EFFECTS OF TREATMENT METHODS ON TOTAL POLYPHENOL CONTENT AND ANTIOXIDANT ACTIVITY OF *POLYGONUM MULTIFLORUM* THUNB ROOT EXTRACT

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Abstract
The study shows the changes of total polyphenol compounds and antioxidant activities of Polygonum multiflorum Thunb root extract during the preservation time by freezing method. In addition, the treatment of initially raw materials by drying process also affects strongly total polyphenol compounds and antioxidant activity. Total phenolic compounds were determined by the Folin Ciocalteu method and antioxidant activity was analyzed by radical scavenging activity on 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. The results point out that Polygonum multiflorum Thunb (fresh samples) can be preserved for 3 months at -20°C but content of polyphenol and antioxidant activity decrease significantly by 60%. Fresh samples were dried at 60°C for 4 hours and methods including magnetic stirrer extraction (MSE), microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), and enzyme (Termamyl SC) assisted extraction (EAE) with deionized water as solvent, were used to extract polyphenol in this research. MAE has the shortest treatment time (6 minutes) and the best results (highest total polyphenol) as 44.37 mg GAE/g DW and antioxidant activity value was 132.73 µmol Trolox/g DW. Conversely, EAE and MSE methods give the worst yield (22.81 mg GAE/g DW, 24.51 mg GAE/g DW and 136 µmol Trolox/g DW, 175.29 µmol Trolox/g DW respectively) and require the longest extraction time (from 1 to 2 hours).

Keywords: α-amylase, antioxidant activity, microwave, root of *Polygonum multiflorum* Thumb, total polyphenol, ultrasonic.

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1.INTRODUCTION

Polyphenols which have one or more aromatic rings with one or more hydroxyl groups are the most important compounds in nature. Over thousands phenolic compounds known exist in a considerable number of plants in their various parts. (Dai and Mumper, 2010). Polyphenols are antioxidant compounds which can prevent diseases. For example, blueberry polyphenols have neuroprotective effect (Giacalone *et al*., 2011); strawberries polyphenols reduce the cardiovascular disease risk (Basu *et al.*, 2010), and chocolate polyphenols can decrease blood pressure (Rimbach *et al.*, 2011). In Vietnam, phenolic compounds were discover in herbal plants with high total polyphenols and antioxidant activity especially *Polygonum multiflorum* Thunb *(Fallopia multiflora)* which is known as Ha Thu O (in Vietnamese). *Polygonum multiflorum* Thunb is the wild plant and distributed on mountainous region in the North Vietnam. The local citizens use the root for treating white hair and improving the health.

Currently, there are many methods of extracting polyphenols from plants. For instance, accelerated solvent extraction, ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), supercritical fluid extraction (SFE) (Barbero *et al.*, 2008), and enzyme-assisted extraction (EAE) (Le *et al.*, 2014) with different solvents such as hexane, methanol, ethanol, acetone, and deionized water (Dezashibi *et al*., 2013; Zhang *et al.*, 2014). The purposes of these methods are to increase the efficiency, enable automation, reduce extraction power, reduce of

organic solvent consumption and shorten extraction times.
This study aims to research some popular extraction methods such as UAE, MAE, EAE and MSE, using deionized water as solvent. The extraction efficiency was evaluated by total polyphenol content (TPC) and antioxidant activity (AA). Furthermore, the chemical composition of root and the changes of phenolic compounds in drying and freezing process were investigated.

2. MATERIAL AND METHODS

Plant material

Roots of *Polygonum multiflorum* Thunb were harvested from Cao Bang province, Vietnam. Each root was from 15-25 cm in length and 5-10 cm in diameter. It weighed approximately 1 kg, was brown and pest free. The roots were washed under tap water and their surface was brushed to remove dust and soil.

