

Received: 2020-01-22

Accepted: 2020-03-15

Available online: 2020-03-31

DOI: 10.2478/hepo-2020-0002

## EXPERIMENTAL PAPER

# Research on polyphenols extraction from *Polygonum multiflorum* Thunb. roots

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## Summary

**Introduction:** *Polygonum multiflorum* Thunb. is a herbal common plant in Asia, with many beneficial health effects for human because it contains many bioactive compounds which can prevent some diseases, for instance such as cardiovascular diseases, cancers, neurodegenerative diseases, etc.

**Objective:** The purpose of this research is to point out the effects of extraction factors such as type of solvent, material/solvent ratio (*w/v*), solvent concentration (% *v/v*), temperature (°C) and extraction time on the extraction yield of phenolic compounds from *Polygonum multiflorum* Thunb. roots, for instance, total polyphenol content (TPC) and antioxidant capacity (AC).

**Methods:** The raw material consisting of *Polygonum multiflorum* Thunb root was extracted by the reflux maceration method. TPC and AC of received extract were evaluated by the Folin-Ciocalteu technique and DPPH method with Trolox as a standard agent.

**Results:** The optimal conditions for the extraction process were acetone-water mixture (60% *v/v*) as a solvent, material/solvent ratio of 1/40, extraction temperature of 50°C and extraction time of 90 minutes. The surface structure of material after extraction process changed insignificantly compared with the initial structure.

**Conclusion:** The results showed that TPC and AC obtained the best values (38.60±0.56 mg GAE/g DW (dry weight) and 298.15±2.99 μmol TE/g DW, respectively) at optimal extraction conditions. In addition, some phenolic compounds were detected in the extract such as gallic acid, catechin and resveratrol.

Key words: *antioxidant, extract, phenolic compounds, Polygonum multiflorum Thunb.*

Słowa kluczowe: *właściwości antyoksydacyjne, związki fenolowe, Polygonum multiflorum Thunb.*

## INTRODUCTION

Polyphenols are precious substances present in various plants. Among them, *Polygonum multiflorum* Thunb. roots also contained a large number of phenolic compounds such as flavonols [1], emodin, physcion [2], torachryson-8- O- $\beta$ -D-glucoside and rhaponticoside [3], etc. This is a wild medical herb also cultivated widely in some Asian countries, especially in North Vietnam. The root has been used for the treatment of many diseases such as cardiovascular diseases, diabetes, osteoporosis, etc. [4] during a thousand years because of the presence of these bioactive compounds, especially polyphenols. In addition, *P. multiflorum* roots has been used as a seasoning in daily Chinese diet [5]. Yet, polyphenols extract from this plant has not been applied in the food industry. This will create a new foundation for further research on *P. multiflorum* roots such as the extraction process.

Currently, the extraction of phenolic compounds from herbal plants has been usually performed with use of various methods, such as maceration, Soxhlet, ultrasonic-assisted extraction (UAE), microwave-assisted extraction (MAE), etc. with many different solvents [6] or enzyme-assisted extraction (EAE) [7]. However, each one has its advantages and disadvantages. The reflux maceration extraction can have a long extraction time but it is a process quite easy to control, of low budget and suitable in lab scale. In Vietnam, herbal plants are almost harvested, processed and the bioactive compounds are extracted by local inhabitants of mountainous region. Although, there are many modern extraction methods (UAE, MAE or EAE) improve the amount of extracted polyphenols from plants, especially from *P. multiflorum* roots [8-10]. However, it is very difficult to scale up for these methods in the current condition in Vietnam. Therefore, maybe the traditional extraction methods are still a primary choice, especially the reflux maceration method.

Besides, no studies were performed using reflux maceration method for the polyphenols extraction from *P. multiflorum* roots. Therefore, the aim of this research was to determine the optimum extraction conditions, for instance, the type and concentration of solvent, material/solvent ratio, extraction time and extraction temperature for the extraction of TPC and AC from this material. In addition, some main phenolic compounds of this extract were determined by high performance liquid chromatography (HPLC) method. Besides, the structure of materials was also observed by scanning electron microscope (SEM).

