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Research Article

QUALITATIVE AND QUANTITATIVE PROFILE OF CURCUMIN FROM ETHANOLIC EXTRACT OF *CURCUMA LONGA*

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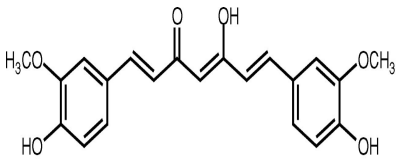
ABSTRACT
Turmeric, derived from the plant *Curcuma longa*, is a gold-colored spice commonly used in the Indian subcontinent, not only for health care but also for the preservation of food and as a yellow dye for textiles. Curcumin, which gives the yellow color to turmeric, was first isolated almost two centuries ago, and its structure as diferuloylmethane was determined in 1910. Since the time of Ayurveda (1900 B.C) numerous therapeutic activities have been assigned to turmeric for a wide variety of diseases and conditions, including those of the skin, pulmonary, and gastrointestinal systems, aches, pains, wounds, sprains, and liver disorders. Extensive research within the last half century has proven that most of these activities, once associated with turmeric, are due to curcumin. Curcumin has been shown to exhibit antioxidant, anti-inflammatory, antiviral, antibacterial, antifungal, and anticancer activities and thus has a potential against various malignant diseases, diabetes, allergies, arthritis, Alzheimer's disease, and other chronic illnesses. Curcumin can be considered an ideal "Spice for Life". Curcumin is the most important fraction of turmeric which is responsible for its biological activity. In the present work we have investigated the qualitative and quantitative determination of curcumin in the ethanolic extract of *C.longa*. Qualitative estimation was carried out by thin layer chromatographic (TLC) method. The total phenolic content of the ethanolic extract of *C.longa* was found to be 11.24 as mg GAE/g. The simultaneous determination of the pharmacologically important active curcuminoids *viz*. curcumin, demethoxycurcumin and bis-demethoxycurcumin in *Curcuma longa* was carried out by spectrophotometric and HPLC techniques. HPLC separation was performed on a Cyber Lab C-18 column (250 x 4.0 mm, 5µ) using acetonitrile and 0.1 % orthophosphoric acid solution in water in the ratio 60 : 40 (v/v) at flow rate of 0.5 mL/min. Detection of curcuminoids were performed at 425 nm.
KEYWORDS: *Curcuma longa*, curcumin, chromatography, spectroscopy

INTRODUCTION

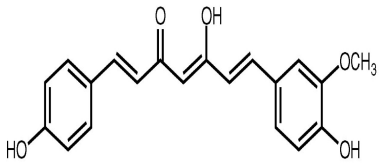
The turmeric (*Curcuma longa*) plant, a perennial herb belonging to the ginger family, is cultivated extensively in south and southeast tropical Asia. The rhizome of this plant is also referred to as the "root" and is the most useful part of the plant for culinary and medicinal purposes. Turmeric is a spice of golden color that is used in cooking in the Indian subcontinent. Because of its color and taste, turmeric was named "Indian saffron" in Europe. Today, India is the primary exporter of turmeric (known as Haldi in India). Although its ability to preserve food through its antioxidant mechanism, to give color to food, and to add taste to the food is well known, its health promoting effects are less well recognized or appreciated. It was once considered a cure for jaundice, an appetite suppressant, and a digestive. In Indian and Chinese medicines, turmeric was used as anti-inflammatory agents to treat gas, colic, toothaches, chest pains, and menstrual difficulties. This spice was also used to help with stomach and liver problems, to heal wounds and lighten scars, and as a cosmetic[1]. The most active component of turmeric is Curcumin, which makes up 2 to 5% of the spice. The characteristic yellow color of turmeric is due to the curcuminoids, first isolated by Vogel in 1842.Curcumin is an orange–yellow crystalline powder practically insoluble in water. The structure of curcumin ($C_{21}H_{20}O_6$) was first described in 1910 by Lampe and Milobedeska and shown to bediferuloylmethane[2]. Turmeric contains a wide variety of phytochemicals including curcumin, demethoxy curcumin, bisdemethoxycurcumin, zingiberene, curcum - enol, curcumol, eugenol, tetrahydrocurcumin, triethylcurcumin, turmerin, turmerones, and turmer - onols[3]. Curcumin is the phytochemical that gives a yellow color to turmeric and is now recognized as being responsible for most of the therapeutic effects. Curcumin is hydrophobic in nature and freely soluble in dimethylsulfoxide, acetone, ethanol, and oils. It has an absorption maxima around 420 nm[4].

