Evaluation of the Phenolic Content in the Buds of *Polygonum cuspidatum* Sieb. et Zucc.

Mădălina LAZURCA¹, Dumitru LAZURCA², Florinela FETEA¹, Floricuta RANGA¹, Carmen SOCACIU¹

¹University of Agricultural Sciences and Veterinary Medicine, 3-5 Mănăștur Street, 400372, Cluj-Napoca, Romania, madalina_lazurca@yahoo.com
²Institute for Research, Development and Innovation, Transilvania University, 10 Institutului Street, Brasov, Romania, dlazurca@unitbv.ro

**Abstract.** *Polygonum cuspidatum* Sieb. et Zucc. (Japanese knotweed) is considered an invasive plant in several countries in Europe, whilst being used in the Traditional Chinese Medicine for various inflammatory diseases, hepatitis, tumors, diarrhea, suppurative dermatitis, gonorrhea, hyperlipidemia, favus athlete’s foot, osteomyelitis. The present study is focused on the characterization of different extracts of *Polygonum cuspidatum* Sieb. et Zucc. buds, by determination of the influence of different solvents on the extraction yield, the phenolic content and their antioxidant capacity. The solvents used were ethanol, propylene glycol, methanol, acid water and alkaline water, in different combinations. The UV-Vis spectra were recorded and the total phenolic content was determined by Folin-Ciocalteu colorimetric method, comparing the extraction yields with the total phenolic contents. The antioxidant activity was evaluated by 1,1-diphenyl-2-hydrazyl (DPPH) assay. The extracts analyzed had total phenolic values from 1048 mg/100g to 1426 mg/100g fresh buds, expressed as gallic acid equivalents, the highest extraction index being 96.3, in good agreement with the antioxidant capacity. These data suggest that *Polygonum cuspidatum* Sieb. et Zucc. buds could be a rich source of natural antioxidants and a good candidate as an ingredient in functional food, cosmetics and drugs for the prevention of the negative effects of oxidative stress on humans.

**Keywords:** *Polygonum cuspidatum*, polyphenols, DPPH, antioxidant capacity, Folin-Ciocalteu.

**INTRODUCTION**

*Polygonum cuspidatum* Sieb. et Zucc. is a plant from the *Polygonaceae* family, originating from China, where it is known as Hu Zhang (Fan et al., 2009). In Europe and North America it is known as Japanese Knotweed, Japanese Bamboo or Mexican Bamboo, and it was first introduced as a decorative plant in gardens and parks. But due to its capacity to spread, it became an invasive plant and a threat for the local flora and ecosystems both in North America and Europe (Weston et al., 2005; Stoll et al., 2012), being on the black list of invasive plants in Switzerland ([http://cps-skew.ch/english/black_list.htm](http://cps-skew.ch/english/black_list.htm)).

The roots and rhizomes of the plant (*Polygonium cuspidati rhizoma*) are used since ancient times in the Traditional Chinese Medicine for the treatment of menoxenia, skin burn, hepatitis, arthralgia, chronic bronchitis, high blood pressure, suppurative dermatitis, hyperlipemia (Ban et al., 2010; Bralley et al., 2008; Chu et al., 2004; Kim et al., 2007) and is officially listed in the Chinese Pharmacopoeia (Fan et al., 2009). The ethanolic extract of *Polygonum cuspidatum* Sieb. et Zucc. was shown to have an inhibitory effect on hepatitis B virus in a HBV-producing cell line (Chang et al., 2005). Also, the extracts of *Polygonum cuspidatum* were found to have potent estrogenic activity (Zhang et al., 2006) and antibacterial activity in vitro (Shan et al., 2008; Song et al., 2006).

