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Nontoxic oil preparation from *Jatropha curcas* L. seeds by an optimized methanol/n-hexane sequential extraction method

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ABSTRACT

Ultrasound-assisted extraction (UAE) of phorbol esters (PEs) from *Jatropha curcas* L. (JC) seed oil using methanol as the extraction solvent was optimized by response surface methodology (RSM). *Jatropha curcas* L. seed oil is an excellent feedstock for biodiesel production, in which the presence of major toxic factor (phorbol esters) has limited its industrial applications. The aim of this work is to prepare the nontoxic oil from *Jatropha curcas* L. whole seeds with the first phorbol esters removal step and the second oil extraction step. The Box-Behnken design was applied to optimize three key parameters in the first phorbol esters removal step including temperature (X_1, °C), extraction time (X_2, min) and liquid-to-solid ratio (X_3, mL:g) for achieving a high extraction efficiency and minimal energy consumption. Ultimately, the optimized conditions were as follows: extraction temperature 36°C, extraction time 52 min and liquid-to-solid ratio 5.78:1. Among these three parameters, temperature had the most significant effect on phorbol esters extraction efficiency followed by extraction time and liquid-to-solid ratio. Maximum content of phorbol esters obtained were 0.33 mg/g *Jatropha curcas* L. seed in the first step and 89.20 ± 2.96% of seed oil could be recovered by *n*-hexane in the second step. A sensitive ultra-performance liquid chromatography method was also established to determine the phorbol esters content within 5 min, with a limit of detection (LOD) of 0.04 μg/g. Since the concentrations of phorbol esters were all below the limit of detection, most of the virtually phorbol ester-free (PE-free) oil was produced to extend the Jatropha biodiesel production chain for household chemicals, cosmetic or jet fuel preparation. Meanwhile, the phorbol esters enriched fraction (PEEF, 71.9 ± 0.3 mg/g) obtained from the first step can be utilized as value-added by-products in pharmaceutical and agro-pharmaceutical fields.

1. Introduction

Jatropha curcas L. (JC), a worldwide cultivated energy crop belonging to the Euphorbiaceae family (Makkar and Becker, 2009; Valdes-Rodriguez et al., 2013), is currently gaining lots of importance for its multipurpose use (Kumar and Sharma, 2008). Jatropha plants have excellent drought tolerance and adaption capacity to a large variety of soil conditions (Achten et al., 2008). The average oil content of dry seed on the mass basis is 34.4%, with the seed kernel containing about 60% oil that can be converted into biodiesel (Goel et al., 2007; Achten et al., 2008). For extraction of the JC oil, two primary methods are identified as mechanical extraction and solvent extraction (Achten et al., 2008). Out of 27-40% oil available in the seed, 60-80% and 70-90% can be recovered respectively by these aforesaid methods (Achten et al., 2007). Moreover, solvent extraction is more economical in a large-scale production, commonly using *n*-hexane to obtain oil from Jatropha seed cake (Achten et al., 2008). The seed cake remaining after oil extraction is a suitable feedstock for by-products of fertilizer, biogas or low-cost industrial enzymes (Staubmann et al., 1997; Gübitz et al., 1999; Mahanta et al., 2008). However, the abundant oil as well as defatted meal of Jatropha plant is not edible because of the toxic phorbol esters (PEs) (Makkar et al., 1998; Baldini et al., 2014). PEs, a group of diterpenes having tigliane skeleton, are the main toxic principle present in JC seed (Makkar and Becker, 2009; Devappa et al., 2010a; Duran-Pena et al., 2014). Li et al. (2010) reported that PEs had a LD50

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of 28 mg/kg body weight in mice. The toxic Jatropha oil exhibited severe histological alterations and inflammatory responses on the reconstructed human epidermis and corneal epithelium (Devappa et al., 2013b). PEs can also act as a potent protein kinase C (PKC) activator to amplify the efficacy of carcinogens (Goel et al., 2007; Devappa et al., 2010a). Seeds from non-toxic Mexican genotype of Jatropha have a low level of PEs concentrations of 0.11 mg/g (Makkar and Becker, 1997b) and normally, the non-toxic oil is edible (Makkar and Becker, 2009). As for toxic genotypes, there is a variety of physical, chemical and biological methods established to extract (Devappa et al., 2010b; Devappa et al., 2013a) as well as detoxify PEs (Kumar et al., 2010; Bu et al., 2012; Sadubthummarak et al., 2013; Wang et al., 2013; Guedes et al., 2014). Besides, PEs are used as a starting material to synthesis the important HIV treatment adjuvant candidate medicine prostratin and DPP (Wender et al., 2008; Devappa et al., 2013d).

Devappa et al. (2010b) established a method to successfully extract 78-80% of PEs present in the crude Jatropha oil at 60°C for 5 min, but to obtain virtually PE-free oil (PEs 23 μg/g oil) it cost 60 min. A central composite rotatable design (CCRD) methodology was applied to optimize the extraction conditions and reduced 97.3% of PEs content in Jatropha seed cake after 8 h (Guedes et al., 2014). In terms of industrial applications, high temperature, long extraction time and large amount of solvent consumption are relatively uneconomic for practical considerations. In addition, PEs are currently determined by qualitative methods such as thin layer chromatography (TLC), gas-liquid chromatography (GLC), mass spectrometry (MS), high performance liquid chromatography (HPLC) (Makkar and Becker, 1997a; Haas et al., 2002; Wang et al., 2012) and ultra-performance liquid chromatography (UPLC) (Hua et al., 2015). Since PEs concentration of TPA equivalent is overestimated compared with that of Jatropha factor C1 (JC1) equivalent (Roach et al., 2012; Wang et al., 2012; Devappa et al., 2013a; Hua et al., 2015), in this work JC1 is used as an external standard for determination of the total PEs content.

