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ULTRASOUND-ASSISTED EXTRACTION OF PHENOLIC COMPOUNDS FROM *POLYGONUM MULTIFLORUM* THUNB. ROOTS

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Abstract

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The goal of this study is to examine the influence of type of solvent (acetone, ethanol, methanol, and water), raw solvent/material ratio (20/1–60/1, v/w), solvent concentration (30–80%, v/v), temperature (30–70°C), time of extraction (10-30 mins) and power consumption of 550 W on the extraction yield of total phenolic compounds (TPC) and antioxidant activity (AC) from *Polygonum multiflorum* Thunb. roots. TPC and AC were determined by the Folin–Ciocalteu assay and DPPH assay. The optimal conditions for extraction process were 60% acetone-water mixture as solvent, solvent/material ratio of 30/1, extraction temperature of 60°C and extraction time of 15 mins. TPC and AC obtained were approximate 43.28±0.54 mg GAE/g DW (Dry weight) and 343.88±3.06 µmol TE/g DW, respectively. The effect of ultrasound-assisted extraction (UAE) on structure of materials of *Polygonum multiflorum* Thunb. root was observed by scanning electron microscopy (SEM). HPLC method was employed to determinate some main phenolic compounds including catechin, gallic acid, and resveratrol.

Key words: antioxidant; *Polygonum multiflorum* Thunb.; polyphenol; solvent; ultrasound

Introduction

Vietnam has the weather that is suitable for many herbal plants, especially *Polygonum multiflorum* Thunb. It is also known as Heshouwu (in Chinese) or Ha Thu O Do (HTOD) (in Vietnamese). This plant is one of the most popular traditional Vietnamese medicines and is widely used in many medicines and prescriptions. It has been used to treat various diseases for many centuries. A study by Lin et al. (2015) has provided comprehensive information on the botany, phytochemistry, traditional uses, pharmacological research and toxicology of *Polygonum multiflorum* Thunb. In addition, a previous study by Lin et al. (2010) indicated the biological composition and antioxidant activity in leaves, stems and roots fresh from this tree. Results also showed that the polyphenol content and the antioxidant activity obtained highest in the roots. This will create a new foundation for further research on *Polygonum multiflorum* Thunb. roots. The phenolic compounds in plants are antioxidant substances and can prevent many diseases. They can apply widely in many food and medicine industry. Nowadays, the scientists discover phenolic compounds can prevent some forms of cancers (Hung et al., 2004), anti-aging effects, antioxidant activity (Wang et al., 2008), etc.

At present, some techniques can be use for extracting phenolic compounds from plants, such as convectional extraction (CE), ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), and accelerated solvent extraction (ASE), etc (Wang and Weller 2006). However, ultrasound-assisted ex-

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traction (UAE) is a new method and has been widely used for extracting the bioactive compounds, especially phenolic compounds. The advantages of UAE compared to other methods is an inexpensive method that does not require complex equipment (Vinatoru, 2001). This method increases the high extraction yield, faster kinetics and can be used with many different solvent (Wang and Weller 2006).

Currently, no studies has researched on UAE method for the extraction of phenolic compounds from HTOD. Therefore, the purpose of study was to determine the extraction conditions including solvent type, extraction time, extraction temperature, solvent concentration, and solvent/material ratio for the extraction of total phenolic content and antioxidant capacity from HTOD. In addition, some main phenolic compounds of the root extracts were analyzed by HPLC method and the structure of materials were observed by scanning electron microscope (SEM).

Material and Methods

Plant material and sample preparation

Polygonum multiflorum Thunb. roots were collected from Cao Bang province (Vietnam). The fresh roots have the weight range 0.5-1 kg, reddish brown color, no diseased or physical injuries. The cleaned roots were sliced 2-3 mm thick pieces and dried at 60°C until < 12% of moisture. The dried samples were ground into a fine powder (< 0.5 mm), packaged in vacuum condition and stored at room temperature for further use.

Chemicals and reagents

Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) reagent was purchased from Sigma-Aldrich (USA). DPPH (2,2-diphenyl-1-picrylhydrazyl) and Folin–Ciocalteu reagent was purchased from Merck (Germany). All organic solvents and other chemicals were of analytical reagent grade.

Extraction process

Dried sample was extracted with solvents (distilled water, 60% ethanol, 60% methanol and 60% acetone) in an ultrasonic bath (ELMA– S60H type, 37 kHz, 550W, Germany) for different times (10-30 mins) at required temperature (30-70°C) and solvent/material ratio (20/1-60/1, v/w). The mixture was filtered for removal of roots particles with supporting of vacuum filtration system, then TPC and AC were analyzed.

