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Evaluation of Curcuminoids in Turmeric Rhizome (*Curcuma longa* L.) Collected from Different Places in India

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ABSTRACT

Turmeric *Curcuma longa* L. is a typical herbaceous plant belongs to Zingiberaceae family, is a major spice widely cultivated in tropical region of Asia. Turmeric is a rich source of curcuminoids, which is responsible for all the physiological properties. The content of curcuminoids may vary in plants grown in different agro-climatic zones. This study was undertaken to evaluate total curcuminoids content in the Ethylene dichloride (EDC) extract of *C. longa* rhizome collected from six different places of India. This study also focused to detect the percentage of three curcuminoids (Curcumin, Demethoxycurcumin and Bisdemethoxycurcumin) present by UV spectrophotometry and HPLC methods. These results provided a clear picture of the percentage of the isomers of Curcumin in various samples collected from different regions of India.

Key words: *Curcuma longa*, Ethylene dichloride (EDC), Curcumin, Demethoxycurcumin and Bisdemethoxycurcumin, UV Spectrophotometry, HPLC.

INTRODUCTION

Turmeric (*Curcuma longa* L.) is a rhizomatous herbaceous perennial plant of the ginger family, Zingiberaceae[1]. It is commonly called as Haldi and is sometimes called "Indian saffron"[2]. It is commonly known for its medicinal values in the Indian traditional systems of medicine[3]. Turmeric is native to tropical South Asia and needs temperatures between 20ºC and 30ºC and a considerable amount of annual rainfall to thrive[4] *Curcuma longa* L. is typical of the herbaceous plant with thick and fleshy rhizomes and leaves in sheaths that characterize the family Zingiberaceae. The underground rhizome, which is processed into the spices, consists of two distinct parts; the egg - shaped primary or mother rhizome, an extension of the stem, and several long cylindrical multi-branched secondary rhizome. Both forms have transverse rings of leaf scars and dents of root scar. Based on their shape, the two forms used to be differentiated in the Western trade, the bulbs as *C. rotunda* and the finger like, cylindrical forms as *C. longa*, though both are from the same plant.[5].

Currently, India is the major producers of turmeric[6]. In India turmeric cultivation is mainly undertaken in Andhra Pradesh, Maharashtra, Assam, Tamil Nadu, Karnataka, Kerala[5]. Other producers in Asia include Bangladesh, Pakisthan, Srilanka, Taiwan, China, Burma and Indonesia[6].

The yellow colour of the rhizomes of the turmeric is due to curcuminoids consisting of curcumin I (diferoyl methane), mono demethoxycurcumin and bisdemethoxycurcumin. The yield is about 5-6%. Besides the above, turmeric also contains 2-4% essential oil and 2-3% of fixed oil. Curcumin I is the main coloring matter and the value of the turmeric products is based on their curcuminoids content. The dye is estimated based

on its absorbance at 416 nms. A large number of publications on curcuminoids have apperared including by WHO - many new properties are being reported[3].

These compounds are polyphenols and produce a pronounced yellow color. They have poor solubility in water at acidic and physiological pH and also hydrolyse rapidly in alkaline solution. Curcuminoids are soluble in dimethyl sulphoxide (DMSO) acetone and ethanol. Curcuminoids are recognized for their broad spectrum of biological activities[8].. The traditional uses of turmeric or natural curcuminoids in folk medicine are multiple, and some of these including antioxidant, anti-inflammatory properties, anti-carcinogenic effects and hypoglycemic effects in humans.[9] The content of total curcuminoids in turmeric powder plays an important role in its antioxidant activity and effectiveness of the product. Thus, a sensitive and accurate analytical procedure is required for the study of Curcumin (C), Demethoxycurcumin (DMC) and Bisdemothoxycurcumin (BDMC) in different turmeric samples[10].

A variety of methods for quantification of the curcuminoids have been reported. Most of these methods are spectrophotometric methods, expressing the total colour content of the sample, gives total curcuminoids content in the sample[8]. HPLC method was sensitive, precise, and accurate for detection and quantification of curcuminoids in the extract of rhizome *Curcuma longa*. [11] Can evaluate percentage composition of each curcuminoids present in the turmeric mixture.

The use of ethylene dichloride has the advantage of being relatively selective extraction of the flavor constituents, water immiscible, nonflammable, and of having sufficiently low boiling point, but requiring no refrigeration.

