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Contributed Paper

Subcritical Water Extraction of Polyphenolic Compounds from *Terminalia chebula* Fruits

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ABSTRACT

Fresh or dried fruit of *Terminalia chebula* Retz. is commonly used as herbal medicine as it contains various phytochemicals including gallic acid (GA), ellagic acid (EA), and corilagin (CG). These polyphenolic compounds also exhibited therapeutic properties such as antioxidant, antihyperlipidemia and anticarcinogenic activities. This study investigated the separation of polyphenolic compounds such as gallic acid and ellagic acid from *T. chebula* fruits by subcritical water extraction (SWE). We considered the effect of extraction temperature (120-220°C) and water flow rates (2-4 ml/min) at the pressure of 4-6 MPa on the amount of compounds extracted and determine the suitable conditions for subcritical water extraction of these compounds. The results showed that the amount of GA and EA extracted increased when the extraction temperature increased and they were the highest at 180°C beyond which the products decreased due to thermal degradation. At a fixed temperature of 180°C, the effect of water flow rate on the amount of desirable compounds indicated that the increase in water flow rate gave higher amount of GA and EA extracted. The suitable condition for subcritical water extraction of gallic acid and ellagic acid from *T. chebula* fruits is at temperature of 180°C and water flow rate of 4 ml/min.

Keywords: Subcritical water extraction, Terminalia chebula, Samor thai, Polyphenolic, Gallic acid, Ellagic acid.

1. INTRODUCTION

Terminalia chebula Retz. is a native plant in India and Southeast Asia. In Thailand, it is known as *Samor thai*. This herbal plant can be used to treat several illnesses due to its various phytochemicals that exhibit various medicinal properties depending on the part used. The most important part of the plants used is the fruit either fresh or dried *T. chebula* which contains many polyphenolic compounds including gallic acid (GA), ellagic acid (EA), and corilagin (CG) [1]. Their chemical structures and molecular weights are shown in Figure1.

These compounds have several therapeutic activities especially against some chronic diseases including cancer and cardiovascular diseases and antioxidant activities [2].

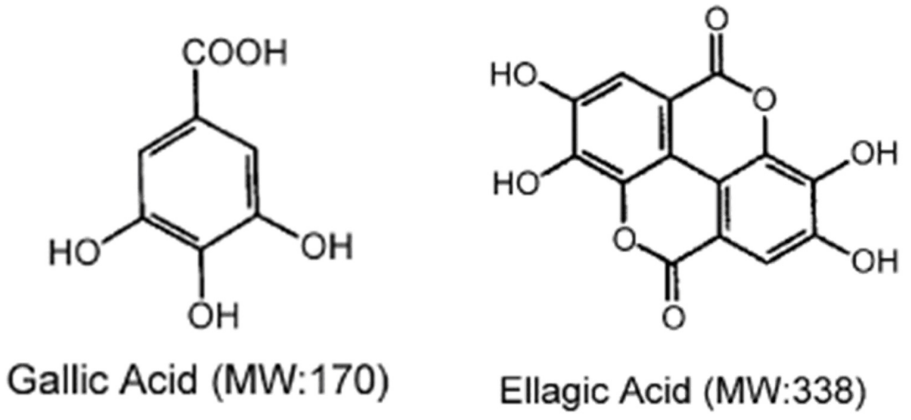

Figure 1. Chemical structures and molecular weights of gallic acid and ellagic acid.

Previously, these phenolic antioxidative components in *T. chebula* were extracted by organic solvents such as ethanol, ethyl acetate [3], ether [4], and 70% methanol [5], but organic solvents would be harmful to the consumer's health if it is not properly removed from the extract. Although phenolic compounds can be extracted with hot water (70-80°C) [6], the process takes a long time due to the low solubility of the compound in water. Alternatively, subcritical water extraction (SWE) can be used, which is the extraction with water at the temperature between boiling (100°C) and critical (374°C) temperature, under high enough pressure to maintain water in the liquid state. At this condition, water polarity decreases, thus increases the solubility of several organic compounds in water. Therefore, this study investigated the suitable conditions for subcritical water extraction of polyphenolic compounds such as gallic acid and ellagic acid from *T. chebula* fruits by considering the effect of extraction temperature and water flow rates.

2. MATERIALS AND METHODS

2.1 Materials

Water used in the experiments was distilled and deionized. The dried fruits of *T. chebula* were obtained from the Chulabhorn Research Institute and then crushed into fine powder using a blender.

2.2 Methods

The apparatus of subcritical water extraction is shown in Figure 2. The system consisted of two HPLC pumps (PU 980, JASCO, Japan) used for delivering water and solvent, a degassing instrument (ERC 3215, CE, Japan), an oven (D63450, HARAEUS, Germany), in which 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).

