Chemia Naissensis, Vol 3, Issue 2, RESEARCH ARTICLE, 89-95

Comparative analysis of HPLC profiles and antioxidant activity of *Artemisia alba* Turra from two habitats in Serbia

Jovana D. Ickovski[1*], Milan N. Mitić[1], Milan B. Stojković[1], Gordana S. Stojanović[1]

1-Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia

Jovana D. Ickovski: jovana.ickovski@pmf.edu.rs
Milan N. Mitić: milan.mitic1@pmf.edu.rs
Milan B. Stojković: milan.stojkovic@pmf.edu.rs
Gordana S. Stojanović: gocast@pmf.ni.ac.rs

ABSTRACT

Artemisia alba Turra was collected at the blooming stage on two different locations in Serbia, Mojinci and Rosomač. Antioxidant activity and HPLC profiles of their methanolic extracts were determined and compared. Flavonoid compounds found in both samples were rutin, apigenin glucoside, quercetin, luteolin, kaempferol and apigenin, while detected phenolic acids were chlorogenic acid, *p*-coumaric acid, cynarine and rosmarinic acid. The only observed qualitative difference was related to the presence of vanillic acid. Extract of *A. alba* from Rosomač was richer in phenolic compounds and flavonoids than *A. alba* from Mojinci. The extract of *A. alba* from Rosomač also possessed higher antioxidant capacity than *A. alba* from Mojinci, which was especially noticed in the 2,2-diphenyl-1-picrylhydrazyl assay. Only the results for the ferric ion reducing antioxidant power assay were higher for extract of *A. alba* from Mojinci, than for the extract of *A. alba* from Rosomač.

Keywords: Artemisia alba, methanolic extract, HPLC, antioxidant activity

Introduction

The genus *Artemisia* L. is one of the largest and most widely distributed over all continents and includes more than 500 species (Bora and Sharma, 2011; Oberprieler et al., 2007). The majority of the *Artemisia* species have been used in folk medicine for the treatment of various diseases such as malaria (Willcox 2009), fever, helminthiasis, hepatitis, cancer, cardiac and digestive problems and neurodegenerative disorders (Bora and Sharma, 2011; Guarrera, 2005). Also, some of them showed antibacterial and antifungal activities (Vajs et al., 2004). *Artemisia alba* Turra (synonyms: *A. lobelii* All., *A. camphorata* Vill.) is an aromatic herb of the Asteraceae family, typical for the southern and south-eastern parts of Europe (Trendafilova et al., 2018). This plant was used in traditional medicine as a digestive, tonic, mineralising agent and decoction of leaves was used to heal burns and contusions (Peron et al., 2017). It was found that the essential oil of *A. alba* has antimicrobial activity (Stojanović et al., 2000). Phenolic compounds (phenolic acids and flavonoids) have antioxidant activity and possess the ability to reduce the risk of cancer and cardiovascular diseases (Fraisse et al., 2011; Zhishen et al., 1999).

There are significant differences in terpenoid profiles of *A. alba* essential oils depending on genetic factors, ecological factors (temperature, light, moisture) and type of soil (Radulović and Blagojević, 2010). Although the composition of methanolic extracts and antioxidant activities were the subject of numerous papers (Nikolova et al., 2014; Radović Jakovljević et al., 2020; Trendafilova et al., 2018), we considered that the enrichment of data is important to assess the impact of geographic factor on chemical composition and antioxidant activity. Therefore, this paper is focused on the comparison of antioxidant capacity and phenolic composition of *A. alba* methanolic extracts obtained from species originated from two different localities.

Experimental

Chemicals and reagents

Folin-Ciocalteu's phenol reagent, ABTS, 2,2-diphenyl-1-picrylhydrazyl, neocuproine were purchased from Sigma Co. (St. Louis, Missouri, USA). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid was purchased from Acros Organics (Morris Plains, New Jersey, USA). TPTZ (2,4,6-tripyridyl-*s*-triazine) was purchased from Merck (Darmstadt, Germany). All chemicals and reagents were of analytical purity.

Plant material and plant material extraction

The plant material (*Artemisia alba* Turra) was collected at blooming stage on August 2016 on two locations Mojinci (A1) and Rosomač (A2), Serbia; a voucher specimen 14323 and 14324, respectively, has been deposited in the Herbarium Moesiacum Niš (HMN), Department of Biology and Ecology, Faculty of Science and Mathematics, University of Niš, Serbia. The coordinates of the sites from which the plants were gathered are available in Table 1.

