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

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Abstract

Polyscias fruticosa (L.) Harms root extracts were obtained and separated using ultrasound-assisted extraction (UAE) with the preset optimal parameters, such as solvent/solid (SS) ratio (50/1, mL g^{-1}), ethanol concentration (40%, v/v), temperature (45 °C), and extraction time (20 min). The best values of the total phenolic content (TPC) and antioxidant capacity (AC) of the extracts under these extraction conditions were 2.13 ± 0.02 mg of gallic acid equivalents (GAE) per gram of dry sample weight (DW) for TPC and 78.13 ± 0.25% for AC. In addition, the structure of the plant material was examined by scanning electron microscopy (SEM): it was revealed that the structure of the residues changed completely as a result of the ultrasound treatment compared to the initial material.

Keywords: ethanol, herb, phenolic compounds, *Polyscias fruticosa*, UAE

Introduction

Recently, evidence has been mounting that phenolic compounds possess many properties beneficial for human health, such as antibacterial and antioxidant activities, thus suggesting their therapeutic utility for preventing cancer, cardiovascular disease, obesity, and diabetes [1]. Phenolics are substances having an aromatic ring linking with one or more hydroxyl substituents and classified as secondary metabolites in plants with different structures and functions. Generally, they can be typed as either soluble in water (phenolic acids, phenylpropanoids, flavonoids, and quinones) or not (condensed tannins, lignins, and cell-wall bound hydroxycinammic acids) [2].

Some plants are known to be rich in phenolic compounds. Among them are species of the genus *Polyscias* (Araliaceae) that are widely used for medicinal purposes (roots and leaves) and as a food source. To date, 97 bioactive compounds from various chemical classes have been isolated from them, and these are mostly phenolic compounds (flavonoids). For instance, quercetin-3-*O*-*D*-glucopyranoside, aglycone luteolin**,** diglycoside tamaraxetin 3, 7-di-*O*-*α*-L-rhamnopyranoside have been isolated from *P. fulva*, *P. nodosa*, and *P. balfouriana*, respectively [3]. In addition, some phenolic acids, including chlorogenic, caffeic, and ferulic acids, have been detected in the extracts of *P. filicifolia* shoots using UHPLC-DAD-MS/MS analysis [4]. Of special interest is *P. fruticosa*, which is widely cultivated in Vietnam. It thrives in environments with medium humidity and temperatures varying from 16 to 29 °C. This plant exhibits antipyretic, anti-inflammatory, and analgesic action, as well as α-glucosidase

inhibitory and antidiabetic activities [5]. An exciting feature of *P. fruticosa* is that specific phenolic compounds in its roots and leaves are strong, yet poorly studied: one of the most important finding related to it was made by Mai [6] – using the HPLC method, she revealed that *P. fruticosa* leaves are a source of high levels of quercetin. The latter is a plant flavonol from the flavonoid group of polyphenols and of medical value due to its antioxidant, antiulcer, anti-inflammatory, antibacterial, and antiviral activities, as well as therapeutic potential in the treatment of cardiovascular and neurodegenerative disorders [7]. According to Do [8], *P. fruticosa* also contains alkaloids, tannins, saponins, amino acids, and B vitamins in high amounts. This adds to many previous studies that reported only the presence of saponins in *P. fruticosa* [9, 10]. Until now, the phenolic composition of this plant material, especially polyphenols extraction, have received little attention from researchers.

Only a few studies have reported the presence of phenolic compounds in *P. fruticosa*. Le et al. [11] measured the concentration of quercetin in *P. fruticosa* leaves (0.332 mg g^{-1} DW), as well as flavonoids and total polyphenols in its roots (86.13 μg QE mg^{-1} DW and 125.37 μg GAE mg^{-1} DW, respectively) [12]. Nguyen et al. [13] showed that the DPPH and ABTS antioxidant activities of *P. fruticosa* root extracts had IC_{50} values of 96.14 μg mL^{-1} and 38.76 μg mL^{-1}, respectively. Hence, they suggested that this material can be used in dietary applications and for reducing oxidative stress. In these studies, the extraction process was almost exclusively performed by the conventional method, and the authors focused on the effects of extraction, cultivation, and storage conditions of *P. fruticosa* on the changes in total phenolic content and antioxidant capacity.

