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Isolation, characterization and formulation of curcuminoids and in vitro release study of the encapsulated particles

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ABSTRACT

In this study, the isolation of curcuminoids from turmeric (*Curcuma longa* L.) was performed using different solvent extraction methods and solvents. Obtained extracts were analyzed for the contents of curcumin, demethoxycurcumin and bisdemethoxycurcumin by HPLC and the radical scavenging and antibacterial activities of extracts were determined. Extract with highest content of curcuminoids was mixed with polyethylene glycol (PEG) and formulated into powder using PGGS™ method. Obtained powder was pressed into tablets and a release study of the curcuminoids from the product was observed in simulated gastric and intestinal fluids.

Results show that the highest yield of extract is obtained using conventional extraction with mixing in ethanol giving the highest concentration of curcuminoids as well. All extracts show strong antifungal properties but weak antibacterial activity. Curcuminoids extract was successfully encapsulated in PEG carrier and a fine powder was obtained, which increased the solubility in aqueous media.

1. Introduction

Curcuma longa L., also known as turmeric, is grown in warm, rainy regions of the world such as China, India, Indonesia, Jamaica and Peru. The rhizome of turmeric is an important source of a yellow natural pigment [1], which in the past has been used as a spice, a coloring agent in the food industry, for household medicine usage and as an insect repellent [2]. Turmeric is one of the most popular medicinal herbs, with a wide range of pharmacological activities, such as antioxidant [3], anti-protozoal and anti-venom activities [4] and properties, that have found to be anti-microbial [5], anti-inflammatory, anti-proliferative, anti-angiogenic [6], antitumor [7] and anti-aging [8].

Turmeric has traditionally been used for medical purposes for many centuries in countries such as India and China [7], for treatment of jaundice and other liver ailments. Also, it has been used to treat ulcers, parasitic infections, various skin diseases (scleoderma, psoriasis), sprains, autoimmune diseases (rheumatoid arthritis, psoriasis, inflammatory bowel disease) and for curing the symptoms of colds and flus [9].

The yellow color, which is characteristic for turmeric rhizome, is due to the presence of 3–5% of curcuminoids [10]. Curcuminoids, represented by curcumin (C) (50–60%), demethoxycurcumin (DMC) (20–30%) and bisdemethoxycurcumin (BDMC) (7–20%) [11] have poor stability and low aqueous solubility [3]. Curcumin (bis-α,β-unsaturated β-diketone), commonly called as diferuloylmethane, is a low-molecular-weight compound [6]. Curcumin is practically insoluble in water at acidic and neutral pH conditions. Although curcuminoids are soluble at alkaline conditions, they do however undergo rapid hydrolytic degradation at these conditions [12].

Curcuminoids have immense biological properties, especially curcumin has been reported to possess many medicinal properties. Recently the analogs of curcumin were also reported for their biological activities. Demethoxycurucmin (DMC) was the best inhibitor of MCF-7 cells. Bisdemethoxycurcumin (BDMC) is active for modulation of MDR-1 gene expression [13]. DMC and BDMC are not commercially available. Therefore to study biological properties of individual curcuminoids they need to be isolated at high purity.

The supercritical fluid extraction (SFE) is an alternative and greener extraction method commonly used to extract chemicals or

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flavors from organic substrates, which attracted increasing interest over the past years [14]. SFE offers several advantages compared to conventional extraction methods, including reduced consumption of hazardous organic solvents, higher sample throughput, cleanliness and safety, environmental friendliness, expeditiousness, simplicity, quantitativeness and favorable solvation capacity which approaches that of a liquids [15].

Table 1 represents a short literature review of studies done in the field of curcuminoids extraction using different extraction techniques and solvents [1,10,16,17].

PGSS^{TM} micronization process is suitable for the compounds or their solution in organic solvents in which SCF is highly soluble (e.g. fats, waxes, polymers).

This process is very useful for the encapsulation of active ingredients in polymer matrices [18]. Pharmaceutical particle formations using $SCCO_2$, such as PGSS^{TM} method has received much attention as an alternative precipitation method to those with organic solvents [19]. Particle formation using $SCCO_2$ is important for drug delivery systems that have been successfully used to obtain composites or encapsulates, which comprise an active compound loaded into a matrix of a carrier material, in order to improve product preservation as well as controlling the dissolution rate of the active compound [20,21].