Determination of total polyphenol content (TPC)

The TPC was determined by the Folin-Ciocalteu method (Siddiqua et al., 2010) with some slightly modifications. Gallic acid was used as the standard reagent. TPC was expressed as mg of gallic acid equivalents per g of dry weight (mg GAE/g DW).

Determination of antioxidant capacity (AC)

The AC was determined by DPPH assay which was described by Soto et al. (2014) with some very little modifications. Trolox was used as the standard. AC was expressed in TEAC (Trolox equivalent antioxidant capacity) which determined as μmol of Trolox per g of dry weight (μmol TE/g DW).

Determination of phenolic compounds by HPLC

HPLC analysis of phenolic compounds in *Polygonum multiflorum* Thunb. roots extracts was carried out on a Agilent 1100 Series HPLC system equipped a diode-array UV-vis detector. Analysis was performed on a reversed-phase column (Kromasil C18, 150 x 2.1 mm, 3.5 μm). The sample was injected into the injection port (loop 20 μL). The UV detector was set at a wavelength of 270 nm and 308 nm for gallic acid, catechin and resveratrol, respectively. The flow rate was always 0.2 mL/min at 30°C. The analysis was performed using a gradient program with a two solvent system (A: hydrochloric acid; B: methanol). The standard phenolic compound solutions were prepared in solvent that used in the extractions.

Scanning electron micrographs (SEM)

Using scanning electron microscope system (Jeol JSM-7401F, USA) examines morphological alterations of materials and residues after extraction.

Data analysis

All assays results were performed in triplicates, the received values were expressed in the form of mean±standard deviation (SD) and analyzed by Statgraphics software (Centurion XV). One-way analysis of variance (ANOVA) at $P < 0.05$ was used to determine significant differences between the means.

Results and Discussion

The effect of solvent type on extraction of phenolic compounds

Some dried samples were extracted with 60% methanol, 60% ethanol, 60% acetone, distilled water under the same extraction conditions including solvent/solid ratio of 30/1, 60°C and 20 mins. Figure 1 showed that using 60% acetone had the best results; TPC and AC of extracts have significant differences ($P < 0.05$) from different solvents. They reached

44.06±0.33 mg GAE/g DW and 329.99±10.06 µmol TE/g DW, respectively; while using distilled water for extraction obtained the lowest yields. This can be explained that enzyme polyphenol oxidase was inhibited by organic solvent, whereas in water they are active and degrade phenolic compounds (Lapornik et al., 2005). TPC increased when changing from solvent extraction of water, ethanol, methanol to acetone but not correlated with AC. This result was similar with studies of Bajpai et al. (2005) and Ruanma et al. (2010).

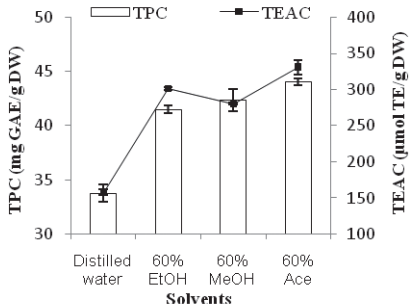

Fig. 1. TPC and AC of extracts at various solvents

Using solvent for extraction is the most common method used to isolate the bioactive compounds, especially polyphenol. The amount and activity of phenolic compounds depends very much on the type of solvent used. The potential antioxidant of phenolic compounds are significantly impacted of the polarity of solvent used in extraction. Besides, TPC and AC depend on the structure of molecular and solubility of phenolic compounds in different polar solvents (Zhang et al., 2014). Phenolic compounds are polar compounds, lowest polarity solvents (chloroform or hexane) are not appropriate for extraction because it gives the bad yields (Liu et al., 2000). According to Chirinos et al. (2007), using highly-polar solvents (water) are also not appropriate for extracting phenolic compounds; water can bring some impurities (such as sugars, organic acids and soluble proteins) that interfere in the identification and quantification of phenolic compounds. Therefore, the extraction process can combine water with other organic solvents and makes a moderately polar medium ensuring the optimal extraction. Many researches also show that aqueous acetone is a good solvent for the extraction of antioxidants polarization (Hismath et al., 2011). Based on the above results, 60% acetone was chosen for the evaluation of next steps.

