

Isolation and Extraction of Curcumin from Three Different Varieties of *Curcuma Longa L* - A Comparative Study

Gokhul. V^{*1}, S. Yuvapriya¹, M. Chandramohan and P. Muthukumar¹

¹Department of Biotechnology, Kumaraguru College of Technology, Coimbatore- 641 049

²Microcore Private Limited, Checkmedu, Erode - 638115 Tamil Nadu

*E-mail: vgokhul2012@gmail.com

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Abstract

Curcuminoids, is one of the naturally occurring yellow pigments which consist of curcumin, demethoxycurcumin and bisdemethoxycurcumin, in *Curcuma longa* rhizome have been popularly used in traditional medicine and some extent, it is one of the active ingredients in drugs and cosmetics. In this study, natural dye curcumin was extracted from 3 different varieties of 10 different rhizomes. Among 3 varieties, salem variety large finger (1.92 %) and bulb(1.88%) shows higher yield of curcumin. Where as No. 8 variety, in large finger (1.81%) and bulb (1.46%) of curcumin. In case of Erode variety, 1.36% from large finger and 1.33% from bulb. For separation of curcumin from demethoxycurcumin and bisdemethoxycurcumin, the mobile phase was prepared using chloroform and methanol in the ratio of 95:5 respectively. The maximum absorbance of the standard curcuminoid was observed at the wavelength of 420 nm with the absorbance of 0.545. Similarly, UV absorption spectra of natural dye solution extracted from *Curcuma longa.L* was 0.743 at 420 nm. From the study, data are useful for further development of standard procedures. Whis this, we can improve extraction efficiency and purification of curcumin from *Curcuma longa. L*.

Keywords: Turmeric rhizome, Curcumin, *Curcuma longa*, Curcuminoids, TLC

1. Introduction

Turmeric (*Curcuma longa L.*) is the shining star among the cornucopia of traditional medicinal plants and it's posses a traditional medicinal value Indian system of medicine [1]. In North India, turmeric is commonly called "haldi," a word derived from the Sanskrit word *haridra*, and in the south it is called "manjal," a word that is frequently used in ancient Tamil literature. It has a long history of usage in traditional medicine in India and China. Ancient Indians have known the medicinal properties of turmeric, thus curcumin, for several millennia. The bio-active polyphenol component of turmeric is curcumin, also known as diferuloylmethane, with an ability to prevent and cure diseases. Turmeric contains about 2-5% curcumin alone. Commercial curcumin contains three main types of curcuminoids, i.e., curcumin (diferuloylmethane or "Curcumin I" about 77%), demethoxy curcumin ("Curcumin II" ~17%) and bis demethoxy curcumin ("Curcumin III" ~3%). Curcumin (diferuloylmethane renders its bright yellow colour to turmeric. These include tetrahydrocurcumin (antioxidative), 4-hydroxy-3-methoxybenzoic acid methyl ester (HMBME), aromatic enone and dienone analogues, metal

chelates of synthetic curcuminoids etc. It has been used for a thousand years as a spice, coloring agent in food processing industries, household medicine and insect repellent [2]. With respect to biological activities of Turmeric, under *in vitro* and *in vivo* studies confirmed that turmeric extracts show Broad biological activities, such as antiinflammatory [3], antibacterial [4], antidepressant [5], antidiabetic [6], antitumor [7], imunomodulatory [8] and gastroprotective properties [9]. Extensive studies carried out by researchers around the globe have clearly demonstrated curcumin's great potential as a thercurcuminapeutic agent, and have paved the way towards conducting clinical trials for a variety of diseases including cancer, cardiovascular, neurological and gastrointestinal disorders, multiple sclerosis, diabetes type II, skin diseases, cystic fibrosis, cataract etc. At present, a variety of methods for quantitative analysis of curcuminoid content were reported. Most of them are spectrophotometric methods [10-12] In this study, 10 different rhizomes from 3 different local varieties of *Curcuma longa* were used to extract curcumin and quantified by spectrophotometrically.

2. Materials and methods

2.1 Plant material collection

Three different turmeric rhizomes such as Erode variety, Salem variety, No.8 variety were collected from the local market of Erode District, Tamil Nadu. Collected rhizome initially washed twice with distilled water and segregated into 10 different rhizomes for further studies (Table 1). Washed rhizomes sliced into small pieces and shade dried for a period of one week.

