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Isolation and characterization of curcumin from powdered rhizomes of turmeric plant marketed in Maragheh city of Iran with soxhlet technique

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Abstract

Turmeric (*Curcuma longa* L.) is extensively used as a spice, food preservative and coloring material. It has been used in traditional medicine for various diseases. Curcumin, the main yellow bioactive component of turmeric has been shown to have a wide spectrum of biological actions. Heretofore, it has been reported that natural colored extracts were isolated from turmeric rhizomes with many methods such as maceration, digestion, microwave and infusion. In this paper, it was tried to isolate and characterize curcumin from the curcumin rhizomes marketed in Maragheh city of Iran by soxhlet extraction technique in methanol solvent. The advantage of this technique is the isolation of large amounts of curcumin (208 mg from 25 g turmeric rhizomes powder) with smaller quantity of methanol.

Keywords: Turmeric; Currcuma longa L.; curcumin; isolation; soxhlet extractor.

Introduction

Turmeric or *Curcuma langa* L. is a tropical perennial plant from the ginger family (Zingiberaceae) [1]. It has a short stem with large longish leaves. It bears egg-shaped or elongated rhizomes, often branched and of a brownish-yellow color [2]. Turmeric is widely consumed in the countries of its origin for a variety of uses, for example, as a dietary pigment, a dietary spice, and a medicine for

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the treatment of various illnesses [3]. It is used in the textile and pharmaceutical industries [4]. Current traditional medicine uses it for biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism, and sinusitis [5-7]. The active ingredient in turmeric is curcumin [8]. Curcumin [diferuloyl methane; 1, 7-bis-(4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-Dione], a yellow pigment isolated from the root of *Curcuma longa* rhizomes, was widely used as a food additive for many years [9]. Curcumin exerts remarkable cytotoxic activity and apoptosis induction upon a variety of cancer cell lines by modulating the activities of numerous transcription factors, growth regulators, adhesion molecules, apoptotic genes and cellular signaling molecules [10-12]. Curcumin is not water-soluble, but it is soluble in methanol, ethanol, DCM or DMSO [13]. Curcumin has been extracted by means of maceration, digestion and infusion [14, 15]. This paper deals with an efficient isolation and characterization of the curcumin. In this work, we first extracted curcumin by soxhlet extractor method. This material can be further purified by recrystallization and characterized by spectrometery analyses.

Experimental

All solvents were purchased from Merck Company. They were distilled before use and stored over a drying agent. IR spectra were recorded with a Shimadzu FTIR-408 spectrophotometer as KBr pells. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Brucker 400 AC spectrometer in $CDCl_3$ as a solvent at room temperature. The λmax and color intensity of lawsone were determined on a Philips PU 8620 UV spectrophotometer in DMSO solvent using a 1-cm quartz cell. Mass spectrum was attained by double-focusing mass spectrometer. Curcuminoids were isolated from together by HPLC apparatus. The chromatographic apparatus consisted of a Jasco (Tokyo, Japan) PU-1580 isocratic pump and a Jasco UV-1575 spectrophotometric detector, a Rheodyne 7725i manual injector equipped with a 20 µL loop (Rheodyne, Cotati, CA, USA). The chromatographic system was controlled by HSS-2000 provided by Jasco using the LC-Net II/ADC interface. TLC was performed by the use of Merck's silica gel.

Isolation and characterization of curcumin from turmeric rhizome powder

25 g of dried turmeric rhizomes powder is placed in a large beaker and 1 L distilled n-hexane is added with a magnetic stirring rod. The suspension is stirred for 3 days. Then, the suspension is filtered and placed in a porous bag or "thimble" made of strong filter

paper, which is placed in chamber of the soxhlet apparatus (Figure 1). 310 mL methanol in flask is heated, and its vapors are condensed in condenser. The condensed extractor drips into the thimble containing the turmeric rhizomes powder, and extracts it by contact. When the level of liquid in chamber rises to the top of siphon tube, the liquid contents of chamber flood into flask. This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated. After 3 days, the solvent is evaporated by rotary apparatus. Then, it is dissolved in 100 mL toluene. The solution is poured into separatory funnel. Then, 100 mL NaOH 0.2 M is added to the solution and shaked for minutes. The aqueous phase is collected and acidified to pH 3 by HCl 0.2 M. The brown extract undergoes a clarification in this step and turns yellow. The filtrate is extracted with diethyl ether (3 × 100 mL). In the final extraction, the ether turns to a very pale yellow, indicating the end of the extraction. The combined ethereal phases are washed with 30 mL water and dried over $MgSO_4$. The ether is removed completely in vacuum to leave a yellow solid as crude curcuminoid products (Scheme 1) [16]. The crude curcumin (Scheme 1C) is purified by thin layer chromatography (208 mg). The product (Figure 2) was chromatographed over silica gel by DCM:methanol in a ratio of 19:1 v/v. The melting point of the isolated, pure curcumin was in the range of 174-177 ^{o}C which is the same as the literature value, 176 ^{o}C [17].