Ultrasound-assisted extraction (UAE) is an alternative technique, in which the extraction process runs with the help of ultrasound waves. Two main factors that enhance the UAE efficiency are cell disruption and effective mass transfer [14]. This method is of major significance in food processing and analysis owing to the growing industrial demand for sustainable development. Ultrasound generates cavitation bubbles in the biological matrix. It has been rated as helpful for achieving high yields and extraction rates of bioactive compounds, especially phenolic compounds [15]. Probe and bath systems are the two ways of applying ultrasound waves to the sample. Bath sonicators can be used for a range of samples simultaneously with high extraction yields and are also suitable for laboratory use. Compared to the conventional methods (maceration, percolation, and Soxhlet method), UAE is simple, inexpensive, applies for various solvents, and easily scaled up for industrial purposes [16]. Particularly, it can offer substantial environmental benefits and has great potential for further advance and application, especially in the food and pharmaceutical fields. Therefore, the purpose of this research was to determine the extraction conditions using UAE in order to obtain the best yield from the extract of *P. fruticosa* root.

1. Material and Methods

1.1. Plant material and sample preparation. For this study, we selected the roots of *Polyscias fruticosa* (L.) Harms. from Tra Vinh province (Vietnam) that were aged 4–5 years. They were washed with tap water, drained, sliced (2–3 mm thick), and dried at 60 °C for 4 h in a convection oven (Shel Lab SGO3-2, USA) until the moisture content was lower than 14%. The latter parameter was measured with a moisture analyzer

(Ohaus MB120, China). The dried samples were then ground into a fine powder (particle diameter < 0.5 mm), vacuum-packed, and kept at room temperature until further analysis.

1.2. Chemicals and reagents. The Folin–Ciocalteu (FC) and DPPH (2,2-diphenyl-1-picrylhydrazyl) reagents were bought from the Merck Company (Germany). All organic solvents and other chemicals were of analytical reagent grade.

1.3. Ultrasound-assisted extraction (UAE) process. The powdered material was extracted using an ultrasonic bath (Elmasonic S60 H, 550 W, Germany) at different extraction temperatures (40, 45, 50, 55, and 60 °C), ethanol concentrations (30, 35, 40, 45, and 50%, v/v), extraction times (10, 15, 20, 25, and 30 min), and solvent/solid (SS, *v*/*w*, mL g^{-1}) ratios (30/1, 40/1, 50/1, 60/1, and 70/1). The extracts were vacuum-filtered to remove any insoluble residue, and their total polyphenols content (TPC) and antioxidant capacity (AC) were measured.

1.4. Total polyphenols content (TPC). The TPC was analyzed according the FC colorimetric assay of Siddiqua et al. [17]. The results were quantitated on a standard curve obtained with gallic acid as a standard agent at 738 nm by an UV-spectrophotometer (Genesys 20, USA). The TPC was expressed as mg of gallic acid equivalents per gram of dry weight (mg GAE g^{-1} DW).

1.5. Antioxidant capacity (AC). The AC was evaluated by DPPH assay using the procedure described by Rahman et al. [18]. It was measured spectrophotometrically at 517 nm and expressed in percent of DPPH radical scavenging capacity (RSC):

$$\mathrm{DPPH_{RSC}} = \frac{\mathrm{OD_0} - \mathrm{OD_1}}{\mathrm{OD_0}} \cdot 100\%,$$

where OD_0 is the absorbance of the control and OD_1 is the absorbance of the sample.

1.6. Scanning electron microscopy (SEM). A scanning electron microscope (Jeol JSM-6400, USA) operating at 5 kV and vacuum pressure of 0.04 Pa was used to determine the structural changes in the *P. fruticosa* root samples before and after extraction.

1.7. Statistical data analysis. All data were statistically processed with analysis of variance to determine the significance level. Fisher's least significant difference (LSD) procedure was used to calculate the significance of differences between the mean scores. Detailed statistical inference was carried out with the help of the Statgraphics software (Centurion XV).

2. Results and Discussion

2.1. Effect of SS ratios on the UAE yield. At the first stage, samples were isolated under the following fixed extraction conditions: extraction time 20 min, temperature 50 °C, and ethanol concentration 50% (v/v). The SS ratio was raised from 30/1 to 70/1 (mL g^{-1}) to determine the efficiency of the extraction process. Table 1 illustrates that the extraction yield increased steadily as the SS ratio grew from 30/1 to 50/1 (mL g^{-1}). Then, both TPC and AC values decreased slightly at higher SS ratios. The best TPC and AC values were 2.03 ± 0.02 mg GAE g^{-1} DW and $77.59 \pm 0.23\%$ at the ratio of 50/1 (mL g^{-1}), respectively.