Table 1: Three different varieties of 10 different rhizomes

Sr. No.	Variety	Rhizome size
	Erode variety	
1.		Bulb
2.		Small finger
3.		Medium finger
4.		Large finger
	Salem variety	
5.		Bulb
6.		Small finger
7.		Medium finger
8.		Large finger
	No.8 variety	
9.		Bulb
10.		Large finger

2.2 Extraction of curcuminoids

0.1g of the sample was taken and 30 ml of ethanol was added. This was Shaken well and put it in the soxhlet in 2 hours 30 minutes. The sample was filtered in a 100 ml measuring flask. The volume was Made up to 100 ml. 20 ml of this was taken and made up to 250 ml.

2.3 Extraction of curcumin from oleo resins

2.3.1 Preparation of Sample

The extract was then filtered. The filtrate thus obtained contains curcuminoids complexed with oleoresins. To separate curcuminoids from oleoresin different solvents were used (hexane, acetone, and chloroform) [13] The oleoresins show best solubility in hexane and curcuminoids are merely not soluble in hexane. Thus curcuminoids were extracted.

2.3.2 Separation of Oleoresin

The oleo resin was extracted from the given sample with the help of hexane and the mixture was run in the rotary vacuum evaporator. Add about 0.5 ml of the oleoresin to 10 ml hexane. Spectrometric reading was taken using hexane as blank. The curcumin is present in the bottom layer of Separating funnel and oleoresin in the top. The curcumin obtained is then dried and powdered.

2.4 Estimations of % curcumin complexed with oleo resin

The turmeric sample was fed with ethanol, such that the concentration of turmeric remains is high. This was then filtered and extracted using a rotary vacuum evaporator. 20 ml of this highly concentrated syrup was taken in a separating funnel. It was then added with 20 ml of hexane. Since oleo resins are highly soluble in hexane and curcuminoids are poorly soluble, oleoresin and curcuminoids were separated. It was then found that nearly 40- 47% of oleoresin complex is composed of curcuminoids. The samples were read spectrophotometrically. The maximum absorbance of curcumin is at 425 nm.

Calculation

$$\text{Absorbance (A)} = a_1 / (L \cdot c)$$

$$\% \text{ curcumin present (along with oleo resin) } B = \frac{a_2 \cdot 125}{L \cdot A \cdot m}$$

$$\% \text{ curcumin present (without oleoresin) } = B \cdot 45\%$$

A=Absorbance of the sample

a1= absorbance of standard curcumin

a2=absorbance of the sample

L=length of the cuvette (path length)

M=mass of the sample

2.5 Estimation of curcuminoids by spectrophotometric analysis:

2.5.1 Preparation of standard

25 mg of the standard curcumin was taken in a 100 ml standard measuring flask. The volume was made up to 100 ml with ethanol (0.25 mg/ml). 1ml of this solution was taken in a 100 ml standard measuring flask and made up to 100 ml (0.0025 mg/ml).

2.5.2 Separation of curcuminoids by TLC

Curcuminoids were profiled using TLC. TLC pre-coated silica gel was taken and separated into 3 columns. The standard curcuminoid was prepared by adding 1 mg of pure curcuminoid and 50 ml of ethanol. The mobile phase was prepared using chloroform and methanol in the ratio of 95:5 respectively. This setup was left for some time until the mobile phase reaches the $\frac{3}{4}$ th of the TLC sheet. The three curcuminoids were separated in to 3 different single bands.

2.5.3 Spectrophotometric analysis

The 10 samples were read spectrophotometric scanning and shows high peak at 420 nm. For spectrometric scanning ethanol is taken as blank, stock standard curcuminoid as reference and sample for analysis.

3. Results and discussion

3.1 Extraction and estimation of curcuminoids

The 3 different varieties of turmeric samples were analyzed for curcumin content. Figure 1 shows the extraction of curcumin from oleoresins.

