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Identification of *Curcuma Longa* Rhizomes by Physicochemical and TLC Fingerprint Analysis

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Abstract: *Curcuma longa* L. rhizomes are widely used by the traditional medical practitioners for curing various diseases. The rhizomes of *C. longa* L. is commonly known as Haldi or Turmeric. Haldi is of special importance to human with the discovery that its rhizome powder, when added to various food preparations, preserves their freshness and imparts a characteristic flavors. The Indian system of medicine comprises of Ayurveda, Siddha and Unani. In these systems many medicine are made up of *C. longa* rhizomes. In Ayurveda, turmeric has been used internally as a stomachic, tonic, blood purifier and externally in the prevention and treatment of skin diseases. The World Health Organization (WHO) in 1999 had given a detail protocol for quality contol of single herbal drugs. We have developed simple and rapid identification method for authentication of *C. longa* rhizome. Physicochemical parameters and TLC fingerprint analysis of *C. longa* rhizomes were also carried out. The study revealed different analytical parameters of the crude drug which will be useful in identification of genuine drug and control of adulterations.
Keywords : *Curcuma longa*, Secondary metabolites, Standardization, phytochemical analysis.

Introduction

Plants have an almost unlimited ability to synthesize diverse scoundary metabolites that have a wide range biological functions that are useful to human *Curcuma longa* Linn., Turmeric (Curcuma longa) and several other species of the curcuma genus grow wild in the forests of Southern Asia including India, Indonesia, Indochina, nearby Asian countries, and some Pacific Islands including Hawaii. All of these areas have traditional culinary and medicinal uses going back to pre-history[1].

In the Indian Ayurveda system of herbal medicine, turmeric is known as strengthening and warming to the whole body. Traditional uses in India include to improve digestion, to improve intestinal flora, to eliminate worms, to relieve gas, to cleanse and strengthen the liver and gallbladder, to normalize menstruation, for relief of arthritis and swelling, as a blood purifier, to warm and promote proper metabolism correcting both excesses and deficiencies, for local application on sprains, burns, cuts, bruises, insect bites and itches, for soothing action in cough and asthma, as antibacterial and anti-fungus, and in any condition of weakness or debility According to Michael Moriarty, "The ancient Hawaiians used this herb for many things, including the prevention and treatment of sinus infections (it is very astringent and appears to pull mucus out), ear infections (swimmers ear) and gastrointestinal ulcers[2].

Turmeric is eaten as a food both raw and cooked throughout Asia. While turmeric root looks much like ginger root, it is less fibrous and is more chewable, crunchy, and succulent[3]. The fresh root (not the powder) has a somewhat sweet and nutty favor mixed with its bitter flavor. As a result, it is not unpleasant to eat and not

difficult to chew. It is sometimes chewed plain or chopped up and put in salads raw. Traditional use includes mashing/grinding it in a mortar to make a paste to mix with other spices for flavoring in curries. In modern times, most common use is of the dried root powder as the base of most curries in India and other nearby countries[4].

The *Curcuma longa*, the yellow root, has been cultivated in India, Southern China and other tropical and subtropical countries. It is conjectured that the yellow root originates from East India, but this is not certain because the plant has never been found in the wild[5]. The plant's flower and growth pattern looks very similar to that of ginger and it is also in the same family as ginger (Zingiberaceae). The yellow root can grow to a height of approximately 1 metre. A bundle of leaves and flower stalk with a 20-cm-long flower rises from the rootstock[6]. Similarly to the ginger plant, side shoots with corm-like swellings develop underground. The yellow root grows best in damp and warm areas[7].

The underground parts of the plants are dug up when the flowers wilt in December and January. The corms and the rootstock are separated from the side shoots. These are then submerged in boiling water and placed in the sun to dry. Through drying in the sunlight, the under-ground parts become yellow in colour: the heat from the sun causes the pigment from the glandular cells to be distributed over the rootstock and corms. Once they are dry, the rootstocks are ground to form a powder which is called "curcumin" or "curcuma". The powder is then sold under the name "fumeric".

Curcuma *longa*

Materials and Methods

Fresh samples of turmeric rhizomes were purchased and authenticated. These are processed prepared and dried and evaluated for pharmacognostical and phytochemical studies were evaluated. In this study, the identity of the drug has been taken into consideration from the plant (genuine) after due taxonomical identification and preparing the rhizomes after collection as done in the case of commercial sample and the characters compared with the market sample both macroscopically and microscopically to detect any changes or adulterants[8].

Microscopy:

Fresh rhizomes of *curcuma longa* were subjected for the microscopical studies. The the sections were cut by free hand sectioning. The numerous temporary and permenant mounts of the microscopical section of the spicemen were made and examined microscopically.

Powder characteristics:

Preliminary examination and behavior of the powder with different chemical reagents was carried out as per reported method.

Quantitative microscopy:

Fresh turmeric showed abundant individual starch grain. They were ovate, simple, with a small beak at one end where the hilum was situated but it was altered during curing due to gelatinisation.