Table 1. Details of the localities from which *A. alba* was harvested

Plant material	Locality name	Longitude	Latitude	WRB soil type
A1	Put Vlkovija, Mojinci	22.90833333	43.08344444	Rendzic Leptosol
A2	Rosomač	22.84122222	43.1535	Rendzic Leptosol

* WRB soil type - World reference base for soil resources 2014

The aerial parts of the plants were used for the experiment. Fresh plant material was dried at room temperature, milled, packed in paper bags, and kept in dry and dark place. Dried plant material (10 g) was macerated with methanol (100 ml) and then was kept for 72 h in the dark, at room temperature, with occasional shaking. The resulting extracts were filtered and evaporated to dryness. The yield of A1 was 5.9%, while the yield of A2 was 9.6%. Dry extracts were dissolved in methanol (20 mg in 1 ml) and the obtained solutions were used for testing the antioxidant activity and HPLC analysis.

HPLC-DAD analysis

The concentration of individual phenolic compounds was determined by HPLC using a direct-injection method developed by Mitić (Mitić et al., 2013). Briefly, Agilent 1200 chromatographic system equipped with a quaternary pump, Agilent 1200 photodiode array detector, automatic injector, Agilent-Eclipse XDBC-18 4.6 × 150 mm column and ChemStation software was used. The column was thermostated at 30° C. Five μL of extract solution in methanol was injected. Two solvents were used for the gradient elution: A (5% formic acid in water) and B (80% acetonitrile + 5% formic acid in water). The elution program used was as follows: from 0 to 10 min 0% B, from 10 to 28 min gradually increases 0-25% B, from 28 to 30 min 25% B, from 30 to 35 min gradually increases 25-50% B, from 35 to 40 min gradually increases 50-80% B, and finally for the last 5 min gradually decreases 80-0% B. The detection wavelengths were 254, 280, 320, and 520 nm. Identification was performed by comparing retention indices and UV / VIS spectra of components with retention indices and UV / VIS spectra of standards. The contents of components in the extracts were quantified using standard curves and expressed as mg per g of dry extract (mg/g).

UV-VIS spectrophotometry

All spectrophotometric analyses of antioxidative potential of *A. alba* Turra extracts were performed on a UV-visible spectrophotometer Perkin Elmer lambda 15 (Massachusetts, USA). Experiments for all applied assays were performed in three independent repetitions and the mean values are presented.

Determination of total phenolic and total flavonoid content

The total phenolic content (**TPC**) of the extract was determined using Folin-Ciocalteu reagent according to the method of Singleton et al. (1999). The total phenolic content was expressed as gallic acid equivalent per milligram of dry extract weight (μg GAE/mg dw). Total flavonoid content (**TFC**) was assayed using the method described by Zhishen et al. (1999). Total flavonoid content was presented as quercetin equivalent (QE) per milligram of dry extract weight (μg QE/mg dw).

Determination of antioxidant activities

Free radical scavenging activity **(DPPH)** was determined using the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical according to the method of Hatano et al. (1988). The results were expressed as micrograms of Trolox equivalents per milligram of dry extract weight (μg TE/mg dw). The method used for determination of the antioxidant free radical capacity was the $ABTS^{\bullet+}$ (radical cation) decolorization assay **(ABTS),** described by Re et al. (1999). The radical scavenging capacity of extracts was expressed as micrograms of Trolox equivalents per milligram of dry extract weight (μg TE/mg dw). The ferric reducing antioxidant power (**FRAP**) assay was based on the methodology of Benzie and Strain (1996). FRAP values were presented as micromoles Fe^{2+} per mg of dry extract weight (μmol Fe/mg dw). Total reducing power test (**TRP**) represents the capacity of extract to reduce iron (III) to iron (II) and was assayed using the method described by Oyaizu (1986). The results were expressed as mg of ascorbic acid per mg of dry extract weight (mg AAE/mg dw). Cupric reducing antioxidant capacity analysis **(CUPRAC)** was used for the determination of antioxidants and hydroxyl radical scavengers, according to the method of Apak et al. (2004). The results of CUPRAC method were presented as Trolox equivalent per milligram of dry extract weight (mg TE/mg dw).

Results and Discussion

The yield of extract from Rosomač was higher (9.6%) than the yield of extract from Mojinci (5.9%), while in the cited papers yields were omitted. The number of identified components was eleven for A1 and ten for A2, as shown in Table 2.