Preparation of extracts

The roots were cut into some small slices (thickness 2-3 mm) and divided into two parts for drying (ranging from 50-80^{o}C) and freezing at -20^{o}C separately. Dried samples were ground into fine powder in the mill. The powder was subsequently passed through a sieve (0.5 mm) and was contained in vacuum plastic bags at room conditions. Samples of 1 g each were transferred to the extraction cuvettes. Deionized water was then added and each extraction method had the different optimal parameters.
For freezing samples, only MSE was used to extract phenolic compounds, whilst four methods (MSE, UAE, MAE and EAE) were utilized to extract phenolic compounds from the dried samples. For MSE, the extraction process has the ratio of sample/solvent of 1/20 at 50^{o}C for 1 hour. UAE has the ratio of sample/solvent of 1/30 at 60^{o}C for 20 minutes; MAE has the ratio of sample/solvent of 1/40, microwave power of 195 W for 5 minutes, and EAE has the ratio of sample/solvent of 1/10, the concentration of enzyme (α-amylase) of 0.3% (v/v), pH of 6 at 85^{o}C for 2 hours. Next, extracts obtained were separated by the vacuum filter, evaporated in vacuum condition at 50^{o}C for 20 minutes and made up to 100 ml by distilled water
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Determination of total polyphenol content (TPC)

The content of phenolic compounds was determined by Folin-Ciocalteu's method which was modified slightly and described by Premakumari *et al.* (2010). Gallic acid was used as the standard chemical. Different concentrations ranging from 1-10 ppm were prepared with deionized water, and the TPC was measured as gallic acid equivalent per gram dry weight basis of fresh sample (mg GAE/g of dry weight). Briefly, 0.1 ml of extract was mixed with 1.5 ml FC reagent (diluted 1/10) and kept for 5 minutes. Then, 4 ml of 20% sodium carbonate solution was added, and filled up to 10 ml with distilled water. After that, this mixture was kept for 30 minutes in dark place, and the absorbance was determined at 738 nm.

Determination of antioxidant activity

The modified DPPH assay was used to measure antioxidant activity of extract (Soto *et al.*, 2014; Chmelová *et al.*, 2015). 4 ml of DPPH solution (0.1 mM ethanol solution) was mixed with 0.1 ml of extract and the mixture was made up to 5 ml with ethanol. The mixture was kept for 30 minutes in the dark, and its absorbance was measured at 517 nm. To achieve the calibration curve, the absorbance values at 517 nm of some concentrations of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were measured.
Results were expressed as Trolox equivalent antioxidant capacity (TEAC) (µmol Trolox/g of dry weight).

Data analysis

Extraction was performed in triplicate, and data obtained were analyzed by Statgraphics software (Centurion XV) with confidence interval p-value of 0.05.

Determination the components of fresh material

Table 1: The components of root of *Polygonum multiflorum* Thunb

Components	Content (%)
Water	78.61±0.54
Protein	2.13±0.12
Lipid	0.42±0.06
Starch	8.88±1.58
Reducing sugar	2.30±0.28
Glucid	12.20±0.14
Ash	1.03±0.03
Heavy metal	-

Water makes up the largest component in fresh roots (78.6%), which is similar to fruits, namely apple (84%), orange (87%), grapefruit (91%), etc (Pennington and Spungen, 1994). Therefore, it is quite difficult to preserve for a long time in normal conditions, and the slices are dried in a dryer for 3-6 hours depending on drying temperature. Protein content is low, approximately 2.13%, and there is a negligible amount of lipid (0.42%) (Table 1). Glucid, mainly starch (8.88%) and reducing sugar (2.3%), constitutes 12.2% of total content. Starch can affect the quantity phenolic compounds yield because there is the adsorption of polyphenols by its starch (Davis and Hoseney, 1979; Deshpande and Salunkhe, 1982). In addition, according to Singleton *et al.* (1974), reducing sugar can interfere with TPC analysis if sugar content is high (more than 2.5%). In this case, the influence of sugar was insignificant. Ash content of Ha Thu O is 1.03%, which is less than that of *Polygonum multiflorum* Thunb roots from China (Jian *et al.*, 2010), and heavy metal was not detected. This difference of chemical components of *Polygonum multiflorum* Thunb from different places has close link to the environment such as soil, climate and harvesting season (Wang *et al.*, 2005).