## MATERIAL AND METHODS

### Sample preparation

*P. multiflorum* roots were obtained from Cao Bang province (Vietnam). The weight of fresh roots was approximately 1 kg. The colour of the skin is reddish-brown and these roots had no damages or physical injuries. Then, they were rinsed with tap water and cut into small pieces (thickness of 2–3 mm) and dried in an oven at 60°C (until the moisture is lower than 12%). Dried slices were ground into fine powder by a disintegrator and sieved through a 0.5 mm screen. Finally, the obtained powdered samples were packaged in vacuum conditions and stored at a room temperature for further use.

### Chemicals and reagents

Chemical agents in this study were obtained from Sigma-Aldrich (USA) and Merck (Germany) such as Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) reagent, DPPH (2,2-diphenyl-1-picrylhydrazyl) and Folin-Ciocalteu reagent. Other chemicals used were of analytical reagent grade.

### Extraction process

Dried powder was extracted with some solvents (distilled water, aqueous ethanol, aqueous methanol and aqueous acetone, solvents concentrations ranged 40–80%, v/v) in a heater for different times (30–120 minutes) at required temperature (30–70°C) and material/solvent ratio (1/10-1/50, v/w). The mixture was mixed by a magnetic stirrer and the solvent was refluxed by the condenser system. After that, it was filtered for removal of roots particles with support of the vacuum filtration system and finally, TPC and AC were determined.

### Determination of TPC in extracts

The TPC was estimated by the Folin-Ciocalteu method with slight modifications [11]. TPC was expressed as mg of gallic acid equivalents per gram of dry weight (mg GAE/g DW), based on a calibration curve of gallic acid.

## Determination of AC in extracts

Based on the method of Soto *et al.* [12] with slight modifications, the AC was assessed by DPPH assay. To obtain the calibration curve, the absorbance at 517 nm of a series of concentrations of Trolox was recorded and AC was expressed in TEAC (Trolox Equivalent Antioxidant Capacity). Trolox was used as a standard and expressed as  $\mu\text{mol}$  of Trolox per gram of dry weight ( $\mu\text{mol TE/g DW}$ ).

## HPLC analysis

The phenolic profiles in optimal extracts were analysed by an Agilent 1100 Series HPLC system equipped with a diode-array UV-VIS detector. A reversed-phase column (Kromasil C18,  $150 \times 2.1$  mm,  $3.5 \mu\text{m}$ ) was used and the UV detector was set at a wavelength of 270 nm for gallic acid and catechin; 308 nm for resveratrol. All samples were injected into the injection port (loop  $20 \mu\text{l}$ ) with a flow rate of 0.2 ml/min at  $30^\circ\text{C}$ . The gradient programme with a two-solvent system (A: hydrochloric acid; B: methanol) was used to analyse and the standard phenolic compound solutions (gallic acid, catechin and resveratrol) were prepared in the solvent used for extractions.

## Scanning electron micrographs (SEM)

A scanning electron microscope system (Jeol JSM-7401F, USA) was used to determine morphological alterations of dried powder samples before and after extraction under high vacuum condition at an accelerating voltage of 5 kV ( $10 \mu\text{m}$ ,  $2000\times$  magnification).

## Statistical analysis

The results reported in this study are the average of at least three measurements and they were expressed as means  $\pm$  standard deviations (SD). The received data were calculated and statistically analysed using the Statgraphics software (Centurion XV). Differences between means were determined by analysis of variance (ANOVA) with Fisher's least significant difference (LSD) procedure on the confidence level superior declared at  $p < 0.05$ .