Micrometry:

Quantitative microscopy of the transverse sections and rhizome powder were performed to determine the size and dimension of tissues, cell and cell content.

Physicochemical Evaluation:

Physicochemical parameters such as foreign organic matter, moisture content, ash values, extractive values, pH etc were determine as per procedures mentioned in accordance with WHO guidelines.

Preliminary phytochemical screening:

The chemical evaluation includes qualitative chemical tests which are used for identification of various phytoconstitients present in the powdered crude drug.

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 (60-800C), 50% sulphuric acid, 50% hydrochloric acid, 50% nitric acid, 10% sodium hydroxide etc.

Chromatographic study

Thin layer chromatography

Alcoholic extract

Alcoholic extract of curcuma which has shown hypoglycaemic effect was subjected to thin layer chromatography.

Stationary phase : Silica gel
Mobile phase : Benzene : Chloroform : Alcohol
45 : 45 : 10
Detection : Boric acid in methanol

Method

Alcoholic extract of turmeric were spotted separately on a TLC plate and developed in a solvent system as stated above. Then the plate was dried and sprayed with the diagnostic agent.

The Rf value of the separate components were calculated

$$\text{Rf value} = \frac{\text{distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Separation of curcurminoids by column chromatography

Absorbent : Silica gel (column chromatography grade)
Solvent : Benzene
Sample : Benzene extract

The column was packed with slurry (silica gel in benzene). It was run for 1-2 hours with benzene. About 1 gram of the benzene extract from the marc obtained after the extraction with pet ether to remove fixed and volatile oils was passed down the column and when the solution has just sunk into the column, fresh benzene equilibrated with water was added for the development of the chromatogram. There is a gradual separation of bands on the column and the complete chromatogram will show 3 distinct zones and a few minor zones development was continued with benzene. Any fixed or volatile oil if turmeric which were not removed completely by petroleum ether passes down the column first as a pale yellow fraction. Evaporation of the solvent leaves a yellow oily residue having the characteristic odour of turmeric. The different zones were collected as liquid chromatogram in separate tubes as each band got washed down the column. The eluates from

the interzones were nearly colourless or only faintly coloured were rejected. The different fractions collected were concentrated by distillation under reduced pressure and further purified by re- chromatography on smaller columns using benzene[9].

Determination of Curcumin content as Curcuminoids by Spcetroscopy method

As per the literatures cited the following method has been adopted using the standard value 1 percent at 430nm – 1560. About 200mg of turmeric powder was weighed accurately and extracted with alcohol and made up to 100ml. From this 5ml was pipette out into 50ml standard flask and made up to the volume. Absorbance was studied at 430nm.

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, 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. The results of morphological characters are mentioned in Table no.1.

Table-1: Morphological Characters

	Fresh sample	**Prepared sample**	**Market sample**
Colour	Pale brown	Yellow-yellowish brown	Yellow-yellowing brown
Taste	Spicy bitter	Initially spicy and later bitter	Spicy bitter
Odour	Aromatic	Aromatic	Aromatic
Shape	Cylindrical	Cylindrical	Cylindrical
Size	3-5cm and 1.8cm	3-5cm and 1.5cm	3-5cm and 1.8cmrs
Fracture	Short	Short	Short
Fractured surface	Yellowish-orange	reddish-brown	reddish-brown
Surface	Notes leaves clearly visible	a few scale leaves and root, branch let, scars seen	Scale leaves, scars of root, branchlet, seen
Bud	Apical bud present	bud scars seen	bud scars seen

T.S of rhizome 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 consisting of thin walled cubical cells of various dimension. The cortex consists of thin walled rounded parenchymatous cells and having olioresin 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[10].

Organoleptic characters

Prepared sample powder

Colour: dark yellow
Odour: aromatic
Taste: spicy, bitter
Texture: fine

Powder characters

1. Fragments of cork cell both sectional view and surface view were seen.
2. Group of rounded parenchyma cells with dark-brown pigment cells were seen
3. Altered pasty masses of starch, coloured yellow (in unstained preparation) were present
4. Parenchymatous cells with unlignified vessels were seen
5. The vessels were found to have spiral, reticulate and annular thickenings
6. **Calcium oxalate crystals were absent**

Power of market sample showed similar characters as above. Hence, from the above power microscopical characters of the powered prepared and market samples of the drug, the genuinely of the market sample was determined

Quantitative microscopy

The measurement of starch grains was made in fresh sample. It was found to conform to standard values specified in brackets. The results were expressed in Table no.2.

Table -2 : Quantitative microscopy

Minimum			**Average**	**Maximum**
Fresh sample	Length	29µ	47.7µ	66.5µ (30-60µ)
Starch grain	width	12.8µ	23.8µ	30.8µ (25-35µ)

By comparing the above observed value with that of standard value, the genuinely of the fresh sample was confirmed

Fluorescence Analysis:

Fluorescence analysis of the powder treated with different solvents and reagents is exhibited in Table no.4. 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 results were expressed in Table No 3 and 4.

