

Phytochemical Evaluation and Curcumin Content Determination of Turmeric Rhizomes Collected From Bhandara District of Maharashtra (India)

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Abstract

Turmeric (*Curcuma longa*) is widely used popular Indian medicinal plant which belongs to the family of Zingiberaceae. Indian turmeric is preferred due to its high Curcumin content as compared to other countries. Curcumin is small molecular weight polyphenolic compound and lipophilic in nature. This active constituent of turmeric is isolated from *curcuma longa* and it provides colour to turmeric. Curcumin has various medicinal properties and shows Anti-inflammatory, anti-oxidant, anti-bacterial and anticancer activities. It has been observed that the chemical composition of most of the herbs changes with geographical region which may be due to climatic conditions and biochemical variations. The present work deals with phytochemical investigation of the turmeric rhizomes obtained from Bhandara District and determination of its curcumin content. Curcumin was isolated from turmeric rhizomes using reported method. The isolated curcumin was characterised by UV, FTIR and TLC methods. The curcumin content of turmeric rhizomes obtained from Bhandara District was determined and compared with other samples collected from different geographical regions. It was concluded that turmeric rhizomes obtained from Bhandara region has highest curcumin content as compare to other regions in Maharashtra (India).

Keywords: Curcumin; FTIR; TLC; *Curcuma longa*; Zingiberaceae

Introduction

In the country like India every kitchen has one of spices named 'haldi' without it food remains incomplete. This 'haldi' is popularly known as turmeric. The biological name of this popular spice is *Turmericor Curcuma longa*, which belongs to the Zingiberaceae or ginger family. Not only in food but turmeric is also used for many medicinal purpose in India in form of Ayurvedic, unani and siddha medicines [1]. India is considered to be the major producer of turmeric as well as the major consumer of this medicinal spice. The appeal of turmeric as a colouring, food preservative and flavouring is Global. According to the Food and Agriculture Organization of the United Nations, over 2400 metric tons of turmeric are imported annually into the USA for consumer use [2]. Although the centre producer of turmeric is India but other countries in Asia that produce this spice are Bangladesh, Pakistan, Sri Lanka, Taiwan, China, Burma (Myanmar), and Indonesia. Turmeric is also produced in Caribbean and Latin America: Jamaica, Haiti, Costa Rica, Peru, and Brazil [3,4].

Turmeric is the boiled, dried, cleaned and polished rhizomes of *curcuma longa* [5]. After harvesting the whole rhizomes are collected [6]. These rhizomes are transported as whole rhizomes. They are usually like fingers 2 to 8 cm long and 1 to 2 cm wide having bulbs and splits [6,7]. The dried rhizomes are further processed and reprocessed to obtain the turmeric powder.

Depending upon the location of the production of turmeric in India turmeric is well known in two forms namely, 'Alleppey' and 'Madras'. Alleppey consists of 3.5% to 5.5% volatile oils, and 4.0% to 7.0% curcumin and mainly imported by United States [4,6,8]. Madras variety consists of only 2% of volatile oils and 2% of curcumin and mainly favoured by British and Middle Eastern countries [6]. Both are used for food purpose. Whereas turmeric produced in Bengal is used as dye in India [3].

The other turmeric producers like Caribbean, Central and South America are not favoured by United States since the concentration of curcumin and volatile oil is low as compared to the Indian varieties and is dark in colour [6,9].

The quality of turmeric is judged at the very beginning by visual appearance of rhizome, its colour uniformity, smooth covering and a distinct breaking snap sound [4].

The main constituent of turmeric is curcumin. Curcumin is a polyphenol that gives turmeric its colour. Curcumin is a polyphenol and is lipophilic in nature, hence insoluble in water and also in ether but soluble in ethanol, dimethylsulfoxide, and other organic solvents [10]. Curcumin is stable at the acidic pH of the stomach [11].

The other constituents present are volatile oils including tumerone, atlantone, and zingiberone and sugars, proteins, and resins [12].

Due to the presence of curcumin as an active constituent and the volatile oils turmeric is widely used as a medicine for the treatment of many ailments. It is vastly used as an anti-inflammatory agent [13-16]. And hence also shows antioxidant property which is even better than the vitamin and E [17]. The rhizome extract had been studied for its anticancer activity [18]. The other activities shown are antimicrobial effect (against bacteria, fungi) [19,20]. Turmeric lowers the cholesterol level and inhibits platelet aggregation and hence shows cardiovascular effects [21,22]. Turmeric has also proven to be effective for the brain conditions like Alzheimer's and therefore India could be considered to be resistant or having less percent of Alzheimer's patients [23,24].

