Evaluation of pharmacognostical variations in eight accession of *Curcuma longa* L.

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Abstract

Introduction: In recent days, usage of medicinal plants as an herbal drug to treat various kinds of ailments has got drastically increased. Conventionally, *Curcuma longa* (rhizome) is used in the treatment of inflammation, cancer, wound healing, diabetes, asthma, antioxidant, anti-aging, antimicrobial, etc. **Materials and Methods:** The present study deals with the pharmacognostic and preliminary phytochemical evaluation of different accessions of *C. longa* (rhizome). The pharmacognostical profiles include organoleptic study, microscopy evaluation of the rhizome, powder microscopy, physiochemical paramters and determination of total phenolic and flavonoid content were carried out. **Results and Discussion:** Results showed that there are significant pharmacognostical variations in different accessions of turmeric. **Conclusions:** The result of the present study is useful in establishing the standards for identification, authentication, and evaluation of the plant material and the variations as well.

Key words: *Curcuma longa*, India, Pharmacognosy, turmeric, Zingiberaceae

INTRODUCTION

Curcuma longa L. (Zingiberaceae) also called turmeric includes >80 species of rhizomatous perennial herbs and has extensive presence in the tropics of Asia, Africa, and Australia.[1] It is a perennial herbaceous plant, which ranges a stature of up to 1 m. There are highly branched, yellow-to-orange, cylindrical, and aromatic rhizomes. *C. longa* is used as a drug in Ayurvedic and Unani system of medicine.[1,2] Others various common name includes *Curcuma* (Sp. It. Fr.), acafrao da India (port.), geelwortel (Dutch), kurkum (Arab), Manjano (East Africa [KiSwahili]), manjal (Tamil), kunyit (Indonesia), temukunyit (Malaysian), and iyu-chin (Chin.). The chemical components of turmeric are a group of compounds called curcuminoids is a polypohenolic which include curcumin (diferuloylmethane), demethoxycurcumin, and bisdemethoxycurcumin.[3-5] Curcuminoids contain curcumin the principal curcuminoids (about 80%), and other two curcuminoids are demethoxycurcumin (about 12%) and bisdemethoxycurcumin along with other

one's protein (6.3%), fat (5.1%), minerals (3.5%), carbohydrates (69.4%), and moisture (13.1%). The essential oil (5.8%) obtained by steam distillation of rhizomes has a-phellandrene (1%), sabinene (0.6%), cineol (1%), borneol (0.5%), zingiberene (25%), and sesquiterpenes (53%).[3] The World Health Organization (WHO) has suggested the use of turmeric as a spice.[6] Literature showed that chemical constituents of turmeric are varied according to geographical location, climatic conditions, etc.[1,4] Previous research reported the variation in chemical constituents, as well as molecular diversity of turmeric but very little work has been done on pharmacognostical variations among different accessions of turmeric. Hence, it is imperative to find out the pharmacognostical variation among different accessions of turmeric collected across subcontinent of India.

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MATERIALS AND METHODS

Collection and Authentication of the Drug Samples

In the present investigation, samples were collected from different geographical regions of India. The fresh rhizomes 8 samples of *C. longa* were collected from various ecological and sub-climatic zones of India during period from October 21, 2011, to January 12, 2012. The collections sites of *C. longa* are given in Table 1. All samples of *C. longa* were authenticated by taxonomist Professor M.P. Sharma, Department of Botany, Faculty of Science, Jamia Hamdard University. Chemicals and reagents used in the study were purchased from Sigma-Aldrich Corporation, St. Louis, MO USA. All voucher specimens (BNPL/T-12) were deposited in Molecular Ecology Laboratory, Jamia Hamdard, New Delhi, India. Rhizomes were cut into thin sections for microscopy study and also cut into pieces, dried, and powdered for other pharmacognostical study.

