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Supercritical CO_2 extraction of *Eucalyptus* leaves oil and comparison with Soxhlet extraction and hydro-distillation methods

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ABSTRACT

Oils were extracted from the leaves of *Eucalyptus loxophleba* ssp. *lissophloia*, also known as oil Mallee, using a laboratory scale supercritical fluid extraction (SFE) system using CO_2. The effect of temperature (40–80 °C), pressure (10–50 MPa) and extraction time (30–150 min) on the oil yield was investigated using a central composite design method to determine the significance and interactions of these parameters. The results showed that pressure had the most significant enhancing effect on the oil yield, while temperature and time showed a lesser impact. There was also pronounced interaction between temperature and pressure and their combined effect on the yield was such that the oil yield increased with increasing temperature at high pressures but decreased at low pressures. For comparison, the *Eucalyptus* leaves were also extracted with Soxhlet extraction, using two different solvents, and hydro-distillation methods. In the Soxhlet extraction, the solvent type had a more significant effect on the oil yield than the extraction time. Overall, the Soxhlet extraction produced the highest oil yield while hydro-distillation the lowest among the three methods. The SFE yield was up to 4.78%, comparable in magnitude to that of the hexane Soxhlet extraction of 7.9%. The chemical compositions of the extracted oils were analysed using a gas chromatography-mass spectrometer (GC–MS). The dominant component identified in the *Eucalyptus* oil was 1,8-cineole. The chemical compositions of the extracts were quite different for the three extraction methods. The oil extracted by hydro-distillation contained only volatile compounds while the oil from the SFE and Soxhlet contained both volatile and higher molecular weight compounds. The effect of the addition of ethanol as a modifier at concentrations from 5 w% to 15 w% on the supercritical fluid extraction of Eucalyptus leaves oil was also investigated. The ethanol addition was shown to increase the efficiency of oil extraction from Eucalyptus leaves and the oil yield increased with increasing ethanol concentrations.

1. Introduction

One of the plants with its various extracts that are extensively used in the cosmetic, perfumery, food and pharmaceutical industry is *Eucalyptus* [1]. *Eucalyptus* is a large genus of the *Myrtaceae* family which includes over 700 species [2]. Although *Eucalyptus* is widely grown in many countries all over the world, most of the species are native to Australia. This plant is a genus of tall, evergreen and magnificent trees cultivated all over the world for its oil, gum, pulp, timber, medicine and aesthetic values [2]. Among the various wood and non-wood products, *Eucalyptus* oil found in its foliage is a most important one [2]. *Eucalyptus* oil is a complex mixture of a variety of monoterpenes, sesquiterpenes, and aromatic phenols,oxides, ethers, alcohols, esters, aldehydes and ketones. They are extracted from the foliage of *Eucalyptus* trees, the quantity and strength of the oil vary across *Eucalyptus* species [2]. *Eucalyptus* oil has been shown to contain very high amounts of 1,8-cineole, which has chemical and physical properties that make it suitable for a range of applications. The most-known compound, terpenoid, gives *Eucalyptus* foliage its characteristic smell. *Eucalyptus* oil is used widely as an ingredient in many general pharmaceutical products (eg liniments, inhalants, expectorants) due to its broad biological properties including anti-inflammatory, anti-allergenic, anti-asthmatic, anticonvulsant, antiseptic, aquaculture antiviral, anti-bacterial and anti-malarial. It has also been used as a flavour and aroma enhancer in food and cleaning products and in cosmetic formulations [3].

Eucalyptus oil can be extracted using a variety of methods. Currently, the most popular method of extraction is steam extraction or hydro-distillation [4]. However, the high temperature operation of this technique can lead to degradation of thermally labile compounds and partial hydrolysis of water sensitive compounds, resulting in the formation of artefact undesirable in the final

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extracts [4]. Solvent extraction using ethanol has also been applied [5], however, separating the solvent from the extracted is often too difficult and some solvent residues would be present in the finished product. Recently, supercritical fluid extraction (SFE) has gained increasing attention particularly in the food, pharmaceutical and perfume industries [6]. The commonly used fluid in SFE is CO_2, which has several unique characteristics and physic-chemical properties, being non-toxic, non-flammable, inexpensive, odourless, and of low critical pressure (7.38 MPa) and temperature (31.1 °C) [6]. The use of CO_2 leaves no residue in the products, thus providing an oil of superior quality. Therefore, SFE using CO_2 has several advantages over traditional extraction techniques including operation at low temperatures thus preservation of the thermally labile components in the extracts. Besides, SFE can bring about environmental benefits as it uses no or significantly less environmentally hostile organic solvents. Although a few studies have explored the possible use of supercritical CO_2 (SC-CO_2) extraction to extract *Eucalyptus* oil [7,8], the conditions have not yet been optimized. In general, several important factors, including pressure, temperature and extraction time, have important effects on the oil yield.

The response surface methodology (RSM) has been demonstrated to be a powerful tool for determining the factors and their interactions [9]. The RSM is a collection of mathematical and statistical techniques useful for analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize this response [9]. This procedure involves fitting a function to the experimental data and then using optimization techniques to determine the optimum parameters [10]. It is much faster and more efficient for gathering research results than the classic, one variable-at-a time or full-factors experimentation approach [11].