A relevant aspect to be taken into account when dealing with

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medicinal plants is the chemical composition variation due to the geographic localization, harvest time, climate conditions, cultivation handling, age of vegetable material, period and storing conditions, among others [25]. The objective of the present study was to evaluate the Turmeric Rhizomes Collected from Bhandara District for its chemical composition and curcumin content. The curcumin content was also determined for turmeric rhizomes collected from local market of Satara, Nashik, Dhule and Mumbai region for comparative study.

Materials and Methods

Chemicals and reagents

All the chemicals and reagents used were of analytical reagent grade. Pure Curcumin (Purity=95%) was obtained from Sigma Aldrich, Mumbai, India.

Collection and processing of plant material

Fresh rhizomes of turmeric were collected from Pavani Taluka of Bhandara District, Maharashtra (India) in the month of January and February. The plant specimen was authenticated by Dr. Rajendra D Shinde, Associate Professor, Department of Botany, St. Xavier's College, Mumbai - 400 001 as *Curcuma Longa L.* (Family: Zingiberaceae). The plant specimen matches with the Blatter Herbarium specimen no. B.R. - 469 of B. Rukmini Bai. The collected rhizomes were boiled in water for about 30 minutes and then dried under sunlight. The dried rhizomes were size reduced and shifted through mesh of fine size.

Extraction process

Turmeric rhizome powder was extracted with 95% ethanol in a Soxhlet assembly until all the colouring matter is extracted. The obtained crude extract was concentrated to semisolid brown coloured mass by evaporating ethanol.

Preliminary phytochemical evaluation of crude extract

The crude extract was evaluated for the presence of various phytoconstituents such as carbohydrates, proteins, Alkaloids, glycosides, terpenes, steroids, flavanoids, tannins and saponins using commonly employed precipitation and coloration reactions reported in standard reference books.

Carbohydrates: The extract was dissolved in 10 ml of distilled water and filtered through Whatmann No.1 filter paper and the filtrate is subjected to tests for carbohydrates.

a) Molish test: 2 ml solution was placed in a test tube. 1 drop of Molish Reagent was added. 2 ml of conc. HCL was added from the sides of the test tube. The test tube was observed for formation of a violet ring. A violet ring at the junction of the two liquids indicates presence of carbohydrates [26].

Protein: The extract was dissolved in 10 ml of distilled water and filtered through Whatmann No.1 filter paper and the filtrate is subjected to tests for proteins and amino acids.

a) Millon's test: To 2 ml of filtrate, few drops of Millon's reagent are added. The result was observed. A white precipitate indicated presence of proteins.

b) Biuret test: An aliquot of 2 ml of filtrate was treated with drop of 2% copper sulphate solution. To this, 1 ml of ethanol (95%) was added, followed by excess of potassium hydroxide pellets. The pink color in ethanol layer indicated presence of proteins.

Alkaloid: About 50 mg of Solvent free extract was stirred with 3

ml of dilute hydrochloric acid and then filtered thoroughly. The filtrate was tested carefully with various alkaloid reagents as follows:

a) Mayer's test: To a 1 ml of filtrate, few drops of Mayer's reagent are added by the side of the test tube. The white or creamy precipitate indicated test as positive.

b) Wagner's test: To a 1 ml of filtrate, few drops of Wagner's reagent are added by the side of the test tube. The color change was observed. A reddish-brown precipitate confirms the test as positive.

c) Dragendorff's test: To 1 ml of filtrate, 2 ml of Dragendorff's reagent are added and the result was observed carefully. A prominent yellow precipitate confirms the test as positive [27].

Glycosides

a) Borntrager's Test: Extract was boiled with dilute sulphuric acid, filtered and to the filtrate chloroform was added and shaken well. The organic layer was separated to which ammonia is added slowly. Presence of glycoside is denoted by pink to red color in the ammoniacal layer.

b) Legal Test: The test is employed for digitoxose containing glycosides. The extract was dissolved in pyridine, sodium nitroprusside solution was added to it and made alkaline. Pink or red color indicates presence of glycosides.

Terpenoid and steroid: Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed for terpenoid and green bluish color for steroids [28].

Flavonoid: Four millilitres of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and orange color for flavones.

Tannins: To 0.5 ml of extract solution 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue color was observed for gallic tannins and green black for catecholic tannins [29].

Saponins: About 0.2 g of the extract was shaken with 5 ml of distilled water and then heated to boil. Frothing (appearance of creamy mass of small bubbles) shows the presence of saponins [30].