*Ethical approval: The conducted research is not related to either human or animal use.*

## RESULTS AND DISCUSSION

### Effect of type of solvent on TPC and AC

The dried powder samples were extracted with various solvents such as 50% methanol, 50% ethanol, 50% acetone and distilled water under the same extraction conditions, including material/solvent ratio of 1/20, and temperature of  $50^\circ\text{C}$  in 60 minutes. The results showed that TPC and AC of extracts differ from other solvents ( $p < 0.05$ ). This pointed out that the solvent strongly affects the polyphenols extraction. The maximum TPC was  $30.43 \pm 0.78$  mg GAE/g DW and AC was  $221.56 \pm 3.83 \mu\text{mol TE/g DW}$  at acetone concentration of 50% (fig. 1).

The solvent choice for the extraction process is quite important mainly because it can increase or decrease TPC and AC levels in the extract. Many studies proved that TPC and AC depend on the polarity of solvent and solubility of phenolic compounds in various solvents [8, 13]. In fact, methanol, ethanol

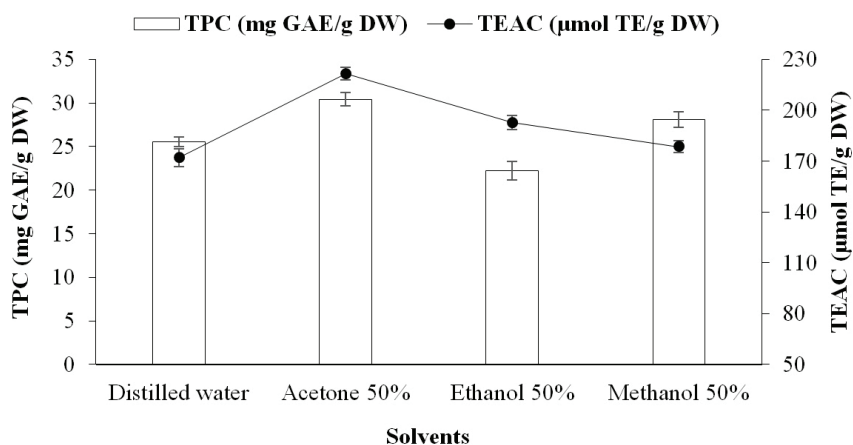


Figure 1

TPC and AC of extracts at various solvents

and acetone were used for extracting phenolic compounds from plants, especially the mixture of organic solvent and water [14]. The highest polar solvent (water) or the lowest polar solvent (hexane or chloroform) is also not suitable for extracting these bioactive compounds because water could dissolve some impurities, such as protein or sugar. They significantly affect the received result [15], especially TPC. At the same time, phenolic compounds are polar compounds. Thus, they cannot dissolve completely in the unsuitable polar solvent. Hence, the yield of the extraction process is low. Many studies also proved that aqueous acetone is an optimum solvent to extract phenolic compounds from plants such as elderberry, grape marc [16] and neem leaves [17]. Accordingly, aqueous acetone was a suitable solvent for the next steps.

### Effect of material/acetone ratio on the TPC and AC

Figure 2 shows that there are significant differences of TPC and AC levels between various material/acetone ratios ( $p < 0.05$ ). However, the material/acetone ratio of 1/40 led to the best values. Both TPC and AC obtained maximum values ( $33.72 \pm 0.44$  mg GAE/g DW and  $235.52 \pm 3.5$   $\mu$ mol TE/g DW). The material/acetone ratio is also the most important factor of the extraction process: it can strongly affect the extraction yield as TPC and AC.

An increase of the amount of solvent can lead to a quick increase of the yield of extraction and then the yield peaked at material/acetone ratio of 1/40, before slow decrease. Increasing the level of solvent

can promote the diffusion of polyphenols into solvent and dissolution of polyphenols can improve rapidly [18]. This process lasts continuously until the equilibrium is reached [19]. If the amount of solvent is too large, the yield gained is insignificant and would not be cost-effective because of time and energy consumed to chase a volume solvent. On the other hand, a small amount of solvent cannot completely extract polyphenols from materials [8].

