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Contributed Paper

Extraction of Phenolic Compounds from Fruits of Bitter Melon (*Momordica charantia*) with Subcritical Water Extraction and Antioxidant Activities of These Extracts

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ABSTRACT

Bitter melon (*Momordica charantia*) is traditionally known for its medicinal properties such as antidiabetic, anticancer, anti-inflammation, antivirus, and cholesterol lowering effects. It contains many phenolic compounds that may have the potential as antioxidant and antimutagen. Although the value of bitter melon is realized, scientific information on phenolic composition of bitter melon and antioxidant and antimutagenic activities of its extracts from food grade solvents are limited. This study were investigated the total phenolic contents of bitter melon obtained by subcritical water extraction (SCWE) and antioxidant activities of these extracts. The effect of extraction temperature was considered and the results were compared with the extracts obtained by solvent extraction and soxhlet extraction. The total phenolic contents of bitter melon obtained by the SCWE at 130 °C, the SCWE at 150 °C, SCWE at 180 °C, the SCWE at 200 °C, the solvent extraction, and soxhlet extraction were 10.571, 25.219, 42.915, 48.177, 7.743, and 4.992 mg gallic acid equivalents (GAE)/g dry weight (DW), respectively. Overall, the extracts obtained by SCWE were significantly higher than solvent extraction and soxhlet extraction. The main phenolic acid contained in bitter melon was gallic acid. The phenolic acid was calculated from HPLC analysis of the extracts that the gallic acids of the extracts from bitter melon obtained by the SCWE at 130 °C, the SCWE at 150 °C, SCWE at 180 °C, the SCWE at 200 °C, the solvent extraction, and soxhlet extraction were 0.0913, 0.3169, 0.5502, 0.6462, 0.0271, and .0120 mg/g DW, respectively. Antioxidant was represented by IC_{50} index which the IC_{50} values of extract obtained with the SCWE at 130 °C, the SCWE at 150 °C, the SCWE at 180 °C, the SCWE at 200 °C, the solvent extraction, and soxhlet extraction were 4.480, 3.970, 5.757, 5.720, 8.426, and 6.107 μg/ml, respectively. The IC_{50} values of the SCWE at all temperatures were lower than that of solvent extraction and soxhlet extraction, which means SCWE gave the extracts with higher antioxidant activity.

Keywords: Subcritical water extraction, Bitter melon, *Momordica charantia*, Antioxidant, Phenolic.

1. INTRODUCTION

Phenolic compounds are categorized as secondary metabolites essential for growth and reproduction of plants. They are known as hydrophilic antioxidants, and are produced as a response for defending injured plants against pathogens. They potentially show antioxidant, antimutagen, antitumor, anti-inflammatory, and anticarcinogenic properties [1]. In general, deep–colored vegetables and fruits including bitter melon are good sources of phenolic compounds.

Bitter melon (*Momordica charantia*) or commonly as *Ma-ra-khee-nok*, is an herbal plant grown in Thailand and other tropical regions. It is traditionally known for its medicinal properties such as antidiabetic, antitumorous, anticancer, anti-inflamatory, antiviral, and cholesterol lowering effects etc. [2, 3, 4]. The main constituents of bitter melon which are responsible for these effects are such as triterpene, proteid, steroid, alkaloid, inorganic, lipid, and phenolic compounds [3]. The protein in bitter melon including protein MAP-30, alpha–momorcharin, and beta–momorcharin were shown to have the ability for fighting against HIV [5].A steroid, charantin, contained mainly in the aerial parts, has been proven for its antidiabetic activity [6]. The phenolic compounds from bitter melon extracted by solvent extraction were reported to exhibit antioxidant activity [7].

Recently, subcritical and pressurized fluids have become an interesting alternative in the extraction of herbal plants and the most benign and available solvent for pressurized solvent extraction is water. The subcritical fluid extraction is a technique for extraction of plants based on the use of solvent whose temperature lies between boiling and critical temperatures as an extractant, and at high pressure enough to maintain the liquid state [8]. The important advantages of this method are its simplicity, reduced extraction time, higher quality of the extract, lower cost of the extracting agent, and an environmentally friendly technique [9]. Extraction with subcritical fluid using water as a solvent has been shown to be effective for several compounds, such as essential oils from majoram [10], oregano [8], and coriander seeds [11] etc. Moreover, Jesada showed that charantin which is a fat soluble steroid could be successfully extracted benignly from fruit of bitter melon using polar solvents at subcritical condition such as acetone and ethanol [12]. Although the authors showed that subcritical water was not suitable for extraction of charantin from fruit of bitter melon, water is an adequately good solvent for extraction of phenolic compounds from bitter melon due to the higher solubility of phenolic compounds in water.