Table 2. The components of methanolic extracts of *A. alba* identified by HPLC-DAD and their masses given in mg/g of dry extract weight

Component	A1, Mojinci	A2, Rosomač	A2/A1
Vanilllic acid	1.29	/	
Chlorogenic acid	2.66	18.6	**6.99**
p-Coumaric acid	0.51	4.28	**8.39**
Cynarine	2.08	16.9	**8.13**
Rutin	0.85	14.7	17.3
Apigenin glucoside	5.36	3.24	0.60
Rosmarinic acid	3.29	22.0	**6.69**
Quercetin	1.91	5.11	2.68
Luteolin	0.75	0.77	1.03
Kaempferol	0.82	0.66	0.80
Apigenin	0.60	1.17	1.95

Apigenin glucoside and rosmarinic acid were the most abundant phenolic constituents in A1 extract (5.36 and 3.29 mg/g of dry extract, respectively), while in A2 extract dominant compounds were rosmarinic acid and chlorogenic acid (22.0 and 18.6 mg/g of dry extract, respectively). Flavonoid compounds found in both samples were rutin, apigenin glucoside, quercetin, luteolin, kaempferol and apigenin, while detected phenolic acids were chlorogenic acid, *p*-coumaric acid, cynarine and rosmarinic acid. The only observed qualitative difference was related to the presence of vanillic acid. Vanillic acid was present only in A1 extract. The total amounts of flavonoids in A1 and A2 were 10.3 and 25.7 mg/g of dry extract, respectively. The contents of phenolic acids in those samples (A1 and A2) were 9.83 and 61.8 mg/g of dry extract,

respectively. Some regularity in the ratios of phenolic acids in these two samples A2/A1 can be noticed. The ratio between A2 and A1 for rosmarinic acid, chlorogenic acid, cynarine and *p*-coumaric acid was from 6.69 to 8.39. As we can see, A2 extract was richer in both flavonoid and phenolic acid constituents.

The phenolic profile of *A. alba* extract was previously published, and the significant similarity was noticed with our work. Research in North-East of Italy has shown that the phenolic compounds of the ethanolic extract of the plant from this territory were chlorogenic acid, rutin, kaempferol, that were also present in our samples, as well as quercetin 3-*O*-glucoside, isorhamnetin 3-*O*-rutinoside, kaempferol 3-*O*-glucopyranoside, isorhamnetin 3-*O*-glucopyranoside, quercetin dihexoside, isorhamnetin dihexoside, and isorhamnetin (Peron et al., 2017). Phenolic constituents of *A. alba* methanolic extract from Bulgaria, which were present in our samples, were chlorogenic acid, rutin, apigenin glucoside, luteolin and apigenin (Trendafilova et al., 2018). Chlorogenic acid, rutin, luteolin and apigenin were also found in the ethanolic extract of *A. alba* from Serbia (Đorđević et al., 2013).

Table 3. Total phenolic (TPC) and total flavonoid (TFC) contents and antioxidant activity (DPPH, ABTS, TRP, FRAP and CUPRAC assays) of *A. alba* methanolic extracts

Extract	TPC (μg GAE/mg)	TFC (μg QE/mg)	DPPH (μg TE/mg)	ABTS (μg TE/mg)	TRP (mg AAE/mg)	FRAP (μmol Fe/mg)	CUPRAC (mg TE/mg)
A1	117	35.5	29.1	83.1	38.1	39.2	152
A2	185	68.6	107	90.6	61.1	27.5	302

Two methanolic extracts of *A. alba* originated from different localities (A1 and A2) were analyzed for their total phenolic and flavonoid contents (Table 3). A2 was richer in phenolic compounds and flavonoids (185 μg GAE/mg; 68.6 mg μg QE/mg) than A1 (117 μg GAE/mg; 35.5 mg μg QE/mg). DPPH and ABTS tests were used for the estimation of antioxidant capacity of the extracts. As shown in Table 3, A2 extract possessed higher radical scavenging activity (107 and 90.6 μM TE/mg, respectively) than the A1 extract (29.1 and 83.1 μM TE/mg, respectively), especially in DPPH assay. Only the results from FRAP test are higher for A1 (39.2 μmol Fe/mg) than for A2 extract (27.5 μmol Fe/mg).

Conclusion

The presented results showed minor differences in the qualitative composition of the methanolic extracts (the only difference was related to the presence of vanillic acid), but significant differences in the quantitative composition, which caused different antioxidant activity. These results indicate that the impact of the site on the chemical composition of the methanolic extract is small if the same soil type is present on both sites.

