UDK 633.88:66.061
Original scientific paper

COMPARISON OF MACERATION AND ULTRASOUND-ASSISTED EXTRACTION OF ANTIOXIDANT COMPOUNDS FROM *VACCINIUM MYRTILLUS* L.

Petar Batinić[1], Aleksandra A. Jovanović[2], Muna Rajab Elferjane[3], Natalija Čutović[1], Milena Milošević[4], Aleksandar Marinković[5], Branko Bugarski[5]

[1]*Institute for Medicinal Plants Research "Dr Josif Pančić", Tadeuša Košćuška 1, 11000 Belgrade, Serbia*
[2]*Univeristy of Belgrade, Institute for the Application of the Nuclear Energy INEP, Banatska 31b, 11080 Zemun, Belgrade, Serbia*
[3]*University of Misurata, Faculty of Nursing and Health Sciences, Misurata, Libya*
[4]*University of Belgrade, Institute of Chemistry, Technology and Metallurgy, Njegoševa 12, 11000 Belgrade, Serbia*
[5]*University of Belgrade, Faculty of Technology and Metallurgy, Karnegijeva 4, 11000 Belgrade, Serbia*

Abstract

Vaccinium myrtillus L. (Ericaceae), a perennial, wild, and small deciduous shrub that grows in the mountains and forests of Europe, contains anthocyanins, phenolic acids, flavonoids, fatty acids, stilbenes, iridoid glycosides, dietary fibers, vitamins, and minerals. The leaves' extracts are widely used in traditional medicine due to their astringent, antiseptic, antioxidant, anti-inflammatory, skin-rejuvenating, lipid-lowering, hypolipidemic, and hypoglycemic activities. The novel extraction techniques, including ultrasound-assisted extraction, provide various benefits, such as reducing solvent consumption and extraction time and increasing extraction yield, and quality. Hence, in the present study, V. myrtillus extracts were prepared using dried leaves (0.66 g), 50% ethanol as the extraction solvent (20 mL), and maceration (60 min) or ultrasound-assisted extraction (ultrasound probe, amplitude of 60% for 5 min). The obtained extracts were examined in terms of total polyphenol content (TPC) and antioxidant activity (ABTS, DPPH, FRAP, and CUPRAC assays). The TPC of the extract prepared using maceration was 55.2±0.7 mg gallic acid equivalents (GAE)/g of plant material, while the TPC of the extract prepared using an ultrasound probe was 55.6±1.0 mg GAE/g. The DPPH radical scavenging activities of the extracts correlated with the TPC and amounted to 1.81±0.05 mg/mL for macerate and 1.79±0.02 mg/mL for the extract obtained using an ultrasound probe, whereas ABTS antioxidant capacity did not correlate with the polyphenol concentration, 31.4±0.9 µmol Trolox equivalents (TE)/g for the macerate and 43.4±1.1 µmol TE/g for the extract from the ultrasound-assisted extraction. According to the results of FRAP and CUPRAC assays, the antioxidant potential was similar for both extracts (15.3±0.2 and 15.5±0.2 µmol Fe^{2+}/g and 45.7±0.5 and 46.0±0.7 µmol TE/g, respectively). Due to higher ABTS radical scavenging potential and significantly shorter extraction time, V. myrtillus extract prepared using an ultrasound probe was favored. The present research was an initial step in the preparation of V. myrtillus extracts which can be potentially implemented in food, pharmaceutical, and cosmetic formulations.

Keywords: *Antioxidant activity, maceration, ultrasound-assisted extraction, polyphenols, Vaccinium myrtillus.*

Introduction

Vaccinium myrtillus L. (Ericaceae), is a perennial, wild, and small deciduous shrub arising from a creeping rhizome that grows in the mountains and forests of Europe and North Asia. The parts used are fruits and leaves (Khan and Abourashed, 2011). The plant contains anthocyanins, phenolic acids, flavonoids, fatty acids, stilbenes, iridoid glycosides, dietary fibers, vitamins, and minerals (Jensen et al., 2002; Khan and Abourashed, 2011). The leaf extracts are widely used in traditional medicine due to their astringent, antiseptic, antioxidant, anti-inflammatory, skin-rejuvenating, lipid-lowering, hypolipidemic, and hypoglycemic activities. *Per os* applied to rats with diabetes mellitus type 1, *V. myrtillus* leaf extracts lowered plasma, glucose, cholesterol, and triglyceride levels (Khan and Abourashed, 2011).