Microscopy of Drug Samples

Transverse section of the fresh rhizome was stained with safranin, mounted with glycerin and was observed under compound microscope.[7]

Identification Test

The individual extract was subjected to the qualitative phytochemical screening for the presence of some chemical constituents. The phytochemical test was carried out adopting standards procedure.[8,9]

Physicochemical Evaluation of Drugs

The physiochemical studies were carried out as per described in the WHO guidelines (WHO, 1991).[10]

Determination of Foreign Matter

The weighed (100 g) samples were spread out as a thin layer on filter paper. The foreign matter was detected by thrice by inspection with the unaided eye or using a lens (×6). All the foreign matter separated from the samples were weighed and calculated as per the percentage present in the samples.

Loss of Drying

The limit of detection determines the loss of moisture as well as the component present in the particular samples. The powdered drug sample (1 g) was placed on the tarred evaporating dish of infrared torsion balance moisture meter (Adair DuttKolkatta, India) and dried at 105°C for 2 h and followed by measurements of their reading after every 20 min. The drying was continued till the successive reading coincides with each other, or the difference between two successive reading did not differ by more the 0.25%.

Ash Values

Total ash

The ash values were determined according to the method described in pharmacopeia guidelines. To determine the ash value, the powdered drugs (1.0 g) were incinerated in a silica crucible at a temperature not exceeding 450°C until free from carbon. It was then cooled and weighed to get the total ash content. The ash value of samples was calculated.

Acid insoluble ash

Total ash found was boiled with 25 mL dilute hydrochloric acid (6.0 N) for 5 min. The insoluble matter collected on an ashless filter paper washed with hot water and ignited at a temperature not exceeding 450°C to a constant weight. After that weight was taken and the acid insoluble ash was calculated.

Water soluble ash

Water soluble ash was also determined by boiling the ash for 5 min with 25 mL of water, collected insoluble matter on ashless filter paper, washed with hot water and ignited for 15 min at a temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water-soluble ash.

Table 1: Different accessions of *C. longa*			
Code No.	**Cultivation regions(Provinces)**	**Geographical Co- ordinate**	**Source**
T1	Trivandrum(Kerala)	8° 29′ 15″ N, 76° 57′ 9″E	Local farmer
T2	Lucknow(U.P)	26° 50′ 49.2″ N,80° 56′ 49.2″ E	Herbal Garden, Integral University.
T3	Nashik(Maharashtra)	20° 0′ 0″ N, 73° 46′ 48″ E	Local farmer
T4	Delhi (Delhi)	28° 36′ 36″ N,77°13′ 48″E	Herbal garden, Hamdard University
T5	Erode (Tamilnadu)	11° 21′ 0″ N, 77° 44′ 0″ E	Local farmer
T6	Guwahati (Assam)	26° 11′ 0″ N, 91° 44′ 0″ E	Local farmer
T7	Surat (Gujrat)	26° 11′ 0″ N, 91° 44′ 0″ E	Local farmer
T8	Patna(Bihar)	25°36′39.6″N,85°8′38.4″E	Local farmer

Determination of Total Phenolic Content

Determination of total phenolic content in the methanolic extract of all samples were carried out using standard UV spectrophotometric method.[11,12] Gallic acid was used as a standard for the determination of total phenol in all the samples. The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of gallic acid equivalent per g dry weight.

Preparation of Methanolic Extract of *C. longa*

Eight different specimens of *C. longa* rhizomes (each of 1 g air-dried fresh rhizomes) procured from various locations such as south, west, east, and north of India and were extracted by reflux using 50 mL of high-performance liquid chromatography grade methanol for minimum of 2 h at temperature 70°C and then filtered through Whatman No#42. Extracts obtained after the reflux were concentrated on hot water bath and finally, the volume was adjusted to 50 mL with methanol.

Reagents

Nearly 10% the Folin–Ciocalteu (FC) reagent and Na_2CO_3 (1M) were prepared with distilled water and standard (Gallic acid) 1 mg/mL solution in methanol. Different dilutions were made with methanol from 25 µg to 300 µg/mL.