Effects of frozen storage of *Polygonum multiflorum* Thunb root extract on its total polyphenol and antioxidant activity.

The quantity of TPC and AA of fresh samples give the highest values (65.47 mg GAE/g DW and 620 µmol Trolox/g DW) (Figure 1). During the frozen storage, TPC and AA decreased sharply for the first 20 days and fluctuated slightly in the late periods. At the 100th days, TPC and AA have the lowest value (26.03 mg GAE/g DW and 220.33 µmol Trolox/g DW), it may lose during storage. The slow freezing process involves freezing the water in the cellular spaces of plant tissue. Water freezes, it expands and the ice crystals cause the cell walls to rupture. Components of cell escape outside quite easy and the product can be reduced nutrient value (Tran *et al.*, 1985), especially phenolic compounds. Therefore, the slow freezing process affects strongly to TPC and AA values.

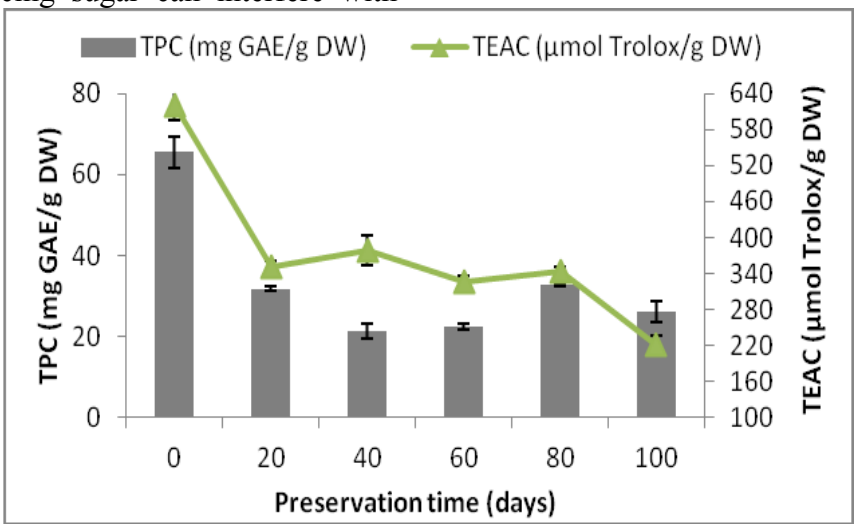

Figure 1. Frozen storage effects on TPC and AA of root extract of *Polygonum multiflorum* Thunb

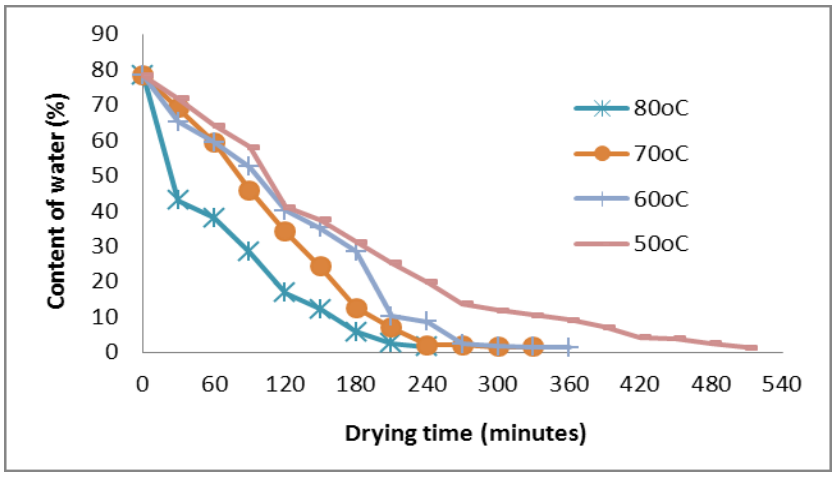

Figure 2. Drying curve of slices of *Polygonum multiflorum* Thunb root at different drying temperature