The material/solvent ratio depends on materials, solvents, extraction methods, *etc.* The received material/solvent ratio in this case is higher than that of Tabaraki *et al.* [20], they extracted polyphenols from *Punica granatum* L. fruit with that of 1/50 by ultrasonic-assisted extraction, or lower than that of Tan *et al.* [21], who extracted polyphenols from *Centella asiatica* with that of 1/15 by maceration method. From the results obtained, the material/solvent ratio of 1/40 was used for further experiments.

### Effect of solvent concentrations on TPC and AC

The results showed that TPC and AC were significantly different at different acetone concentrations ( $p < 0.05$ ). The highest TPC and AC were  $36.5 \pm 3.14$  mg GAE/g DW and  $281.39 \pm 3.21$   $\mu$ mol TE/g DW at acetone concentration of 60%, respectively (fig. 3). That means that the polarity of polyphenols in the extract was medium. The extraction yield increased dramatically at acetone concentration from 40% to 60%, then decreased rapidly with an increase of acetone concentration of 80%. The combination of acetone and water is the

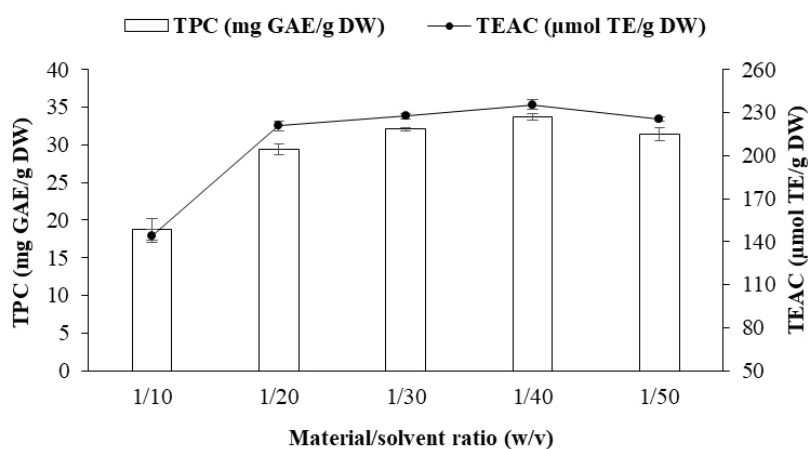


Figure 2

TPC and AC of extracts at various material/solvent ratios

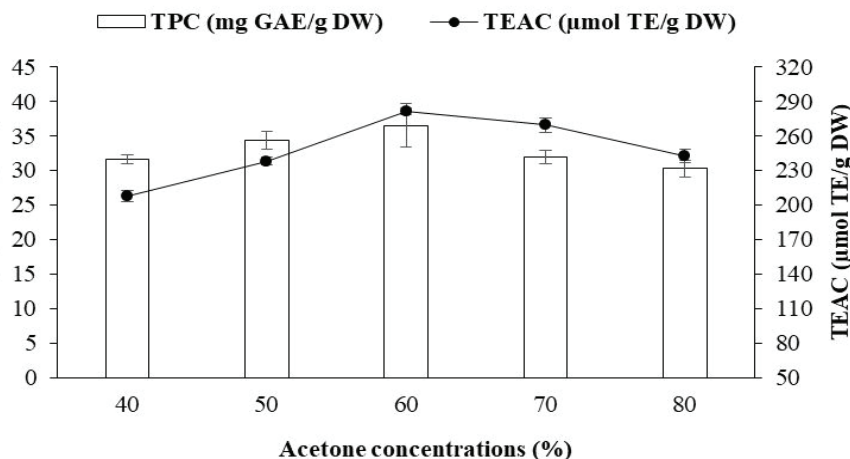


Figure 3

TPC and AC of extracts at various acetone concentrations

optimum solvent to extract phenolic compounds in this study. Water can diffuse easily into the plant cell, lead to facilitating the movement of bioactive compounds into the extracting solvent [22].