In this work different extraction techniques were applied for the isolation of curcuminoids from turmeric, namely supercritical fluid extraction, ultrasound-assisted solvent extraction and conventional solvent extraction with mixing and Soxhlet extraction. Supercritical extraction experiments were performed by semi-continuous flow apparatus using supercritical carbon dioxide ($SCCO_2$) at pressures 200 bar and 300 bar and temperatures 40 °C, 60 °C and 80 °C. Solvent extractions were performed using ethanol and hexane, whereas ultra-sound assisted extraction was performed only with ethanol. Amount of curcuminoids present in curcuma extracts were determined by high performance liquid chromatography (HPLC). The aim of this study was also to investigate the antimicrobial (antifungal and antibacterial) activities of curcuminoids extracts obtained under the various extraction conditions using agar-well diffusion methods, and to evaluate their radical scavenging activities using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging spectrophotometric method. Additionally, the encapsulation of extract containing the highest amounts of curcuminoids in a polyethylene glycol (PEG) carrier was done using batch PGSS^{TM} in order to obtain a powdered product. The formulated product was pressed into tablets and a preliminary in vitro release study of the encapsulated forms of curcuminoids extract in simulated gastric (SGF) and intestinal fluid (KH_2PO_4) was performed.

2. Materials and methods

2.1. Materials

Turmeric rhizomes were purchased from the Slovenian market. CO_2 (>99.5% purity) was obtained from Messer (Slovenia). Solvents, absolute ethanol and hexane were purchased from Sigma–Aldrich (Germany) and J.T. Baker (Netherlands). Analytical-grade curcumin (≥65%) (C), demethoxycurcumin (≥98%) (DMC) and bisdemethoxycurcumin (≥98%) (BDMC) were purchased from Sigma–Aldrich. Acetonitrile and acetic acid used for HPLC analysis were obtained from Merck (Germany). For the antifungal activity tests, potato dextrose agar (PDA; Merck, Cat. No. 1.10130.0500) was used and for the anti-microbial tests nutrient agar, based on meat, was used and was prepared from the following chemicals: sodium chloride (Cat. No. 1.06404.1000), meat extract (Cat. No. 1.03979.0500), peptone from meat (Cat. No. 1.07214.1000), which were purchased from Merck. D-(+)-glucose (Cat. No. G-5400) was supplied from Sigma–Aldrich. Polyethylene glycol (M_w = 1500 g/mol) was provided by Merck. KH_2PO_4 aqueous solution was used for the phosphate buffer, 0.2 M NaOH was used for pH adjustment. HCl and NaCl were used for the preparation of simulated gastric fluid (SGF).

2.2. Methods

2.2.1. Supercritical fluid extraction

The extraction experiments with dense CO_2 were performed on a semi-continuous flow apparatus [22,23]. The apparatus was home build for a maximum pressure of 500 bar and a temperature of 100 °C. Approximately 10 g of powdered turmeric rhizome was charged into the extractor (V = 60 mL). The temperature of the water bath was regulated and maintained constant (±0.5 °C, LAUDA DR. R Wobser GmbH & Co. KG, Lauda Königshofen, Germany). Liquefied CO_2 was continuously pumped with a high pressure pump (ISCO syringe pump, model 260D, Lincoln, Nebraska, P_{max} = 450 bar) through the preheating coil and over the bed of sample in extractor. The solvent flow rate was measured with a flow meter (ELSTER HANDEL GmbH, Mainz, Germany). The product precipitated in a glass trap, where the separation was performed at 1 bar and at 0 °C. The product collected in the glass trap was weighted (±0.1 mg) and yield was calculated by using Eq. (1).

$$\text{Yield}\,(\%) = \frac{m_{\text{extract}}}{m_{\text{raw material}}} \times 100 \quad (1)$$

2.2.2. Soxhlet extraction

10 g of powdered turmeric was placed in a thimble, which was inserted into a Soxhlet apparatus and extracted with 250 mL of

Table 1
Short literature review of studies done in the field of curcuminoids extraction using different extraction techniques and solvents.

Extracted compounds	Solvent	Pressure (bar)	Temperature (°C)	Yield of extraction (%)	Purity of extract [a] (%)	Reference
Curcuminoids and essential oils of turmeric	$SCCO_2$	250–300	45	4.50–6.51		[1]
	$SCCO_2$-ethanol	250–300	45	13.42–22.58		
Extraction and purification of curcuminoids	$SCCO_2$-ethanol	300	50		69.37[c]	[10]
	Microwave				15.57–90.96[c]	
	UAE[b]				6.57–71.47[c]	
	Soxhlet extraction				2.1[c]	
Extraction of turmeric	$SCCO_2$	260–300	47–77	0–6.47	0–71[d]	[16]
Extraction and purification of turmerones	$SCCO_2$	28.2–208	40–60	6.98	91.8[d]	[17]

[a] Curcumin or Turmerones content in extract after purification process.
[b] Ultra-sound assisted extraction.
[c] Turmerones content.
[d] Curcumin content.