Table -3 Fluorescence Analysis of Extracts

Material	**Extracts**	**Day light**	**UV light**
Turmeric	Petroleum ether	Yellow	Light yellowish green
	Benzene	Yellow	yellowish green
	Alcohol	yellowish green	yellowish green
	Chloroform	yellowish green	yellowish green
	Water	Nil	Nil

Table -4 Fluorescence Analysis of Turmeric

Material	**Reagents**	**Day light**	**UV light**
Turmeric	Powder	Yellow	yellowish green
	Powder+1N NaOH(aqu)	Red	yellowish green
	Powder+1N NaOH (Alc)	Reddish orange	yellowish green
	Powder + 50% H_2SO_4	yellowish	yellowish green

Physicochemical Evaluation

The results of the physicochemical constants of raw material within the limit which is mentioned in Table no.5. 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 0.23±0.015%w/w, it indicates that there 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 7.42±0.021%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 4.5±0.03%w/w. The amount of acid-insoluble siliceous matter present was 0.86±0.61%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 18.28±0.01%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 9.42±0.57%w/w which signifies the nature of the phytoconstituents present in plant.

Table -5: Physicochemical parameters of *Curcuma longa* (rhizome)

S.No	Parameters	Analysis % w/w	Remark
1	Foreign matter	Nil	NMT -2.0%
2	Total ash	4.5±0.03	NMT-9%
3	Acid insoluble ash	0.86±0.61%	NMT-1%
4	Alcohol soluble extractive value	9.42±0.57%	NLT-8%
5	Water soluble extractive value	18.26±0.01%	NLT-12%

Preliminary Phytochemical Screening

500g of the coarse powder of Curcuma (market sample) was subjected to extraction by cold percolation petroleum ether (40-60^{0}c), benzene, chloroform, ethanol(70%) and chloroform water. percentage yield of various extracts were mentions table No 6.

Table-6: percentage yield of various extracts

Extracts	Turmeric			
	Yield in % w/w	Colour	consistency	Yield in w/w
Petroleum ether (40-60^{0}c)	2.1	Yellow	Viscous	2.2
Benzene	10.3	Brown	Solid	2.8
Chloroform	6.8	Reddish	Solid	3.8
Ethanol	10.2	Reddish	Solid	9.8
water	2.8	Pale Yellow	Solid	15.8

Table-7: qualitative chemical examination of various extracts

Plant constituent test	TURMERIC				
	P.E	BEN	CHE	Alc	AQ
Alkaloids Mayers's reagents			+		
Carbohydrates and glycosides Molisch reagent					
Phytosterols Liebermann's test					
Fixed oil and fats Spot test	+				
Saponins Foam test					
Phenolic Ferric chloride		+	+	+	
Proteins Millons's					+
Resin With water		+	+	+	
Volatile oil Test for aroma	+				
Vitamins Ascorbic acid					

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 were expressed in Table No 7.

Chromatographic study

Thin layer chromatography

A chromatographic study of curcuminoids present in Curcuma longa was undertaken, since during the screening for the bio- active substances, it was found, that the curcumin has an effect on bringing down blood sugar level in normal fasting rabbits. Hence it was thought that curcumin may be having a major role to play in the fall of blood –sugar level[11,12,13]. Standard sample of curcumin was not available for comparison. Hence isolation was taken up of curcumin. Through curcumin is highly soluble in alcohol, since tannins also may interfere, it was considered curcumin be isolated from benzene extract, taking into account the past work done of a chromatographic study of curcuminoids in Curcuma longa. The results were expressed in Table No 8.

Table-8: Thin layer chromatography

SPOTS	Rf value		Colour	
	As per Ref	As per TLC	As per Ref	As per TLC
1.Spot I	0.25-0.35	0.26	Yellow	Yellow
2. spot II (Curcumin derivative)	0.35-0.45	0.39	Orange	Light orange
3. Spot III (Curcumin)	0.40-0.45	0.42	Orange	Dark orange

Column Chromatography

Fraction 1:

This is the eluate containing the orange –yellow zone. As the benzene solution is evaporated, curcumin is thrown out of solution in the form of orange –red crystals. Recrystallization from acetone gave curcumin in the form of needles of crystals having a melting point of 180^0c. The yield was 50mg. This was confirmed by means of chemical test. This was used as standard curcumin which is adsorbed lowest on the column and forms by far major constituent.

Spectroscopy method

Average weight of the one tablet was found out from 20 ml tablets. It was extracted with alcohol and made upto 100ml. From this 5ml was pipette out into 50ml standard flask and made up to volume. Measured at 430nm using (Shimadzu – 1201 – spectrophoto meter)

Calculation

For 1560 Absorbance - 1 gram
For 0581 absorbance - x

The content of curcumin as Curcuminoids in each tablet of average weight has been calculated after incorporating appropriate dilution factor. The percentage of Curcumin content = 0.756%

Conclusion

Summary the standardization, qualitative and quantitative determination of phytochemicals and TLC profiling were successfully accomplished. Furthermore, the result may be useful in developing potential phytopharmaceutical products based on *C. langa*. However there is a need to further identify specifically the flavonoid, saponin and alkaloid compounds that are present in the extracts**.**

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