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Physicochemical and Phytochemical Investigation of *Curcuma Longa* linn. Rhizome

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ABSTRACT

In recent year there has been rapid increase in the standardization of selected medicinal plant of potential therapeutic significance. The rhizome 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, Physico-chemical constants and Phytochemical screening. This information will be used for further pharmacological and instrumental evaluation of the species and will assist in standardization for quality, purity and sample identification. Physicochemical parameters such as total ash value, acid insoluble ash value and water soluble ash value were determined which were 14.69, 1.20 and 4.12% w/w respectively. The alcohol soluble extractive and water soluble extractive were also determined. Preliminary phytochemical analysis of ethanol extract was carried out. The results were positive for steroids terpenoids, phenols, tannins and flavonoids. HPTLC analysis confirms the presence of Curcuminoids. These secondary metabolites were the active constituents of *Curcuma longa* Linn. that may be responsible for its pharmacological activities.

Keywords: *Curcuma longa,* Curcuminoids, Rhizome, Physicochemical constants.

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INTRODUCTION

The use of the medicinal plants is increasing in many countries where 35% of drugs contain natural products[1]. Turmeric is one of most essential spices all over the world with a long and distinguished human use particularly in the Eastern civilization. In India, *Curcuma longa* has been in use as a culinary ingredient since 3000 BC[2]. Turmeric (*Curcuma longa* L.) is a medicinal plant extensively used in Ayurveda, Unani and Siddha medicine as home remedy for various diseases. *Curcuma longa*, botanically related to ginger belongs to the Zingiberaceae family[3]. Turmeric is native to the monsoon forests of South East Asia. It is a perennial herb, 1m 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[4].

Current traditional Indian medicine uses it for biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism, and sinusitis[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. Due to its increasing demand and overexploitation without ensuring its regeneration, the plant has recently been categorized as an endangered species. The plant is also having some amount of antifungal protein against drug- resistant *Candida albicans*[6.] There is a need for documentation of research work carried out on traditional medicines. With this backdrop, it becomes extremely important to make an effort towards standardization of the material to be used as medicine. The main aim of the present work is to study the macro, physico-chemical standards and phytochemical analysis of the Rhizome of the Plant Curcuma Longa Linn which could be used for the proper identification of this drug.

MATERIALS AND METHOD

Plant material

The dried plant specimen for the proposed study was purchased from a commercial source, at Chennai, Tamil Nadu. It was identified and authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Center, (PARC), Chennai, where the voucher specimen has been deposited (PARC/2007/2161) in the Pharmacognosy herbarium.

Macroscopical characterization

Macroscopical studies of rhizome was done by naked eye and shape, color, taste and odor of rhizome were determined and reported.

Physico-chemical evaluations

Physicochemical parameters such as moisture content, ash values, extractive values, crude fibre content etc. were determined as per procedures mentioned in accordance with WHO guidelines[7,8].

Fluorescence Analysis

The fluorescence analyses of the drug powder as well as extract were carried out by using the method of Chase and Pratt[9]**.** The powder and extract were treated with different chemical reagents with color changes under ordinary day light and UV light by the standard method.

Extraction

Fresh rhizomes were cleaned and cut into small pieces and air dried for 2 days. The dried sample was again dried in a hot air oven at 50°C for 24 hrs, then ground into powder and pass through a sieve with nominal mesh size of 2 mm. in diameter. About 10 g of turmeric powder was steam distilled using Clavenger-type apparatus at a rate of 2-3 ml/min for 05 hours. After extraction of volatile oil by hydro-distillation, the marc was further macerated with 600 ml of 95% ethanol on a shaker with 210 rpm at room temperature until the last extract was colorless[10]. The ethanol extracts were combined and filtered. The filtrates were concentrated under reduced pressure at 50°C using a rotary vacuum evaporator. The extract was further evaporated on a boiling water bath until constant weight was obtained.

Preliminary phytochemical screening

The ethanol (95%) extract of *Curcuma longa* was subjected to the following chemical tests for identification of phytochemical constituents[10].

HPTLC analysis

The ethanolic extract of the rhizome of *Curcuma longa* was further subjected to HPTLC for the conformation of the active constituents under the following conditions[11].

Stationary Phase	:	Pre coated silica gel 60F 254
Mobile Phase	:	Chloroform: Ethanol: Glacial Acetic Acid (95:5:1)
Developing Chamber	:	Twin trough Chamber 20x10
Sample	:	total ethanol extract was dissolved in 1ml ethanol.
Sample Applicator	:	CAMAG Linomat 5
Dosage Speed	:	150nl/s
Syringe Size	:	10 µl
Number of tracks	:	1
Scanner	:	CAMAG TLC Scanner 3
Wavelength	:	254nm

RESULTS AND DISCUSSION

Macroscopical Study

The central or primary rhizomes are ovate, irregularly ovoid, cylindrical or fusiform, curved, sometimes slightly branched into a Y - shape, 1.1-10.3 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. (Figure. 1)

Figure 1: Macroscopy of *Curcuma longa* rhizome

Physicochemical Parameters

The rhizome of the plant was having 14.69% w/w of total ash, 4.12% w/w of water soluble ash and acid insoluble ash is about 1.20% w/w. The water soluble extractive value was 19.64% w/w which was high as compared to alcohol soluble extractive value which was 12.84% w/w. So the plant shows high water soluble ash and water soluble extractive value. The loss on drying was about 7.56% w/w. Crude fibre content was 4.53% w/w. These data's were helpful for identifying and ascertaining the quality of the collected crude drug. (Table. 1).