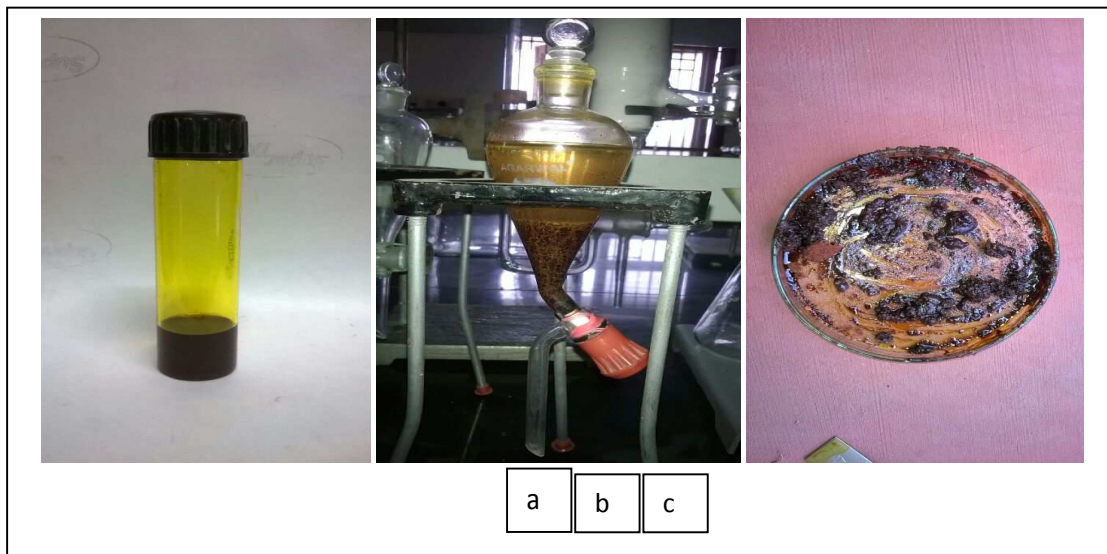


Figure 1: Extraction of curcumin from oleoresins
(a. Oleoresin; b. Extracted curcumin; c. Powdered curcumin)

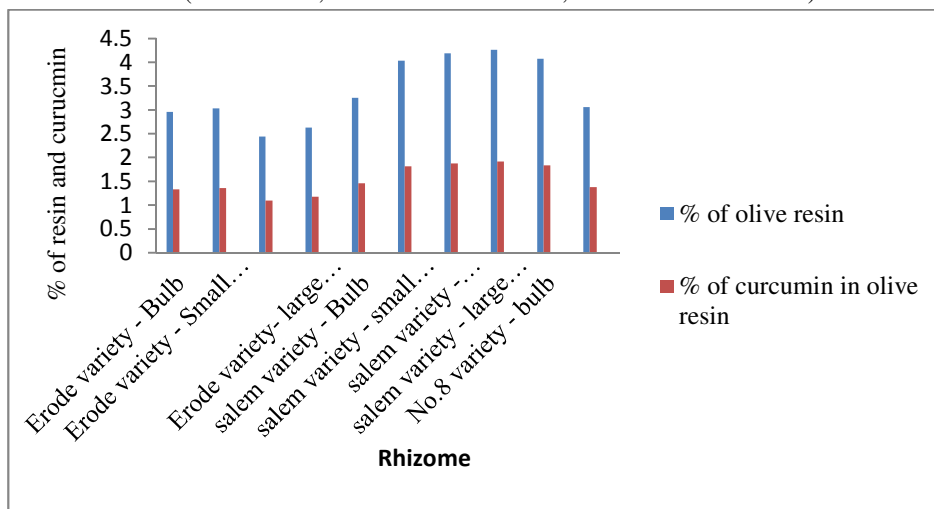


Figure 2: Percentage of curcumin from 3 varieties of 10 different rhizomes

From the analysis it was found that the curcumin content was found to be higher in Salem variety. The other 2 varieties contain the curcumin percentage from 1.10% - 1.50% whereas the Salem variety is found to contain the higher value of curcumin content. It contains around 1.90% in most of its categories.

Figure 2 shows total curcumin content of 3 varieties of 10 different rhizomes. It is clear that the large fingers of all the varieties contain large amount of curcumin respective to all the other categories of the same variety. Next to the large finger, the bulb of all varieties was found to have large amount of curcuminoids. From the above data, it was clear that

Salem variety is found to have high amount of curcuminoids. And also, in all varieties, large finger category is found to have high amount of curcuminoids. Also the oleoresin compound is found to have maximum absorption at ultraviolet region from 260 nm - 290 nm.

3.2 Separation of curcumoids by TLC

The mobile phase was prepared using chloroform and methanol in the ratio of 95:5 respectively. Sample was run on TLC (figure:3) we got three different single spots of Curcumin, Demethoxycurcumin, and Bisdemethoxycurcumin.