In this study, we investigated the total phenolic contents of bitter melon obtained by subcritical water extraction. The effect of extraction temperature was considered and the results were compared with the extracts obtained by solvent extraction and soxhlet extraction. Moreover, the antioxidant activities of these extracts were determined.

2. MATERIALS AND METHODS

2.1 Materials and chemicals

The fruits of bitter melon were obtained from the market in Bangkok, Thailand. Gallic acid was obtained from Sigma Chemical Co. (St Louis, Mo, USA). Methanol was purchased from Fisher Scientific, UK. Water used in the experiments was distilled and deionized water.

2.2 Sample preparation

The fruits of bitter melon were cleaned and cut into small pieces, and then oven dried at 50 ^{o}C for a day. The dried sample was then pulverized into fine powder in a grinder, which was then stored at 4 ^{o}C until use.

2.3 Subcritical water extraction (SCWE)

The subcritical water extraction was carried out in a laboratory-built apparatus shown in Figure 1. The extraction system consisted of two HPLC pumps (PU 980, JASCO, Japan) used to deliver the water and solvent through the system at constant flow rates, a degassing instrument (ERC 3215, CE, Japan), an oven (D63450, HARAEUS, Germany), where the extraction vessel (10 ml, Thar Design, USA) was mounted, a pressure gauge, and a back pressure regulator valve (AKICO, Japan). All connections were made with stainless steel capillaries (1/16 inch inside diameter).

Water was passed through a degassing equipment to remove dissolved oxygen, The degassed water was then delivered to preheating coil, made from 3 m length stainless steel tubing, installed in the oven, and delivered through to the extraction vessel, which was preloaded with 1.0 g of sample. The back pressure regulator valve placed at the outlet of the extraction system was used to maintain the system pressure to ensure that the water was in liquid state at the temperatures tested. Before starting the extraction, all connections were checked for possible leakage. The second pump was then turned on to deliver ethanol at constant flow rate of 1 ml/min to wash off any residual product in the outlet line behind the extractor. The extract was cooled in a coil immersed in a water bath to prevent possible product degradation, and the extract was collected in fractions in sample collecting vials every 10 minutes in a first hour and every 20 minutes in the second hour. After extraction, the compound remained in the sample residue was extracted repeatedly in 30 ml methanol until the extract was clear. The samples were then evaporated under vacuum to remove the water and methanol until volume of the samples were remained about 10 ml and stored at 4 °C until analysis.

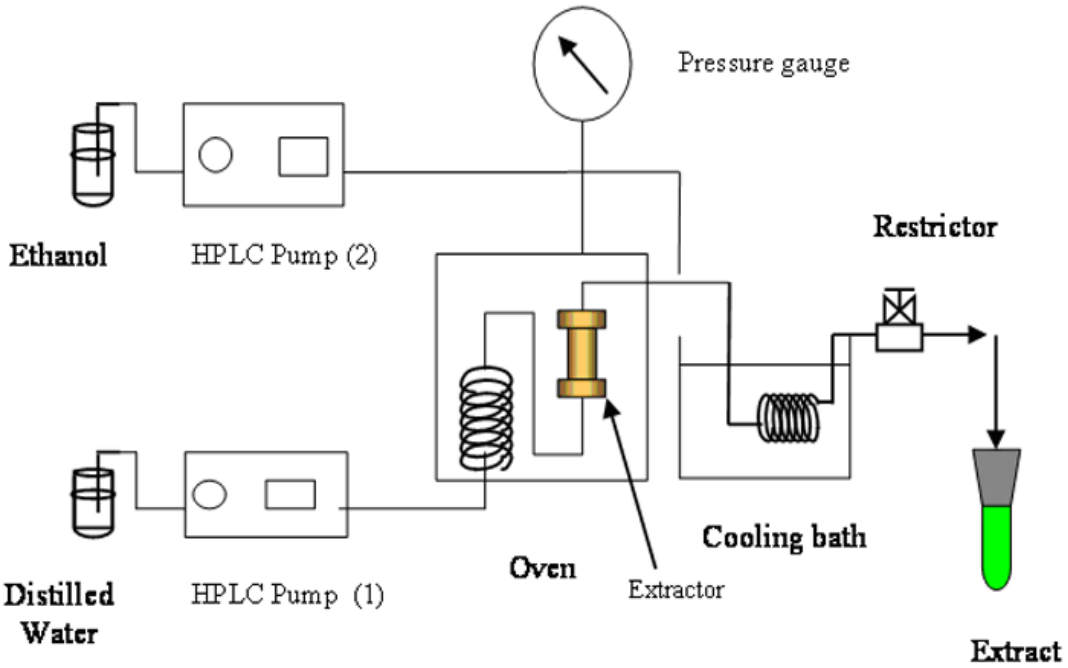

Figure 1. Diagram of experimental setup subcritical water extraction.