Before heating the extraction system, all connections were checked for possible leakage. The oven was preloaded with 1 g of ground *T. chebula* fruits and the temperature was set to the desired operating condition

(120-220°C). When the temperature reached the set point, the extraction started. The degassed water (distilled water without dissolved oxygen) was then delivered at a constant flow rate (2-5 ml/min) with the first HPLC pump to a 3-m preheating section installed in the oven to heat it to the required temperature before passing through the extraction vessel. The pressure of the system was adjusted to the desired condition using the back-pressure regulator valve at the outlet coil. Because pressure has no effect on the water polarity [7], thus in this study, we used the pressure of 4-6 MPa to ensure that water was in liquid state at the temperatures tested. The second pump was then turned on to deliver degassed water 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 was then collected in fractions in collecting flasks. After that, the extract was analyzed by HPLC with UV detection at the wavelength of 270 nm.

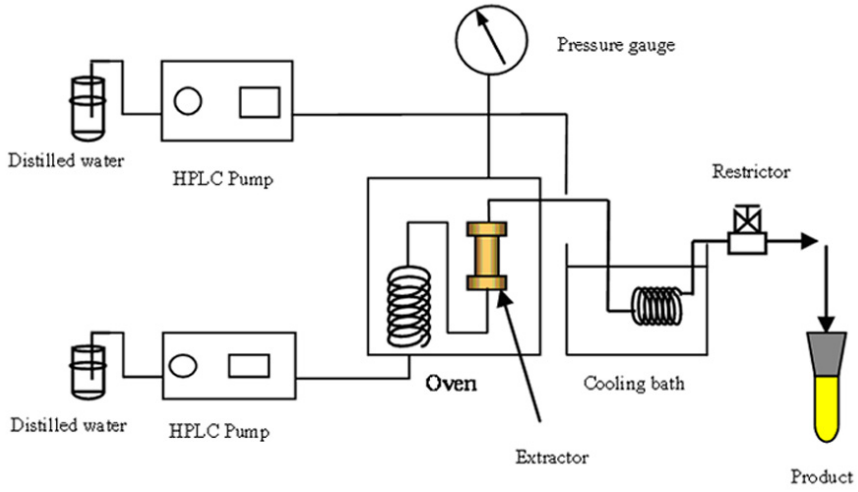

Figure 2. Diagram of experimental setup subcritical water extraction.

After each extraction, the amount of polyphenolic compounds remaining in the fruit residue was determined by solvent extraction with distilled water. The fruit residue was taken out of the extractor and placed into a 100 ml Erlenmeyer flask, containing 30 ml of distilled water. It was then allowed to release the products into the solvent overnight. The solution was then replaced with 10 ml of fresh distilled water daily for 5 days.

3. RESULTS AND DISCUSSION

3.1 Effect of extraction temperature

In this work, the effect of subcritical water extraction temperature in the range 120-220°C on the amount of gallic acid and ellagic acid at a fixed pressure of 6 MPa and a fixed flow rate of 5 ml/min was determined. To get the maximum possible product, we operated this extraction for 4 hr.

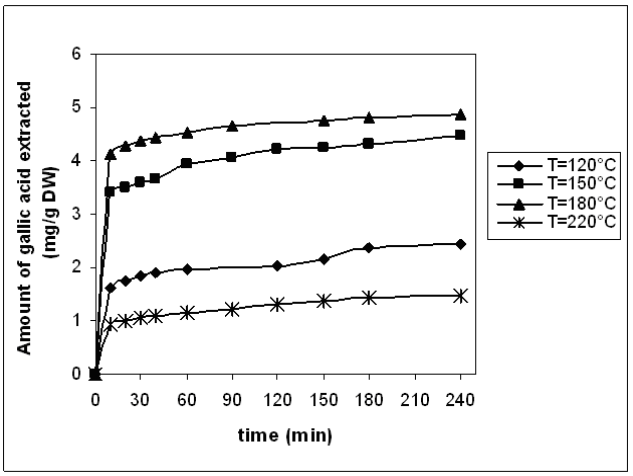

Figure 3. Effect of subcritical water extraction temperature on the amount of gallic acid extracted

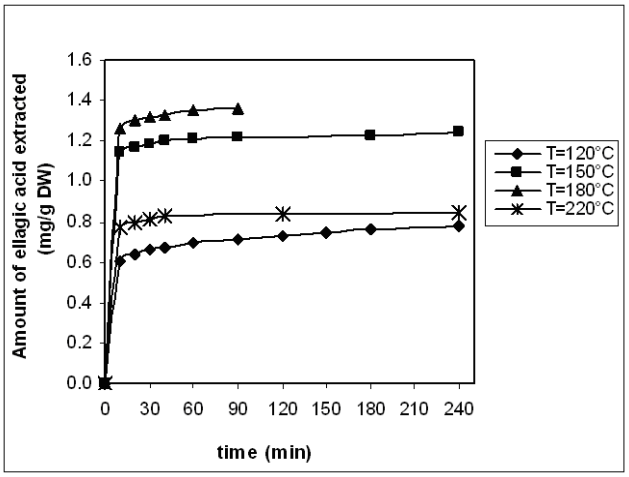

Figure 4. Effect of subcritical water extraction temperature on the amount of ellagic acid extracted.

