

PHARMACOGNOSTIC AND PHYTOCHEMICAL EVALUATION OF THE RHIZOMES OF *CURCUMA LONGA* LINN.

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ABSTRACT

In recent year there has been rapid increase in the standardization of selected medicinal plant of potential therapeutic significance. Despite the modern techniques, identification of plant drug by Pharmacognostic study is more reliable. The rhizomes of *Curcuma longa* reported to have good medicinal values in traditional system of medicines. The present study deals with pharmacognostic parameters for the rhizomes of *Curcuma longa* which mainly consist of Macromorphology, Cytomorphology, Physico-chemical constants and Phytochemical screening. This information will be of use for further pharmacological and instrumental evaluation of the species and will assist in standardization for quality, purity and sample identification.

KEYWORDS: *Curcuma longa*, Pharmacognostic study, Cytomorphology, Standardization.

INTRODUCTION

India has a rich history of using plants for medicinal purposes. Turmeric (*Curcuma longa* L.) is a medicinal plant extensively used in Ayurveda, Unani and Siddha medicine as home remedy for various diseases. *C. longa*, botanically related to ginger belongs to the Zingiberaceae family.^[1] Turmeric is native to the monsoon forests of South East Asia. It is a perennial herb, 1 meter tall with underground rhizomes. Curcuma species contain turmerin (a water-soluble peptide), essential oils (such as turmerones, atlantones and zingiberene) and curcuminoids including curcumin [1, 7-bis-(4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione]. Curcuminoids can be defined as phenolic compounds derived from the rhizomes of Curcuma species (Zingiberaceae).^[2,3] Current traditional Indian medicine uses it for biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism, and sinusitis.^[4,5] Powder of turmeric mixed with slaked lime is a household remedy for the treatment of

sprains and swelling caused by injury, applied locally over the affected area. The old Hindu texts have described it as an aromatic stimulant and carminative.^[6] In some parts of India, the powder is taken orally for the treatment of sore throat. This nonnutritive phytochemical is pharmacologically safe, considering that it has been consumed as a dietary spice, at doses up to 100 mg/day, for centuries.^[7]

MATERIAL AND METHODS

Collection and Identification of Plant Material

The plant material was collected from the Khari Baoli market, Old Delhi. And was identified as *Curcuma Longa lin* (Rhizomes) family (Zingiberaceae), by Dr. Anju Pal (Scientist), G. B. Pant University of Agriculture and Technology, Pant Nagar by correlating their morphological and microscopic characters with those given in literature. The organoleptic characters of fresh rhizomes and dried rhizome powder like colour, odour and taste and the macroscopic characters like size, shape, surface, fracture were evaluated as per standard WHO guidelines.

Pharmacognostic evaluation

Total ash value^[8]

It depicts the total amount of material produced after the complete incineration of the ground drug above 400°C to remove all the carbon atoms. 2g of powdered drug was weighed and placed in the crucible and heated at about 400°C. The crucible was cooled and the % of the total ash with reference to the air-dried sample of the crude drug was calculated.

Acid insoluble ash^[8]

Total ash obtained was dissolved in 1N HCl solution and heated for 5 min. The insoluble matter was filtered in whatman filter paper; the filter paper was further dried at 70°C and then cooled. The residue was weighed and the percentage of insoluble ash of the crude drug w.r.t. the air dried sample of crude drug was calculated.

Water soluble ash^[8]

To the total ash crucible, 25ml double distilled water was added and boiled for about 5min. Insoluble matter was collected on an ash less filter paper in a crucible, washed with hot water and ignited for about 15min above 45°C. The weight of the residue is subtracted from the weight of the total ash. Content of water soluble ash in mg/g of the air dried material was calculated.

Determination of solvent extractive value^[8]

2g of the air- dried coarsely powdered drug was macerated with 100ml of different solvents in closed flasks for 24h, and are shaken frequently. The solvent was filtered and the filtrate was weighed in a Petri dish. The dish was evaporated on a water bath and then dried in an oven at 100°C. The dish was cooled and extractible value was calculated as % (w/w) with reference to air dried drug.