2.4 Solvent extraction

One gram of the fine ground sample was weighed into a test tube and 10 ml methanol was added and the sample was then extracted for 2 h in an ultrasonic bath (275DAG, Crest, Malaysia) at 65 °C. After extraction, the sample was cooled to room temperature and then centrifuged at 1500 rpm for 15 min. The sample residue was extracted repeatedly with 30 ml of methanol. The extract was filtered with filter paper (No.4, Whatman, England) and evaporated under vacuum to remove the methanol. Then the concentrated extract was stored at 4 °C until use.

2.5 Soxhlet extraction

One gram of the fine ground sample was weighed into a thimble and was extracted with 200 ml of methanol for 4 hr. The sample residue was removed from the thimble and extracted repeatedly with 30 ml of methanol using ultrasonication. The extract was filtered with filter paper (No.4, Whatman, England) and evaporated under vacuum to remove the methanol. Then the concentrated extract was stored at 4 °C until analysis.

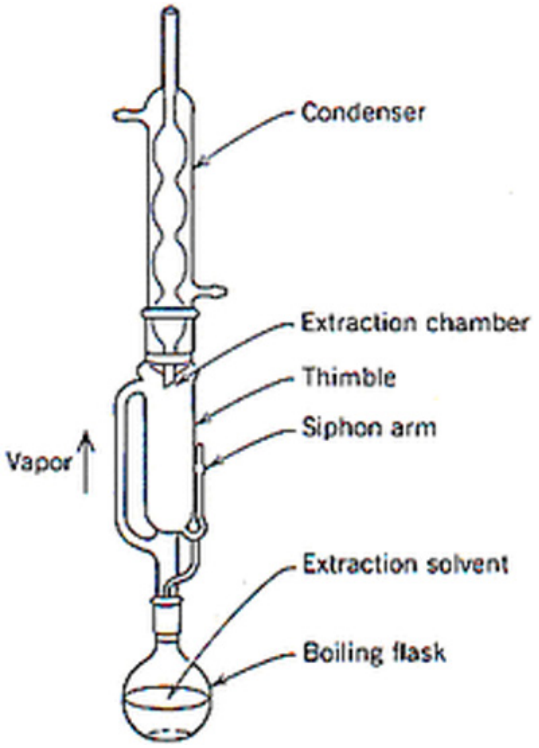

Figure 2. Diagram of soxhlet apparatus.

2.6 Total phenolic contents analysis

The total phenolic content was analyzed with the Folin-Ciocalteau method modified from [13]. 0.1 ml of the extract was mixed with 2.8 ml of distilled water, 0.1 ml of 50% Folin-Ciocalteau reagent, and 2 ml of Na_2CO_3 (2 g/100ml). The mixture was incubated at room temperature for 30 minutes. The mixture absorbance was measured spectrophotometrically at wavelength 750 nm. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram dry material.

2.7 Phenolic acid constituent analysis

The phenolic acid constituents of the extracts were determined using HPLC by modified from the method of Cai et al. [14]. HPLC were performed with a C-18 Inertsil ODS-3 column (5 μm particle, 4.6 x 250 mm ID) and equipped with UV detector. The UV detector absorbance was monitored at 254 nm. The mobile phases consisted of solvent A (0.1% trifluoroacetic acid in acetonitrile), solvent B (0.1% trifluoroacetic acid in HPLC grade water), and solvent C (100% methanol, HPLC grade). Flow rate was set at 1.0 ml/min, and column temperature was maintained at 37 °C throughout of the test. The initial solvent composition was 0% solvent A and 100% solvent B. A linear gradient was used to increase solvent A from 0% to 10% within 7 minutes. This solvent composition was maintained at an isocratic flow for 3 min. The solvent A was then increased from 10% to 40% using a 20-min linear gradient. This composition was maintained for 2 min and returned to the initial composition in 3 min. Solvent C was used for washing the column after each run. The sample injection volume was 10 μl. The concentrations of phenolic acids in the sample were calculated from standard curves, from a plot of peak areas versus concentrations for a series of standard solutions.