As shown in Fig.3 and Fig.4, as expected the results showed that increasing temperature caused increasing product solubility. In addition, the increased solubility could be also be caused by the decreasing water polarity at higher temperature. At 180°C, the amount of GA and EA extracted was the highest. However, at 220°C, thermal degradation of the product occurred and this result was confirmed by HPLC analysis which showed that no GA or EA remained in the sample residue. Moreover, it can be seen that the volume of water used and the extraction time about 2 hr were enough for the extraction process.

3.2 Effect of water flow rates

The effect of water flow rate (2-4 ml/min) was determined for extraction at 180°C and at 4 MPa. The results for the amount of gallic acid and ellagic acid extracted versus volume of water used are shown in Fig.5 and Fig.6, respectively. It can be seen from these figures that the amount of the desired

compounds increased with an increase in volumetric flow rate up to 4 ml/min. The reason is possibly because at low flow rate, the residence time of the extract in the extractor was higher and thus caused the decomposition of products. The amount of the GA and EA extracted at 4ml/min at 4 MPa was actually higher than that obtained with water at the flow rate of 5 ml/min and at the the pressure of 6 MPa as previously reported. Possible reasons for this should further be investigated.

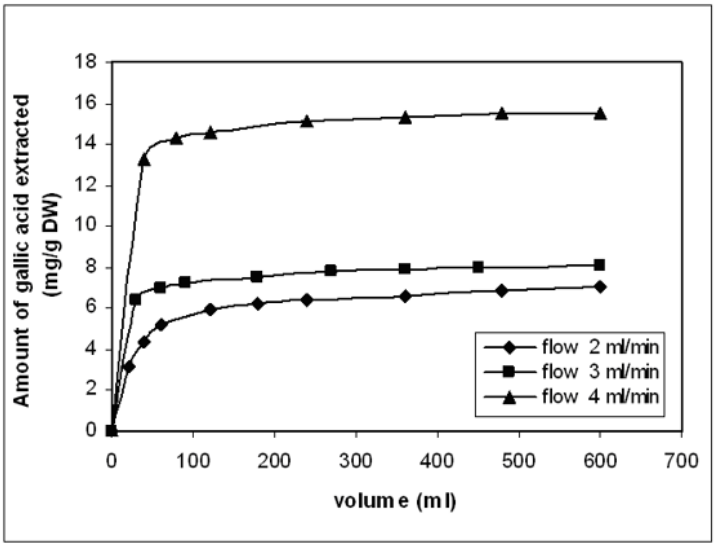

Figure 5. Effect of water flow rate on the amount of gallic acid extracted versus volume of water for SWE at 180°C.

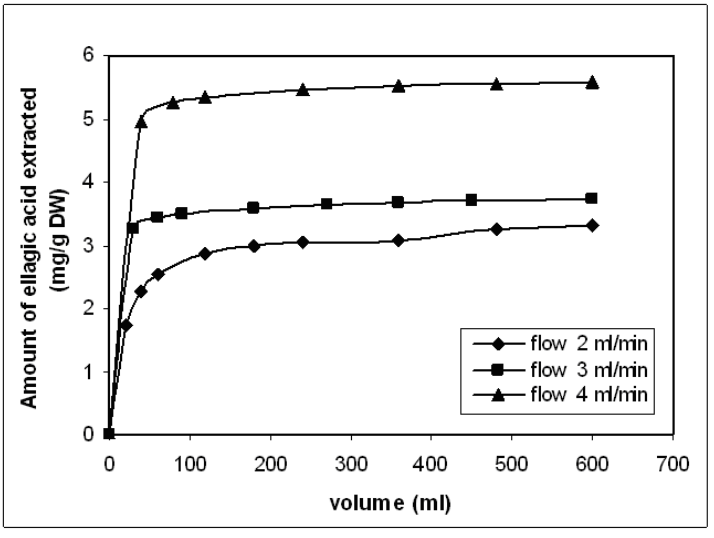

Figure 6. Effect of water flow rate on the amount of ellagic acid extracted versus volume of water for SWE at 180°C.

4. CONCLUSIONS

The results in the present study showed that the higher the temperature, the greater the amount of phenolic compounds extracted, especially at the temperature of 180°C which gave the highest amount of the extracts. However, the possible degradation of gallic acid and ellagic acid occurred at high extraction temperature. In the same way, increasing water flow rate increased the products. These results could be concluded that the suitable condition for subcritical water extraction of gallic acid and ellagic acid from *T. chebula* fruits is at temperature of 180°C and water flow rate of 4 ml/min. From these results, it could be suggested that subcritical water extraction is an alternative method for extraction of polyphenolic compounds from *T. chebula* fruits.

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