The chemical composition of *Polygonii cuspidati rhizoma* includes various classes of compounds, to mention anthraquinones (Chu et al., 2004; Zhao et al., 2005; Beňová et al., 2007).
Recent studies focused on the determination of benefits of *Polygonum cuspidatum* bioactive compounds revealed important therapeutic potential (Cai et al., 2004; Chang et al., 2005; Chen et al., 2007; Delmas et al., 2005; Hsu et al., 2007; Jeong et al., 2010; Lin et al., 2010; Pan et al., 2007; Shan et al., 2008; Song et al., 2006; Xing et al., 2009). In the last few years resveratrol, a stilbene found in the roots of *Polygonum cuspidatum* Sieb.et Zucc., has become interesting due to its antioxidant capacity (King et al., 2006; Pandey and Rizvi, 2011), inhibition of carcinogenesis (Chachay et al., 2011; King et al., 2006; Pezzuto, 2008; Shukla and Singh, 2011), atherosclerosis prevention agent (Delmas et al., 2005), cardioprotective (Smoliga et al., 2011; King et al., 2006; Petrovski et al., 2011; Wu and Hsieh, 2011), stimulant of insulin secretion (Chen et al., 2007), prevention of neurodegenerative diseases (Rocha-González et al., 2008). A recent study showed an antidepressant-like activity of resveratrol with involvement in the serotonin and noradrenaline systems (Xu et al., 2010). Emndin, an antraquinone isolated from *Polygonum cuspidatum* Sieb.et Zucc., is a thyrosinase inhibitor and can be used as agent for skin whitening (Leu et al., 2008). It also showed anti-viral activity and *in vitro* antibacterial activity (Zhao et al., 2005; Shan et al., 2008). Various pharmacological studies support emodin’s potential as purgative, hepatoprotective, cardiotonic, anticancer agent (Srinivas et al., 2007). Piceid has shown inhibitory activity on thyrosinase and melanogenesis, therefore being a possible skin whitening agent (Jeong et al., 2010). Some studies have shown the regulating effects of piceid (also known an polydatin) on the lipid levels, improving the LDL/HDL rapport and lowering the levels of cholesterol, suggesting that piceid may be effective in the prevention of hyperlipidemias associated with complication of adiposity (Du et al., 2009; Xing et al., 2009).

To our knowledge there are few literature data regarding the buds of *Polygonum cuspidatum* Sieb.et Zucc. and their chemical composition. The purpose of the present study is the evaluation of the phenolic content and antioxidant capacity of *Polygonum cuspidatum* Sieb.et Zucc. buds and also the influence of different solvents on the extraction yield and phenols recovery.

**MATERIALS AND METHODS**

**Chemicals**

The standard compounds, including 6-hydroxy-2,5,7,8-tetramethylchrooman-2-carboxilic acid (Trolox) 98% purity and 2,2-diphenyl-1-picrylhydrazyl (DPPH) 95% purity, were obtained from Sigma-Aldrich (Darmstadt, Germany). Folin-Ciocalteu’s reagent, Na$_2$CO$_3$, CH$_3$OH and HCl were purchased from Merck (Darmstadt, Germany). Solvents used for the extraction, ethanol and polypropylene glycol, were obtained from local producers. The water used for extraction, acid or alkaline, was obtained with a LeveLuk SD 501 apparatus purchased from Enagic (Dusseldorf, Germany).

**Sample extraction**

The buds of *Polygonum cuspidatum* Sieb.et Zucc. used in this study were harvested from the roots at the end of March, from the wild flora in Brasov area, Romania. After collection, the buds were washed with water to remove any trace of soil. Buds were grinded manually and samples were stored in a freezer at -20°C until further analysis. Aliquots of 130 g buds were mixed with 1300 ml solvent, realizing a 10% concentration of plant in solvent.

Several solvents were used for extraction, in order to determine the most appropriate one for the extraction of phenolic compounds: polypropylene glycol and acidic water (1:1 v/v), polypropylene glycol, acidic water and ethanol (1:1:1 v/v/v), polypropylene glycol and
alkaline water (1:1 v/v), ethanol and acidic water (1:1 v/v) and methanol acidulated with hydrochloric acid 1% as presented in Table 1.

Tab. 1.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Solvent used</th>
</tr>
</thead>
<tbody>
<tr>
<td>I A</td>
<td>Polypropylene glycol : acid water 1:1 (v/v)</td>
</tr>
<tr>
<td>II A</td>
<td>Polypropylene glycol : acid water : ethanol 1:1:1 (v/v/v)</td>
</tr>
<tr>
<td>III A</td>
<td>Polypropylene glycol : alkaline water 1:1 (v/v)</td>
</tr>
<tr>
<td>IV A</td>
<td>Ethanol : acidic water 1:1 (v/v)</td>
</tr>
<tr>
<td>V A</td>
<td>Methanol acidulated with hydrochloric acid 1%</td>
</tr>
</tbody>
</table>

The extraction method for polyphenols from *Polygonum cuspidatum* Sieb.et Zucc. buds is presented in Fig. 1.

Fig. 1. Extraction protocol for *Polygonum cuspidatum* Sieb.et Zucc. buds using ultrasounds and filtration (ExA) or followed by a second extraction (ExB) by Timatic system.