This change has significant difference at p-value=0.05, and was similar to research about some vegetables under frozen condition as spinach, broccoli, carrot, etc. TPC and AA values reduce rapidly after storage time except onion (Ninfali and Bacchiocca, 2003). However, for raspberry fruit, TPC and AA remain unchanged after frozen storage (365 days) (Cano *et al.*, 2000). Different types of phenolic compounds and the various polyphenol compositions available in each plant contribute to the differences in the change of TPC and AA values after frozen storage. The study shows that people use frozen samples will have a 60-65% drop in the TPC and AA with respect to the people who use an identical amount of fresh samples.

Our results indicate that *Polygonum multiflorum* Thunb should not be stored in slow freezing process. TPC and AA value reduce drastically in 3 months. Therefore, other methods such as drying of raw materials or consuming the fresh roots after harvest, which can storage samples for a long time, avoid the reduction of TPC and AA values.

Drying curve of slices of Polygonum multiflorum Thunb *root*

Fresh sample was dried ranging from 5 to 12% content of water at different oven temperature and dried samples stored easy in plastic bags. At 50°C, drying time is about 300-400 minutes. Conversely, drying time at 60°C, 70°C and 80°C was shorter than 50°C, ranging from 150-240 minutes. In addition, the initial water content is high (nearly 80%) and the slices are quite thick (2-3 mm); it is difficult for water to escape from materials. Therefore, it can affect strongly the drying time.

The figure 2 shows that the fastest drying rate is occurred when slices are dried at higher temperature. Drying temperature was similar with study of Madrau *et al.* (2009) which examines the effect of drying temperature on polyphenol content and antioxidant activity of apricots at 55°C and 75°C and with study of Abdullah *et al.* (2011) which investigates drying characteristics and herbal metabolites composition of misai kucing leaves at 40°C, 55°C and 75°C. Drying temperature also strongly influence total polyphenol and antioxidant activity (Malik and Bradford, 2008; Abdullah *et al.*, 2011), and phenolic compounds can be destroyed at high temperature.

Effect of drying of Polygonum multiflorum Thunb *root on its total polyphenol and antioxidant activity*

Figure 3 and 4 show fresh samples which have the highest content of TPC and AA values; MAE was the most efficient method, TPC obtains 99.55 mg GAE/g DW and AA was 606.6 µmol Trolox/g DW. Conversely, EAE has the lowest yield (45.28 mg GAE/g DW and AA was 571.6 µmol Trolox/g DW). TPC was drastically reduced by high temperature and long treatment time. This changes has significant differences at p-value = 0.05.

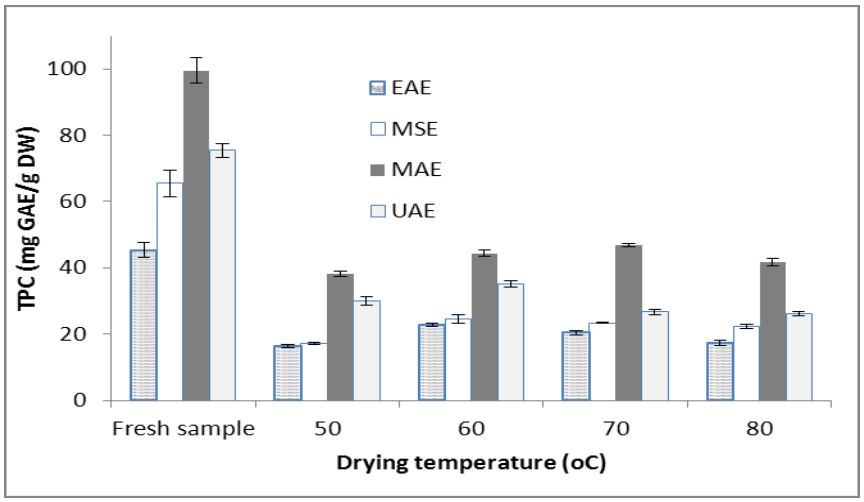

Figure 3. Total polyphenol of *Polygonum multiflorum* Thunb root extracts at different drying temperature and extraction methods

