PHYTOCHEMICAL AND ANTIOXIDANT PROPERTIES OF *ATHAMANTA TURBITH* (L.) BROT COLLECTED FROM SERBIA

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INTRODUCTION

Plants are a remarkable source of different bioactive compounds with proved antioxidant properties. In order to obtain valuable natural compounds from plants it is important to apply adequate extraction procedures including appropriate solvents as well as extraction techniques. Among several procedures classical solvent extraction (SE) and ultrasound-assisted extraction (UAE) stand out as the most present. Recently there has been a trend of examining endemic plants as novel valuable sources of different bioactives. Among them is *Athamanta turbith* (L.) Brot., flowering plant from the Apiaceae family that grows in Đetinja Canyon. River Đetinja springs on the slopes of Tara Mountain, near village Kremna in Western Serbia (Figure 1). This distinguished area has great historical, cultural, geological, ecological and biological importance. *A. turbith* L. prefers chalky, dry soils and gravel, which are exposed to sun. The average height of the plant is 30 cm. Stems are branched, bright green leaves are triangular, 2 to 4 times pinnated. In summer months, this lithophyte forms inflorescences that belong to compound umbel type with tiny, white, star-shaped flowers as shown in Figure 1.

Figure 1. Location of Djetinja Canyon (left) and *Athamanta turbith* L. Brot (right).

The main objective of this study was to determine the content of selected bioactive compounds present in three distinct plant parts – rhizome, vegetative shoot and inflorescence by application of usual spectrophotometric methods. For this purpose, two extraction techniques were performed: solvent extraction (SE) and ultrasound-assisted extraction (UAE) in order to obtain maximal yield of bioactive compounds. After that, antioxidant properties of prepared extracts were analyzed and correlated with determined bioactive compounds of *A. turbith* L.

MATERIAL AND METHODS

Plant material (rhizome, vegetative shoot and inflorescence) of *A. turbith* was collected from Djetinja Canyon (western Serbia) and further used to prepare extracts. Extraction was performed in 80% methanol as a solvent with two different approaches: powdered plant material was extracted with solvent for 3 h in ratio 1:10 without (classical solvent extraction, SE,) and with application of ultrasound (ultrasound-assisted extraction, UAE). Analysis of total phenolic content (TPC), total flavonoid content (TFC) and total hydroxycinnamic acid derivative content (HCA) was performed via spectrophotometric methods. Additionally, antioxidant properties of extracts were determined with five assays: $ABTS^{\cdot+}$, $DPPH^{\cdot}$, ferric reducing power (FRP), *in vitro* phosphomolybdenum total antioxidant capacity (TAC) and cupric reducing antioxidant capacity (CUPRAC). Statistical analyses of the data were performed using the STATISTICA 12.0. Statistical significance was evaluated employing Tukey's test.

RESULTS AND DISSCUSION

The content of total phenolics (TPC), total flavonoids (TFC) and total dihydroxycinnamic acid derivatives (HCAs) observed in rhizome, vegetative shoot and in-florescence extracts of A. turbith is shown in Table 1. The inflorescence had the highest TPC in UAE obtained-extract, as well as the highest TFC and HCA in SE-prepared extract. In general, the extract of inflorescence obtained by UAE had significantly higher ($p< 0.05$) content of TPC and TFC than extract obtained by SE. However, there was no significant difference in the content of HCAs, achieved in the inflorescence, for both extracts. The lowest amount of TPC and HCAs was detected in the rhizome regardless of the extraction methods ($p< 0.05$), while the flavonoids were not detected.

Table 1. Phytochemical composition of *Athamanta turbith*

Sample	Extraction technique	TPC* [mg/g GAE]	TFC [mg/g QE]	HCAs [mg/g CGAE]
Inflorescence	UAE	2.73±0.13 ^{a**}	1.36±0.02 ^{a}	1.41±0.004 ^{a}
	SE	1.95±0.15 ^{b}	1.56±0.02 ^{b}	1.45±0.11 ^{a}
Vegetative shoot	UAE	1.06±0.02 ^{c}	0.70±0.002 ^{c}	1.07±0.009 ^{b}
	SE	0.87±0.01^{c}	0.53±0.05 ^{d}	0.85±0.008 ^{c}
Rhizome	UAE	0.37±0.03 ^{d}	n.d.	0.71±0.00 ^{d}
	SE	0.40±0.01 ^{d}	n.d.	0.66±0.00 ^{d}

*TPC- total phenolic content; TFC- total flavonoid content; HCA- total dyhydroxicinnamic acid derivative content; GAE- gallic acid equivalents; QE- quercetin equivalents; CGAE- chlorogenic acid equivalents; n.d. – not detected.