Our aim is to evaluate curcuminoid content in ethylene dichloride extract of turmeric grown in different agro-climatic conditions using UV spectrophotometric method and HPLC analytical methods.

MATERIAL AND METHODS

Turmeric samples from different places in India were collected. All solvents/Chemicals used were of AR/HPLC grade and obtained from E-Merck. The reference standard of Curcumin was purchased from sigma chemicals co. USA.

Extraction of turmeric

The rhizomes were washed properly with deionised water and dried under sunlight. This was then made into small flakes using a pulveriser and labeled accordingly. Approximately 15gm of samples were taken into a thimble and placed in a soxlet apparatus, 150ml of Ethylene dichloride was added and extracted at 70°C for 6 hours. After completion of extraction the dark brown extract was then filtered, concentrated using rotary evaporator, and finally by vaccum suction gave a crude dried extract which was black orange in color[9]. Each raw sample of turmeric was extracted by the same method. The yield was calculated for each sample.

Estimation of total curcuminoids content using UV spectroscopy analysis methods

Sample Preparation

The sample solution had been prepared by taking an accurately weighed amount of the extract in the range of 0.5g – 1.0g and into a 100 ml volumetric flask and dissolved with acetone, the solution were sonicated for 5 minutes and then madeup to the mark using acetone. Pipetted out 1 ml of this solution in a 50 ml volumetric flask and made up to the mark using acetone. Finally pipette out 1ml portion from 50 ml volumetric flask into a 25 ml volumetric flask and diluted to volume with acetone [12].

Estimation of total curcuminoids:

Determined the absorbance of each solution in 1 cm cells at the wavelength of maximum absorption at about 420 nm using acetone as blank.

Calculation

$$\text{Percentage of curcumin} = \frac{\text{Absorbance of sample x Dilution}}{\text{standard x Wt. of sample}}$$

E1 % of standard = 1.5772 (value for pure curcumin from sigma).

Total curcminoids is calculated using formula:

$$\text{Total curcuminoids} = \frac{\text{Absorbance} \times 100 \times 50 \times 25}{1.5772 \times \text{wt of sample taken}}$$

The content of total curcuminoids in *C. longa* collected from different places is given in table. 1.

Procedure for Estimation of curcuminoids using HPLC

Preparation of Sample

Weighed accurately 25mg sample and dissolve in 25ml acetone. From this pipetted out 1ml and dilute to 5 ml with acetone. Filtered through 0.2µm membrane filter before injection.

Chromatographic Conditions

Samples were analysed by HPLC in a Shimadzer LC 20A0 liquid chromatograph system with SPD-M20AuV detector in isocratic mode. 20µl of sample was injected and the elution was carried out with gradient solvent systems with a flow rate of 1.0ml/min at ambient temperature. Column used was C18.(250X4.6mm), mobile phase 40% THF and 60% water containing 1% citric acid, pH adjusted to 3.0 using concentrated potassium hydroxide solution, measured in wave length 420nm[13].

Percentage of each curcuminoids was calculated using formula

$$\text{Percentage (\%) of curcumin oil} = \frac{\text{Peak area of sample} \times \text{Weight of Std} \times \text{Purity of Std}}{\text{Area of Std} \times \text{weight of the Sample}}$$

Percentage of each curcuminoids present in different turmeric samples collected from different places is shown in table. 2.

RESULTS AND DISCUSSION

Commercially available turmeric rhizomes were collected from different places in India. These samples were extracted with ethylene dichloride and the total curcuminoids were estimated. This study was done to compare total curcuminoids content in turmeric collected from different places using UV spectroscopy with that of HPLC method. The total curcuminoids in different variety of *C. longa* by UV spectroscopy is given in Table. 1. It gives quantification of total color content of sample, spectrophotometric methods lack precision due to interference by other pigments preset in the plant. HPLC method was sensitive, and accurate for quantification of curcuminoids in the ethylene dichloride extract of rhizome *Curcuma longa* and percentage composition of each curcuminoids by HPLC was summarized in table. 2. Curcumin was found to be the major compound in all of the tested varieties. It was found that Rajapuri and Duggirala variety have greater amount of total curcuminoids contents. The HPLC profile of Rajapuri is shown in figure. 2. Nizam variety was found to be the least amount of curcuminoids, the percentage of curcuminoids varying from different varieties of turmeric, may be due to turmeric grown in different agro-climatic conditions like temperature, salt content in water, rainfall, soil condition.