Table 1: Physico-chemical constants of *Curcuma longa rhizome*

S. No	Parameters	Results (%w/w)
1.	**Ash values**	
	Total ash	14.69
	Water soluble ash	4.12
	Acid insoluble ash	1.20
2.	**Extractive values**	
	Petroleum ether extractives	1.38
	Chloroform extractives	2.10
	Alcohol soluble extractives	12.84
	Water soluble extractives	19.64
3.	**Loss on drying**	7.56
4.	**Crude fibre content**	4.58

Fluorescence analysis

Fluorescence studies revealed specific fluorescence in visible light and white light with different chemical treatment; which helps in identification and standardization of the plant within the species. Fluorescence is the phenomenon exhibited by numerous phytoconstituents present in the plant material. In fluorescence, the fluorescence light is always of greater wavelength than the exciting light. Light rich in short wavelength is very active in producing fluorescence and for this reason ultraviolet light produces fluorescence in many substance which do not visible fluoresce in day light. The powder of *Curcuma longa* rhizome showed yellow fluorescence with methanol in UV light at 366nm, which indicates the presence of chromophore in the drug. (Table 2a, 2b)

Table 2a: Fluorescence analysis of the powder of *Curcuma longa rhizome*

S.No	Reagents	Visible light	Short UV (254 nm)	Long UV (366 nm)
1	Powdered drug	Yellow	Greenish yellow	Dark yellow
2	Powder + Conc. HCl	Yellow	Pale green	Yellow
3	Powder + Conc. H_2SO_4	Brownish black	Dark Brown	Greenish Black
4	Powder +Conc. HNO_3	Orange	Green	Brown
5	Powder + 10% NaOH	Reddish Brown	Yellowish Brown	Dark Brown
5	Powder + Dil. Ammonia	Brown	Brownish Black	Yellowish Green
6	Powder + Picric acid	Yellow	Pale green	Dark Green

Table 2b: Fluorescence analysis of various extracts of *Curcuma longa rhizome*

S. No	Reagents	Visible light	Short UV (254 nm)	Long UV (366 nm)
1	Petroleum ether	Light yellow	Light Yellow	Faint yellow
2	Chloroform	Faint Yellow	Light green	Greenish yellow
3	Ethyl acetate	Yellow	Pale green	Greenish yellow
4	Methanol	Yellow	Pale green	Yellow

Table 3: Preliminary Phytochemical screening of total ethanol extract of *Curcuma longa*

Chemical Test	Total ethanol extract
Carbohydrates	-
Steroids	+
Saponins	-
Phenolic compound	+
Tannins	+
Proteins	-
Terpenoids	+
Flavonoids	+
Glycosides	-

+ = Present; - = Absent.

Preliminary Phytochemical Analysis

Qualitative preliminary phytochemical analysis was performed initially with different chemical reagents to detect the nature of phytoconstituents and their presence in the extract. Preliminary

phytochemical analysis of the ethanol extract of *Curcuma longa* indicates the presence of various secondary plant metabolites like, phenols, tannins, flavonoids, terpeneoids and steroids. (Table 3)

HPTLC analysis

HPTLC fingerprint is one of the versatile tools for qualitative and quantitative analysis of active constituents. It is also a diagnostic method to find out the adulterants and to check the purity. Curcuminoids contain three different diarylheptanoids Curcumin (diferuloylmethane), Demethoxycurcumin (phydroxycinnamoylferuloymethane), and Bisdemethoxycurcumin (di-phydroxycinnamoylmethane) [12]. Different compositions of mobile phase were tested in HPTLC for the separation of individual curcuminoids and its Rf values were determined shown in Figure 2. The desired resolution of separation was achieved using Chloroform: Ethanol: Glacial Acetic Acid (95:5:1) as the mobile phase. The Rf value of Curcuminoids were 0.75, 0.45, and 0.27, for Curcumin, Demethoxy curcumin, Bisdemethoxy curcumin respectively.

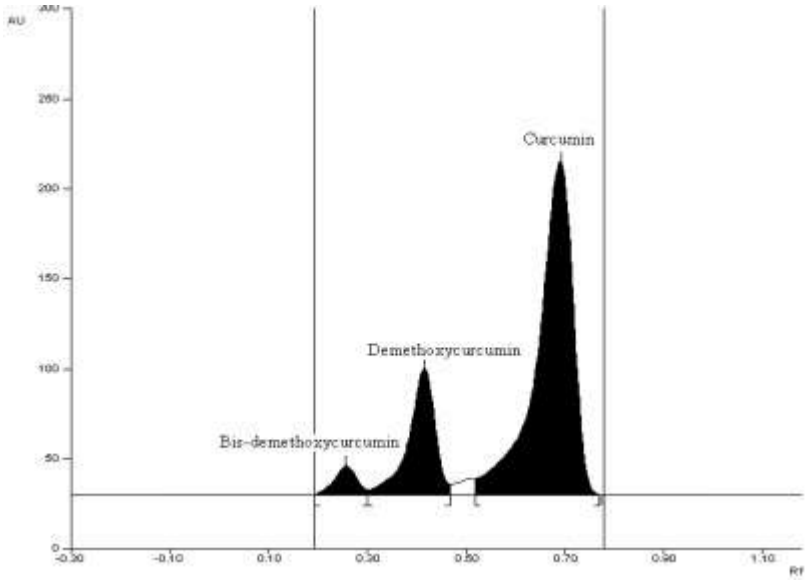

Figure 2: HPTLC fingerprint of ethanol extract of *Curcuma longa* rhizome

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 confirms 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. Presence of high amount of Curcuminoids is recommended as potential for various pharmacological effects of the rhizome of *Curcuma longa* Linn.

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