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MICROWAVE-ASSISTED EXTRACTION OF PHENOLIC COMPOUNDS FROM *Polyscias fruticosa* (L.) Harms ROOT

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Abstract

Polyscias fruticosa (L.) Harms is an herbal plant that possesses many medicinal uses. The interest in this material and its applications have been increasing rapidly in recent years. However, changes in phytochemical components and antioxidant properties of *P. fruticosa* roots when using microwave-assisted extraction (MAE) methods have not been well understood. The aim of this study was to determine the best extraction conditions for total phenolic content (TPC) and antioxidant capacity (AC) of *P. fruticosa* roots using MAE method. Four factors of the extraction process (solvent/material (SM) ratio, solvent concentrations, microwave power, and extraction time) were investigated. DPPH method was used to determine free radical scavenging activities while TPC was estimated by the Folin-Ciocalteu assay. The results pointed to the optimal extraction parameters which were ethanol concentration of 50% (v/v), ethanol/material ratio of 60/1 (mL/g), microwave power of 265 W, and extraction time of 5 min. TPC and AC obtained were approximately 2.31±0.01 mg GAE/g DW and 76.62±0.23%. In addition, the initial material was completely destroyed under microwave treatment. The results also indicated that MAE could be a fast and reliable method for quantitative analysis of phenolic compounds in *P. fruticosa* roots.

INTRODUCTION

Polyscias fruticosa belongs to the family *Araliaceae* which is widely cultivated in Asia for medicinal and food purposes, especially in Vietnam. All parts of this plant are useful for human health. For instance, the leaves are used as a tonic and for their anti-inflammatory, antitoxin, and antibacterial activities and as digestive support. The root extract has been traditionally used for the treatment of ischemia, dysentery, inflammation, neuralgia, and rheumatic pains [1]. Particularly, *P. fruticosa* root can be applied in the beverage industry in Vietnam, especially in traditional alcohol processing. The previous study found that this plant possesses a large number of bioactive compounds such as phenolic compounds, saponins, and triterpenoids [2]. However, nowadays, much of the published literature is focused on studying saponins due to the pharmacological activities of this plant related to the presence of saponins [3], while the phenolic compounds in *P. fruticosa* can prevent the growth of prostate and lung cancer, improve vascular health, and also exhibit anti-mutagenic, anti-asthma and anti-cancer activities [4-6]. Until now, few studies have reported about the extraction of phenolic compounds from this plant in Vietnam. Almost these studies only performed the extraction process using the conventional method and authors have focused on the effects of extraction, cultivation, and storage conditions of *P. fruticosa* on changes in TPC and AC. As we know, the TPC and AC of the material significantly depend on extraction methods. Hence, finding out an optimal extraction method to enhance TPC and AC is quite important and necessary in many fields including the food industry, biotechnology, and the medicinal field.

There are many methods to extract phenolic compounds from plants, and one of them is microwave-assisted extraction (MAE). Using MAE techniques to extract biological compounds has received more and more attention

in recent years because this method has advantages over traditional methods. It provides short extraction time, high recovery efficiency, lower cost, and reduction of solvent consumption [7]. Therefore, the main objective of this study is to determine some extraction parameters using the MAE method to obtain the highest amount of TPC and AC from the extract of *P. fruticosa* root.

MATERIALS AND METHODS

Materials and Sample Preparation

P. fruticosa roots were harvested from Tra Vinh province (Vietnam). The roots were cleaned, sliced (2-3 mm thickness), and dried at 60°C until the moisture level was less than 14%. The slices were ground into a fine powder (diameter of the particle is less than 0.5 mm), vacuum-packed, and stored at room temperature (28-30°C) until analyses.

Chemicals and Reagents

Folin-Ciocalteu (FC) and DPPH (2,2-diphenyl-1-picrylhydrazyl) reagents were purchased from Merck (Germany) and all other chemicals, and organic solvents were of analytical grade.

Extraction Process

Root powder (3 g) was extracted using aqueous ethanol as a solvent under microwave irradiation (Electrolux EMM20K18GM, China). The extraction parameters consist of SM ratios (40/1, 50/1, 60/1, 70/1, and 80/1, mL/g), ethanol concentrations (35, 40, 45, 50, 55, and 60%, v/v), extraction times (1, 3, 5, 7, and 9 min), and various microwave powers (97, 188, 265, 411, and 439 W). The extracts obtained were filtered through Whatman (No. 4) filter paper, then the TPC and AC content of the extracts were determined.