Turmeric rhizomes

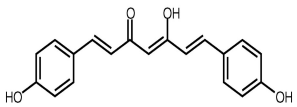
Turmeric powder



Curcumin

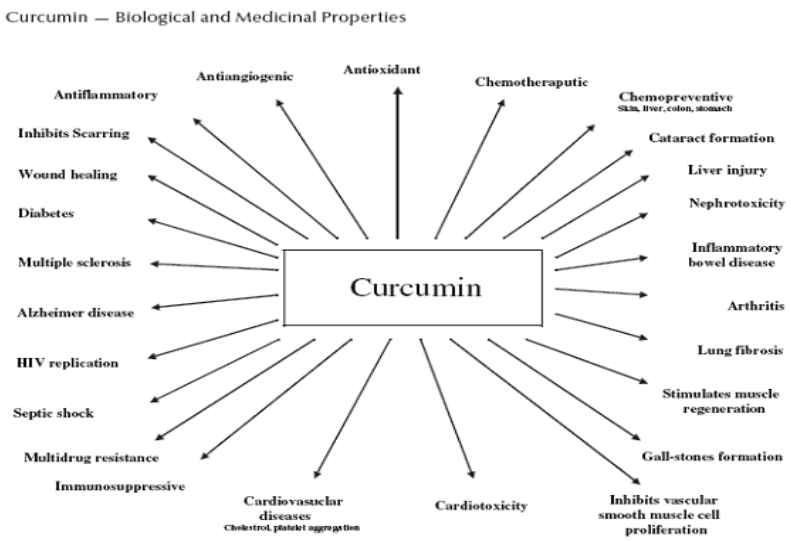


Demethoxycurcumin



Bisdemethoxycurcumin

Turmeric has been used as a food additive in curries to improve palatability and storage stability. Curcuminoids, a natural coloring agent, is recognized as a rich source of phenolic compounds, consisting of three different compounds: curcumin, demethoxycurcumin and bisdemethoxycurcumin. It also has potential as a pharmaceutical excipient, since it possess antioxidant, anti-inflammatory, antimutagenic and anti HIV properties and can reduce blood glucose[5]and LDL[6]. Various biological and medicinal uses of Curcumin are listed below .



MATERIALS AND METHODS

Plant Material

The powder of *C. longa* was obtained from the Pharmacognosy research lab.L.N.C.P. Bhopal (M.P.). A voucher specimen was deposited. The physiochemical studies were carried out using standard methodology[7].

PREPARATION OF EXTRACT

Approximately 70g of *C.longa* powder was extracted with ethanol by soxhelation and the solvent was recovered by distillation. The extract was concentrated under reduced pressure and air dried.

Physio chemical Tests

Description Reddish brown thick paste with characteristics odour & characteristics taste.

Physical Evaluation In physical evaluation, ash values viz., total ash, acid insoluble ash and water soluble ash, and extractive values viz., alcohol soluble extractive value and water soluble extractive were determined. The ash values represent the inorganic salts present in the drug.

Determination of Total Ash Value Two gram of *C.longa* powder was taken in a tared silica crucible and incinerated at a temperature not exceeding 450 °C until free from carbon. The resultant ash was cooled and weighed. The percentage of ash was calculated with reference to the air-dried drug.

Acid Insoluble Ash Value The total ash obtained from 2g of *C.longa* powder was boiled for 5 minutes with 25 ml of dilute hydrochloric acid and the insoluble matter was collected on an ashless filter paper. It was washed with hot water, ignited and weighed. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

Water Soluble Ash Value The total ash obtained from 2g of *C.longa* powder was boiled for 5 minutes with 25 ml of water and the insoluble matter was collected on an ashless filter paper. It was washed with hot water, ignited and weighed. The percentage of water-soluble ash was calculated with reference to the air-dried drug.