Table 1

Effect of the SS ratio on TPC and AC of the extract

SS ratios (mL g^{-1})	30/1	40/1	50/1	60/1	70/1
TPC (mg GAE g^{-1} DW)	1.53 ± 0.02^{a}	1.77 ± 0.05^{c}	2.03 ± 0.02^{e}	1.86 ± 0.02^{d}	1.69 ± 0.05^{b}
AC ($DPPH_{RSC}$, %)	70.67 ± 0.36^{a}	75.59 ± 0.22^{b}	77.59 ± 0.23^{c}	77.30 ± 0.21^{c}	75.66 ± 0.28^{b}

Values are the mean ± standard deviation of triplicate analyses. Different superscript letters (a, b, c, etc.) in the same row indicate significant differences between the SS ratios ($p < 0.05$) as measured by Fisher's LSD test.

The positive effect of increasing the SS ratio was also reported by Quoc and Muoi [19] who isolated polyphenols from *Polygonum multiflorum* Thunb. roots using UAE: the highest process yield was achieved when the SS ratio was increased from 20/1 to 30/1 (mL g^{-1}). This trend has been also observed in other studies [20, 21]. However, when equilibrium is reached, the soluble compounds no longer diffuse into the solvent [22]. Therefore, if the solvent volume is too large, the extraction yield increases insignificantly, which is time consuming. At lower SS ratios, it is impossible to extract the material completely. On the other hand, the SS ratio depends on the type and size of materials and the level of soluble components.

Based on the above results, the SS ratio of 50/1 (mL g^{-1}) was considered suitable to evaluate the TPC and AC for the subsequent experiment.

2.2. Effect of ethanol concentration on the UAE yield. The experiment was performed at the SS ratio of 50/1 (mL g^{-1}), temperature 50 °C, and extraction time 20 min. Ethanol concentrations varied from 30 to 50% (v/v). The results in Table 2 show that the TPC values slightly increased, reached 2.03 ± 0.02 mg GAE g^{-1} DW at the ethanol concentration of 40% (v/v), and then slowly decreased at higher ethanol concentrations; the AC value remained unchanged at all concentrations studied. This indicates that the AC values do not depend on the ethanol concentrations from 30 to 50% (v/v).

Ethanol is a promising environmentally friendly solvent and thus an important ingredient in food production. The presence of water in the ethanol feed increases the mixture polarity; in this case, ethanol concentrations have a medium polarity and can improve the TPC value of the extracts. Compared to a pure solvent, water can penetrate easily into the plant cells, raise the polarity of the solvent, and reduce its viscosity; with suitable polarity, soluble chemical components are easily released, and the extraction efficiency becomes enhanced significantly [23]. The successful use of aqueous ethanol as a solvent to extract polyphenols from *P. fruticosa* roots has been described in the literature [12, 13]. The optimal ethanol concentration obtained in this study was essentially different from those found in other works. Tabaraki et al. [24] and Wang et al. [25] isolated polyphenols from pomegranate peel and *Sparganii rhizoma* using UAE at the ethanol concentrations of 70% (v/v) and 80% (v/v), respectively. These variations can be explained by the different polarities and soluble components of the materials. Therefore, the ethanol concentration of 40% (v/v) was set for the next step, which aimed to evaluate the UAE yield.

2.3. Effect of temperature on the UAE yield. Temperature is one of the important parameters in the UAE process. In this study, temperatures were evaluated in the range from 40 to 60 °C with other fixed parameters: SS ratio 50/1 (mL g^{-1}), ethanol concentration 40% (v/v), and extraction time 20 min.