Determination of Total Polyphenol Content (TPC)

The TPC was determined by the FC colorimetric method [9]. The results were based on a standard curve obtained with gallic acid as a standard agent at 738 nm, UV-spectrophotometer (Genesys 20, USA). TPC were expressed as mg of gallic acid equivalents per gram of dry weight (mg GAE/g DW).

Determination of Antioxidant Capacity (AC)

The AC in the extracts was determined by DPPH assay and this method was described in the procedures of Rahman et al. [8]. The AC was measured spectrophotometrically at 517 nm and expressed in percent DPPH radical scavenging capacity (RSC).

$$\%DPPH_{RSC} = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 is the absorbance of the control, and A_1 is the absorbance of the samples.

Scanning Electron Micrographs (SEM)

A scanning electron microscope system (Jeol JSM-6400, USA) was used to examine morphological changes in the dried powder of *P. fruticosa* root before and after extraction at 5 kV and at a vacuum pressure of 0.04 Pa.

Statistical Analysis

Experimental results were analyzed by the one-way analysis of variance (ANOVA) method and significant differences among the means from triplicate analyses at (p<0.05) were determined by Fisher's least significant difference (LSD) procedure using Statgraphics software (Centurion XV). The values obtained were expressed in the form of mean±standard deviation (SD).

RESULTS AND DISCUSSION

Effect of SM Ratio on TPC and AC of the Extract

The powdered samples of *P. fruticosa* roots were extracted with aqueous ethanol under the following extraction conditions: ethanol concentration of 50% (v/v), extraction time of 5 min, and microwave power of 265 W; while SM ratios (40/1, 50/1, 60/1, 70/1, and 80/1, mL/g) were investigated in this study.

Figure 1 shows that the SM ratios strongly affects the extraction efficiency as well as the AC of polyphenol compounds. Both TPC and AC slightly increased at first and then decreased after peaking at the ratio of 60/1 (mL/g). The highest values of TPC and AC were 2.25±0.07 mg GAE/g DW and 75.75±0.14%, respectively.

Increasing the SM ratio promotes a greater diffusion of phenolic compounds into the solvent [10]. However, these components will not continue to increase when equilibrium is reached [11], leading to an increase in TPC and AC. Therefore, if the amount of solvent is too large, the improvement in extraction efficiency will be insignificant, and the process will take more time and be more costly. With a low SM ratio, the material will be hardly exposed to the solvent, and will not be possible to completely extract phenolic compounds. The SM ratio obtained is different from that of previous studies. For instance, Quoc and Muoi [12] reported that the best extraction yield from *Polygonum multiflorum* Thunb. roots using MAE was at an SM ratio of 40/1 (mL/g). This difference was attributed to differences in the initial materials and their chemical components.

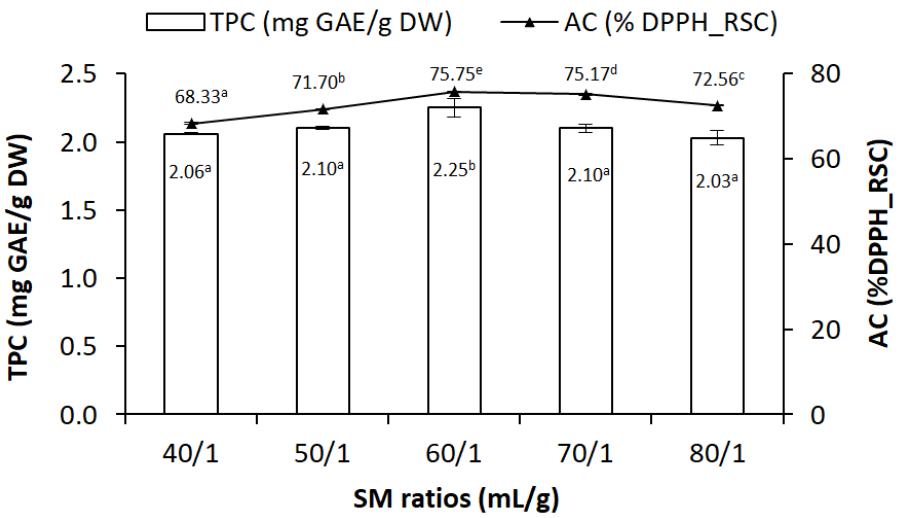

Figure 1. Effect of SM ratios on TPC and AC of the extract

Consequently, the SM ratio of 60/1 (mL/g) was considered as suitable to gain maximum TPC and AC for polyphenol extraction from *P. fruticosa* roots.