The optimum acetone concentration in this experiment is higher than that of other researches, for instance, Hismath *et al.* [17] extracted polyphenols from neem leaves with acetone concentration of 48.49% by water bath shaker extraction and Addai *et al.* [23] used acetone concentration of 50% to extract polyphenols from papaya by maceration method. However, this result is also similar to that of Quoc and Muoi [8], who extracted these compounds from *P. multiflorum* roots with acetone concentration of 60% by ultrasonic-assisted extraction. This means that TPC and AC depend on solvent concentration, extraction method and initial material. Accordingly, the suitable acetone concentration for the next experiment was 60%.

### Effect of extraction time and extraction temperature on TPC and AC

Extraction time and extraction temperature are the most important factors, which affect directly the TPC and AC. TPC and AC in the extract were significantly different for all extraction time and temperature ( $p < 0.05$ ). The yield obtains the maximum value at 50°C for 90 minutes, the TPC and AC values dramatically increased to  $38.60 \pm 0.56$  mg GAE/g DW (fig. 4) and  $298.15 \pm 2.99$  µmol TE/g DW (fig. 5), respectively. In general, the yield of the extraction process increases quickly with increasing extraction time from 30 to 90 min and extraction temperature from 30 to 50°C. Then, it sharply drops for the rest of time and temperature.

The bioactive compounds are easily degraded at high temperatures and long extraction time,

