RESEARCH ARTICLE Am. J. PharmTech Res. 2012; 2(6) ISSN: 2249-3387

AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Enzymatic Extraction of Curcumin from *Curcuma Longa* Rhizome

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ABSTRACT

The selective enzyme assisted extraction of curcumin was developed and optimized from C*urcuma longa*. When water was used as solvent, 3% of curcumin were extracted representing a search for an interesting alternative for the extraction in industrial processes. A selective extraction process after the treatment with enzymes is proposed by using 30% (v/v) methanol which releases up to 15% of the curcumin, present in the rhizome. The optimal conditions were as follows: pH value was 5.5, concentration of cellulase solution was 2.5 mg/mL, incubation time was 8 h, incubation temperature was 50 ºC and solid:solvent ratio was 1:8 . Enzyme-assisted extraction was proved to be environment-friendly and economical, and could be used in natural product extraction in large scale.

Keywords: *Curcuma longa*, Total curcuminoids, enzymatic extraction

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Received 30 October 2012, Accepted 21 November 2012

Please cite this article in press as Yadav S *et al.*, Enzymatic Extraction of Curcumin from *Curcuma Longa* Rhizome. American Journal of PharmTech Research 2012.

INTRODUCTION

Turmeric (Curcuma longa), a common Indian dietary pigment and spice has been shown to possess a wide range of therapeutic utilities in the traditional Indian Medicine. It's role in wound healing, urinary tract infection, liver ailments are well documented[1]. The active component of turmeric identified as curcumin exhibits a variety of pharmacological effects including antioxidant, adaptogenic, anti-inflammatory and anti-infectious activities[2-3]. However, there are few studies presently available that document its cardioprotective potential[4]. The major and characteristic active components of the herb are three curcuminoids (Fig. 1) which have been studied for their anti-inflammatory, anti-Alzheimer, anti-cancer, anti-oxidant, hypoglycemic and anti-microbial features[5-10]. The curcuminoids are the basis for the quality control of *C. longa* and other plant-derived drugs from the herb[11].

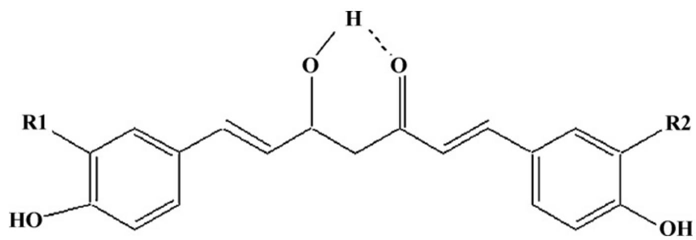

Figure. 1. Chemical structure of Curcuminoids. Curcumin: R1 =R2 =OCH3; desmethoxycurcumin:R1 =H, R2 =OCH3; bisdesmethoxycurcumin: R1 =R2 =H.

Although many reports about extracting curcuminoids by using different extraction methods have been published[12-13], some disadvantages still exist such as large organic solvent consumption, and low efficiency. Therefore, high efficient extraction method of curcumin represents a hot spot in C*urcuma longa* research. Enzyme-assisted extraction is a method applied to the study of secondary metabolites releasing from biogenic materials. It possess the advantages of environmental- friendship, high efficiency and easy operation process. It has been represented as an alternative way for natural product extraction. Hydrolytic enzymes including cellulase, beta-glucosidase and pectinase, which are commonly used in extraction[14-16], can interact on cell wall, break down its structural integrity so as to increase the releasing of curcumin notably.

The main aim of the present study is to examine and optimize the process of enzyme-assisted extraction of curcumin from the rhizomes of *Curcuma longa*. For this purpose, the selection of enzyme type, pH and the concentration of enzyme solution, incubation time and temperature were studied, in order to obtain high yields of above natural products economically and environmental friendly.