The effect of solvent/material ratios on extraction of phenolic compounds

Based on the Figure 2, the solvent/material ratio of 30/1 was the best result. TPC and AC have the highest values with 45.78±0.75 mg GAE/g DW and 348.29±5.4 µmol TE/g DW, respectively; and effect of solvent/material ratios on the values of TPC and AC have a significant differences ($P < 0.05$).

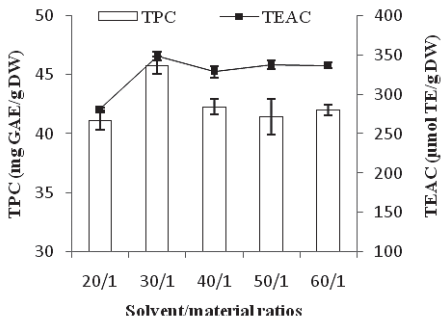

Fig. 2. TPC and AC of extracts at various solvent/material ratios

The purpose of these experiments was to extract TPC and TEAC and reach the highest efficiency. The solvent/material ratio is the most important factor of extraction process. Increasing the solvent/material ratios promote the phenolic compounds which diffuse into solvent greater (Cacace and Mazza. 2003). However, these components will not continue to increase once equilibrium is reached (Herodež et al., 2003). If amount of solvent is too large, the performance gained is negligible and would not be cost effective because of time and energy to chase a volume solvent, while the prolonged pursuit of high temperature solvent would significantly reduce polyphenol antioxidant activity. A low solvent/material ratio could not extract completely phenolic compounds from materials. Therefore, determining the suitable solvent/materials ratio for extraction to achieve the highest economic efficiency is necessary, to ensure no shortage and wastage that may occur when using less or too much solvent extractable. Therefore, the solvent/material ratio of 30/1 was used for further experiments.

The effect of acetone concentration on extraction of phenolic compounds

The values of TPC and AC from extracts are performed in Figure 3 and the effect of acetone concentrations on TPC and AC has a significant difference ($P < 0.05$). The optimal result peaks at acetone concentration of 60%, TPC and AC are 45.83±1.07 mg GAE/g DW and 340.42±4.98 µmol TE/g DW, respectively.

When increasing the concentration of acetone, TPC and TEAC also increased and reached for the highest values at 60% acetone, but both TPC and TEAC decreased when increasing the concentration increases to 80%. The efficiency of extract-

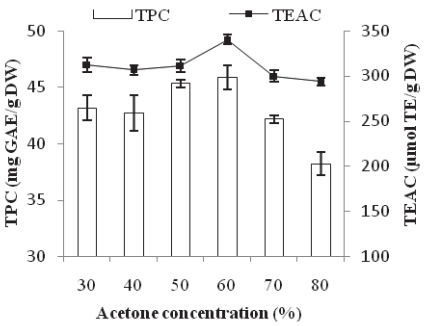

Fig. 3. TPC and AC of extracts at various acetone concentrations

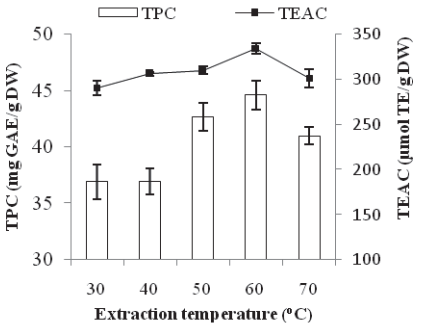

Fig.4. TPC and AC of extracts at various extraction temperature

ing phenolic compounds from plant material depends on type of solvent used, and especially the polarity of solvent because there are many kinds of phenolic compounds in plants (polar and non-polar) that were dissolved in many different polarity of solvents. According to this opinion, adjusting polarity of solvent is quite necessary. According to Veggi et al. (2013), increasing amount of water in solvent could increase the diffusion of water into the cells of plant, the bioactive substances transport easily into the solvent. Besides, the large amounts of water can dissolve many different organic compounds as sugar, protein which affects the accuracy of measuring TPC and AC (Chirinos et al., 2007). Increasing the acetone concentration in solvent solution decreases the polarity, but its impellent extraction of other components. Besides that, it is activation of the breakdown of membrane cells, promotes abilities of solvent into solid mixture (Zhang et al., 2006). However, the high concentration of acetone (low polarity) of the solvent is not suitable for extracting antioxidants compounds in some cases. It depends on type of phenolic compounds in materials. Accordingly, the suitable acetone concentration for next experiment was 60%.

The effect of extraction temperature on extraction of phenolic compounds

The analyzable results showed that the temperatures have a significant effect on extracting the phenolic compounds ($P < 0.05$). According to Figure 4, TPC and AC achieve the maximum values of 44.62±1.25 mg GAE/g DW and 334.47±6.08 µmol TE/g DW at 60°C.