Table 2

Effect of the ethanol concentration on TPC and AC of the extract

Ethanol concentration (%, v/v)	30	35	40	45	50
TPC (mg GAE g^{-1} DW)	1.73 ± 0.03^{a}	1.86 ± 0.02^{b}	2.03 ± 0.02^{c}	1.82 ± 0.02^{b}	2.01 ± 0.02^{c}
AC ($DPPH_{RSC}$, %)	77.44 ± 0.26^{a}	77.46 ± 0.42^{a}	77.51 ± 0.26^{a}	77.46 ± 0.15^{a}	77.36 ± 0.34^{a}

Values are the mean ± standard deviation of triplicate analyses. Different superscript letters (a, b, c, etc.) in the same row indicate significant differences between the ethanol concentrations ($p < 0.05$) as measured by Fisher's LSD test.

Table 3

Effect of the extraction temperature on TPC and AC of the extract

Temperature (°C)	40	45	50	55	60
TPC (mg GAE g^{-1} DW)	1.82 ± 0.01^{a}	2.13 ± 0.03^{d}	2.02 ± 0.01^{c}	1.89 ± 0.04^{b}	1.85 ± 0.04^{ab}
AC ($DPPH_{RSC}$, %)	77.16 ± 0.24^{a}	78.06 ± 0.16^{b}	77.53 ± 0.33^{a}	77.16 ± 0.24^{a}	77.12 ± 0.09^{a}

Values are the mean ± standard deviation of triplicate analyses. Different superscript letters (a, b, c, etc.) in the same row indicate significant differences between the ethanol temperatures ($p < 0.05$) as measured by Fisher's LSD test.

As seen in Table 3, the extraction yield increased slowly at the temperature from 40 to 45 °C; the TPC and AC values at 45 °C were 2.13 ± 0.03 mg GAE g^{-1} DW and 78.06 ± 0.16%, respectively. Thereafter, they declined slightly.

High temperature reduces the surface tension and viscosity of the solvent and enables the solvent to easily penetrate into the plant cells, thereby resulting in a high extraction yield [26]. However, bioactive compounds are thermally sensitive and get degraded at high temperatures. According to Vinatoru [27], UAE generates compressed cavitation bubbles and leads to their collapse at extremely high local temperature and pressure; the cell matrix is opened, and extractives are released. In general, the extraction temperature used in this study was relatively lower than the temperatures used in previous studies. For example, Aybastıer et al. [28] and Sahin et al. [29] isolated phenolic compounds from blackberry leaves and *Artemisia absinthium* by using UAE at 66–68 °C and 64–70 °C, respectively. This demonstrates that the phenolic compounds contained in *P. fruticosa* are more sensitive to temperature than those in other plant materials.

Consequently, the extraction temperature of 45 °C is the best choice to gain the maximum extraction yield for the next step.

2.4. Effect of extraction time on the UAE yield. The variations in the TPC and AC values were examined at various time intervals (10, 15, 20, 25, and 30 min), solvent concentration of 40% (v/v), SS ratio of 50/1 (mL g^{-1}), and temperature of 45 °C. The influence of the extraction period on the yield was significant ($p < 0.05$). The optimal TPC and AC values were 2.13 ± 0.02 mg GAE/g DW and 78.13 ± 0.25%, respectively, for the interval of 20 min (Table 4). These values were much higher than those obtained by Nguyen [30] who also extracted polyphenols from *P. fruticosa* roots (0.124–0.313 mg GAE g^{-1} DW for TPC and 20.36–29.42% for $DPPH_{RSC}$).

Table 4

Effect of the extraction time on TPC and AC of the extract

Extraction time (min)	10	15	20	25	30
TPC (mg GAE g^{-1} DW)	1.66 ± 0.03^{b}	1.88 ± 0.04^{c}	2.13 ± 0.02^{d}	2.13 ± 0.04^{d}	1.09 ± 0.02^{a}
AC ($DPPH_{RSC}$, %)	78.01 ± 0.16^{c}	78.12 ± 0.16^{c}	78.13 ± 0.25^{c}	77.44 ± 0.16^{b}	76.71 ± 0.17^{a}

Values are the mean ± standard deviation of triplicate analyses. Different superscript letters (a, b, c, etc.) in the same row indicate significant differences between the extraction times ($p < 0.05$) as measured by Fisher's LSD test.

