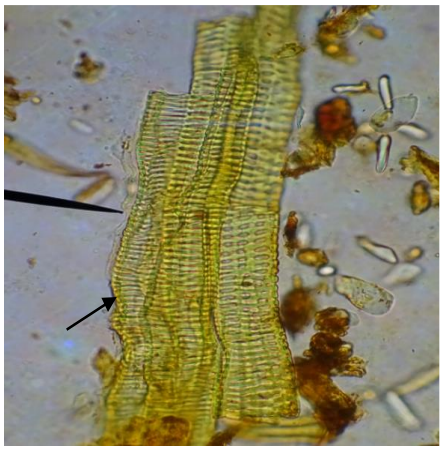

Figure 7: Presence of vesicle in 40x

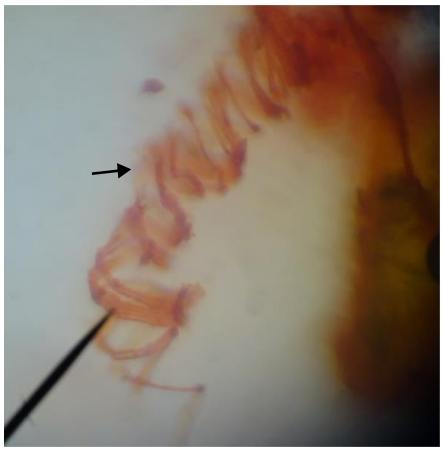

Figure 8: Spiral vessel

Transverse section of the fresh rhizome was stained with saffranin, mounted with glycerine and was observed under compound microscope[4].

Powder Analysis

Fresh rhizomes of *Curcuma angustifolia* Roxb. were collected and washed under running tap water and then with distilled water. The rhizome was cut approximately 1cm long and was shade dried. The sample was pulverized in a mixer and was used for organoleptic study, fluorescent study and for physicochemical characterisation.

Organoleptic Evaluation

The selected plant drug is assessed for the colour, odour and texture. The organoleptic evaluation is done according to the textual methods[5].

Table 1: Fluorescence behaviour of drug powder of *Curcuma angustifolia* Roxb

S.No	Treatment	Day light	UV Light	
			Short 254nm	Long 365 nm
1	Powder	Mustard yellow		Brown
2	Powder+H_2O	Orange yellow	Yellow	Yellow
3	Powder+1N HCl[1]	Mustard yellow	Yellow	Neon yellow
4	Powder+1N HNO_3[2]	Mustard yellow	Yellow	Mustard yellow
5	Powder+1N H_2SO_4[3]	Mustard yellow	Yellow	Mustard yellow light
6	Powder+1N NaOH[4]	Cherry red	Yellowish brown	Brownish yellow
7	Powder+Alc. NaOH[4]	Cherry red light	Yellow	Mustard yellow
8	Powder+1N KOH[5]	Cherry red	Yellowish brown	Brownish yellow
9	Powder+Alc. KOH	Cherry red	Yellow	Mustard yellow dark
10	Powder+ Ammonia	Cherry red	Yellow	Mustard yellow dark

[1,2,3] Spectrum reagents and chemical Pvt. Ltd.,Cochin; [4] s.d fine-chem Limited., Mumbai; [5] Merck specialities Pvt. Ltd., Mumbai

Table 2: Fluorescence behaviour of Crude Extract of *Curcuma angustifolia* Roxb

S.No	Extract	Day light	UV light	
			Short 254nm	Long 365 nm
1	Petroleum ether	Lemon yellow	Light green	Mustard yellow pale
2	Chloroform	Brownish yellow	Mustard yellow	Mustard yellow
3	Ethanol	Reddish brown	Mustard yellow dark	Dark pink

Physicochemical parameters

Various physicochemical parameters namely moisture content, total ash, acid insoluble ash, water-alcohol extractive values were recorded in percentage according to the method prescribed by Indian pharmacopeia[6,7]. The average percentage w/w of ash content, moisture content and the extractive values were determined.

Fluorescent study

A small quantity of dried powder of the rhizome were placed on the grease free microscopic slide and treated with 1-2 drops of chemical reagents like 1N HCl, 1N HNO_3, 1N H_2SO_4, 1N NaOH, Alcoholic NaOH, 1N KOH, Alcoholic KOH and Ammonia separately with gentle tilting. The slides were placed in the ultra violet viewer chamber and viewed in day light, short (254nm), long (365nm) ultraviolet radiation and the colour changes of the powdered drug when treated with different chemical reagents were recorded based on different chemical constituents[8].