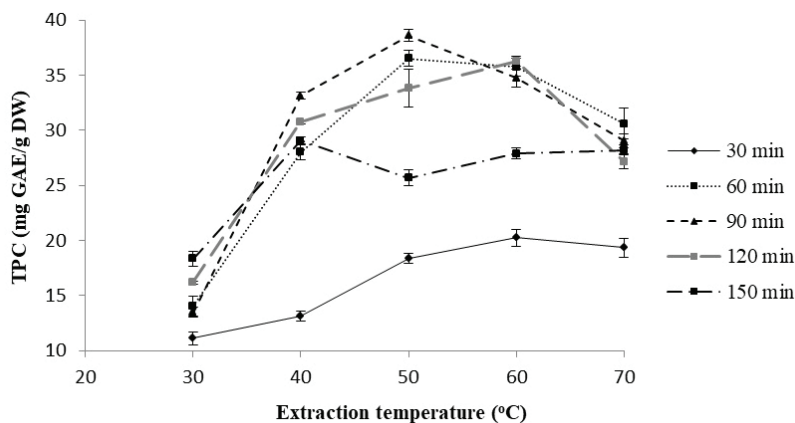


Figure 4

TPC of extracts at various extraction temperatures and extraction times

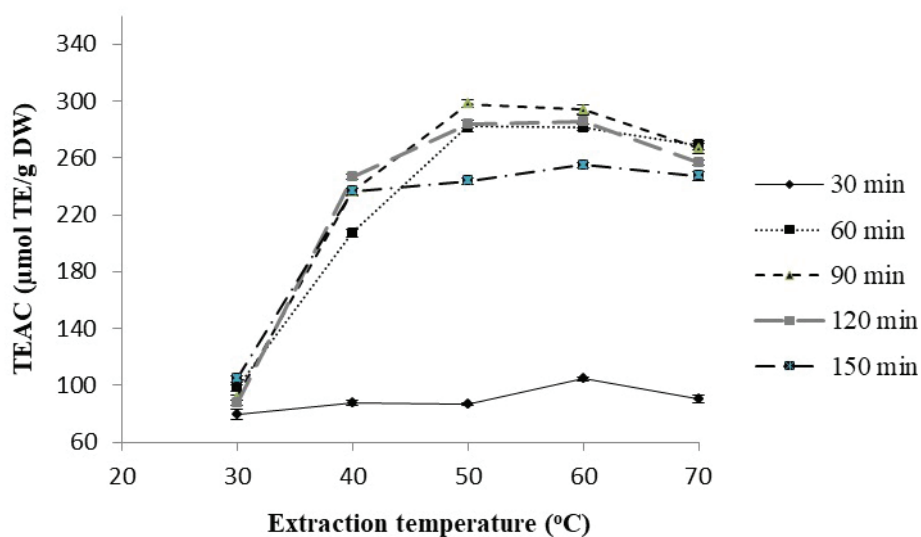


Figure 5

AC of extracts at various extraction temperatures and extraction times

especially phenolic compounds. They can be also degraded by oxygen, light and high temperature in the surrounding environment. However, high temperatures can improve the extraction yield because of promoting the diffusion of polyphenols and reducing the solvent viscosity [24]. In this case, the extraction temperature is the best choice. It is lower than that of Quoc and Muoi [8] who extracted polyphenol from *P. multiflorum* roots by ultrasonic-assisted extraction at 60°C.

Extending the extraction time can improve TPC and AC mainly because they were extracted completely. However, in some cases, the yield increases insignificantly with longer extraction time [25]. Suitable extraction time saves time and costs of the implementation process [26]. The extraction time in this study was longer than that of the study of Quoc and Muoi [9], who also extracted polyphenols from *P. multiflorum* roots by microwave-assisted extraction for 5 minutes or shorter than that of the study of Zheng *et al.* [7] who used visczyme L to extract polyphenols from unripe apples for 12 hours. For these reasons, extraction time and extraction temperature depend on the extraction method and material used. Based on the achieved results, the optimal treatment conditions in this study are the extraction temperature of 50°C and the extraction time of 90 minutes.

#### Determination of the structure of material before/after the extraction process and individual polyphenols content

Figure 6 points out that initial samples include some parts of cell plants and starch which have the various diameter and egg/oval/sphere shape. After the extraction process, starch is not gelatinized at 50°C and a few wrinkles and fragments appear on the surface of the cell wall. This proved that this extraction process affects insignificantly the initial material.

Figure 7 and 8 show that some specific phenolic compounds were also detected in the extract such as catechin (1.98 mg/g), gallic acid 0.58 (mg/g) and resveratrol (0.023 mg/g). These results are not similar to those of Quoc and Muoi [8, 9] in the same material; although, the materials were harvest in the same place. This means that the extraction methods affect the content of the specific phenolic compounds strongly.

#### CONCLUSIONS

The results recorded show that acetone is the best solvent that can increase the yield of polyphenols extraction from *P. multiflorum* roots. The best TPC and AC values were  $38.60 \pm 0.56$  mg GAE/g DW and  $298.15 \pm 2.99$   $\mu$ mol TE/g DW at the optimal extraction conditions (60% aqueous acetone, material/solvent

