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Short communication

Phenolic acids significantly contribute to antioxidant potency of *Gynostemma pentaphyllum* aqueous and methanol extracts

Dunja Šamec[a], Lidija Valek-Žulj[b], Sanja Martinez[b], Jiři Grúz[c,d], Ante Piljac[e], Jasenka Piljac-Žegarac[a,*]

[a] Department of Molecular Biology, Institute Ruđer Bošković, Bijenička c. 54, 10000 Zagreb, Croatia
[b] Faculty of Chemical Engineering and Technology, University of Zagreb, Savska c. 16, 10000 Zagreb, Croatia
[c] Laboratory of Growth Regulators, Palacký University and Institute of Experimental Botany ASCR, Šlechtitelů 11, CZ-783 71 Olomouc, Czech Republic
[d] Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University, Šlechtitelů 11, 783 71 Olomouc, Czech Republic
[e] University Clinic for Diabetes, Endocrinology and Metabolic Disorders Vuk Vrhovac, Dugi Dol 4a, 10000 Zagreb, Croatia

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ABSTRACT

Cyclic voltammetry, as well as four well-established spectrophotometric and fluorimetric assays, were applied to study the contribution of phenolic compounds to the antioxidant capacity of aqueous and methanol extracts of Jiaogulan tea (*Gynostemma pentaphyllum*). In addition, individual phenolic acid profiles of *G. pentaphyllum* extracts, determined using UPLC–MS/MS, were reported for the first time in this study. Methanol extraction resulted in higher contents of phenolic compounds, as well as antioxidant capacity of tested extracts. Our results suggest that *G. pentaphyllum* aqueous and methanol extracts have high antioxidant capacity and are rich in phenolic compounds, such as chlorogenic acid, which might play a role in the prevention of type 2 diabetes mellitus and cardiovascular disease.

1. Introduction

Gynostemma pentaphyllum (fam. *Cucurbitaceae*) is a herbaceous vine that grows wild in China, and in many other countries throughout Asia. Jiaogulan tea, made from *G. pentaphyllum* leaves, has been used as an energizing agent, for treatment of common colds and infections, and for general health (Blumert, 1999). Pharmacological studies of Jiaogulan have pointed to its immunomodulatory (Suntararuks et al., 2008), antitumor (Chen et al., 2011), anti-gastric ulcer (Rujjanawate et al., 2004), anti-hyperlipidemic, hypoglycemic (Megalli et al., 2006), hepatoprotective (Lin et al., 2000) and cardiovascular disease protective effects (Circosta et al., 2005). Most of these activities are associated with the presence of antioxidants, such as saponins, flavonoids, polysaccharides and gypenosides in Jiaogulan tea (Kao et al., 2008). Earlier published studies have focused on *G. pentaphyllum* flavonoids (Kao et al., 2008; Tsai et al., 2008), although phenolic acids also contribute to the overall bioavailable antioxidants. This study reports for the first time the content of individual free phenolic acids in aqueous and methanolic extracts of *G. pentaphyllum* and their contribution to the antioxidant capacity of this important medicinal plant.

2. Material and methods

2.1. Chemicals and instruments

Chemicals and reagents were supplied by Sigma Chemical Co. (St. Louis, MQ, USA), Kemika (Zagreb, Croatia) or Laphoma (Skopje, Macedonia). Bio-Spec-1601 UV–vis spectrophotometer (Shimadzu Corporation, Kyoto, Japan) and fluorescence microplate reader (Multiskan Ascent 354, Labsystems) were used for spectrophotometric and fluorimetric measurements, respectively. ACQUITY Ultra Performance LC™ system (Waters, Milford, MA, USA), linked to a Micromass Quattro micro™ API benchtop triple quadrupole mass spectrometer (Waters MS Technologies, Manchester, UK) was used for UPLC–MS/MS.

* Corresponding author.
E-mail address: jpiljac@irb.hr (J. Piljac-Žegarac).

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Table 1
Total phenol (TP) and flavonoid (TF) content and ferric reducing/antioxidant power of aqueous and metanol Jiaogulan tea extracts.

Sample	Extract	TP mg GAE/g DW	TF mg CE/g DW	FRAP mmol Fe^{2+}/g DW
Malaysian	MeOH	11.57 ± 0.35[a]	5.39 ± 0.38[a]	141.50 ± 6.70[a]
	W	8.11 ± 0.25[b]	2.76 ± 0.19[b]	72.96 ± 4.88[b]
German	MeOH	10.42 ± 0.70[a]	4.75 ± 0.16[a]	130.85 ± 2.89[a]
	W	6.61 ± 0.66[c]	1.89 ± 0.21[c]	60.72 ± 2.73[b]

Numeric values with different superscript letters across each column represent significantly different values.