As extraction temperature increases from 30°C to 60°C, the values of TPC and AC also increase. The high temperature extraction can promote the diffusion of phenolic compounds, reduces the viscosity of the solvent (Wissam et al., 2012), opens cell matrix and easily release phenolic compounds (Zhang et al., 2013). However, upon reaching 70°C, the TPC and AC decline rapidly because they are quite sensitive with heat treatment. Some phenolic compounds were destroyed by high temperature extraction. The others convert insoluble phenolic compounds to soluble phenolics (Xu et al., 2007). Phenolic compound are multiform with different quantity and antioxidant activity and they depend on the species. Therefore, the effective heat treatment to release antioxidant compounds from different plant species may be different (Kim et al., 2006). In addition, the high temperature may increase solvent losses through evaporation, which increases the cost of extraction and change the solvent/material ratio. Thereby, the temperature is one of the most important factors which affect strongly TPC and AC in extraction process. Based on the achieved results, the optimal extraction temperature was 60°C.

The effect of extraction time on extraction of phenolic compounds

The timelines were investigated from 10 to 30 minutes. Figure 5 shows that the optimal TPC and AC achieved after extraction time of 15 minutes with 43.28±0.54 mg GAE/g DW and 343.88±3.06 µmol TE/g DW, respectively. Effect of extraction times on the yield have a significant difference ($P < 0.05$). TPC and AC tend to increase with increasing extraction time from 10 to 20 minutes then drops rapidly from 20 to 30 minutes. During extraction time, the structure of material particles are broken by the ultrasonic cavitation and the process of diffusion of phenolic compounds in solvents will be faster (Melecchi et al., 2006).

Extraction time of 10 minutes is not sufficient for solvents to penetrate into the cells, solubilize and extract phenolic compounds. Then the extraction yield obtained will be low. Therefore, extraction time is very important in the extraction of phenolic compounds using solvent. Suitable extraction time saves time and costs of the implementation process (Benmeziane et al., 2014). Due to increased extraction time, the extracted sample is heated longer, extraction

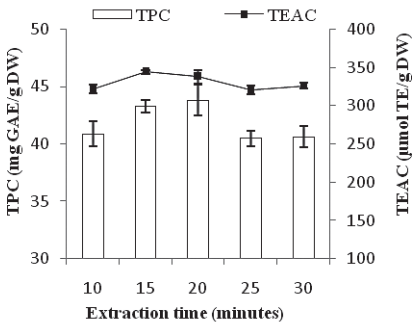

Fig. 5. TPC and AC of extracts at various extraction time

yield decreased because of the decomposition of phenolic compounds, the product might polymerize into insoluble compounds (Shi et al., 2003). In addition, phenolic compounds are oxidized by environmental factors such as light and oxygen (Hismath et al., 2011). Based on the obtained results, the optimal extraction time was 15 minutes for extraction process.

The effect of ultrasound-assisted extraction on structure of material

Figure 6A shows that initial materials consist of many pieces of cell plants and starch which are dry and absolutely separate. The starch has many different diameter range 8-20 µm, egg (or oval) shape and many rift on surface. Conversely, surface of cell was an intact and smooth surface. After treatment by UAE, starch was gelatinized at high temperature and it is also one of the causes of sticky cell wall. In addition, a large number of wrinkles appear on surface of cell wall (Figure 6B) because the ultrasound destroys the cuticular layer (Chemat et al., 2004). The localized pressure and temperature increase quickly by the UAE-generated cavitation bubbles, the thin cell walls were broken and damaged. However, these changes might allow for the easy entry of the solvent into the cellular channels (Aspé and Fernández, 2011).

Identification of some phenolic compounds in the extract

HPLC method was utilized to identify some phenolic compounds from the extracts at the optimal conditions. The detected phenolic compounds were resveratrol (0.028 mg/g), gallic acid (0.35 mg/g) and catechin (1.83 mg/g) (Figure 7). Detecting these components is similar with research of Quoc and Muoi (2015) that also recognized that these bioactive compounds existed in HTOD from Vietnam. However, the contents have the significant differences by environmental factors and extraction technology.