There are several factors such as operating pressure, temperature, solvent flow rate, extraction time and sample particle size that can affect the performance of supercritical fluid extraction. The effect of particle size on the supercritical fluid extraction of *Moringa oleifera* seeds was studied by Zhao and Zhang [12]. It was found that the extraction process was characterized by two periods. The first period features a constant extraction rate which can be explained by the extraction of solute more accessible to the solvent and the particle size has little effect in this period. In the second period, the oil yield increases with a decrease in particle size. This is because the intra-particle diffusion has taken control of the oil transfer during this period. Besides, the effect of the solvent flow rate on the supercritical fluid extraction of *M. oleifera* seeds oil was also investigated [13]. It was found that in the case of *M. oleifera* seeds, the oil yield increased with increasing the solvent flow rate at the same extraction time. However, the solubility of *M. oleifera* seeds oil was not a function of the CO_2 flow rate but an indication of the mass transfer limitation. The effects of sample particle size and solvent flow rate have been well addressed in the literature and were not a primary objective in the present work as described in this paper.

The objective of this study was to optimize the process parameters (pressure, temperature and extraction time) for the extraction of *Eucalyptus* oil using the response surface methodology, and to evaluate the effect of these parameters on the oil yield. In addition, the yield and chemical profiles of the extracts obtained by SFE were also analysed and compared to those achieved by hydro-distillation and Soxhlet extraction methods.

2. Materials, experimental design and analytical methods

2.1. Materials

Leave samples of a *Eucalyptus loxophleba* ssp. *lissophloia*, also known locally as oil Mallee, were collected from the Narrogin region, southwest of Western Australia. The samples were air dried for two days and the final moisture content was determined, by drying the samples in an oven (Model 8050, Contherm, New Zealand) set at 103 °C for 5 h [14], to be 12.3%. The air dried samples were ground with a knife grinder (Model 3383-L30, Thomas Scientific, USA) and the ground samples were sieved using a sieved shaker (model EFL2000/2, Endecotts Ltd., London, England). The fraction of particles under 400 μm was selected for all subsequent extraction experiments. The final samples were kept in a sealed container and placed in a refrigerator before experimentation.

2.2. Supercritical fluid extraction

The supercritical fluid extraction (SFE) experiments were carried out using an SFT Custom SCW-SFE system (Newark, DE, USA) as described elsewhere [12,15]. In this work, 5 g of *Eucalyptus* leaves were carefully weighed and loaded into the 50 ml SC-CO_2 extraction vessel. About 1 g of glass wool was packed at both ends of the extractor to stop entrainment of the substrate. SFE started as soon as the desired pressure and temperature had been reached. The flow rate of the expanded gas CO_2 (under atmosphere pressure and room temperature of 20 °C) was set at 2 L/min in all runs, determined based on a set of preliminary experimental trials where the solvent flow rate was varied from 1.0 to 4.0 L/min and the flow rate of 2 L/min was judged to be appropriate for this study.

The extract was collected in ethanol in an amber bottle. In order to improve the collection efficiency, the bottle was placed in an ice bath during the dynamic extraction stage, which also acted as a freezing-trap to minimise the loss of volatile compounds as the sublimation of CO_2 decreases the temperature of the collection solvent. The precipitates in all the connection tube lines were washed out with ethanol and then mixed with the collected extract in the amber bottle. The mixture was made up to 10 ml with ethanol, and 1 ml was taken for GC–MS analysis, the rest was put in a rotary evaporator (model N-1000S-W, EYELA, Tokyo, Japan) to remove the ethanol solvent, and then the weight of extracts was measured. Finally, the extraction yield was estimated as follows:

$$\textit{Extraction yield}\,(\%) = \frac{\textit{mass of total extracted}}{\textit{mass of dried leaves}} \times 100\% \quad (1)$$

A three-factor central composite design (CCD) combined with response surface methodology (RSM) was applied to determine the best combination of process variables for SFE of the *Eucalyptus* leaves oil. The independent variables studied here were pressure (X_1: 10–50 MPa), temperature (X_2: 40–80 °C) and extraction time (30–150 min), while the response variable was the oil yield. Table 1 shows the arrangement of the CCD performed in this investigation, where 20 randomized experiments including six replicates at the centre points were employed to fit the full quadratic equation model. The yields presented in Table 1 were the average of at least three measurements under otherwise the same conditions. The polynomial equation employed in this study is shown as Eq. (2), with which the linear (X_1, X_2, X_3), quadratic (X_1^2, X_2^2, X_3^2) and interactive (X_1X_2, X_1X_3, X_2X_3) effects of independent variables of pressure (X_1), temperature (X_2), and time (X_3) on dependent variable (Y) can be determined:

$$Y = \beta_0 + \sum_{i=1}^{3}\beta_i X_i + \sum_{i=1}^{3}\beta_{ii}X_i^2 + \sum\sum_{i<j=1}^{3}\beta_{ij}X_iX_j \quad (2)$$

where Y is the response (extraction yield of *Eucalyptus* oil), β_0 β_i β_{ii} β_{ij} are constant coefficients of intercept, linear, quadratic and interaction terms, respectively. X_i and X_j are independent variables (pressure, temperature and extraction time). The actual levels of the independent variables used in the experimental design and the observed response for *Eucalyptus* oil were shown in Table 1.