Isolation of curcumin from turmeric rhizomes

Crude extract was dissolved in alcohol and was filtered. The filtrate was concentrated. The concentrate hence obtained was dissolved in benzene. Sodium hydroxide (0.1% w/v) was added to benzene solution. Using a separating funnel curcumin was partitioned between the two layers. Sodium hydroxide layer was taken and curcumin was precipitated by adding dilute hydrochloric acid solution. The precipitate obtained was filtered using vacuum filtration unit and dried. The isolated curcumin was used for further evaluation.

Evaluation of Isolated curcumin by thin layer chromatography (TLC)

TLC of the isolated curcumin was performed on pre-coated silica gel G plates (Stationary Phase) using mixture of n-hexane and ethyl acetate in the ratio 7:3 as solvent system (Mobile Phase). Curcumin was used as standard. Detection was done by spraying the plate with vanillin-sulphuric acid reagent. The R_f values for the separated spots were calculated and compared with R_f value of pure curcumin and values reported in the literature.

Fourier transform infrared spectroscopy (FTIR) of isolated curcumin

An infrared spectrum of isolated curcumin was recorded using FTIR Spectrophotometer (IR Affinity-1, Shimadzu, Japan). The scanning range was 500 to 4000 cm^{-1} and the IR spectra of samples were obtained using potassium bromide disc method.

Estimation of curcumin content in turmeric rhizome

The curcumin content of turmeric rhizome collected from Bhandara district was determined using following procedure:

Preparation of calibration curve for curcumin: Standard curve was obtained using the standard solution in the range of 1 $\mu\text{g}/\text{ml}$ to 4 $\mu\text{g}/\text{ml}$ (about 40%-160% of the standard concentration of 2.5 $\mu\text{g}/\text{ml}$). Absorbances of these solutions were taken at 425nm using UV-Visible spectrophotometry (Model No.1700, Shimadzu Corporation, Tokyo, Japan) having two matched quartz cells with 1 cm path length.

Preparation of Test Solution: Test solution was prepared by adding about 100 mg of crude turmeric rhizome powder in 50 ml of alcohol (95%) contained in 100 ml volumetric flask. The solution was sonicated for about 10 minutes and the volume was made upto 100 ml by alcohol (95%). The solution was filtered and 2 ml of this solution was diluted upto 25 ml by alcohol (95%). Absorbances of the resultant solution was taken by UV spectrophotometry at 425 nm. The procedure was repeated 3 times. The percentage of curcumin content was found out from calibration curve of standard curcumin.

The similar procedure was followed for determination of curcumin content in turmeric rhizomes collected from different regions/districts of Maharashtra, India (Satara, Dhule, Nasik and Mumbai).

Results and Discussion

The results of preliminary phytochemical evaluation of turmeric rhizomes collected from Bhandara district are summarized in Table 1.

The results indicated presence of carbohydrates, Proteins and Amino-acids, Alkaloids, Terpenoids and Flavonoids.

The isolated curcumin powder was bright yellow in colour. The results of TLC are depicted in Table 2. The R_f value intense spot obtained with test solution was matching with R_f value of the standard curcumin. Two more less intense spots were observed with test solution. These spots may be due to the presence of other curcuminoids in the isolated curcumin.

The chemical structure of curcumin is represented in Figure 1 which gives idea about various functional groups present the compound.

The identity of isolated curcumin was further confirmed by FTIR. FTIR spectrum of curcumin showed a characteristic stretching band of O-H at 3512 cm^{-1} . The peak at 3014 cm^{-1} represents C-H Stretching and 1602 cm^{-1} peak was assigned to C=C symmetric aromatic ring stretching. The peak at 1506 cm^{-1} represents C=O, while enol C-O peak was obtained at 1280 cm^{-1} and benzoate trans-C-H vibration was at 962 cm^{-1} . The FTIR spectrum of the curcumin was matching with the FTIR spectrum reported in literature [31]. The FTIR spectrum of isolated curcumin from turmeric rhizome collected from Bhandara district is represented in Figure 2.

The results of TLC and FTIR study confirmed that the isolated compound is curcumin.

The calibration curve constructed for standard curcumin for the estimation of curcumin in the turmeric rhizome collected from different geographical region of Maharashtra is represented in Figure

3. Calibration curve was plotted using different concentration values of curcumin versus their respective absorbances. The regression equation obtained for the straight line was $y=0.1595x-0.0229$, where x is concentration and y is the absorbance. The correlation coefficient was 0.9964, indicating good linearity.

The curcumin content of turmeric rhizomes collected from different geographical regions of Maharashtra were determined using UV-spectroscopy. The results of Curcumin content for the turmeric rhizomes obtained from Bhandara district and other regions of rest of Maharashtra are summarized in Table 3.