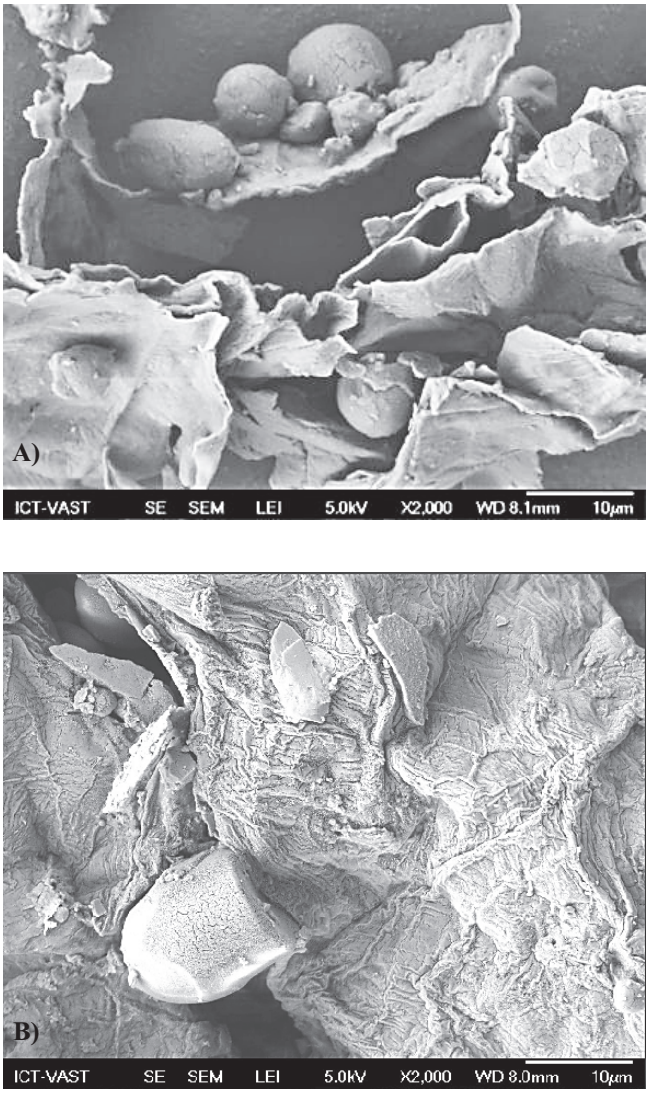

Fig. 6. Structure of material before (A) and after (B) treatment by UAE

Conclusion

The result showed that acetone was the optimal solvent for the phenolic compounds with ultrasound-assisted extraction from *Polygonum multiflorum* Thunb. roots. The highest TPC and AC in the extract were 43.28±0.54 mg GAE/g DW and 343.88±3.06 µmol TE/g DW, respectively. The optimal conditions were acetone concentration of 60%, solvent/material of 30/1, extraction time of 15 minutes and extraction temperature of 60°C. Many cells of material were destroyed by UAE and had wrinkles on surface and some main phenolic compouds were detected as catechin, gallic acid, and resveratrol.

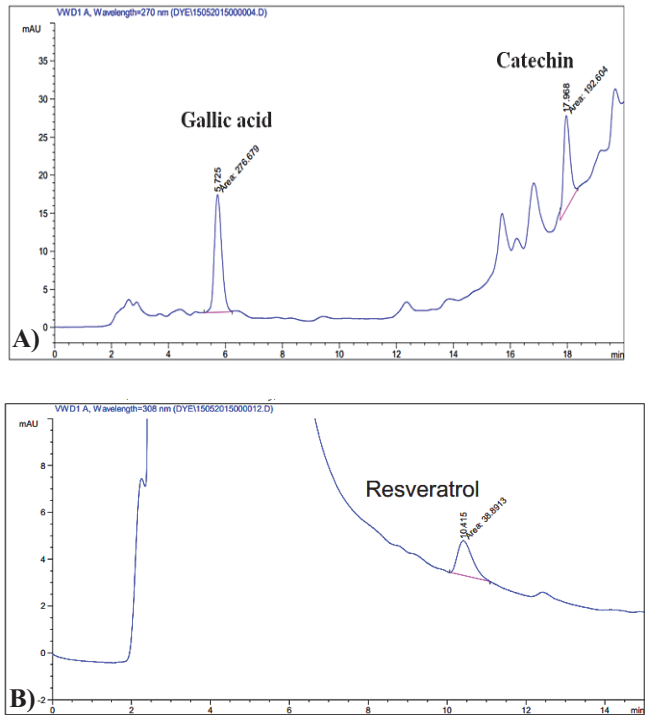

Fig. 7. HPLC chromatograms of a sample of Polygonum multiflorum Thunb. root extracts acquired at 270 nm (A) and 308 nm (B)

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