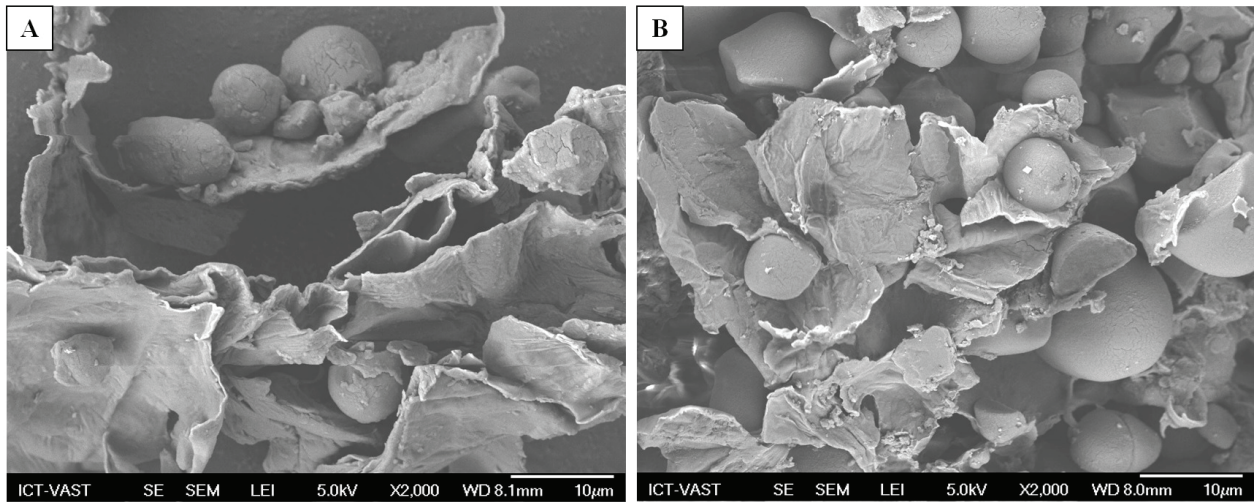


Figure 6

Structure of material before (A) and after (B) extraction process

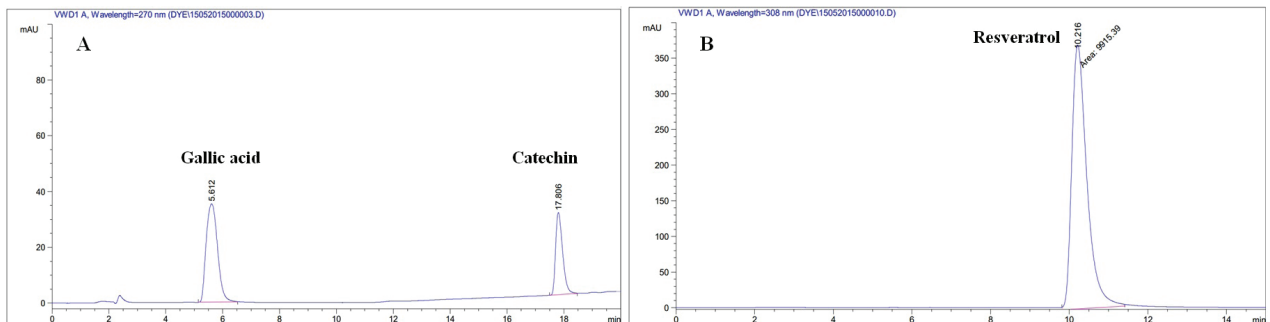


Figure 7

HPLC chromatograms of a mixed standard solutions of gallic acid, catechin and resveratrol

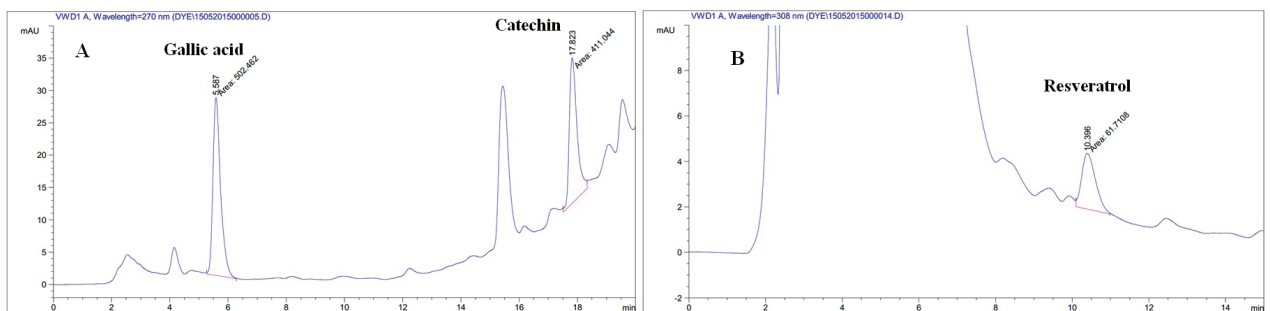


Figure 8

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

ratio of 1/40, extraction time of 90 minutes and extraction temperature of 50°C). Cell wall was not affected significantly by acetone and the received extract has the presence of the main phenolic compounds as catechin, gallic acid and resveratrol.

*Conflict of interest: Authors declare no conflict of interest.*

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