Effect of Ethanol Concentration on TPC and AC of the Extract

The experiments were carried out with some of the extraction parameters above, including the SM ratio of 60/1 (mL/g), extraction time of 5 min, and microwave power of 265 W, while ethanol concentrations (35, 40, 45, 50, and 55%, v/v) were examined in this study.

Figure 2 illustrates that an increase in ethanol concentration, can lead to an increase in TPC and AC. Both TPC and AC obtained the highest values of 2.33±0.01 mg GAE/g DW and 75.75±0.12% at the ethanol concentration of 50%, then their values decreased at the higher ethanol concentrations. The optimal ethanol concentration obtained in this study is similar to that of other studies. For instance, Simíc et al. [13] and Milutinovíc et al. [14] extracted phenolic compounds from chokeberries and waste *Equisetum arvense* using MAE at ethanol concentrations of 53.6% and 54.5%, respectively. In this study, the used solvent concentration remained relatively low, it pointed that

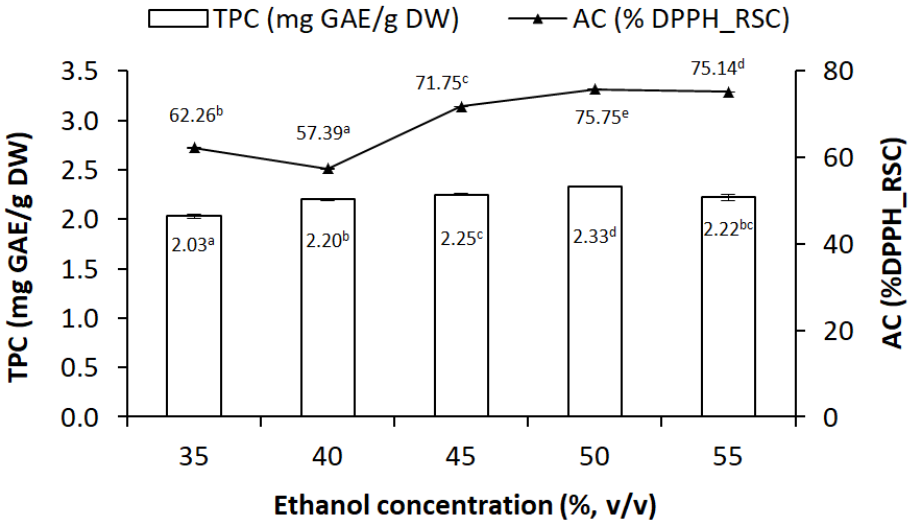

Figure 2. Effect of ethanol concentration (%, v/v) on TPC and AC of the extract

MAE could reduce ethanol consumption.

According to Kaderides et al. [15], the presence of water in ethanol seems to enhance the extraction yield when compared to the pure solvent. The water in the solvent diffuses easily into the plant cell's matrix, increases solvent polarity, decreases the viscosity of the solvent, and allows bioactive compounds to be easily isolated. In addition, the presence of water can also improve the mass transfer in the extraction process and increase extraction efficiency [16]. Based on the above statements, 50% ethanol was chosen as the appropriate concentration for conducting further studies.

Effect of Microwave Power on TPC and AC of the Extract

From the results above, the experiments were continued with fixed factors, including ethanol concentration of 50% (v/v), SM ratio of 60/1 (mL/g), and extraction time of 5 min. The microwave power experiments were performed at 97, 188, 265, 411, and 439 W.

