Extraction and purification of curcuminoids from
Turmeric (curcuma longa L.)

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Abstract - Turmeric comes from the root of the Curcuma longa plant and has a tough brown skin and a deep orange flesh. Turmeric has long been used as a powerful anti-inflammatory in both the Chinese and Indian systems of medicine. Turmeric was traditionally called “Indian saffron” because of its deep yellow-orange color and has been used throughout history as a condiment, healing remedy and textile dye. Turmeric is rich in curcuminoids. Curcuminoids vary in chemical structures, Physico-chemical characteristics. The present work reports on extraction method using Soxhlet extractor. Isolation and purification of curcuminoids was carried out by column chromatography. The quantification of curcumin in maximum resultant extract (by methanol) was performed using pre validated HPLC methodology. Percentage yield of curcumin by HPLC was 12.39%.extracted curcuminoids were subjected to spectrophotometer to check it’s percentage amount in extracted sample. Different solvent were used for extraction, among them methanol showed maximum yield of each curcuminoids. Separation of curcuminoids were tested in TLC chloroform: methanol at 95:5 showed RF value at 0.67, 0.6, 0.506 as curcumin, dimethoxycurcumin, bis demethoxycurcumin respectively. the methanol extract was subjected to silica gel column chromatography with chloroform: methanol at increasing polarity followed by TLC to check purity of extracted curcumin.

I. INTRODUCTION

Turmeric is commonly known for its medicinal values in the Indian traditional systems of medicine. Turmeric has been used traditionally in “ayurvedic medicine” as an antiseptic, wound healing, and anti inflammatory compounds. Curcumin, dimethoxycurcumin and bis demethoxycurcumin is a dietary photochemical obtained from dried rhizomes of the turmeric plant (curcuma Longa). Curcumin is a main coloring substance in Curcuma longa and two related compounds, demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC), are altogether known as curcuminoid. The value of the turmericic products is based on their curcuminoid content. Quantitative estimation of curcuminoids can be carried out photometrically based on its absorbance at 420 nm .The principal colouring components of curcumin exhibit a keto-enol tautomerism and antioxidative properties.

The antioxidant property of curcumin can prevent rancidity of foods and provide foodstuffs containing less oxidized fat or free radicals. The powerful anti-oxidation property of curcumin has an important role in keeping curry for a long time without it turning rancid. Curcuminoids are poorly soluble in the hydrocarbon solvents. Curcumin is an oil soluble pigment, practically insoluble in water at acidic and neutral pH, soluble in alkali. Preparations of water-soluble curcumin by incorporation into various surfactant micellar systems (acetone, methanol, and ethanol) have been reported [1]. It is stable at high temperatures and in acids, but unstable in alkaline conditions and in the presence of light.

Curcumin is widely used to colour many foods [2]. Curcumin is listed for use in dairy products, fats, oils and fat emulsions, edible ices, fruit and vegetable products, confectionery, cereal products, bakery wares, meat and meat products, fish and fish products, eggs and eggs products, spices, soups, sauces and protein products. Curcumin, the most active curcuminoid found in turmeric, has been shown to possess a multitude of beneficial effects in the treatment of cancers, cardiovascular disease, and inflammation [3]. A daily dose of 2 grams of Curcuma domestic extract was found to provide pain relief that was equivalent to ibuprofen for the relief of pain associated with osteoarthritis of the knee[3]. Commercial capsules of curcumin contain piperine, a compound found in
pepper which aids absorption of curcumin into the blood stream. Curcumin might be potentially useful in some kidney diseases by preventing renal inflammation.[12]

The most conventional method for extraction of curcumin has been Soxhlet extraction with heating time ranging as long as up to 12 h. the Soxhlet extraction process is a time consuming, laborious and makes use of bulk amount of organic solvents, as the heating process continues for long hours, the approach possibly involves high risk of thermal decomposition of target molecules .Soxhlet extraction method operate through cell permeation followed by solubilizing the active constituents by the extracting solvent. Curcumin present inside the oleoresin cells which in turn is covered by tightly packed cork cells probably makes the entry route for the solvent and time consuming. A number of studies are undertaken to separate curcuminoid pigments by thin layer chromatography (TLC), column chromatography. HPLC method was sensitive, precise, and accurate for detection and quantification of curcuminoids in the extract of rhizomes curcuma longa.

II. MATERIALS AND METHODS

Curcuma longa (turmeric) collected from Satara dist (Rajapuri). All solvents /chemicals used were of AR/HPLC grade and obtained from SRL. The reference curcumin purchased from HIGHMEDIA Company in India.

METHODS

1. Extraction of curcuminoids

Fresh rhizomes were cleaned, washed with deionised water, sliced and dried in the sun for one week and again. Dried at 50˚c in a hot air oven for six hours. Dried rhizomes were cut in small pieces, powdered by electronic mill. Six gm of sample were taken into a thimble and placed in a Soxhlet apparatus, were set up with various solvent from non polar to polar. 250 ml of solvent was added and extracted according to their boiling point for seven hours. The extract was concentrated in rotary evaporator .this crude curcuminoids mixture contained curcumin, demethoxycurcumin and bis demethoxycurcumin.