2.2. Extraction

Methanol extracts of Jiaogulan teas (*G. pentaphyllum*), produced in Malaysia and Germany, were prepared by mixing 60 mg of dried material with 2 mL of 80% methanol and shaking for 2 h on a rotary homogenizer at 24 ± 2 °C. Methanol has been chosen as one of the extraction solvents because it was earlier found to be more efficient in extraction of low molecular weight polyphenols. Following, the extracts were sonicated (Iskra, Zagreb, Croatia) for 15 min and centrifuged at 10,000 × *g* (Centrifuge 5415C, Eppendorf, Germany) for 15 min. Aqueous extracts were prepared by infusing 1.5 g of tea with 50 mL of deionised water, heated to 95 °C for 8 min. The supernatant was used for analyses. For electrochemical measurements, 1 g of powdered sample was mixed with 50 mL of 80% methanol, shaken for 1 h at 125 rpm on a Cole-Parmer shaker (Vernon Hills, IL) at 24 ± 2 °C, sonicated (Iskra, Zagreb, Croatia) for 15 min and centrifuged on a Multifuge 3S-R (Kendro, Germany) at 10,000 × *g* for 15 min. Aqueous tea extracts were prepared by infusing 2 g of tea leaves with 100 mL of deionised water and heating for 8 min to 95 °C. All extractions were performed in triplicate.

2.3. Polyphenolic content

The Folin–Ciocalteu assay (Singleton and Rossi, 1965), adapted to small volumes, was used to determine the total phenol (TP) content of aqueous and methanol tea extracts. The total flavonoid content (TF) was determined according to the $AlCl_3$ colorimetric assay (Zhishen et al., 1999), adapted for small volumes (Šamec et al., 2011). Gallic acid and catechin were used to prepare the calibration curves, respectively. The UPLC–MS/MS analysis of phenolic acids was performed as described earlier (Gruz et al., 2008).

2.4. Antioxidant capacity

The FRAP assay (Benzie and Strain, 1999) was used to estimate the antioxidant potential of tested infusions, expressed in mmol Fe^{2+}/g DW. The DPPH• radical scavenging capacity was determined according to the method outlined by Brand-Williams et al. (1995), while the ABTS•+ radical cation decolorization assay was performed according to Re et al. (1999). The results of both assays were reported in μmol Trolox/g DW.

The ORAC assay for performed according to Prior et al. (2003), with small modifications. The excitation and emission wavelengths were 485 nm and 525 nm, respectively. Fluorescence readings were taken on a fluorescence microplate reader (Multiskan Ascent 354, Labsystems) every 3 min for 90 min. AAPH was used as the peroxyl generator, and Trolox as the standard, in the concentration range 12.5–250 μM. All dilutions were carried out in 75 mM phosphate buffer (pH 7.4). The results were reported in μmol Trolox/g DW.

2.5. Cyclic voltammetry

CV measurements were performed in the potential range 0–900 mV. A Saturated Calomel Electrode (SCE) was used in conjunction with a platinum counter electrode and a glassy carbon working electrode. Aqueous tea infusions were diluted 4× in 0.1 M acetate buffer, pH 3. Methanol extracts were diluted 4× with 80% methanol/water solution and 0.1 M $LiClO_4$ was added to increase solution conductivity. The scans were taken in the potential range 0–900 mV, at a scan rate 100 mV s^{-1}. Prior to each measurement, background currents measured in the acetate buffer and the methanol/water/$LiClO_4$ solution, were subtracted from the currents measured in tea solutions. The area beneath the major anodic peak up to 800 mV (Q_{800}), proportional to antioxidant capacity, was used to calculate the TEAC values based on a calibration curve, Q_{800} vs. *c*, obtained for Trolox in the concentration range 2–100 μmol dm^{-3}.

2.6. Statistical and mathematical analyses

All presented values are means of three measurements ± standard deviation (SD). Analysis of Variance (ANOVA) with Tukey post-hoc test was performed using StatistiXL (Nedlands, Western Australia). Differences at $p < 0.05$ were considered to be significant.

3. Results and discussion

3.1. Phenolic acids and other phenolic compounds in G. pentaphyllum

The levels of 12 free phenolic acids in Jiaogulan tea extracts are shown in Table 2. A higher concentration of individual phenolic acids was found in methanol compared to aqueous extracts, indicating that 80% methanol is a more suitable extraction solvent. Significantly different content of phenolic acids in samples from different producers was observed; salicylic and chlorogenic acids were significantly higher in the Malaysian Jiaogulan tea extracts, while German tea extracts had a higher content of 4-hydroxybenzoic and 4-coumaric acids.

The TP and TF contents of Jiaogulan tea extracts are presented in Table 1. Methanol extracts have shown significantly higher TP and TF contents than aqueous extracts. Similar observation was reported earlier for 112 Chinese medicinal plants by Cai et al. (2004). The TP content of Jiaogulan tea extracts obtained in this study (11.57 ± 0.35 and 10.42 ± 0.70 mg GAE/g DW for methanol, 8.11 ± 0.25 and 6.61 ± 0.66 mg GAE/g DW for aqueous extracts, respectively) was significantly higher than the TP content of *G. pentaphyllum* whole plants reported by Cai et al. (2004), (0.44 and 0.24 g GAE/100 g DW for 80% methanol and aqueous extracts, respectively). In that particular study, flavonols, especially rutin, were reported as major phenolic constituents of *G. pentaphyllum*. In our study, the TF contents of methanol and aqueous extracts of Jiaogulan tea from two producers were 5.39 ± 0.38 (M) and 4.75 ± 0.16 (G) mg CE/g DW, and 2.76 ± 0.19 (M) and 1.89 ± 0.21 (G) mg CE/g DW, respectively.

3.2. Antioxidant capacity

Four established spectrophotometric and fluorimetric assays, as well cyclic voltammetry (CV), were applied to elucidate the antioxidant potency of *G. pentaphyllum* aqueous and methanol extracts,