From Figure 3, it was observed that the highest TPC value was 2.31±0.03 mg GAE/g DW at a microwave power of 265 W, while the highest AC value was 80.88±0.18% at a

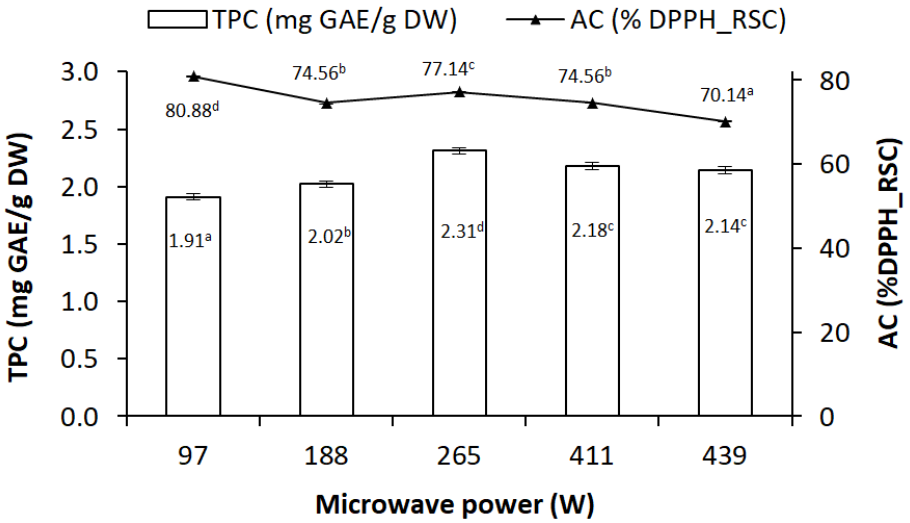

Figure 3. Effect of microwave power (W) on TPC and AC of the extract

microwave power of 97 W. In general, the extraction efficiency at a microwave power of 265 W is optimal, compared to 97 W, the TPC value increases by nearly 21%, while the AC value only decreases 4.6%.

Basically, as the microwave power increased, the yield of polyphenols also increased. The higher the microwave power is, the higher the temperature of the extract is. At optimal temperatures, the solubility and diffusion coefficients of the chemical components to be isolated increase, and the viscosity of the solvent decreases, thus facilitating the solvent's passage through the solid substrate mass. At the suitable microwave power, it can easily destroy the plant cell and accelerate the release of bioactive compounds into the solvent [17]. However, at higher microwave powers (or higher temperatures), the phenolic compounds were decomposed because they are quite thermally sensitive. On a laboratory scale, most submitted studies reported that the microwave power varied from 127 W to 800 W [12, 18]. These differences are attributed to the various materials used and their chemical components. Hence, the suitable microwave power of 265 W was selected for the next step in this study.

Effect of Extraction Time on TPC and AC of the Extract

The irradiation times (1, 3, 5, 7, and 9 min) were investigated, and all extraction parameters were unchanged (SM ratio of 60/1, mL/g; ethanol concentration of 50%, v/v; and microwave power of 265 W). The phenolic compound recovery, in parallel with antioxidant activity, significantly increased with irradiation time, increasing from 1 to 5 min, then both TPC and AC values strongly decreased for longer extraction times. The best results were achieved at an extraction time of 5 min (TPC and AC values were 2.31±0.01 mg GAE/g DW and 76.62±0.23%, respectively) (Figure 4).