MATERIAL AND METHODS

Plant material

The authenticated dry turmeric was collected and ground into fine powder using a high-speed blender. The dry, ground turmeric was packed in a plastic bag, sealed and kept in the refrigerator (5°C) until used.

Chemicals and reagents

Curcumin, and Cellulase were provided by Radiant Research Pvt. Ltd as gift sample. Methanol of analytical grade were purchased from Rankem Ltd. and double-distilled water was used in all experiments

Enzyme-assisted extraction and pretreatment

Cellulase was quantified accurately and dispersed in deionized water to obtain enzyme solutions of certain concentrations (0.25-4 mg/mL). 100 g dry powder was added to the enzymatic solution and adjusted to certain pH (3.5-7.0) with 0.1 M HCl solution and shaken on a flat-bed orbital shaker for a period of time (1-10 hr) at certain temperature (30-55ºC). After the treatment fulfilled, the extract was filtered through Whatmann filter paper no 1. Filtrate collected was concentrated in vacuo (55ºC) in a rotary evaporator and analyzed by spectrophotometer. All the experiments were performed in triplicate.

Quantification of Curcumin

Quantitative determination of total curcuminoids content in each sample of *C. longa* was performed by the described method[17-19] with a spectrophotometer in the visible range at 420 nm using a 1.0 cm quartz cell and absorbance was measured. Total curcuminoids content was calculated using a standard curve. Analysis of each sample was done in triplicate. For preparation of standard solution, standard curcumin (2.00 mg) was accurately weighed and transferred to a 5-ml volumetric flask. Methanol was added and adjusted to a final concentration of 400 □ g/ml. From this solution, concentrations of 0.8, 1.6, 2.0, 2.4 and 3.2 □ g/ml were prepared and used for preparation of the calibration curve. For preparation of sample solution from turmeric, the extract (300.00 mg) of each sample was separately transferred to a 10-ml volumetric flask. Tetrahydrofuran was added to volume and mixed. The mixture was set aside at room temperature for 24 h with frequent shaking. One milliliter of the clear supernatant liquid was transferred and diluted with methanol to 25 ml volume. This solution (1 ml) was then transferred to a 50-ml volumetric flask, and diluted to volume with methanol and % curcumin was calculated.

Statistical analysis

All results were subjected to statistical analyses. Mean values of all data were obtained from triplicate experiment and significance of differences was evaluated.

RESULTS AND DISCUSSION

Cellulase catalyzes the breakdown of cellulose into glucose, cellobiose and higher glucose polymers; therefore, it is used to extract curcuminoids from turmeric rhizome.

Effect of pH value of enzyme solution

It has been reported that the activity of cellulase can be influenced by pH very much, and it is believed that it works better with pH<7 [20]. The effect of pH was studied in this experiment in order to pick out the proper pH value which would make the cellulase work best. Figure.2 shows the effect of pH on the extraction yields of the curcumin. It can be observed that the yields of curcumin varied unregularly with different pH value. The yields of curcumin achieved the maximum at pH 5.5.