Extracted curcuminoids were compared with the standard by HPLC apparatus (Scheme 2). Compounds were separated on a 250 mm × 4.6 mm ID, 5-µm particle, Perfectsil Target ODS-3 column (MZ-Analysentechnik, Germany) with a ODS-3 precolumn (10×4.0 mm I.D., 5-µm), which was maintained at ambient temperature. The isocratic mobile phase consisted of acetonitrile-5% acetic acid, 52:48 (v/v), and the flow rate was 1 mL min^{-1}. The mobile phase was filtered, before using, through a 0.45-µm Millipore filter and degassed ultrasonically. The detection wavelength was 425 nm.

UV-Vis spectrum of curcumin is recorded in ethanol solvent (Scheme 3). The transitions are obtained in 423 and 442 wavelengths. The commonly observed transitions are n to π^* or π to π^*. We saw conjugation causes absorption signatures shift to longer wavelengths because the π to π^* transitions are more intense than n to π^* transitions.

IR (neat, cm^{-1}): 3421 (stretching phenolic and enolic O-H), 3091 (stretching C-H_{sp2}), 2920 and 2848 (stretching C-H_{sp3}), 1695 (stretching CO), 1627-1509 (C=C vibrational

bands), 1281 (bending CO) and 1153 (stretching C-O).

^{1}H NMR (FT-400 MHz, $CDCl_3$): δ; 3.716 (s, $6H_{11}$), 5.812 (s, $2H_1$), 6.463-6.515 (d, $2H_3$), 7.575-7.627 (d, $2H_4$), 6.931-6.958 (d, $2H_9$), 7.06 (s, $2H_6$) and 7.119-7.141 (d, $2H_{10}$).

^{13}C NMR (FT-400 MHz, $CDCl_3$): δ; 56 (C_{11}), 101 (C_1), 111 (C_6), 115 (C_9), 121 (C_3), 123 (C_{10}), 126 (C_5), 141 (C_4), 148 (C_7), 149 (C_8), 183 (C_2).

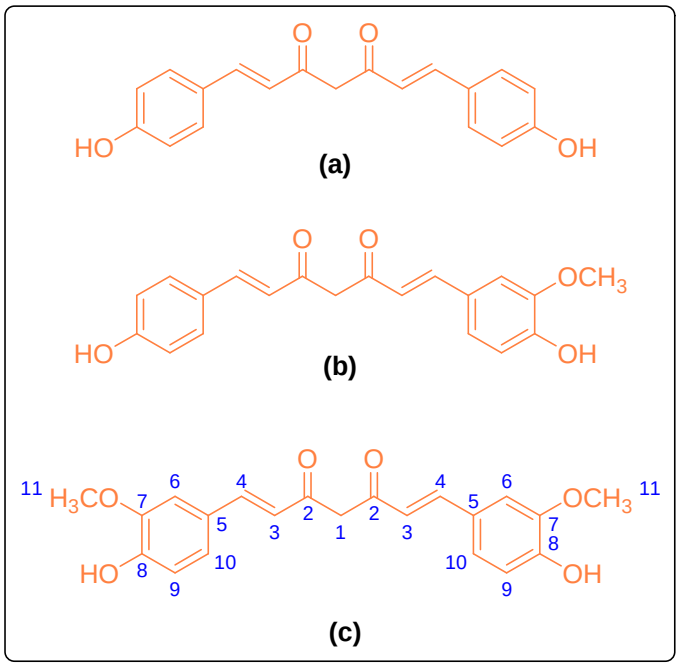
MS: m/z= 368 $[M]^+$, 350 $[M - H_2O]^+$, 217 $[A]^+$, 191 $[B]^+$, 177 $[C]^+$, 160 $[B - OCH_3]^+$ and 147$[C - OCH_3]^+$ (Scheme 4).

