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Comparison of different extraction methods for the extraction of major bioactive flavonoid compounds from spearmint (*Mentha spicata* L.) leaves

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ABSTRACT

Different bioactive flavonoid compounds including catechin, epicatechin, rutin, myricetin, luteolin, apigenin and naringenin were obtained from spearmint (*Mentha spicata* L.) leaves by using conventional soxhlet extraction (CSE) and supercritical carbon dioxide (SC-CO_2) extraction at different extraction schemes and parameters. The effect of different parameters such as temperature (40, 50 and 60 °C), pressure (100, 200 and 300 bar) and dynamic extraction time (30, 60 and 90 min) on the supercritical carbon dioxide (SC-CO_2) extraction of spearmint flavonoids was investigated using full factorial arrangement in a completely randomized design (CRD). The extracts of spearmint leaves obtained by CSE and optimal SC-CO_2 extraction conditions were further analyzed by high performance liquid chromatography (HPLC) to identify and quantify major bioactive flavonoid compounds profile. Comparable results were obtained by optimum SC-CO_2 extraction condition (60 °C, 200 bar, 60 min) and 70% ethanol soxhlet extraction. As revealed by the results, soxhlet extraction had a higher crude extract yield (257.67 mg/g) comparing to the SC-CO_2 extraction (60.57 mg/g). Supercritical carbon dioxide extract (optimum condition) was found to have more main flavonoid compounds (seven bioactive flavonoids) with high concentration comparing to the 70% ethanol soxhlet extraction (five bioactive flavonoids). Therefore, SC-CO_2 extraction is considered as an alternative process compared to the CSE for obtaining the bioactive flavonoid compounds with high concentration from spearmint leaves.

Keywords: Spearmint (*Mentha spicata* L.); Bioactive flavonoid; Supercritical carbon dioxide (SC-CO_2) extraction; Conventional soxhlet extraction (CSE); High performance liquid chromatography (HPLC)

1. Introduction

Spearmint belongs to the genus *Mentha* in the family *Labiateae* (*Lamiaceae*) (Sweetie et al., 2007). A number of studies have found that herbs of the *Lamiaceae* family are a potential source of natural antioxidants (Choudhury et al., 2006). Rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.), thyme (*Thymus vulgaris* L.) and lavender (*Lavendula angustifolia Mill.*) are native to the Mediterranean region; balm (*Melissa officinalis* L.) and spearmint (*Mentha spicata* L.) are common plants in Britain and other European countries (Wang et al., 2004; Paranjpe, 2001).

Different surveys have shown that herb extracts are useful as stabilizers of edible oils. Most studies on antioxidant compounds in the *Lamiaceae* family are directed to phenolic diterpenes, flavonoids and phenolic acids (Kivilompolo and Hyotylainen, 2007). Flavonoids which are widely distributed in the leaves, seeds, bark and flowers of plants are a broad class of low molecular weight compounds. Flavonoids are a kind of highly effective antioxidant and less toxic than syn-

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thetic antioxidants such as BHA and BHT. Therefore, these secondary plant phenolics have received the greatest attention and have been studied extensively (Heim et al., 2002; Naczk, 2004; Syed and Sharma, 2001). Effective separation of antioxidants (high extraction yield and concentration of bioactive compounds) from a complex plant matrix is a difficult procedure due to co-extraction of other various compounds, which are undesirable in antioxidant extract. Different extraction techniques such as percolation, soxhlet and supercritical fluid extraction have been used to isolate antioxidants from plants. Conventional soxhlet extraction (CSE) is a standard technique which has been used for a long time. The main disadvantages of conventional soxhlet extraction are long extraction time and consumption of large amounts of used solvents. This extraction method is also not suitable for the extraction of thermo-sensitive compounds due to the possibility of thermal decomposition of target compounds as extraction usually occurs at the boiling point of used solvent for a long time. However, conventional soxhlet extraction comparing to supercritical fluid extraction (SFE) is still widely used due to its simplicity (Grigonis et al., 2005). Supercritical fluid extraction with CO_2 is an attractive method for food industry applications due to its unique properties (Lin et al., 1999).