2.8 Antioxidant activity determination

Antioxidatant activity was detemined using ABTS (2, 2'-azinobis-(3 EthylbenzoThiazoline 6-sulfonic acid) radical scavenging assay which was carried out following the method of Re et al. [15] with some modifications. The extract was diluted in series in water and each diluted samples were added with the $ABTS^{o+}$ stock solution ,which included 7mM ABTS and 2.45 mM potassium persulfate, with the volume ratio of 1:10 (sample solution: $ABTS^{o+}$ stock solution). The $ABTS^{o+}$ stock solution had absorbance of 0.70 ± 0.02 units at 734 nm using the spectrophotometer. The solutions were mixed using a vortex and the mixtures were incubated at room temperature for 10 minutes, and then the absorbance was taken at 734 nm using the spectrophotometer.

For comparing the antioxidant activity of the extracts obtained at various conditions, concentration of sample producing 50% reduction of the radical absorbance (IC_{50}) was used as an index. The IC_{50} values for various extracts were found from the plots of percent inhibition (PI) versus the corresponding concentration of the sample. The values of PI were calculated using the following equation:

$$PI\ (\%) = [1-(A_t / A_r)] \times 100 \qquad (1)$$

Where A_t and A_r are absorbance of test sample and absorbance of the reference, respectively.

3. RESULTS AND DISCUSSION

3.1 Total phenolic and phenolic acid contents analysis

The total phenolic contents of the extracts obtained by the SCWE at 130 °C, the SCWE at 150 °C, SCWE at 180 °C, the SCWE at 200 °C, the solvent extraction, and soxhlet extraction were 10.571, 25.219, 42.915, 48.177, 7.743, and 4.992 mg GAE/g dry weight (DW), respectively. Overall, the extracts obtained by SCWE were significantly higher than solvent extraction and soxhlet extraction as shown in Fig. 3. Temperature is expected to have a significant effect on extraction efficiency. The amount of the total phenolic contents of the extracts obtained by the SCWE increased when the temperature increased and the total phenolic contents of each collected samples were found the most among in 10 min as shown in Figure 4.

The main phenolic acid contained in

bitter melon was gallic acid. The phenolic acid was calculated from HPLC analysis of the extracted that this results indicated the extracts obtained by SCWE for 2 hr contained higher amount of gallic acids were than that obtained by solvent extraction at 65 °C for 2 hr and soxhlet extraction for 4 h. The gallic acids of the extracts from bitter melon obtained by the SCWE at 130 °C, the SCWE at 150 °C, SCWE at 180 °C, the SCWE at 200 °C, the solvent extraction, and soxhlet extraction were 0.0913, 0.3169, 0.5502, 0.6462, 0.0271, and 0.0120 mg/g DW, respectively.

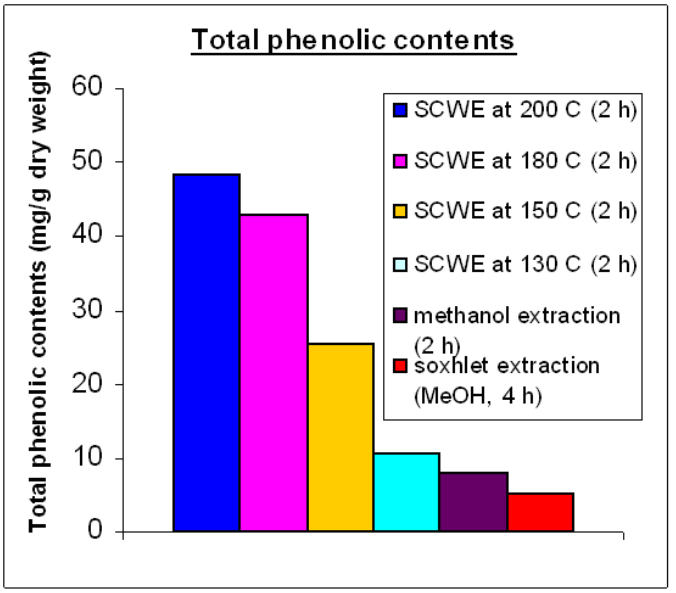

Figure 3. Total phenolic contents of bitter melon extracts by the SCWE at 130 °C, the SCWE at 150 °C, the SCWE at 180 °C, the SCWE at 200 °C, the solvent extraction and soxhlet extraction, respectively.