** Different superscript letters$^{(a-e)}$ in a same column indicate significant differences at $p< 0.05$.

Antioxidant properties of *A. turbith* extracts determined with five assays are shown in Table 2. The inflorescence had the highest antioxidant activity in both quencher assays (at $\gamma= 0.1$ g/mL) with 92.1% of inhibition for $ABTS^{\cdot+}$ (UAE extract,) and 77.7% inhibition of $DPPH^{\cdot}$ (for both extracts). In addition, it exhibited the highest FRP (18.4 mg/g AAE, SE extract), CUPRAC (~40 mg/g AAE for both extracts) and TAC (~35 mg/g AAE for both extracts). The inflorescence extracts obtained by SE and UAE show a statistically significant difference ($p< 0.05$) between antioxidant activity, only in the $ABTS^{\cdot+}$ and FRP assays. The rhizome had the lowest values for all antioxidant assays concerning both SE and UEA. Differences in antioxidant activity between SE and UAE-obtained rhizome extracts were statistically significant ($p< 0.05$) for $ABTS^{\cdot+}$ and $DPPH^{\cdot}$ assays.

Table 2. Antioxidant properties of *A. turbith* extracts

Sample	Extraction tehnique	ABTS$^{\cdot+}$* [% inh.]	DPPH$^{\cdot}$ [% inh.]	TAC [mg/g AAE]	CUPRAC [mg/g AAE]	FRP [mg/g AAE]
Inflorescence	UAE	51.43±0.06 ^{a**}	77.68±0.55 ^{a}	3.60±0.07 ^{a}	39.15±3.03 ^{a}	11.06±0.52 ^{a}
	SE	92.11±0.48 ^{b}	77.77±0.57 ^{a}	3.53±0.29 ^{a}	41.83±1.29 ^{a}	18.37±1.70 ^{b}
Vegetative shoot	UAE	23.67±0.00 ^{c}	33.86±0.14 ^{b}	1.75±0.00 ^{b}	12.52±1.10 ^{b}	1.59±0.09 ^{cd}
	SE	34.00±0.06 ^{d}	50.34±0.41 ^{c}	1.54±0.01 ^{b}	8.42±1.03 ^{b}	3.33±0.30 ^{c}
Rhizome	UAE	13.91±0.13 ^{e}	10.00±0.17 ^{d}	1.72±0.14 ^{b}	n.d.	0.46±0.06 ^{d}
	SE	9.14±0.66 ^{f}	5.67±0.31 ^{e}	1.78±0.007 ^{b}	n.d	0.46±0.05 ^{d}

*$ABTS^{\cdot+}$- 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation; $DPPH^{\cdot}$- 2,2-diphenylpicrylhydrazyl cation; TAC - total antioxidant capacity determined via *in vitro* phosphomolybdenum assay; CUPRAC- Cupric Reducing Antioxidant Capacity; FRP - Ferric Reducing Power; AAE- ascorbic acid equivalents.

** Different superscript letters $^{(a-e)}$ in a same column indicate significant differences at $p< 0.05$.

CONCLUSION

Phytochemical analysis of different parts of *A. turbith* revealed distinctions in phenolic composition with inflorescence as the best source of bioactive compounds. There was no clear influence of ultrasound assisted extraction on the content of total phenolics, flavonoids and dyhydroxicinnamic acid derivatives. All examined extracts exhibited singificant antioxidant activity examined through five different assays. Correlation analysis confirmed strong connection between phenolics (in particular flavonoids and dyhydroxicinnamic acid derivatives) and several antioxidant assays such as CUPRAC and $DPPH^{\cdot}$ assays.

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