Results and discussion

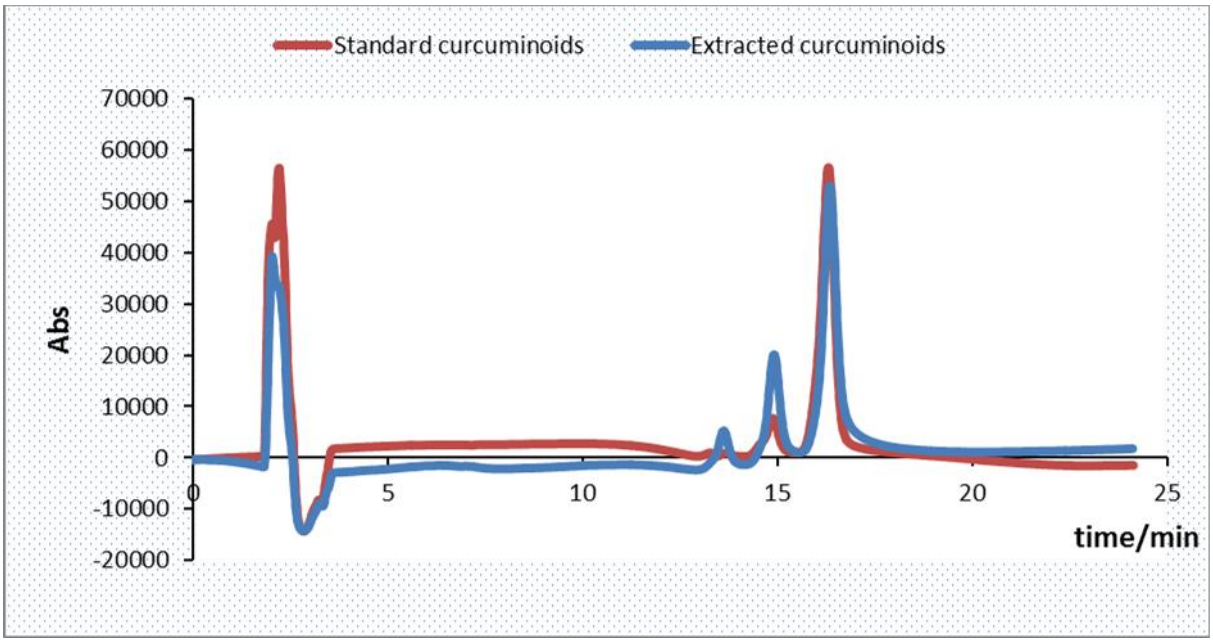
We have demonstrated that the color of turmeric rhizomes powder is due to the curcumin which is the main compound in this plant. This natural compound has many usages such as medicine. Before this time, curcumin had been extracted by means of maceration, digestion, microwave and infusion. In this work, we extracted the curcumin by soxhlet extractor method. It is characterized by spectroscopy methods such as HPLC, UV-Vis, FTIR, Mass and NMR analysis. The advantage of this method, compared to previously described methods, is the large amounts of curcumin that can be extracted from the powder by usage of less solvent amount. This affects tremendous economy in terms of time, energy and consequently financial inputs.