Samples Preparation

About 1 mg/mL solution of each extract in methanol was prepared followed by withdrawn of 0.5 L of each one. To this, added 5 mL FC reagent and 4 mL of Na_2CO_3 solution and kept for 15 min. Finally, absorbance was taken at 765 nm of all the samples.

Preparation of Standard

Different dilutions of the standard reference were prepared in the range of 25–300 (µg/mL). 0.5 mL of each standard solution was taken followed by their addition of 5 mL of FC reagent and 4 mL of Na_2CO_3 solution and kept for 15 min. Absorbance was taken at 765 nm.

Blank Solution

It was prepared by adding 0.5 ml of methanol to 5 mL of FC reagent and 4 mL Na_2CO_3 solution.

Determination of Total Flavonoid Content

Determination of total flavonoid content in the methanolic extract of all samples were carried out using standard UV spectrophotometric method.[11,12] Rutin was used as a standard for the determination of total flavonoid in all the samples. The total flavonoid content was calculated from a calibration curve, and the results were expressed as mg rutin equivalent per g dry weight.

Preparation of Methanolic Extract

The same process was adopted as mentioned above for phenolic content.

Reagents

$AlCl_3$ (0.1 mg/mL) and CH_3COONa (1M) were prepared along with the dilution of Rutin (Standard) from 10 µg/mL to 100 µg/mL.

Sample Preparation

About 1 mg/mL solution of each extract in methanol was prepared followed by withdrawal of 0.5 mL of each one. To
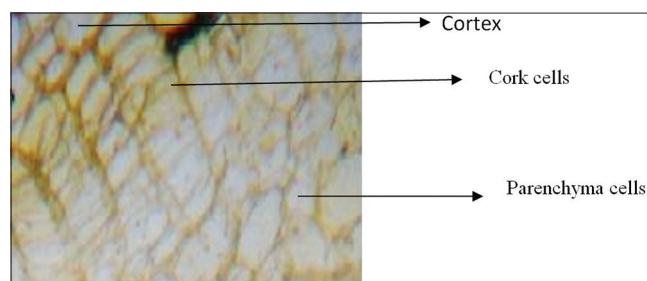

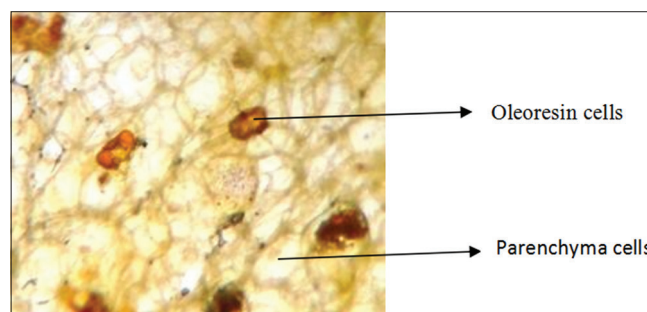**Figure 1:** Transverse section of the outer part (one sector) of rhizome of *Curcuma longa*

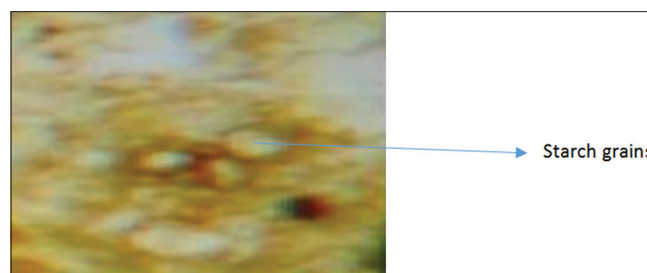**Figure 2:** Transverse section of rhizome of *Curcuma longa* (one-sector)

Figure 3: Distribution of starch cells in rhizomes of *Curcuma longa*

this, 0.1 mL AlCl$_3$ and 0.1 mL of CH$_3$COONa reagent were added and finally added of 2.8 mL distilled water and kept for 30 min. The absorbance of all samples were taken at 415 nm.