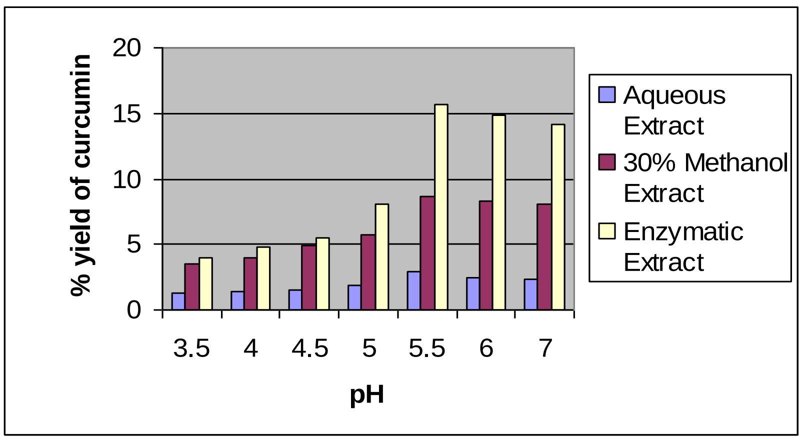

Figure. 2. Effect of pH on the yield of curcumin

Effect of enzyme concentration

The effect of concentration of cellulase on the extraction yields of curcumin was studied and the results are shown in Figure. 3. According to the results, it is obvious that with the increasing of cellulase concentration, the yields of these curcumin increased gradually until 2.5 mg/mL. Comparing with the yields of curcumin at the concentration of 2.5 mg/mL, 4.0 mg/mL did not show distinct advantage. Considering the economic influence, 2.5 mg/mL was selected for the pretreatment of the extraction process.

Effect of incubation time

Figure 4 showed the results of the effect of cellulase incubation time on the extraction yields of curcumin. The yields of these curcumin increased notablely along with the extending of incubation time. The yields of curcumin reached the peak (15.68 mg/g) at 8 h. And the yields began to decrease in additional time. Thus, 8 h was considered to be enough for cellulase to catalyze the hydrolysis of cell wall in rhizome of *Curcuma longa*.

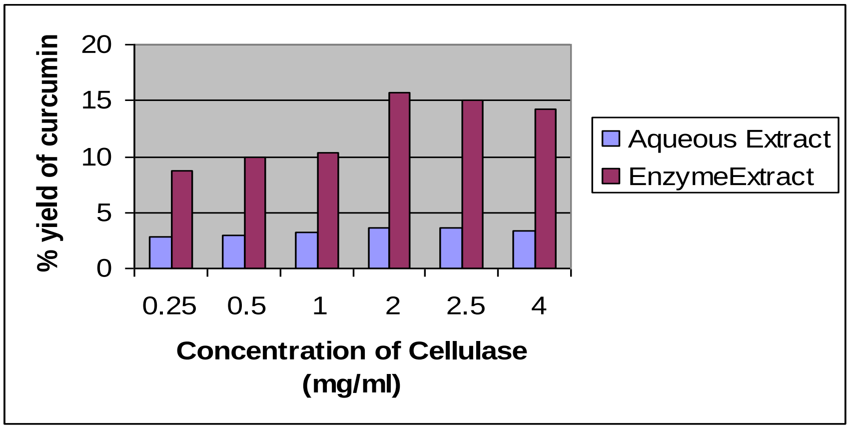

Figure. 3. Effect of conc. of Cellulase on yield of curcumin

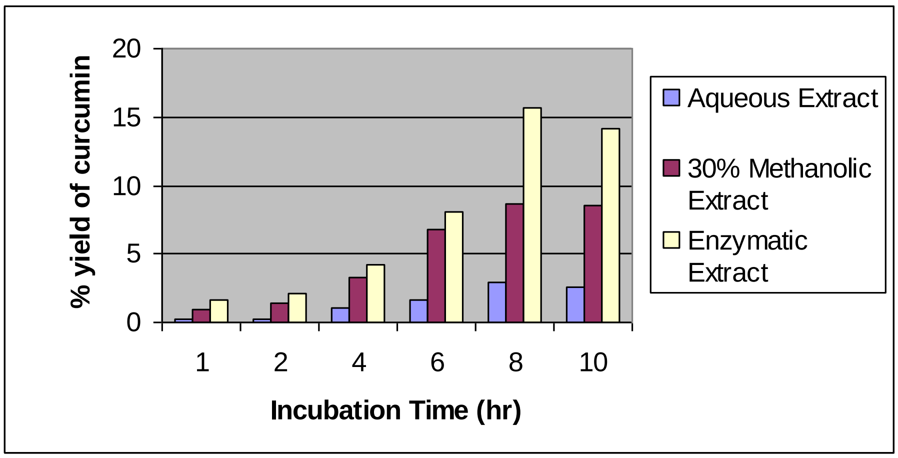

Figure. 4. Effect of Incubation time on the yield of curcumin

Solid Solvent Ratio

Different solid: solvent ratios ranging from 1:2 to 1: 12 were studied and the optimum ratio for the extraction of curcumin was found to be 1:8 g/ml (Figure 5).