Study of crude fibre content

To the 2gm of powder drug 50ml of 10% v/v nitric acid is added and brought to boiling with constant stirring. The solution is strained through cotton cloth on a Buchnner funnel. To the residue 50ml of 2.5% w/v NaOH solution is added, boiled, strained and washed with hot water[4]. The residue is transferred in a clean and dried crucible to determine the weight of the crude fibre.

Preliminary Phytochemical analysis

The preliminary Phytochemical analysis of petroleum ether, chloroform and ethanolic extracts were performed. The extracts were dissolved in their mother solvents and were screened for phytochemicals by standard method[4, 9,10].

Estimation of total phenolics and Flavonoids

Total phenolics were measured by the Folin-Ciocalteau assay (FC) by singleton and Rossi using Gallic acid as the calibration standard to express result in mg/gm Gallic acid equivalents (GAE). The hydroxyl group of gallic acid brings about reduction of oxygen atoms from tungustic and or molybdate in FC reagent and there by producing reactive species which has characteristic blue colour exhibiting λmax at 738nm[11]. Estimation of Flavonoids was done by aluminium chloride colorimetric method using Quercetine and results were expressed in mg/gm of sample equivalent to Quercetine. Aluminium chloride forms an acid stable with flavones which is read at 320nm colorimetrically[12].

Table 3: Physicochemical parameters of *Curcuma angustifolia* Roxb.

S.No	Parameter	Results
1	Moisture	0.67%
2	Total Ash	10%
3	Acid-insoluble ash	6.20%
4	Water soluble ash	7.21%
5	Alcohol-soluble extractive	18%
6	Water- soluble extractive	10%
7	Crude Fibre Content	42%

RESULTS

Brief description of the planT

Curcuma angustifolia Roxb. rootstock is small, emitting long fleshy fibres terminating in pale oblong pendulous tubers. Leaves with petiole measures 30-45cm. Flowering spike are lateral, apart from usually appearing earlier than the leafy spike, crowned by several enlarged empty pink bracts (Fig 1). Flower are longer than their bracts, 3 or 4 together in the axil of each bract, sheaths of pseudo stem is pale green. Calyx is 3-toothed. Corolla tube is 13mm which are longer than the 2 lateral ones. Lateral staminodes are oblong, united to the filament and capsule is ovoid[1].

Table 4: Preliminary Phytochemical analysis of crude extract of *Curcuma angustifolia* Roxb.

S.No	Test	Petroleum ether extract	Chloroform extract	Ethanol extract
1	Alkaloids	−	+	+
2	Carbohydrates	−	+	+
3	Glycosides	−	−	−
4	Saponins	−	−	−
5	Phytosterols	−	−	+
6	Fixed oils and fats	+	−	−
7	Resins	+	+	−
8	Tannins	+	+	+
9	Phenols	−	+	+
10	Flavonoids	−	+	+
11	Proteins and amino acids	−	−	+
12	Terpenes	−	−	+
13	Gums and mucilage	−	−	−

+ indicated the presence of ; − was not determined

Macroscopic studies

Macroscopically rhizome is externally buff coloured cylindrical with short lateral branch. The size of the rhizome varies from 4-8cm long and 1-2 cm width. The organoleptic characters of the rhizome has aromatic odour with agreeable and characteristic taste.

Microscopic studies

Microscopically the transverse section of the rhizome shows (Fig: 3) the few layers of dark brown coloured thin walled brick shaped irregularly arranged paranenchymatous cells followed by inner cork. Cortex has thin walled paranenchymatous cells with scattered fibro vascular bundles (Fig: 7).The parenchyma of the pith and the cortex contains starch grains (Fig: 4) of 11.4 µ to 39.9 µ size average being 25.7 µ. The section also showed the presence of oil cells have subersied walls and contains orange to yellow globules of volatile oil (Fig:5). Cortical vascular bundles are scattered and collateral type. Fibres are lignified pitted septate measuring from 193.8 µ to 877.8 µ size and average being 535.8 µ.