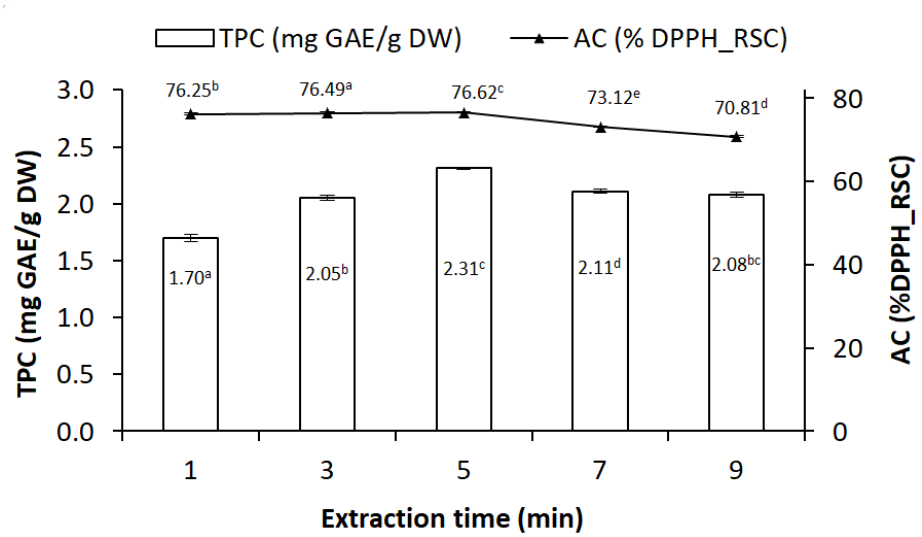

Figure 4. Effect of extraction time on TPC and AC of the extract

The extraction time is one of the important factors in the MAE process, it significantly affects the extraction efficiency as well as the AC of polyphenol compounds. An increase in the extraction time can lead to an increase in the yield of MAE. However, there is also an associated risk of degradation of thermolabile bioactive compounds [19]. In addition, these compounds can be oxidized if extraction time is excessive. The irradiation time in this study is similar to that reported by Quoc and Muoi [12] and Simíc et al. [13]. They also extracted polyphenols from plants using the MAE technique. Compared with other extraction methods, the extraction time of MAE is lower than that of ultrasonic-assisted acid hydrolysis (UAAH) and conventional extraction (CE) methods. For instance, Hoang et al. [20] and Nguyen et al. [21] also extracted polyphenols from *P. fruticosa* leaves and roots using UAAH and CE methods for 90 and 180 min, respectively. This showed that MAE method provides high extraction efficiency in short time.

Based on the above statements, an extraction time of 5 min was the best choice for the next experiment.

Effect of MAE on the Structure of the Material

The initial powdered sample has a small diameter (5-10 µm) with many cracks and tiny pores on the surface. After the MAE treatment, it can be seen that the cells were severely damaged by the impact of microwaves. The structure of the surface of the sample strongly changes; it is rough, tightly sticky, and forms a large amorphous block. Also, the initial pores seem to be bigger (Figure 5). This phenomenon was observed for other materials such as caraway seeds [22], ashwagandha root [23], and *P. multiflorum* Thunb. roots [12]. This proves that the plant cell matrix was collapsed under the microwave irradiation and easily released bioactive compounds into the solvent.

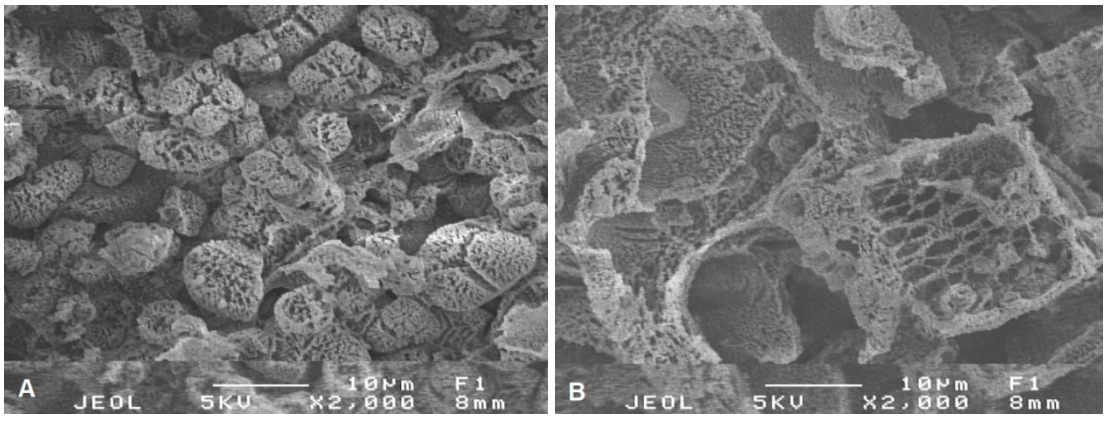

Figure 5. Structure of the material before (A) and after (B) treatment by MAE

CONCLUSION

In this study, an optimized procedure based on MAE has been developed for the extraction of total polyphenols from *P. fruticosa* roots. In MAE, the solvent to material ratio, microwave power, ethanol concentration, and extraction time were found to affect TPC and AC of this material significantly. The extraction conditions of phenolic compounds in this study were determined to be the following: ethanol concentration of 50 % (v/v), SM ratio of 60/1 (mL/g), extraction time of 5 min, and microwave power of 265 W. Compared with other methods, MAE of phenolics from *P. fruticosa* roots was more time efficient and provided high extraction efficiency. Therefore, MAE can be applicable to all natural products and if explored properly, can prove to be an efficient tool for sample preparation and large-scale industrial application.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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