It has been observed that the climatic conditions have significant impact on curcumin content of turmeric rhizome. Because of diverse weather conditions and soil type the curcumin content was found to be varied for samples collected from different geographical source. The curcumin content of turmeric obtained from Bhandara district was found to be highest as compared to other geographical regions of Maharashtra.

Conclusion

The medicinal value of turmeric depends upon curcumin content.

Sr. No.	Test	Observation
1	Carbohydrates	+ve
2	Proteins and Amino-acids	+ve
3	Alkaloids	+ve
4	Glycosides	-ve
5	Terpenoids and Steroids	+ve (Terpenoid)
6	Flavonoids	+ve
7	Tannins	-ve
8	Saponins	-ve

+ve = Detected, -ve = Not detected

Table 1: Preliminary phytochemical evaluation.

Sample	R_f value
Standard curcumin	Spot = 0.467
Test sample (Isolated curcumin)	Spot 1 = 0.074
	Spot 2 = 0.224
	Spot 3 = 0.448 (Intense spot)

Table 2: Results of TLC study of curcumin.

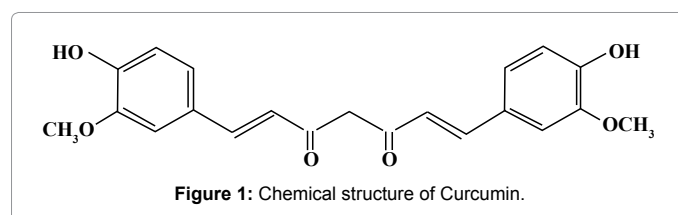


Figure 1: Chemical structure of Curcumin.

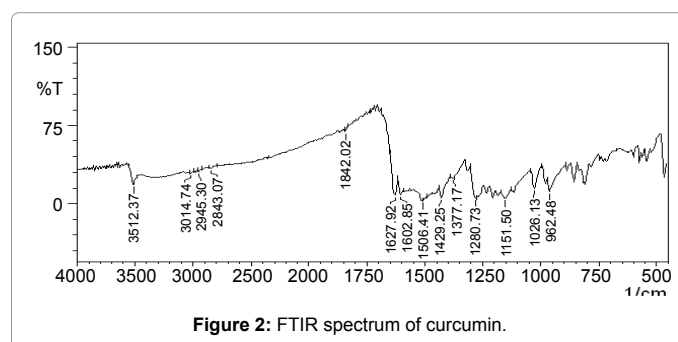


Figure 2: FTIR spectrum of curcumin.

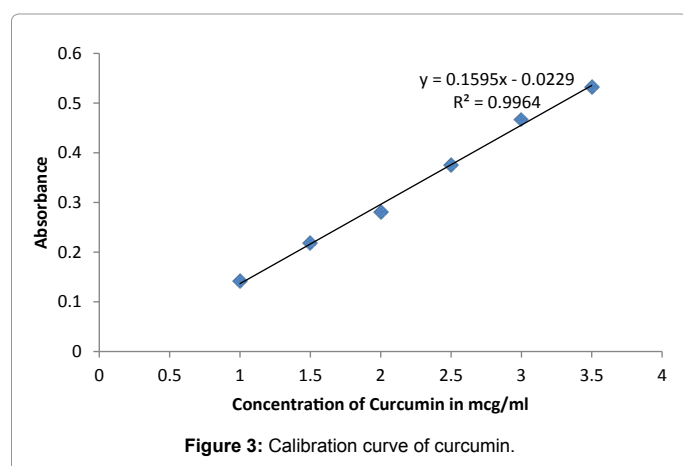


Figure 3: Calibration curve of curcumin.

Region (District)	Bhandara	Satara	Dhule	Nashik	Mumbai
% Curcumin content (w/w)	4.32	3.97	2.85	3.46	3.11

Table 3: Results of curcumin contents of various samples of turmeric rhizomes.

The content of curcumin in turmeric determines its colour, quality, therapeutic utility and hence its cost. Therefore the curcumin content of turmeric is very important economically as well as therapeutically. Samples of turmeric rhizomes were collected from various districts and its curcumin content was determined. Highest curcumin content was found in turmeric rhizomes obtained from Bhandara region of Maharashtra in India. This data will be useful to ayurvedic and pharmaceutical industries for deciding the source /supply of turmeric. Further research work need to be carried out to study the impact of different factors such as geographic localization, harvest time and climate conditions etc. on cultivation in terms of chemical composition of turmeric rhizome.

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