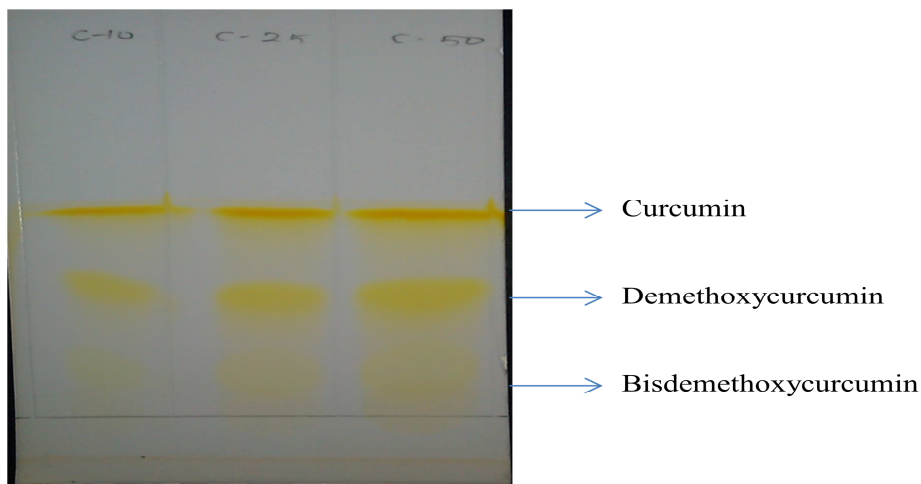


Figure 3: TLC result of Ethanolic extract of standard curcumin

3.3. Spectrophotometric analysis

The graph for the standard solution is obtained as:

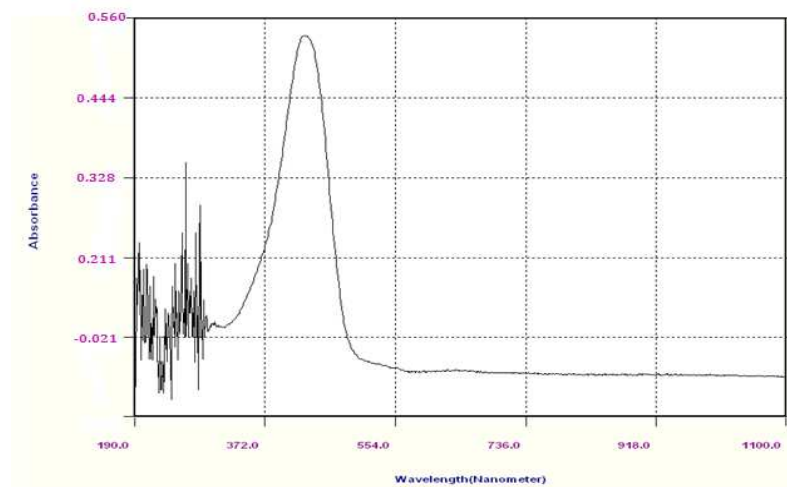


Figure 4: UV absorption spectra of curcumin standard

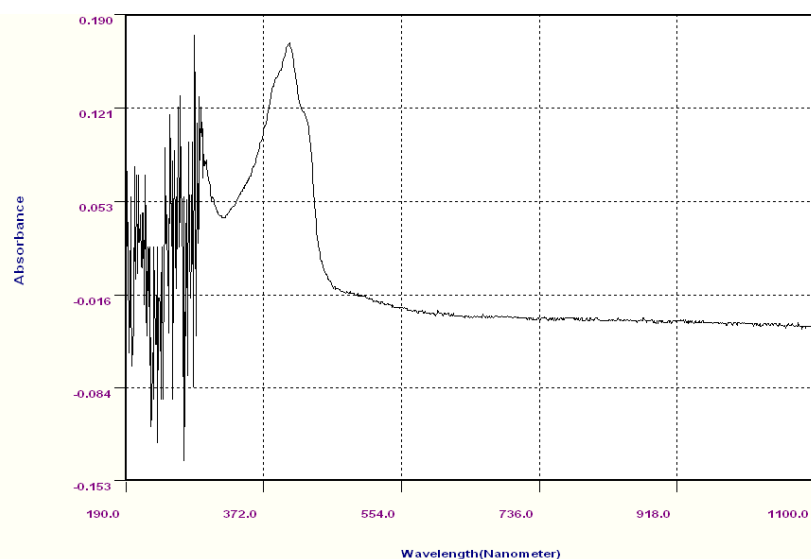


Figure 5: UV absorption spectra of natural dye solution extracted from *Curcuma longa. L*

The above graph shows the maximum absorbance of the standard curcuminoid to be at the wavelength of 420 nm and absorbance of 0.545. Similarly, UV absorption spectra of natural dye solution extracted from *Curcuma longa.L* was carried out (figure 4). Figure 5 shows maximum absorbance of the sample is at 420 nm and absorbance is about 0.743. Of all the samples analyzed, Salem variety-large finger shows maximum absorbance.

4. Conclusion

In this study, it is confirmed that large finger of salem variety having higher amount of curcumin compare to other two varieties of rhizome. With respect to curcumin extraction, solvent (ethanol) play a major role for maximum recovery of curcumin. Results are encouraging; it is felt that, we need to improve extraction procedure as well as conditions for maximum extraction of curcumin from rhizome of *Curcuma longa.L*. From the results obtained, we conclude that, Among these 3 varieties, salem variety, both bulb and Large fingers shows maximum curcumin content. Not only salem variety, all three varieties, bulb and large finger shows maximum content of curcumin per unit weight of rhizome.

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