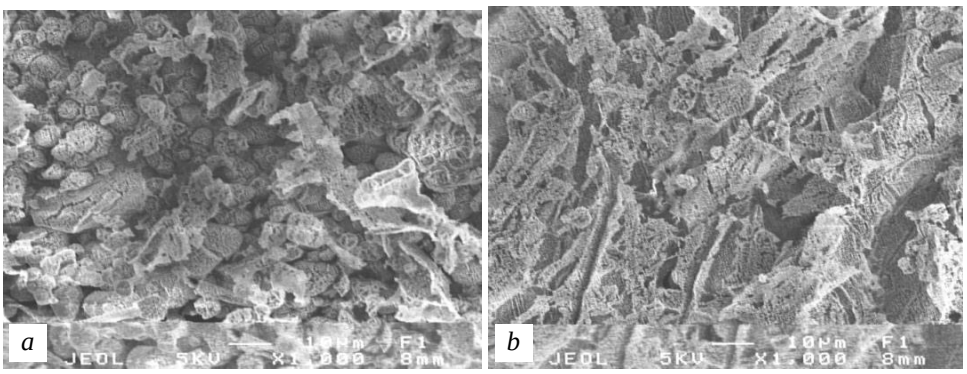

Fig. 1. Structure of the plant material before (*a*) and after (*b*) UAE

The extraction time also plays a major role in the UAE process. If it is short, the extractives are not completely released into the solvent, which results in a low extraction efficacy. In contrast, if the extraction time is long, the bioactive compounds in the extract are exposed to high temperature or oxygen in the surrounding environment, leading to their degradation. The extraction time of 20 min used in this study can be regarded as relatively short; it is similar to that used in the extraction of polyphenols from the seed shells of *Euryale ferox* (21 min) [31] and shorter than that selected for blackberry leaves (105–107 min) [28]. This can be explained by the fact that the extraction time strongly depends on the content of extractives in the material, as well as on the size and structure of the sample. Hence, the extraction time of 20 min was chosen for the experiments in this study.

2.5. Effect of UAE on the structure of the material. At the optimum extraction parameters determined above, the powdered sample and the residue after the UAE treatment were analyzed using SEM. Fig. 1, *a* shows that the sample particles were quite small (< 10 μm) and some plant cells were larger and considerably dispersed. The surface of the sample particles had many small pores. After the UAE treatment, the residue micromorphology changed completely as compared to the initial sample. Many large cleft, rough surfaces (Fig. 1, *b*) appeared. Our results are similar to those reported by Chemat et al. [32] and Ho et al. [33] who extracted polyphenols from caraway seeds and misai kucing, respectively. Fig. 1, *b* shows the plant cells damaged by ultrasound waves. Ultrasonic energy may affect the diffusion boundary layer by surface vibration and expansion of the material that influence the mass transfer [34]. As a result, the extraction efficiency increases.

Conclusions

The results obtained by us confirm that aqueous ethanol can be recommended as the best solvent to extract phenolic compounds from *P. fruticosa* roots. In our study, the extraction yield was determined by all extraction factors. The most suitable conditions for UAE were as follows: SS ratio 50/1 (mL g^{-1}), ethanol concentration 40% (v/v), temperature 45 °C, and extraction interval 20 min. The highest TPC and AC values were 2.13 ± 0.02 mg GAE g^{-1} DW and 78.13 ± 0.25%, respectively. The SEM examination showed that ultrasound waves cause destruction of plant cells and increase the TPC and AC values.

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ОРИГИНАЛЬНАЯ СТАТЬЯ

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Экстракция с ультразвуковой обработкой фенольных соединений из корня *Polyscias fruticosa* (L.) Harms

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Аннотация

Получены экстракты корня *Polyscias fruticosa* (L.) Harms с помощью метода экстракции с ультразвуковой обработкой (Э-УЗ) при следующих экспериментально установленных оптимальных параметрах: соотношение растворитель/твердое вещество – 50/1, мл/г; концентрация

этанола – 40%, об.; температура – 45°C; и время экстракции – 20 мин. Для исследуемых экстрактов определены наилучшие значения общего содержания фенольных соединений (ОФ) и антиоксидантной емкости (АОЕ), которые составили 2.13 ± 0.02 мг эквивалентов галловой кислоты (ГК) на грамм сухого веса (СВ) образца для ОФ и 78.13 ± 0.25% для АОЕ. Кроме того, с помощью сканирующей электронной микроскопии (СЭМ) показано, что обработка ультразвуком полностью изменяет структуру растительного материала по сравнению с его исходным состоянием.

Ключевые слова: этанол, лекарственное растение, фенольные соединения, *Polyscias fruticosa*, Э-УЗ

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