The unique characteristic of this system is application of gases above their critical points to extract selective soluble components from a raw material (Cavero et al., 2006). However, CO_2 is not a suitable solvent for the extraction of polar compounds because it behaves as non-polar fluid for certain conditions of temperature and pressure. Therefore, this is the main limiting step in its use for the separation of polar phenolic compounds. But, in order to increase the polarity of extraction solvent some food grade modifiers like ethanol can be used (Qingyong Lang, 2001). The objectives of the present work were to investigate the effect of different parameters, such as pressure, temperature and dynamic extraction time on the supercritical fluid extraction of spearmint leaves flavonoids and to compare the extraction yield and concentration of flavonoids in the extracts obtained under conventional soxhlet and supercritical fluid extraction. To the best of our knowledge, no report has been yet appeared on the supercritical fluid extraction (SFE) of Malaysian spearmint leaves flavonoid compounds.

2. Materials and methods

2.1. Materials

The leaves of spearmint (*M. spicata* L.) were obtained from Cameron Highland in Pahang, Malaysia. After harvesting, the leaves were separated and washed under tap water. Leaves were dried at 40 °C in a ventilated drying oven (1350FX, USA) for 24 h and then stored at ambient temperature (22 °C) in the dark. The samples were ground in grinding mill (MX-335, Panasonic, Malaysia) for 10 s to produce a powder with an approximate size of 0.525 mm.

2.2. Reagents

Carbon dioxide (CO_2, SFE grade), contained in a diptube cylinder, was purchased from MOX Company in Malaysia. Methanol (MeOH, analytical grade), ethanol (EtOH, 99.5%, analytical grade) and petroleum ether (analytical grade) were purchased from Scharlau chemical, European Union. Methanol (MeOH, HPLC grade) was purchased from Fisher scientific chemical, USA. TFA (trifluoroacetic acid, ≥98%) was obtained from Sigma–Aldrich, Germany. All flavonoid standards including (+)-catechin, (−)-epicatechin, apigenin, rutin, luteolin, myricetin and naringenin were purchased from Sigma–Aldrich, Germany.

2.3. Conventional soxhlet extraction (CSE)

Three grams (3 g) of dried and ground spearmint leaves were placed in a soxhlet apparatus. Extraction was performed with 150 ml of an appropriate solvent for 6 h. After extraction, a rotary vacuum evaporator (Eyela, A-1000S, Japan) at 40 °C was used in order to remove solvent. In this experiment four solvents were used: pure ethanol, methanol, petroleum ether and 70% ethanol. All extractions were performed in duplicate.

2.4. Supercritical carbon dioxide (SC-CO_2) extraction

Supercritical CO_2 (SC-CO_2) extraction was performed on a supercritical fluid extractor (ABRP200, Pittsburgh, PA, USA) with the extractor volume 500 ml (Fig. 1). The flow rate of CO_2, the extraction temperature and pressure were adjusted by the

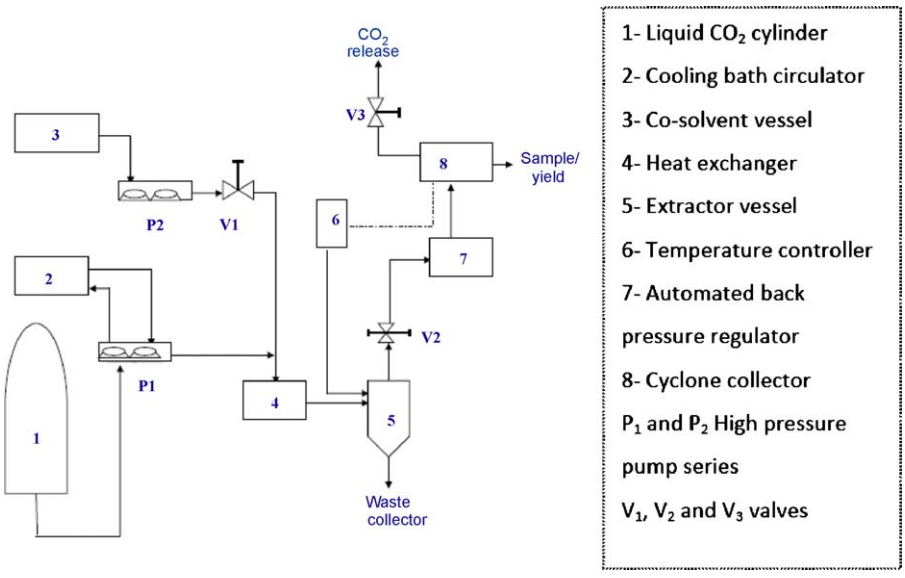

Fig. 1 – Schematic diagram of supercritical fluid extractor.