Loss on drying (LOD)

LOD is the loss in weight in % (w/w) resulting from water and volatile matter of any kind that can be driven off under specified conditions. 1g sample is transferred to a shallow bottle and weighed. Sample was distributed evenly and dried in a hot air oven at 105°C for 1h with the stopper open. After 1h, the stopper was closed and cooled at room temperature and the bottle was weighed.

Preparation of the extract

The rhizomes of *Curcuma longa* were collected and dried in sun for 3 days, cut into small pieces and again dried. The upper bark of the rhizome was removed to obtain the fresh rhizome. The dried rhizome was then grinded to obtain a fine powder. The powder was again dried and was ready for use.

Ethanollic extraction

The grinded powder was extracted with 500ml of dehydrated ethanol and 1000ml double distilled water respectively by Soxhlation for 72h. The extract was concentrated at temperature <45°C. The residue was dried and refrigerated.

Aqueous extraction

The grinded powder was then extracted with 1000ml double distilled water containing 3-4 drops of chloroform for 48h. The extract was then concentrated at temperature less than 45°C. The residue was then dried and refrigerated.

Qualitative chemical tests**Test for alkaloids^[9]**

(a) **Mayer's test:** To 2ml test solution, 2N HCl was added. The aqueous layer formed was decanted and Mayer's reagent (Qualigens, India) was added to it. A cream coloured precipitate indicates the presence of alkaloids.

(b) Dragendroff's test: To 2ml test solution, and Dragendroff's reagent (Qualigens, India) was added to it. A reddish brown precipitate indicates the presence of alkaloids.

(c) Wagner's test: To 2ml test solution, and Wagner's reagent (Qualigens, India) was added to it. A reddish brown precipitate indicates the presence of alkaloids.

(d) Hager's test: To 2ml test solution, and Hager's reagent (Qualigens, India) was added to it. A yellow coloured precipitate indicates the presence of alkaloids.

Test for glycosides

(a) To 2ml test solution, equal quantity of Fehling's solution A and B was added and solution was heated. A brick red precipitate indicates the presence of glycosides.

(b) Legal's test: To 2ml test solution, pyridine (Merck, India) and alkaline sodium nitroprusside was added to obtain a blood red colour.

Test for flavonoids^[9]

(a) Shinoda test: To 2ml test solution, few fragments of Magnesium ribbon (Loba Chemie, India) were added and to it conc. H₂SO₄ was added drop wise. Pink scarlet or crimson red colour appears.

(b) Zinc chloride reduction test: To 2ml test solution, a mixture of zinc dust (Merck, India) and conc. HCl (Qualigens, India) was added. A red colour is obtained after few minutes.

(c) Alkaline reagent test: To 2ml test solution, sodium hydroxide (Qualigens, India) solution was added to give a yellow or red colour.

Test for tannins

(a) Gelatin test: To 2ml test solution, 1% Gelatin solution containing 10% sodium chloride was added to obtain a white precipitate.

(b) Ferric chloride test: To 2ml test solution, ferric chloride was added to give a blue green colour.

Test for proteins and amino acids^[9]

(a) Millon's test: To 2ml test solution, Millon's reagent is added which gives a white precipitate, which on heating changes to red.

(b) Ninhydrin test: To 2ml test solution, ninhydrin solution was added and the solution was boiled. Amino acids and proteins when boiled with 0.2% ninhydrin reagent show a violet colour.

Test for fats and fixed oils^[9]

(a) **Stain test:** Small amount of the extract was pressed between two filter papers; the stain on the filter paper indicates the presence of fixed oils.

(b) **Saponification test:** Few drops of 0.5N alcoholic potassium hydroxide was added in small quantity to the extract solution with a drop of phenolphthalein and heated on a water bath for 1-2h. The formation of soap or partial neutralization for the alkali indicates the presence of fats and fixed oils.