Figure 1. The soxhlet extractor

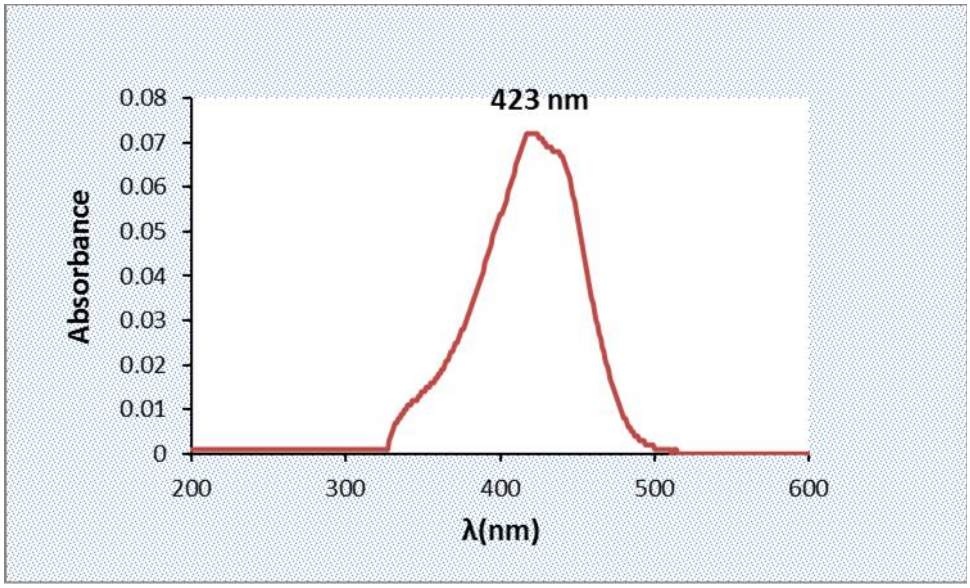
Figure 2. Curcumin compound powder



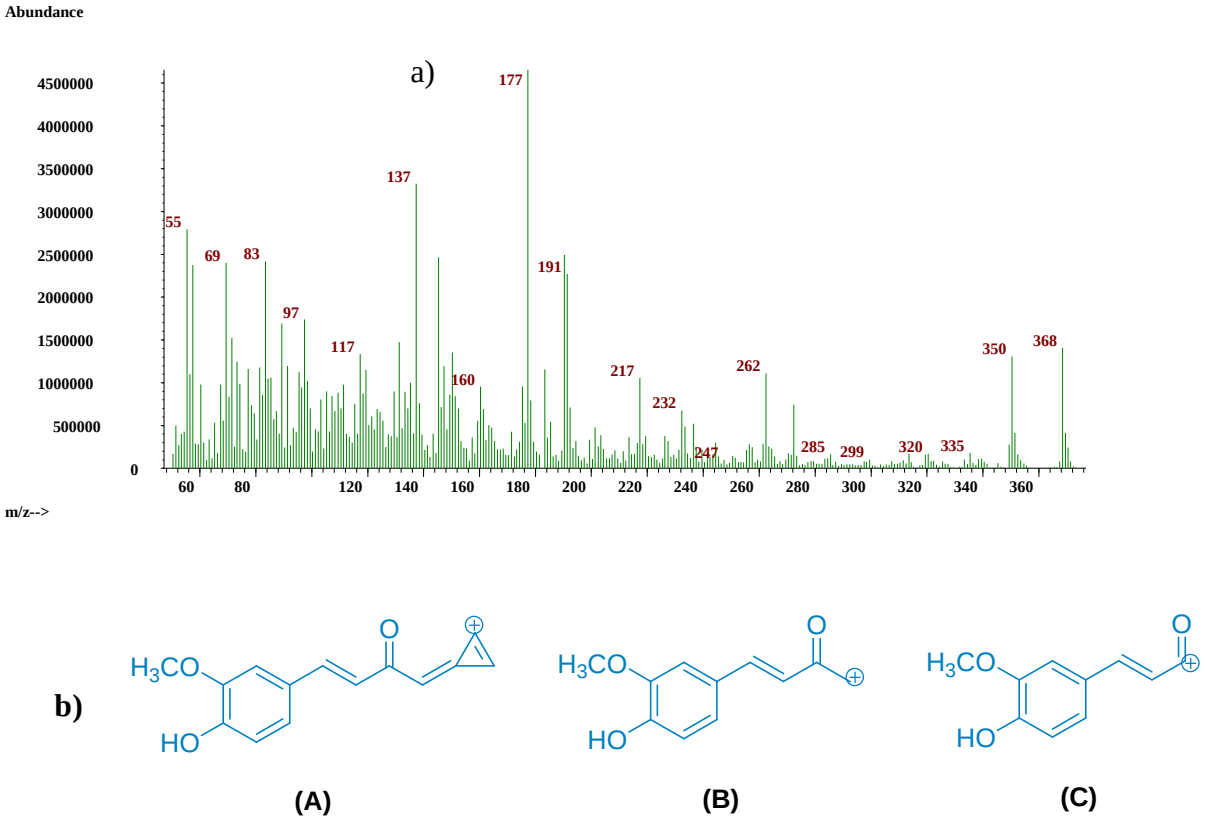
Scheme 1. Structure of curcuminoids



Scheme 2. HPLC analysis of curcuminoids



Scheme 3. UV-Vis analysis of curcumin



Scheme 4. (a) Curcumin mass spectrum; (b) Fragments of curcumin mass spectrum

Conclusion

In this study, we extracted natural curcuminoids by soxhlet extractor. Then, they were isolated and characterized by HPLC analysis. The curcumin was purified by TLC and characterized with spectroscopy methods. The advantage of our method is the extraction of curcumin with smaller quantity of methanol.

Acknowledgments

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