After ultrasounds extraction, a fraction of the extracts was submitted to further extraction using a Timatic Lab Extractor with a capacity of 1L purchased from TechnoLab (Spello, Italy). The Timatic Lab Extractor uses as extraction process a repeated percolation under controlled pressure (8 bar). The extracts obtained after filtration were marked as ExA and the double extraction using Timatic Lab Extractor, as ExB.
UV spectra and extraction yield
The UV spectra for the ExA and ExB extracts were recorded using a T70+ UV/VIS Spectrometer (from PG Instruments Ltd, Great Britain), in the UV range of 200- 400 nm. The yield of extraction was evaluated as “extraction index” according to the UV absorption units at 280 nm multiplied with the dilution.

Total phenolic content determination
The total content of polyphenols in the knotweed extracts was determined using modified Folin-Ciocalteu colorimetric method (Hsu et al., 2007). Shortly, 15 µl of extract were mixed with 2835 µl of distilled water, oxidized with 150 µl of Folin-Ciocalteu reagent and homogenized. After 5 minutes the reaction was neutralized with 450 µl of Na₂CO₃. The absorbance was measured at 750 nm, after 2 hours storage in the dark at room temperature, using a Synergy HT Multi-Mode Microplate Reader (from BioTek Instruments). The results were expressed as milligram of gallic acid equivalents per 100 grams of fresh buds.

DPPH assay
The DPPH antioxidant activity was done according to a method presented by Brand-Williams et al (1995). A solution of DPPH (80 µM(Brand-Williams et al., 1995)) was freshly prepared in methanol 95%. A volume of 1750 µl of this solution was allowed to react with 250 µl of sample and the absorbance was measured at 515 nm after 30 min. Trolox was used as pure standard for the calibration curve and the results were expressed as µM trolox/ g fresh weight buds. The antioxidant activity was calculated as average of triplicate samples.

Statistical analysis
The results are expressed as mean ± standard deviation (SD) from three parallel measurements. Pearson’s correlation coefficient (R²) was calculated using Microsoft Excel 2010.

RESULTS AND DISCUSSION

UV spectra and extraction yields
The UV-Vis spectra recorded for the extracts are presented in Fig. 2. All the samples showed similar shapes of the spectra, with different intensities of the peaks at 280 nm, suggesting the presence of phenolic compounds, in some cases with flavonoids.

![UV spectra for Polygonum cuspidatum samples](image)

Fig.2. UV spectra recorded for Polygonum cuspidatum Sieb.et Zucc. samples, differentiated by the extraction procedure.

The absorbance data are presented in Table 2.
Evaluation of extraction index as a measure of extraction yields, comparatively for ExA and ExB, considering the absorbance at 280 nm and the dilution (1:100).

<table>
<thead>
<tr>
<th>Solvent type</th>
<th>ExA Absorbance at 280 nm</th>
<th>ExB Absorbance at 280 nm</th>
<th>ExA Extraction index (A x dil. factor)</th>
<th>ExB Extraction index (A x dil. factor)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.741</td>
<td>0.963</td>
<td>74.1</td>
<td>96.3</td>
</tr>
<tr>
<td>II</td>
<td>0.636</td>
<td>0.780</td>
<td>63.6</td>
<td>78.0</td>
</tr>
<tr>
<td>III</td>
<td>0.510</td>
<td>0.518</td>
<td>51.0</td>
<td>51.8</td>
</tr>
<tr>
<td>IV</td>
<td>0.644</td>
<td>0.795</td>
<td>64.4</td>
<td>79.5</td>
</tr>
<tr>
<td>V</td>
<td>0.553</td>
<td>0.655</td>
<td>55.3</td>
<td>65.5</td>
</tr>
</tbody>
</table>

It is relevant that absorbance of ExB samples was superior in all extracts, increased by 1.2-1.4 times. The highest extraction index was observed when solvent I was used, followed by solvents II and IV, for both ExA and ExB procedures. According to these findings, propylene glycol–based solvent in acidic water proved to be the most appropriate, followed by ethanol.

Total phenolic content determination

The total phenolic content (TPC) determined for ExA and ExB are presented in Table 3.

Total phenolic content (TPC) in *Polygonum cuspidatum* Sieb.et Zucc. buds extract determined using Folin-Ciocalteu method

<table>
<thead>
<tr>
<th>Solvent type</th>
<th>Extract line A*</th>
<th>Extract line B*</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1080±58</td>
<td>1240±33</td>
</tr>
<tr>
<td>II</td>
<td>1292±2</td>
<td>1398±14</td>
</tr>
<tr>
<td>III</td>
<td>1048±13</td>
<td>1066±6</td>
</tr>
<tr>
<td>IV</td>
<td>1236±8</td>
<td>1426±15</td>
</tr>
<tr>
<td>V</td>
<td>1150±53</td>
<td>1202±33</td>
</tr>
</tbody>
</table>

*GAE – galic acid equivalents, ExA are obtained by ultrasonication, ExB were submitted to further extraction using Timatic Lab Extractor. Values represent mean ± SD.