Fresh samples was dried at 50°C, 60°C, 70°C and 80°C, TPC and AA obtain the highest values at 60°C. TPC values of fresh samples were more than that of dried samples by 49 to 62 %, and AA values of fresh samples were higher than dried samples by 71 to 78 % at 60°C. According to Madrau *et al.* (2009), as the drying temperature increases, TPC also decreases. Besides, TPC can reduce at low drying temperature because enzyme polyphenol oxidase (PPO) was not degraded (Malik and Bradford, 2008). In addition, the evaporation of water during drying process concentrates enzyme PPO. Therefore, PPO activity increases (Kim and Jung, 2011). AA value can fluctuate during drying process depending on temperature and materials (Madrau *et al.*, 2009; Abdullah *et al.*, 2011).

In general, MAE was the optimal method because TPC and AA value were high. Treatment time was also short, and it was easy to practice. TPC at 60°C using MAE were higher than other methods (44.37 mg GAE/g DW), UAE (35.08 mg GAE/g DW), MSE (24.51 mg GAE/g DW) and EAE (22.81 mg GAE/g DW). TPC of samples from MAE and UAE methods were higher than samples from China which have the extracted solvent as deionized water (33.9 mg GAE/g DW) and methanol (24.2 mg GAE/g DW) (Chen *et al.*, 2006).

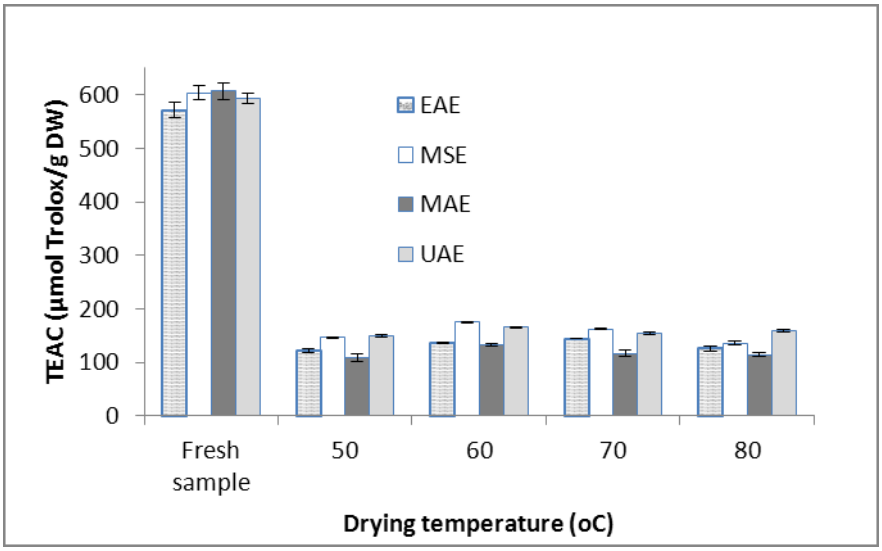

Figure 4. Antioxidant activity of *Polygonum multiflorum* Thunb root extracts at different drying temperature and extraction methods.

The AA values at 60°C with MSE are 175.29 µmol Trolox/g DW in comparisons to UAE (165.4 µmol Trolox/g DW), EAE (136.09 µmol Trolox/g DW) and MAE (132.73 µmol Trolox/g DW). All values in this study were lower than results of Li *et al*. (2013) (265.34 µmol Trolox/g DW) with deionized water as solvent. The difference of extraction methods, land, gender, etc which cause the changes about TPC and AA values.

4. CONCLUSIONS

The herbal plant, *Polygonum multiflorum* Thunb, should not be stored by frozen methods because the TPC and AA values are reduced sharply. The roots should be dried at 60°C, ground and kept in plastic bag for longer storage time. MAE was the optimal method due to higher extraction yield and shorter irradiation time in comparison with the other methods.

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