Separation of Curcumin by Thin layer chromatography

For thin layer chromatographic studies of curcumin, precoated Silca gel F_{254} aluminum plates (20 X 20cm) were used[8].The Curcumin was separated using n-hexane : ethyl acetate[7:3]. The colour and R_f values were recorded using spraying the plates with 1% alcoholic KOH solution.

Determination of Total Phenolic contents

The Folin-Ciocalteu reagent assay was used to determine the total phenolic contents. The extract 1ml (10mg/ml) was mixed with 0.5ml Folin-Ciocalteu reagent previously diluted with 7ml deionized water. The solution was allowed to stand for 3min. at 25^0C before adding 0.2ml of saturated sodium carbonate solution. The mixture was allowed to stand for another 120 min and absorbance was measured at 725nm.Gallic acid was used as standard for the calibration curve. The total phenolic contents of the extract were calculated in terms of Gallic acid equivalent [GAE][9].

$$C = c \times V/m$$

Where, C= total phenolic compound in mg/gm of the extract

c = concentration of gallic acid (established via calibration curve)

V = volume of the extract in ml

m = wt. of extract in gm

Determination of % Curcumin and Color value by UV/Visible spectroscopic method

0.1 gm of dried extract was dissolved in 25ml of ethanol. This solution was filtered and volume made upto 100ml.Then 10ml of above solution was taken in volumetric flask and again volume made upto 100ml with ethanol. The absorbance was measured at 425nm. % Curcumin and colour value were determined[10].

A standard Curcumin 0.25g/lit give absorbance at 425nm = 0.42

Absorptivity of Curcumin (A) = 0.42 / 1 x 0.025

= 16.8

% Curcumin = a x 100 / L x A x W

Where, a = absorbance of sample at 425nm

L = path length (1cm)

A = Absorptivity

Colour value = a x 1000

Estimation of Curcumin by HPLC

The HPLC analysis was performed using a LC-100, Cyberlab™, Salo Torrace, Millburry, MAO 1527, USA with LC-UV-100 UV detector. A CAPCELL (C-18) HPLC-packed column (4.6 mm I.D.X 250 mm), type MG 5 µm, number AKAD/05245 was used for the chromatographic separations. The mobile phase consisted of solvent [methanol]. The separation was performed using isocratic elution (0-15 min) with a flow rate of 1.2 ml/min and a column temperature of 25°C. The injection volume was 25µl, and UV detection was effected at 254 nm. HPLC grade solvents were obtained from Shyam brothers, 27- sindhi market, Bhopal. After phytochemical analysis the ethanolic extract (10µg/ml) were subjected to HPLC column and the obtained record were superimposed on the retention time values of these extract[10].

RESULTS AND DISCUSSION

Keeping in view of the ethno-pharmacological importance of powder *C.longa*, preliminary studies were undertaken for standardization. Organoleptic evaluation (Table:1) showed the following characters: colour-brown, sensation - coarse and odour - odourless. The organoleptic studies indicated important characteristics such as typical tongue sensitizing aromatic taste, aromatic odour, etc, which are useful diagnostic characters. The physiochemical parameters are shown in Table 1. Mean ash values (%) were found to be 4.98(total), 1.4(acid insoluble ash). Total ash value was relatively high, which may be due to high content of carbonates, phosphates, silicates and silica. Ash value is useful in determining authenticity and purity of drug and also these values are important quantitative standards. The solubility study and loss on drying of sample were determined (Table 1). Different active constituents of Turmeric powder such as Curcumin and curcuminoids were successfully detected directly from the ethanolic extract of *C.longa*. When a turmeric extract was separated on a TLC plate, each band produced molecular ion peaks corresponding to curcumin, demethoxycurcumin and Bis-Demethoxycurcumin (Table:2). The Quantative determination of phenolic content ,% Curcumin and colour value from ethanolic extract of *C.longa* was found to be 11.24 as mg GAE/g ,10.23 % & 172 respectively(Table 3).The extracts analyzed displayed were reported on the figure 1, components and a number of peaks were superimposed on the standard extract at same conditions . On the basis of these profiles, three major constituent curcumin, Demethoxycurcumin and Bis demethoxy Curcumin were fractionated from ethanolic extract of *C.longa* by open column chromatography over C-18 silica gel, subsequently separated by preparative HPLC (Table 4)