Preparation of Standard

Different dilutions of standard reference were prepared in the range of 10–100 (μg/mL). 0.5 mL of each standard solution was taken into 1.5 mL of methanol, then added 0.1 mL of AlCl$_3$, 0.1 mL of CH$_3$COONa, and 2.8 mL of distilled water and kept for 30 min. The absorbance of all samples were taken at 415 nm.

RESULTS AND DISCUSSION

Microscopic Observation

Transverse section of the rhizome of turmeric showed the following microscopical characters [Figures 1-3].
a. Epidermis
   It is thick-walled having cubical cells of various dimensions.
b. Cortex
   These cells are characterized by the presence of mostly thin-walled rounded parenchyma cells.
c. Cork cells
   These are composed of 4–6 layers of thin-walled and brick-shaped.
d. Vascular bundles
   Vascular bundles are collateral and found to be scattered.
e. Parenchyma cells
   These are cells of the ground tissue, and it contains starch grains of 4-μm in diameter.
f. Oil cell
   It is suberized walls containing orange-yellow globule of volatile oil or amorphous resinous matter.
g. Vessels
   Vessels are mainly spirally thickened and having few reticulate and annular structures.
h. Pericycle
   It consists of three to four layers of rectangular cells.
i. Vascular tissue
   These cells are radially arranged. Phloem patches and xylem are seen to be arranged alternately; xylem is found to be in exarch condition.
j. Pith
   Well-developed pith is found and is made up of thick-walled parenchymatous cells.

Powder Study of Rhizome of *C. longa*

Powder study of the rhizome of *C. longa* showed the following characteristics features:

The powder is brownish black with camphoraceous odor. The taste is bitter; it includes powder fibers and small granules of vessels. Parenchyma cell is spherical to angular cells in the forms of grains. The grains are clumps of the parenchymatous cell. They are filled with starch grains, which become blue with iodine solution. Oleoresin crystals are originally impregnated in the parenchyma, they become free in powder and are found in dispersed condition. Vascular elements large number of vessels elements either entire or in the form of fragments. Vascular elements show spiral and pitted thickenings, most of the elements are of vessel category. Large numbers of fibers are found to be scattered in parenchyma cells.

Phytochemical study results showed that carbohydrate, alkaloid, phenolic, flavonoid, tannin, protein, saponin, mucilage, sterols, anthraquinone and terpenoid were present in all samples of *C. longa* [Table 2].

S.N	Phytoconstituents	Samples							
		T1	T2	T3	T4	T5	T6	T7	T8
1	Carbohydrate	+	+	+	+	+	+	+	+
2	Alkaloid	+	+	+	+	+	+	+	+
3	Anthraquinone	+	+	+	+	+	+	+	+
4	Phenolic and Tannin	+	+	+	+	+	+	+	+
5	Flavonoid	+	+	+	+	+	+	+	+
6	Protein	+	+	+	+	+	+	+	+
7	Saponin	+	+	+	+	+	+	+	+
8	Mucilage	+	+	+	+	+	+	+	+
9	Sterols	+	+	+	+	+	+	+	+
10	Terpenoids	+	+	+	+	+	+	+	+
11	Resins	+	+	+	+	+	+	+	+