The objective of this study was to establish a reliable method to obtain virtually PE-free oil, wherein RSM was utilized to optimize the conditions of PEs extraction step. Meanwhile, a rapid and sensitive detection method using UPLC was also built for determination of JC1 as well as the total PEs content in a relatively short time.

2. Materials and methods

2.1. Materials and chemicals

Jatropha curcas L. (JC) seeds of toxic genotype were collected from cultivated trees (mature, approx. age 8 years) existing in places around Yongren County, Yunnan, China in 2015. Jatropha factor C1 (JC1, 98.3% purity) was prepared and purified by our laboratory (Hua et al., 2015). Water (ultrapure grade) was prepared by an ultrapure water system (WSD, Chengdu, China). Acetonitrile and formic acid were HPLC grade reagents (Tedia, Anhui, China). Methanol, *n*-hexane and ammonium formate were analytical grade reagents (Kelong, Chengdu, China)

2.2. UPLC method and analysis

A UPLC method was built to determine the concentrations of PEs on an ACQUITY UPLC H-class system (Waters, Miford MA, USA) equipped with a QSM quaternary solvent system, a FTN auto sampler manager and an eLambda photodiode array detector. PEs were separated using a CORTECS UPLC C18+ column (1.6 μm, 2.1 mm × 150 mm, Waters, USA) at 40°C. The conditions of binary solvent system were as follows: solvent A, acetonitrile-water (90/10, v/v) containing 25 mM ammonium formate and 0.1% formic acid; solvent B, ultrapure grade water containing 25 mM ammonium formate and 0.1% formic acid. A program of 88% A and 12% B was performed from 0 to 5 min at a flow rate of 0.5 mL/min. The sample injection volume was 2 μL with a detection wavelength set at 280 nm (λ_{max} of the UV absorbance of PEs).

Table 1
Independent variables and the actual experimental values applied for optimization.

Independent variable	Symbol	Coded values		
		−1	0	+1
Temperature	X_1	30	45	60
Time	X_2	5	32.5	60
Liquid-to-solid ratio	X_3	10	30	50

2.3. Single factor experiments

UAE experiments were conducted on an ultrasound processor (SB-300DTY, Scientz, Ningbo, China). The effect of temperature on PEs extraction efficiency was determined at 30, 40, 50 and 60°C with an extraction time of 20 min, a liquid-to-solid ratio of 30:1 and a frequency of 59 kHz. The effect of time on PEs extraction efficiency was determined at 5, 10, 20, 30 and 50 min with a temperature of 40°C, a liquid-to-solid ratio of 30:1 and a frequency of 59 kHz. Finally, the effect of liquid-to-solid ratio on PEs extraction efficiency was determined at 5:1, 10:1, 20:1, 40:1 and 60:1 with a temperature of 40°C, an extraction time of 50 min and a frequency of 59 kHz. All the samples of these three parameters (temperature, time and liquid-to-solid ratio) were done in triplicate using methanol as the solvent. PEs extraction efficiency was calculated by the relative content of JC1 as follows:

$$PEs\quad extraction\quad efficiency = \frac{m_0}{m_1} \times \frac{Peakarea\ JC1}{10^5} \qquad (1)$$

where m_0 was the theoretical weight of JC seeds (g) and m_1 was the actual weight of JC seeds (g). The 10^5 was a correction coefficient in this equation to normalize the data of PEs relative contents.

2.4. RSM design and statistical analysis

UAE parameters for PEs extraction were optimized using RSM (Bezerra et al., 2008) on Design-Expert v8.0.5b software according to the single factor experiments. Box-Behnken design used a three-level-three-factor method with seventeen experimental runs including five replicates at the center point. The extraction variables were temperature (X_1, °C), time (X_2, min) and liquid-to-solid ratio (X_3, mL:g). Their coded values are given in Table 1. The relative content of JC1 was chosen as a response variable to measure the PEs extraction efficiency. Table 2 shows the run order, variable conditions, and the actual experimental values, which is fitted with a second-order polynomial model as follows:

$$Y = \beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_{11}X_1^2+\beta_{22}X_2^2+\beta_{33}X_3^2 +\beta_{12}X_1X_2+\beta_{13}X_1X_3+\beta_{23}X_2X_3 \qquad (2)$$

where β_0, β_1, β_2, β_3, β_{11}, β_{12} ...are the regression coefficients. Y is utilized to represent the response variable (relative content of JC1) while X_1, X_2 and X_3 are the non-coded values for extraction temperature, time and liquid-to-solid ratio, respectively. To determine the interactive effects of variables on PEs extraction efficiency, the experimental data were analyzed by ANOVA with the lack of fit for the quadratic model. A 3D response surface plot was finally generated by Design-Expert software.