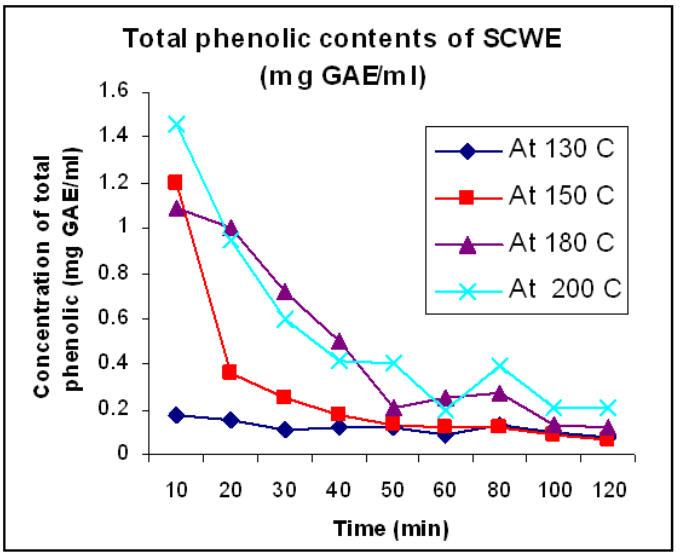

Figure 4. Effect of temperature on extraction efficiency of SCWE.

3.2 Antioxidant activity

Antioxidant was represented by IC_{50} index which is the concentration of sample producing 50% reduction of the radical absorbance. The IC_{50} values of each collected sample of the SCWE at 130 °C, at 150 °C, at 180 °C, at 200 °C for 2 h and IC_{50} values of the extracts obtained with solvent extraction at 65 °C for 2 hr and soxhlet extraction for 4 h were 4.480, 3.970, 5.757, 5.720, 8.426, and 6.107 μg/ml, respectively as shown in Figure 5. The IC_{50} values of the SCWE at all temperatures were lower than that of solvent extraction and soxhlet extraction, which means SCWE gave the extracts with higher antioxidant activity. There was no significant difference in the antioxidant activities of the SCWE from bitter extracted with different extraction time but the extraction temperature gave significantly different results as shown in Figure 6. Even though the total phenolic contents of the extracts obtained by different extrac-tion methods and extraction temperatures were significantly different, their different antioxidant activities indicated that antioxidant activity was determined not only by their total phenolic contents but also by other compounds extracted from the sample.

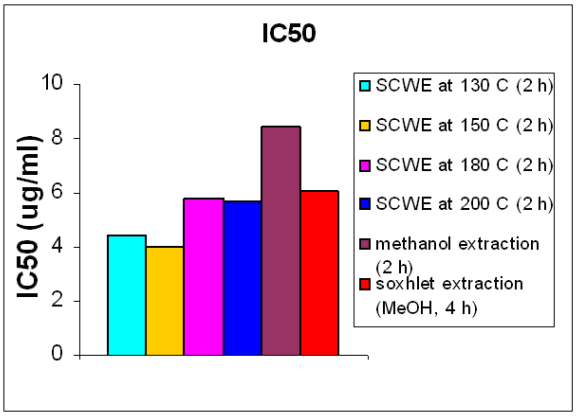

Figure 5. Antioxidant activity (IC_{50}) of the bitter melon extracts by the SCWE at 130 °C, the SCWE at 150 °C, the SCWE at 180 °C, the SCWE at 200 °C, the solvent extraction and soxhlet extraction, respectively.

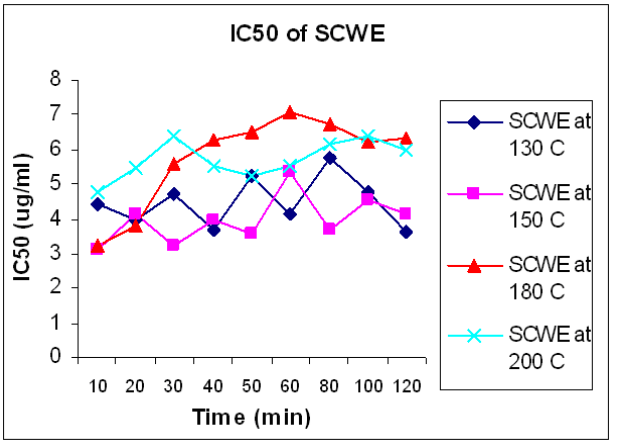

Figure 6. Effect of temperature on antioxidant activity (IC_{50}) of the bitter melon extracts by the SCWE.

4. CONCLUSIONS

Bitter melon is a good source of phenolic compounds which possess potent antioxidant activity. The extracts obtained by SCWE had higher total phenolic contents and antioxidant activity than that obtained by solvent extraction and soxhlet extraction. And these results indicated that the SCWE is a promising alternative for extraction of the antioxidative phenolic compounds from bitter melon.

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