Effect of temperature on enzyme activity

The study of the thermal effect on the extraction yields was also carried out in this work. The results are presented in Figure.6. The yields of curcumin varied with the change in temperature. With the increase in temperature, the yields of curcumin increased gradually until 50 ºC. The yields of curcumin increased upto 15.68 mg/ml. Therefore, 50 ºC was chosen for cellulase incubation temperature in this assay.

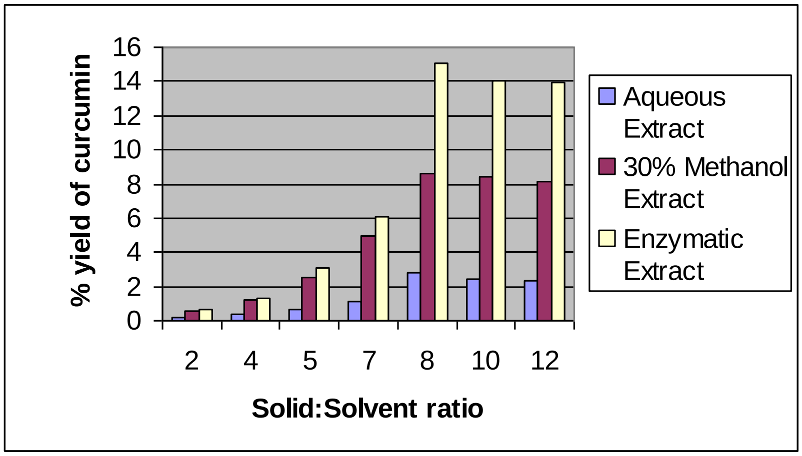

Figure. 5. Effect of Solid: Solvent ratio on the yield of curcumin

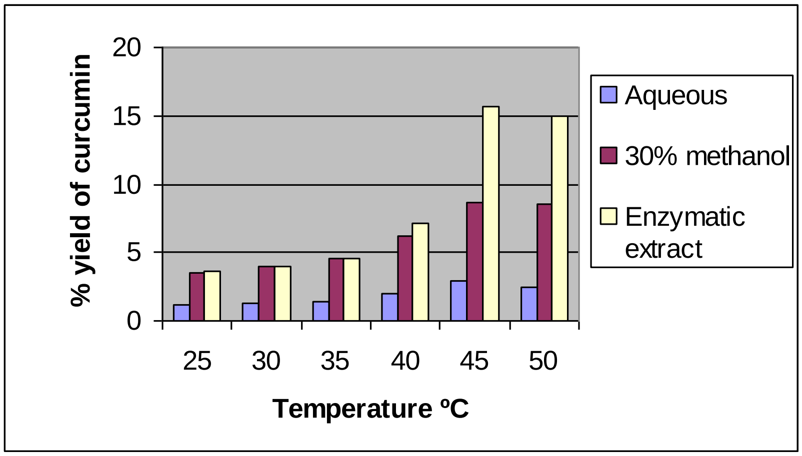

Figure. 6. Effect of Temperature on the yield of curcumin

CONCLUSION

Enzyme–assisted extraction of curcumin, from rhizomes of *Curcuma longa* was carried out in present study. The effect of hydrolytic enzyme was studied and it was proved that cellulose at a concentration of 2.5mg/ml to be most effective for extracting curcumin from rhizome of *Curcuma longa*. As per the economic effect, cellulase was chosen for the treatment of the rhizome. Enzyme-assisted extraction may provide a feasible way for the extraction of curcumin from *Curcuma longa* and other species of turmeric, it has the advantages of environment friendship, lower cost, easy operation and higher efficiency, and it is promising for industry application broadly.

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