2. Estimation of curcuminoids: by spectrophotometric analysis Procedure

i) Preparation of standard: 1mg of pure curcumin was dissolved into methanol and water such that concentration was 60µg/ml, got reading at 420 nm. They are shown in graph no (3). Refer this graph as standard.

ii) Spectrophotometric analysis:

To find out concentration of extracted sample by using spectrophotometer, 1 mg of sample were mixed with methanol and water same as standard solution. OD was taken at 420 nm. Because all three components of curcuminoids has λmax at 420 nm.

iii) Separation of curcuminoids by TLC:

Methanol extracts were tested on TLC for presence different curcuminoids. The TLC pre-coated silica gel. Plate were developed using glass beaker , which was pre-saturated with mobile phase for 20 min and each plate was developed up to a height of about 6.8 cm. chloroform : methanol mobile phase was used with composition 95: 5. After development, plates were removed and dried. spots were analyzed.

III. COLUMN CHROMATOGRAPHY

A. Sample preparation

Six gram of fine powdered rhizomes were subjected to Soxhlet extraction and solvent used were methanol for seven hours. The extract was concentrated in rotary evaporator .this crude curcuminoids mixture contained curcumin, demethoxycurcumin and bis demethoxycurcumin.

B. Silica gel Column chromatography

Methanol extract was subjected to Column chromatography in silica gel glass column. About 1gm of crude curcuminoids were mixed with 1 ml methanol and loaded on to the column (34x1.5cm) and eluted with chloroform : methanol followed by methanol with increasing polarity. All the collected fractions were subjected to TLC and detected as yellow Spots.

IV. RESULT AND DISCUSSION:

After drying Soxhlet extract were weighted and weight percentage of curcuminoids were calculated those are shown in table (no.1). Maximum concentration of curcuminoids was obtained in methanol extract in the form of dark black orange colour .

Concentrations of extracted dried sample were analyzed on spectrophotometer at 420 nm with respect to standard graph (fig 3). We got regration values such as 0.998, 0.997, 0.986, and 0.991 for methanol (with conc. 60µg/ml), acetone (100µg/ml), ethyl acetate (200µg/ml) and chloroform (100µg/ml) respectively.

Sample was run on TLC (fig.4), we got three different spots of C, DMC, BDNC. RF values for these spots were calculated and found to be similar that of reported values [10] (Table no.2). According to the RF values curcumin was analyzed by running standard
curcumin along with the sample. The HPLC profile of extracted crude curcuminoids showed curcumin and its analogous DMC and BDMC to present in chromatogram (fig 2B.) Spiking in methanol extract was compared to that of standard curcumin HPLC profile (fig 2A)

**Screening of solvent for extraction**

Different solvents with varying polarity were used for extraction of curcuminoids from turmeric rhizomes. Various solvents used were chloroform, ethyl acetate, methanol, acetone. After concentrating each extract total yield were determined and percentage yield of curcuminoids in the extract were analyzed by spectrophotometer at 420nm. Extract was also subjected to the TLC and got RF values as 0.67, 0.6, 0.506 for C, DMC, BDMC respectively. According to this, curcumin was found to be the major compound followed by DMC and BDMC. Hence methanol used as extracting solvent for curcumin extraction.

**Table 1. weight % extract of curcuminoids**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>% wt. extract</th>
<th>Dry wt. (from 6 gm turmeric)</th>
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<tbody>
<tr>
<td>Acetone</td>
<td>4.6%</td>
<td>0.28gm</td>
</tr>
<tr>
<td>Chloroform</td>
<td>4.3%</td>
<td>0.26gm</td>
</tr>
<tr>
<td>Methanol</td>
<td>5.6%</td>
<td>0.34gm</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>4.5%</td>
<td>0.27gm</td>
</tr>
</tbody>
</table>

Total weight % extract of curcuminoid was determined as describe in the text.

**Table 2. Separation of curcuminoids by TLC**

<table>
<thead>
<tr>
<th>Sr no</th>
<th>TLC Mobile phase</th>
<th>Ratio</th>
<th>RF values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chloroform:</td>
<td></td>
<td>C</td>
</tr>
<tr>
<td>1</td>
<td>methanol 19:1</td>
<td></td>
<td>0.67</td>
</tr>
<tr>
<td>2</td>
<td>methanol 19:1</td>
<td></td>
<td>0.75</td>
</tr>
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</table>

Each plate was developed to a height of about 6.8cm

C=curcumin, DMC=demethoxycurcumin, BDMC=bisdemethoxycurcumin

**Fig. 3. Standard calibration curve of curcumin**

\[ y = 0.128x \]
\[ R^2 = 0.998 \]

**Fig. 4. TLC result of methanol extract**
REFERENCES:


2. I.Stanconic.”curcumin,chemical and technical assessment”(CTA).