The TPC values for the extracts analyzed range from 1048 to 1426 mg GAE/100 g fresh weight for ExA. The highest TPC values were obtained for the extracts using as solvents ethanol: acidic water 1:1 (v/v) (IV) and polypropylene glycol:acidic water: ethanol 1:1:1 (v/v/v), (II) with a TPC of 1236 mg GAE/100g fresh weight and respectively, 1292 mg GAE/100 g fresh weight for ExA.

The results suggest that the most appropriate solvents for the extraction of polyphenolic compounds from the buds of *Polygonum cuspidatum* Sieb.et Zucc. are ethanol:acidic water 1:1 (v/v) and polypropylene glycol:acidic water:ethanol 1:1:1 (v/v/v).

For ExB, the TPC values obtained were higher than for ExA, ranging from 1066 to 1426 mg GAE/100 g fresh weight. According to these results, using the Timatic Lab Extractor
increases the extraction yield for phenolic compounds. The highest TPC values were obtained, same as for ExA, using ethanol:acidic water 1:1 (v/v) (IV) and polypropylene glycol:acidic water:ethanol 1:1:1 (v/v/v) (II) as solvents. The TPC was 1426 mg GAE/100 g fresh weight and respectively, 1398 mg GAE/100 g fresh weight.

These results imply that the Polygonum cuspidatum Sieb.et Zucc. extracts contain high amounts of phenolic compounds.

**DPPH assay**

The mean values of antioxidant activity (AO) obtained for all studied extracts are summarized in Table 4.

Tab. 4. Antioxidant activity (AO) (expressed in µM trolox/g fresh weight) of buds extracts of Polygonum cuspidatum Sieb.et Zucc.

<table>
<thead>
<tr>
<th>Extract line A</th>
<th>Extract line B</th>
</tr>
</thead>
<tbody>
<tr>
<td>I 145.586±4.5</td>
<td>I 159.759±7.18</td>
</tr>
<tr>
<td>II 182.483±7.84</td>
<td>II 233.977±6.13</td>
</tr>
<tr>
<td>III 115.276±2.89</td>
<td>III 181.448±3.54</td>
</tr>
<tr>
<td>IV 154.471±4.88</td>
<td>IV 228.034±6.77</td>
</tr>
<tr>
<td>V 89.034±2.45</td>
<td>V 95.506±1.55</td>
</tr>
</tbody>
</table>

Values represent mean ± SD.

All the extracts presented good antioxidant activity, in good agreement with the total phenolic content, but not significant ($R^2 = 0.5274$). Antioxidant activity ranged from 89.034 to 182.483 µM trolox/g fresh weight buds for the extracts obtained using ultrasounds extraction and from 95.506 to 233.977 µM trolox/g fresh weight buds for the extracts obtained using the Timatic Lab Extractor. The highest values for the antioxidant activity were obtained for the extracts in ethanol : acidic water 1:1 (v/v) (IV) and polypropylene glycol : acidic water : ethanol 1:1:1 (v/v/v) (II), with values of 228.034 µM trolox/g of fresh buds and respectively, 233.977 µM trolox/g of fresh buds.

**CONCLUSION**

The extraction of phenolic compounds from Polygonum cuspidatum Sieb.et Zucc. buds was done using two different procedures (ultrasounds and a Timatic Lab extractor) and with different mixtures of solvents. The data presented show that the Timatic Lab Extractor is more appropriate for extracting phenolic compounds from Polygonum cuspidatum Sieb.et Zucc. buds.

The mixture of ethanol:acidic water 1:1 (v/v) (IV) and polypropylene glycol:acidic water:ethanol 1:1:1 (v/v/v) (IV) proved to be the most adequate for the extraction of phenolic compounds, in both extraction systems, having the highest values of extraction index, the total phenolic concentration and the highest antioxidant activity.

These data suggest that Polygonum cuspidatum Sieb.et Zucc. buds could be a rich source of natural antioxidants and a good candidate as an ingredient in functional food, cosmetics and drugs for the prevention of the negative effects of oxidative stress on humans.
According to these data we can recommend the solvent II, polypropylene glycol:acidic water:ethanol 1:1:1 (v/v/v) to be very appropriate (considering the lower concentration of ethanol) for these purposes.

REFERENCES