Table 1: UV spectroscopic analysis of six different varieties of turmeric rhizome in India

S. No	Sample	Total extract yielded	Absorbance [O.D] at 430 nm	Weight (g) of extract	Total % of total curcuminoids
1.	Erode	0.889	0.382	0.7756	39.03
2.	Nizam	1.98	0.699	1.8609	29.76
3.	Rajapuri	0.96	0.475	0.7694	48.92
4.	Duggirala	0.76	0.378	0.6605	45.35
5.	Allepey	0.82	0.300	0.7174	33.16
6.	Assam	1.05	0.459	0.9449	38.49

The total extract of the turmeric samples were determined as described in the text.

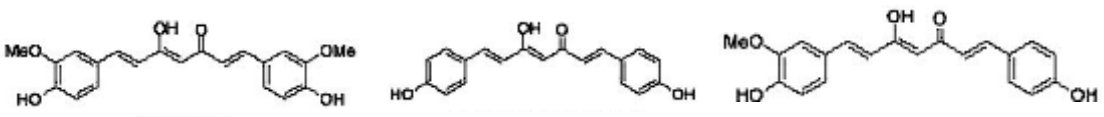
The total curcuminoid content was also estimated and the results are the average of three experiment.

Table 2: HPLC analysis of six different varieties of turmeric rhizome in India

S. No	Sample (from districts)	Curcumin [C]	Demethoxy curcumin [DMC]	Bis-Demethoxy curcumin [BDMC]	Total % of curcuminoids
1.	Erode	24.74%	10.63%	6.34%	41.7
2.	Nizam	13.91%	6.64%	7.30%	27.8
3.	Rajapuri	27.46%	10.24%	5.96%	43.7
4.	Duggirala	27.36%	9.41%	4.75%	41.5
5.	Allepey	23.80%	8.7%	5.04%	37.6
6.	Assam	19.47%	8.86%	8.07%	36.4

The total extract of the turmeric samples were determined as described in the text. The percentage composition of each curcuminoid was estimated and the results are the average of three experiment

UV spectroscopy method and HPLC fingerprints of extract from different location showed a similar pattern of which curcumin was a major component. The average content of curcumin in all extract was 22.79%. Demethoxycurcumin was the second major constituent, whereas bisdemethoxycurcumin was the minor one. The HPLC method appeared to be a recommended method for quantitative analysis of the active compounds in *C. longa* extracts. Rajapuri and



Curcumin Bisdemothoxycurcumin Demethoxycurcumin

Fig. 1: The chemical structures of three curcuminoids

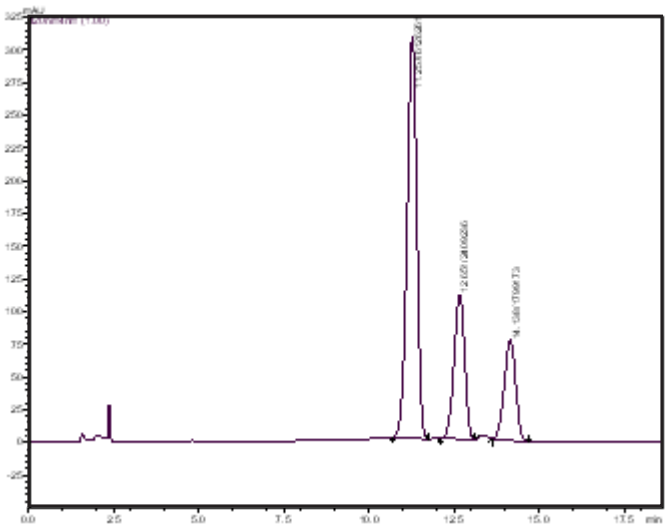


Fig. 2: The HPLC profile of Rajapuri variety turmeric peak showing Curcumin, Demethoxycurcumin, Bisdemethoxycurcumin

Duggirala variety have greater amount of curcuminoids in both the method. Hence these two varieties which have higher curcuminoids content may be good sources for the isolation of each curcuminoids. And after separation and purification of each curcuminoids it can be tested for pharmacological activity such as antioxidant, anti-inflammatory, anticancer activity.

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