Test for Sterols^[10]

Liebermann-Burchard test: To the test solution, 3-4 drops of acetic anhydride was added, the solution was boiled cooled and conc. Sulphuric acid (3 drops) was added. A brown ring appears at the junction of the two layers. The upper layer turns green showing the presence of steroids

Test for triterpenoids^[11]

Salkowski test: To the test solution 2ml chloroform (Qualigens, India) was added with few drops of conc. Sulphuric acid (3ml), and shaken well. Appearance of reddish brown colour at lower layer indicates presence of steroids and that of yellow colour shows the presence of triterpenoids.

Microscopy

Cytomorphology: Fresh rhizomes of *curcuma longa* were subjected for the microscopical studies. The the sections were cut by free hand sectioning. The numerous temporary and permanent mounts of the microscopical section of the spicemen were made and examined microscopically. Photomicrographs of the microscopical section were taken with the help of MOTIC Digital Microscope, provided with MOTIC IMAGE PLUS 2.0 software.^[12,13,14]

Powder characteristics: Preliminary examination and behavior of the powder with different chemical reagents was carried out as per reported method.^[15,16]

Micrometry: Quantitative microscopy of the transverse sections and rhizome powder were performed to determine the size and dimension of tissues, cell and cell content.^[17,18]

Fluorescence analysis: Dried rhizomes were powdered and observed under visible light, short ultra violet light, long ultra violet light after treatment with different reagents like

chloroform, ethyl acetate, methanol, petroleum ether (60-80°C), 50% sulphuric acid, 50% hydrochloric acid, 50% nitric acid, 10% sodium hydroxide etc.^[19]

RESULTS AND DISCUSSION

Macromorphological description: The central or primary rhizomes are ovate, irregularly ovoid, cylindrical or fusiform, curved, sometimes slightly branched into a Y-shape, 4-5 cm long, 5-30 mm in diameter to, rough, with wrinkled striations, distinct cyclic nodes, and rounded scars of root branches and rootlets. The organoleptic evaluation of the rhizomes revealed that the rhizomes were Yellowish to yellowish-brown in colour, with characteristic and aromatic odour and slightly bitter and pungent in taste. The results of morphological characters are mentioned in Table no.1.

Cytomorphological Description: The transverse section of the rhizome shows cork as an outer layer followed by epidermis, cortex, and endodermis and ground tissue. Cork composed of thin walled brown cells which is large and polygonal in shape. Epidermis is consist of thin walled cubical cells of various dimension. The cortex consist of thin walled rounded parenchymatous cells and having oil cells. These cells are filled with gelatinized starch grains and yellow colouring matter. The ground tissue is parenchymatous and cells filled with gelatinized starch grains and yellow pigment. Fibrovascular bundle and oil cells scattered throughout ground tissue.

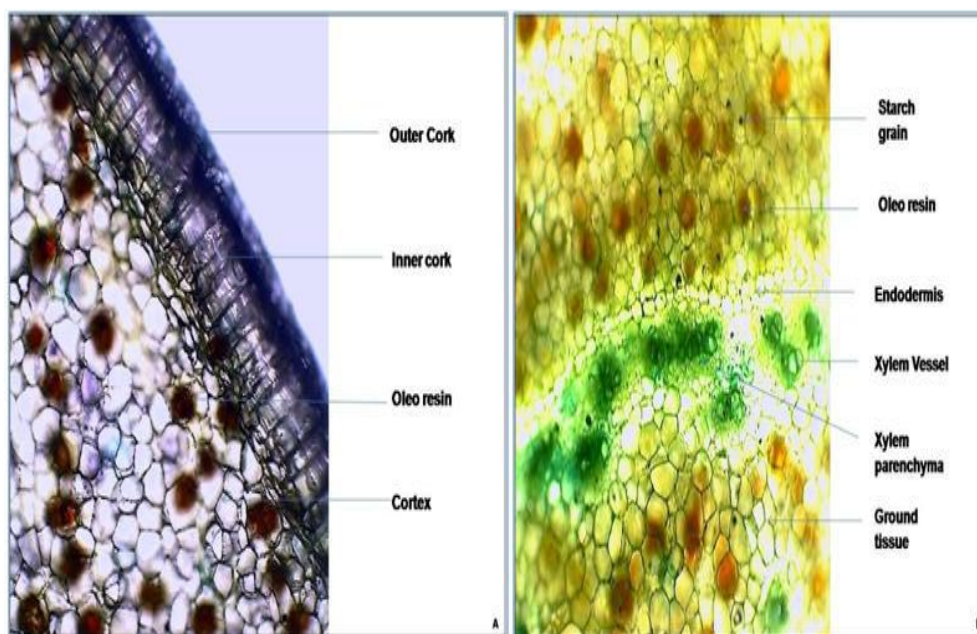
Powder Characteristics: The Powder of *Curcuma longa* rhizome is yellowish brown, with aromatic and characteristic odour and slightly bitter in taste consist of cork, cortex, fragments of parenchymatous cells filled with starch grains and oleoresins.