Powder analysis

Powder is yellowish, with aromatic odour and fibrous; when treated with chloral hydrate solution and stained in safranin mounted with glycerine showed the following characters starch granules (Fig4), oil cells (Fig 5), fibres (Fig 6), vessels (Fig 7) and spiral vessel (Fig 8)

Physico-chemical studies

The physico-chemical parameters are very important in detecting adulteration in the herbal drug. If the water content is high it provides a way for microbial contamination. In the present study water content indicates less chances of microbial contamination during the storage period of crude drug. Ash value represents the presence of inorganic salts naturally occurring in the rhizome. Total ash value is important in evaluating purity in terms of presence of metallic salts and silica. Water soluble ash is the amount of water soluble portion of total ash[13, 14]. Acid in-soluble is the portion of the total ash which is insoluble in dilute HCl represents the amount of silica. The values obtained for total ash, acid-insoluble ash and water soluble ash is within the acceptable standards for herbal plants. The alcohol extractive value was found to be more than water extractive value which indicates the presence of more of alcohol soluble compounds. Determination of crude fibre content signifies the presence of fraction of carbohydrates. The percentage of physico-chemical parameters were determined and summarized in the Table No.3.

Table 5 Estimation of Total phenol and Flavonoids

Extract	Total Phenolics (mg of GAE)/ gm	Total flavonoids (mg of Quercetine/gm)
Petroleum ether	19.8±15	14.97±0.16
Chloroform	43..5±0.4	27.70±0.34
Ethanol	61.1±0.45	41.20±0.19

Fluorescent study

The detection of various chemical constituents which behaves as an chromophores present in the drug on treatment with chemical reagents is an important tool in pharmacognosy which helps in the standardisation of the herbal drug. The characteristic colour observed under ultraviolet radiation were recorded and the results were summarized in Table No.1

Study of crude fibre content

The percentage crude fibres obtained from powder after treating with dilute acid and alkali was found to be 42%

Phytochemical studies

A known quantity of the powder was extracted by Soxhlet apparatus using petroleum ether, chloroform and ethanol successively and tested for the presence of different Phytoconstituents like alkaloids, flavonoids, terpenes, carbohydrates, fixed oils and fats, phenols, tannins. The preliminary Phytochemical screening reveals the presence and as well as absence of certain Phytoconstituents. The pharmacological nature of the medicinal plants is based on the nature of secondary metabolites present in the plant. Therefore the Phytochemical profile of the rhizome was evaluated and the results are presented in the Table No.4. The amount of total phenol in the three extracts varied from 19.8mg/gm of GAE to 61.1mg/gm of GAE in the following order petroleum extract< chloroform extract < ethanol extract. Similarly the amount of total flavonoids

were in the range from 14.97mg/gm of quercentine to 41.20mg/gm of quercentine in the order of petroleum extract< chloroform extract < ethanol extract. The results are summarized in the Table No.5.

DISCUSSION

At present *Curcuma angustifolia* Roxb is used for the treatment of various kinds of ailments without proper pharmacognostic standardisation. World health organisation has set certain standards for the herbal drugs before it is included in the herbal pharmacopeia. Therefore the results of this investigation will help in the proper identification of the plant. The macroscopic, microscopic, physic-chemical study, fluorescent study, crude fibre content helps in the standardisation of the rhizome of *Curcuma angustifolia* Roxb. The plant material absorbs moisture and gets deteriorated very quickly in the presence of water. Therefore analysis of loss on drying is very important which determines the stability of the crude drug. In the present study loss on drying was negligible and hence reveals the stableness of the drug[7, 14]. The ash content represents the presence of inorganic salts present the plant material or present due to adulteration which is an important parameter for drug standardisation. The plant material was subjected to successive solvent extraction using petroleum ether, chloroform and ethanol to get diversified non-polar to polar phytoconstituents which has different degree of solubility and were subjected to various preliminary phytochemical analysis which helps to determine, estimate and identify the important molecules present which are of pharmacological importance. Presence of phenols plays a vital role against oxidative stress in the cell and thereby acts as antioxidant and protects the human system from various ailments including cancer, cardiovascular diseases[13]. Flavonoids have a significant role in pharmacological activities such as antimicrobial, anti-inflammatory, anticancer, antioxidant activities[16-18].

In this regard the pharmacognostic studies of the rhizome of *Curcuma angustifolia* Roxb is important and is further progressed for the pharmacological evaluation and toxicity studies to establish as a herbal drug.

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