Acknowledgment

The research was supported by Ministry of Education, Science and Technological Development of the Republic of Serbia (Project No. 451-03-68/2020-14/200124) and the Serbian Academy of Sciences and Art (SASA), Branch of SASA in Nis (Project No. 0-13-18).

References

Apak, R., Güçlü, K., Özyürek, M., & Karademir, S. E. (2004). Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. Journal of Agricultural and Food Chemistry, 52, 7970-7981.

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant Power": The FRAP assay. Analytical Biochemistry, 239, 70-76.

Bora, K. S., & Sharma, A. (2011). The genus *Artemisia*: A comprehensive review. Pharmaceutical Biology, 49, 101-109.

Đorđević, S., Stanisavljević, D., Ristić, M., Milenković, M., Veličković, D., Stojičević, S., & Zlatković, B. (2013). Chemical, antioxidant and antimicrobial analysis of the essential oil and extract of *Artemisia alba* Turra. Digest Journal of Nanomaterials & Biostructures, 8, 1377-1388.

Fraisse, D., Felgines, C., Texier, O., & Lamaison, J. L. (2011). Caffeoyl derivatives: major antioxidant compounds of some wild herbs of the Asteraceae family. Food and Nutrition Sciences, 2, 181-192.

Guarrera, P. M. (2005). Traditional phytotherapy in Central Italy (Marche, Abruzzo, and Latium). Fitoterapia, 76, 1-25.

Hatano, T., Kagawa, H., Yasuhara, T., & Okuda, T. (1988). Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. Chemical and Pharmaceutical Bulletin, 36, 2090-2097.

Mitić, M. N., Obradović, M. V., Mitić, S. S., Pavlović, A. N., Pavlović, J. LJ., & Stojanović, B. T. (2013). Free radical scavenging activity and phenolic profile of selected Serbian red fruit wines. Revista de Chimie, 64, 68-73.

Nikolova, M., Gussev, Ch., & Nguyen, T. (2010). Evaluation of the antioxidant action and flavonoid composition of *Artemisia* species extracts. Biotechnology & Biotechnological Equipment, 24, 101-103.

Oberprieler, C., Himmelreich, S., & Vogt, R. (2007). A new subtribal classification of the tribe Anthemideae (Compositae). Willdenowia, 37, 89-114.

Oyaizu, M. (1986). Studies on products of browning reaction. Antioxidative activities of products of browning reaction prepared from glucosamine. Japanese Journal of Nutrition, 44, 307-314.

Peron, G., Baldan, V., Sut, S., Faggian, M., Roccabruna, L., Zanini, D., Manzini, P., Maggi, F., & Dall'Acqua, S. (2017). Phytochemical investigations on *Artemisia alba* Turra growing in the North-East of Italy. Natural Product Research, 31, 1861-1868.

Radović Jakovljević, M., Grujičić, D., Tubić Vukajlović, J., Marković, A., Milutinović, M., Stanković, M., Vuković, N., Vukić, M., Milošević-Đorđević, O. (2020). *In vitro* study of genotoxic and cytotoxic activities

of methanol extracts of *Artemisia vulgaris* L. and *Artemisia alba* Turra. South African Journal of Botany, 132, 117-126.

Radulović, N., & Blagojević, P. (2010). Volatile profiles of *Artemisia alba* Turra from contrasting serpentine and calcareous habitats. Natural Product Communications, 5, 1117-1122.

Re, R., Pellegrini, N., Proreggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine, 26, 1231-1237.

Singleton,V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymology, 299, 152-178.

Stojanović, G., Palić, R., & Mitrović, J. (2000). Chemical composition and antimicrobial activity of the essential oil of *Artemisia lobelii* All. Journal of Essential Oil Research, 12, 621-624.

Trendafilova, A., Todorova, M., Genova, V., Peter, S., Wolfram, E., Danova, K., & Evstatieva, L. (2018). Phenolic profile of *Artemisia alba* Turra. Chemistry & Biodiversity, 15, 1872-1880.

Vajs, V., Trifunović, S., Janaćković, P., Soković, M., Milosavljević, S., & Tešević, V. (2004). Antifungal activity of davanone-type sesquiterpenes from *Artemisia lobelii* var. *conescens*. Journal of the Serbian Chemical Society, 69, 969-972.

Willcox, M. (2009). *Artemisia* species: from traditional medicines to modern antimalarial and back again. The Journal of Alternative and Complementary Medicine, 15, 101-109.

Zhishen, J., Mengecheng, T., & Jianming, W. (1999). The determination of flavonoid contents on mulberry and their scavenging effects on superoxide radical. Food Chemistry, 64, 555-559.