Table 2: Phytochemical analysis of *C. longa*

Table 3: Physiochemical data of *C. longa*					
Samples	Foreign organic Matter Mean ±SD	Total Ash value Mean ±SD	Acid insoluble value Mean ±SD	Alcohol soluble Extractive value Mean ±SD	Water soluble Extractive value Mean ±SD	Moisture content Mean ±SD
Delhi	1.99±0.095	7.24±0.511	0.56±0.017	12.61±0.600	9.03±0.846	6.91±0.262
Patna	1.78±0.023	7.7±0.31 8	0.85 ± 0.028	12.68±0.400	9.46±0.622	5.93±0.491
Lucknow	1.3±0.045	7.43±0.481	0.533±0.028	12.80±0.489	10.56±0.304	7.43±0.248
Erode	1.97±0.024	8.0±0.325	0.466±0.125	13.70±0.518	10.73±0.643	7.56±0 .501
Trivandrum	1.78±2.024	6.8±0.264	0.433±0.017	13.02±0.303	10.21±0.771	5.0±0.173
Surat	1.92±0.041	5.0±0.173	0.566±0.075	10.63±0.485	9.63±0.250	7.7±0.182
Nashik	1.23±0.032	6.45±0.217	0.516±0.076	12.50±0.687	10.36±0.198	6.45±0.217
Guwahati	1.57±0.092	7.56±0.240	0.55 ± 0.129	13.52±0.904	10.6±0.174	8.16±0.551

Table 4: Total phenolic content in all samples of *C. longa*				
Samples	Absorbance (mean+S.D)	Concentrations (mean+S.D) (µg/mL)	mg/g X=cV/m	% w/w
Surat	0.742+0.064	66.25+5.342	66.25	6.62
Nashik	0.767+0.045	69.37+6.843	69.37	6.93
Lucknow	0.965+0.083	94.25+0.456	94.25	9.42
Delhi	1.025+0.984	101.625+10.231	101.625	10.16
Trivandrum	1.161+0.124	118.625+9.674	118.625	11.86
Guwahati	1.223+0.152	126.375+12.562	126.375	12.63
Patna	1.489+0.132	159.625+14.231	159.625	15.96
Erode	1.862+0.127	206.250+17.453	206.250	20.62

X=cV/m X= Total phenol content c=concentration of gallic acid obtained from calibration curve m=weight of plant extract V=volume used (mL)

Table 5: Total flavonoid content in different accession of *C. longa*				
Samples	Absorbance (mean+S.D)	Concentrations (mean+S.D)(µg/mL)	mg/g X=cV/m	% w/w
Surat	0.449+0.023	20.83+2.032	20.83	2.83
Nashik	0.528+0.012	34.00+2.045	34.00	3.40
Lucknow	0.621+0.045	49.50+4.183	49.50	4.95
Delhi	0.739+0.076	69.166+5.674	69.166	6.91
Trivandrum	0.614+0.045	48.333+4.124	48.333	4.83
Guwahati	0.695+0.034	61.833+5.785	61.833	6.18
Patna	0.825+0.067	83.500+6.342	83.500	8.35
Erode	0.818+0.089	82.33+4.764	82.33	8.23

X=cV/m X= Total phenol content c=concentration of gallic acid obtained from calibration curve m=weight of plant extract V=volume used (mL)

Physiochemical constant such as foreign organic matter, total ash value, acid insoluble value, alcohol soluble extractive value, water-soluble extractive value, and moisture content of all samples were found to be in the range of I.P. prescribed limit [Table 3]. The total phenolic content of different rhizome samples were quantified using standard UV spectrophotometric method employing gallic acid as standard at 765 nm. The standard calibration curve was found to be linear in the range of 25–300 µg/mL with $r^2 = 0.995$ and regression equation y=0.008x+0.212 [Table 4]. Data showed that total phenol was found to be highest in a sample collected from Erode (206.62% w/w) followed by Patna (159.62%w/w).

The sample of Surat was reported to contain least phenolic content (6.62% w/w). The results of total flavonoid content of different rhizomes samples were quantified using standard UV spectrophotometric method employing rutin as standard at 415 nm. The standard calibration curve was found to be linear in the range of 10–100 µg/mL with $r^2 = 0.997$ and regression equation y=0.006x+0.324. Results showed that the Patna contained the highest content of flavonoid (8.35% w/w) whereas lowest content was found in the samples of Surat (2.83 % w/w) [Table 5]. *C. longa* is the valued medicinal plants and possesses broad range of medicinal uses. These plants are distributed mainly in India, Pakistan, and