Micrometry: The results of micrometric characters of tissue, cells and cell contents were depicted in Table no.2. Measurements of different cells are frequently necessary for the quantitative identification of closely allied substances. In most cases these allied substances are mixed with the original drugs as adulterants and substituent's. Thus, the adulterants and/or substituent in crude drugs can be distinguished by this way with the aid of optical microscopy. (fig.4)

Physicochemical Evaluation: The results of the physicochemical constants of raw material lie within the limit which is mentioned in Table no.3. This signifies that the quality and purity of raw material was good enough; the results of foreign organic matter denote presence of

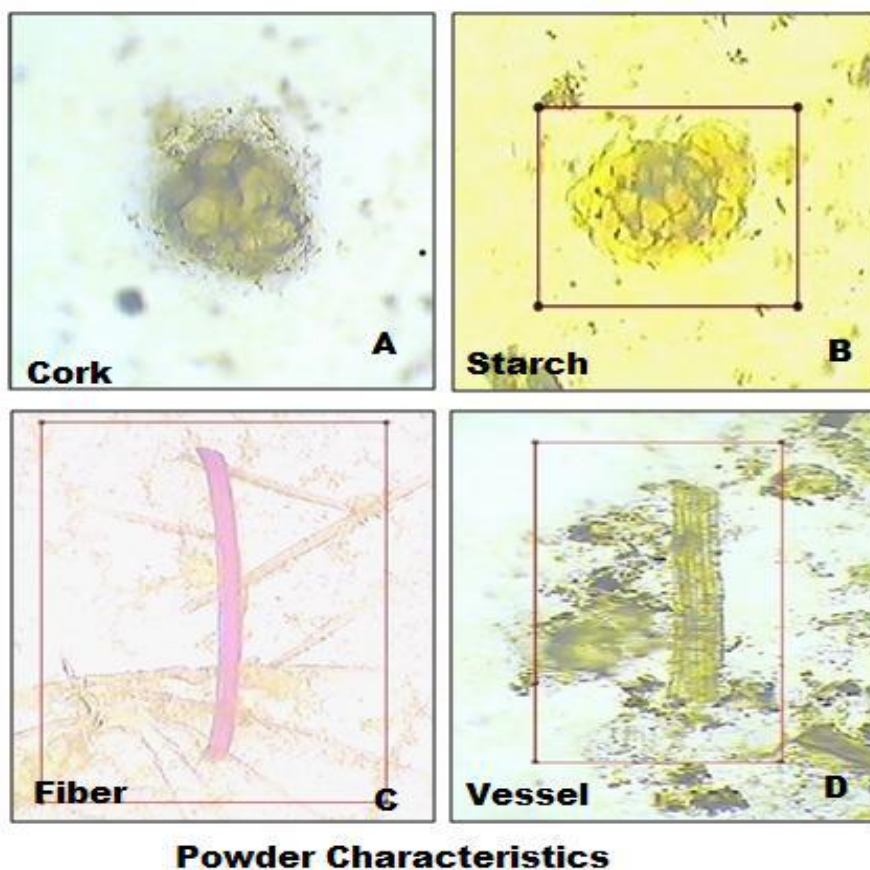
any organism, part or product of an organism other than that named in the specification and description of the herbal material concerned which was found to be $0.25 \pm 0.015\%$ w/w, it indicates that their may be present of part or product of an organism in less amount. Insufficient drying favors spoilage by molds and bacteria and makes possible the enzymatic destruction of active principles.