CONCLUSION

A simple chemical profiling and semi-quantitative method for natural products using analytical method might be applied to diverse field related quality control of medicinal plants or food ingredients.

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Table 1: Physiochemical Evaluation of Powder of *C. longa*

S.No.	Characteristics	Observation	Test method
1.	Identification	(+)ve	TLC
2.	Appearance	Fine powder	Visual
3.	Colour	Yellowish orange	Visual
4.	Odour	characteristics	Organoleptic
5.	Taste	characteristics	Organoleptic
6.	LOD	5.07	I.P 1996
7.	Total Ash value	4.98	1.P 1996
8.	Acid insoluble ash	1.4	1.P 1996
9.	Solubility in water	(-)ve	1.P 1996
10.	Solubility in alcohol	(+)ve	1.P 1996

Table:2 TLC Profile of ethanolic extract of *C. longa*

S. No.	Solvent System	R_f of Std	R_f of Sample	Inference
1.	n-hexane : ethylacetate[7:3]	0.35	0.34	Demethoxycurcumin
2.	n-hexane : ethylacetate[7:3]	0.48	0.46	Bis-Demethoxycurcumin
3.	n-hexane : ethylacetate[7:3]	0.28	0.26	Curcumin

Table.3 Quantative estimation of Curcumin from ethanolic extract of *C. longa*

S.No.	Quantitative analysis	Inference
1.	Total Phenolic content	11.24 as mg GAE/g
2.	% Curcumin(UV/ visible spectroscopy)	10.23%
3.	Colour value	172

Table.4 HPLC analysis of ethanolic extract of *C.longa*

ID	NAME	RT(min.)	Height	Area	Conc.	Half width(s)	Res	Theo. Plate	Tail.factor
1.	Mobile phase	0.290	5125	52726.3	99.8109	10.29	000	15.82	0.56
2.	Bisdemethoxy curcumin	3.828	16	73.3	0.1388	4.58	16.80	13906.22	1.56
3.	Demethoxy curcumin	7.035	4	14.1	0.0267	3.52	27.93	79478.27	1.19
4.	Curcumin	9.292	3	12.5	0.0236	4.16	20.75	99671.23	1.09

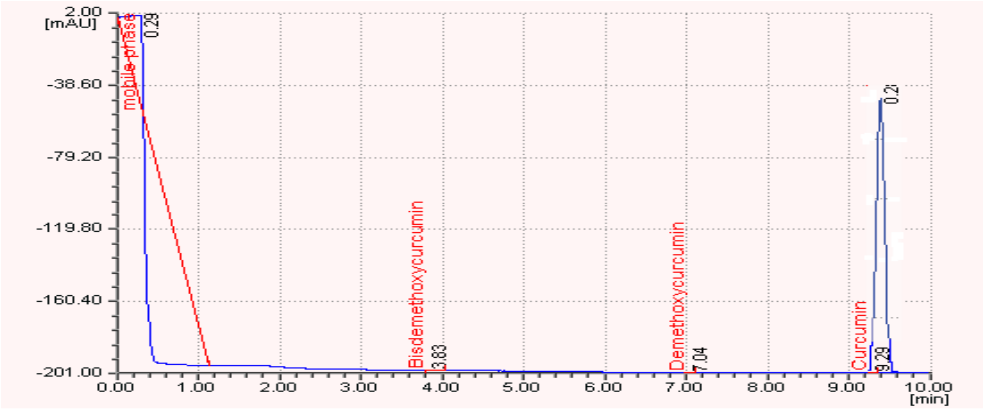

Fig:1 HPLC chromatogram of ethanolic extract of *C. longa*

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