Indonesia, etc. Medicines derive exclusively from natural sources and pharmacognostical evaluation of drugs referred to the confirmation of its identity and determination of its quality, purity, and detection of nature of adulterant present in them. The details pharmacognostical account of the plant included macroscopic, microscopic characters for the correct botanical identifications. Histological characters were studied. Microscopy study of turmeric showed epidermis with thick-walled, cubical cells, and cortex cells characterized by the presence of scattered oleo-resin cells with brownish contents. There are no significant variations observed by macroscopical and microscopical evaluation in different accessions of turmeric.

Physiochemical parameters such as determination of foreign organic matters, total ash value, acid insoluble ash value, alcohol extractive value, water extractive value, and moisture content have also been determined to know the quality of drugs. Extractive values are useful for the evaluation, especially when the constituents of the drugs cannot be readily estimated by any other means. It also helps to indicate the nature of chemical constituents present in the drug.[8,13]

The water and alcohol extractive values were determined to see the solubility of constituents in their respective solvents, as extractives values were primarily useful for the determinations of exhausted or adulterants drugs. On ignition of crude drugs ash remained, comprises of the cell wall and its content. Ash constitutes the inorganic residues obtained after complete combustion of a drug. It represents the inorganic salts naturally occurring in the drugs and was useful for determining the authenticity of drugs. Thus ash value is a validity parameter describe and to assess the degree of purity of a given drug. Acid insoluble ash limit was imposed specially incase where foreign silica might be present.[14,15] In the case of *C. longa* the total ash value, alcohol extractive values were found to be highest in the sample of Erode whereas total ash content is lowest in the sample of Surat. The high extractive value may be due to the presence of high fibrous content is this sample. Chemo profiling of herbal drugs is one of the most important tools for quality assessment. The WHO recommended the use of chromatography techniques for the identification and standardization of quality of plant products. As phenolic and flavonoid content are present in turmeric and these are responsible for the antioxidant and anti-inflammatory activities and allowing them to be used as potential chemopreventives. The diversity among accessions of turmeric revealed that phenolic and flavonoid content were varied significantly. Results showed that total phenolic content was highest in a sample of Erode (20.20% w/w) whereas Patna contains the highest flavonoid content (8.35% w/w) of *C. longa*.

Paliwal *et al*.[16] analyzed phytochemical constituent curcumin and compared it with other samples and found variations among different samples. Paliwal *et al*. have carried pharmacognostical evaluation in *Curcuma caesia* and reported distinct features of *C. caesia*.[16] Our results are also confirmatory with the finding of Paliwal. In another experiment, the efficacy of various organic solvents for extracting antioxidant phenolic and flavonoid compounds of turmeric was compared and reported significant variation.[17] Our finding was also supported by this experiment. Phadungcharoen and Theanphong[18] carried out a microscopic investigation of *Curcuma petiolata* Roxb. rhizome and found several distinct features.

CONCLUSIONS

Correct identification and purity of plant materials is very important. As per the WHO, the macroscopic and microscopic determination of the drug plants is the first step toward establishing the correct identity and purity of the source materials. Microscopic study of the rhizomes and powdered materials acts as a trustworthy source for detecting adulteration. Genuineness of the powdered materials studied showed that there are resemblances in the anatomical or microscopical features of both fresh and dry, powdered samples of *C. longa* which is the base of assessment to evade adulteration of the drug plants. *C. longa* is is a potential and functional food ingredients, herbs and natural ingredient for our daily needs. Food supplementation with *C. longa* may be considered as a novel nutritional approach to reduce chronic diseases as well as mineral deficiency. Experimental data from the literature and as revealed herein indicates adequate information about the use of turmeric as spices with an enormous number of chemical components apart from their several medicinal uses. Large-scale clinical studies are required to rationalize these plants as a suitable phytopharmaceutical drug even though initial data and results from this work seem to be auspicious.

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