Not only the ultimate dryness of the drug is important equally important is the rate at which the moisture is removed and the condition under which it is removed thus the determination of moisture content also provide the method of preparation of drug; and it is observed that the moisture content of the drug was found to be $9.52 \pm 0.020\%$ w/w which signify that the drug is properly dried and properly stored. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign matter such as metallic salts or silica. An analytical result for total ash was found to be $8.35 \pm 0.02\%$ w/w. The amount of acid-insoluble siliceous matter present was $0.952 \pm 0.050\%$ w/w; As the ash values of the crude drugs lies within the fair limit which signify its quality and purity and gives idea about the total inorganic content. The water soluble extractive value indicated the presence of sugar, acids and inorganic compounds; the water soluble extractive value found to be $25.15 \pm 0.055\%$ w/w and alcohol soluble extractive values indicated the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids. The alcohol soluble extractive value was found to be $6.75 \pm 0.565\%$ w/w which signify the nature of the phytoconstituents present in plant. As the pH was determined this was near to 3 that is in acidic range and may be because of acidic salts present in the rhizomes. **Preliminary Phytochemical Screening:** The Preliminary Phytochemical Investigations of Aqueous extract, acetone extract, ethanolic extract and methanolic extract of *Curcuma longa* rhizome were preformed which reveals the presence of Phenolic compound, Tannins, Alkaloids, Terpenes, Saponin type of major secondary metabolites which revealed their potent therapeutic activity. The results of the screening were expressed in Table no.4.



T.S of Cork and Cortex

T.S of Endodermis and Vascular bundle

**Table 1: Macromorphological Description**

Character	Observations
Ogganoleptic Characters	
Color	Yellow or Yellowish brown
Odour	Aromatic and Characteristics
Taste	Slightly bitter
Quantitative Macromorphology	
Size	5-30 mm in diameter
Length	4-5 cm in lenght
Macroscopical Features	
Shape	Finger shape
Surface	Smooth or slightly rough
Texture	Hard and Heavy
Fracture	Short

Table 2: Micrometry of some cells

Type of cells	Dimention Area
Cork Cell	5.36±0.55
Cortex	30.5± 9.05
Oleoresins	3.25 ± 0.15
Epidermis Cells	3.56±0.05
Fragments of Ground Tissue	35.5 ± 5.14

*Values are expressed as mean ± standard deviation

Table 3: Physicochemical parameters

Parameters(%W/W)	Observation
Foreign Organic Matter	0.25 ± 0.015
Moisture Content (LOD)	9.52 ± 0.020
Ash Value	
Total Ash	8.35 ± 0.02
Acid Insoluble Ash	0.95 ± 0.050
Water Soluble Ash	-----
Extractive Value	
Water Soluble Extractive value	25.15 ± 0.055
Alcohol Soluble Extractive value	6.75 ± 0.565
pH	03.00 ± 0.00

* Values are expressed as mean ± standard deviation

Table 4: Preliminary Phytochemical Screening

Parameters	Observations			
	Aqueous Extract	Acetone Extract	Ethanollic Extract	Methanolic Extract
Carbohydrates	+	+	+	+
Phenols	+	+	+	+
Tanins	+	+	+	+
Terpenes	+	+	+	+
Saponins	+	+	+	+
Alkaloids	+	+	+	+

* + indicates presence

CONCLUSION

Standardization is essential measure for quality, purity and sample identification. Macromorphology and microscopy along with the Quantitative analytical microscopy is one of the simplest and cheapest methods to start with for establishing the correct identity of the source materials. Physicochemical and chemical analysis of rhizome confirm the quality and purity of plant and its identification. The present study was useful for further pharmacological and therapeutic evaluation along with the standardization of plant material.

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