Antioxidant compounds can postpone, limit, or completely halt oxidation by scavenging free radicals and lowering oxidative stress. Antioxidants (natural or synthetic) are necessary for these circumstances to counteract the harmful effects of oxidative stress. Polyphenols (natural antioxidants from plant sources) can stop a free radical chain reaction and cause the suppression of free radical formation by controlling enzyme activity or chelating metal ions (Zheng & Wang, 2001).

Biologically active components can be effectively extracted from various plant matrixes using traditional and novel extraction procedures, such as maceration and ultrasound-assisted extraction, respectively. The mentioned traditional method (maceration) is widely used due to its simple operation and low costs (Jovanović et al., 2017). On the other hand, ultrasound-assisted extraction, as a modern extraction technique, provides an increase in the extraction yield and quality of the extracts, faster kinetics, and a wide range of used extraction solvents (Čutović et al., 2022; Jovanović et al., 2017).

In the present study, ethanol *V. myrtillus* extracts were prepared using dried herb and two extraction techniques (maceration and ultrasound-assisted extraction by ultrasound probe). The obtained extracts were characterized *via* analysis of total polyphenol content (TPC) and antioxidant potential (ABTS, DPPH, FRAP, and CUPRAC assays).

Materials and Methods

Plant material and reagents

Vaccinium myrtillus (blueberry) leaves were purchased from Institute for Medicinal Plants Research "Dr Josif Pančić", Belgrade, Serbia. The following reagents were used: Folin-Ciocalteu reagent and gallic acid (Merck, Germany), ethanol (Fisher Scientific, UK), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) - ABTS, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid - Trolox, 2,2-diphenyl-1-picrylhydrazyl - DPPH, iron(III) chloride, potassium ferricyanide, methanol, ammonium acetate, and iron(II) sulfate (Sigma-Aldrich, USA), neocuproin (Acros Organics, Belgium), cuprum chloride (Fluka, Germany), and ultrapure water.

Extraction

Blueberry extracts were prepared using dried leaves (0.66 g), 50% ethanol as the extraction solvent (20 mL), and maceration (60 min in the incubator shaker at 200 rpm, KS 4000i control, IKA, Germany) or ultrasound-assisted extraction (ultrasound probe, amplitude of 60% for 5 min, Sonoplus, Bandelin, Germany). All extracts were filtered through a cellulose filter (fine pore, 0.45 μm) and stored at 4°C until further analyses.

Measurement of total polyphenol content

The TPC of ethanol blueberry extracts was determined spectrophotometrically using the

modified Folin-Ciocalteu method (Galván d'Alessandro et al., 2012). The results are expressed as milligrams of gallic acid equivalents per gram of plant material (mg GAE/g).

Measurement of antioxidant activity

ABTS assay

The ABTS assay was based on the procedure described by Re et al. (1999) with a slight modification and the absorbance was measured at 734 nm. The antioxidant activity was expressed as mmol Trolox equivalent per g of plant material (mmol TE/g).

DPPH assay

The DPPH assay was based on the procedure described by Horžić et al. (2012) with a slight modification and the absorbance was measured at 517 nm. The results were expressed as IC_{50} (mg/mL), defined as the concentration of the extract required to scavenge 50% of the initial DPPH concentration.

FRAP assay

The ferric reducing antioxidant potential of ethanol blueberry extracts was examined as well (Guo et al., 2003). The absorbance of was measured at 593 nm and the results were expressed as μmol Fe^{2+} equivalents per g of plant material (μmol Fe^{2+}/g).

CUPRAC assay

The cupric ion-reducing antioxidant activity of ethanol blueberry extracts was also measured (Petrović et al., 2019). The absorbance was read at 450 nm and the results were expressed as μmol Trolox equivalents per g of plant material (μmol TE/g).

All spectrophotometric measurements were performed in a UV-1800 spectrophotometer (Shimadzu, Japan).

Statistical analysis

The statistical analysis was done by using analysis of variance (one-way ANOVA) and Duncan's *post hoc* test in STATISTICA 7.0. The differences were considered statistically significant at $p<0.05$.

Results and Discussion

The influence of two different extraction procedures (maceration and ultrasound-assisted extraction) on TPC and antioxidant capacity (ABTS and DPPH radical scavenging activity, ferric-reducing antioxidant potential, and cupric ion-reducing antioxidant capacity) of ethanol blueberry extracts was investigated. The results are shown in Figure 1 (for TPC) and Figure 2 (for antioxidant capacity).

As can be seen in Figure 1, there was no statistically significant difference between the TPC of the extracts prepared using different extraction procedures. Namely, the TPC amounted to 55.2±0.7 mg GAE/g in maceration and 55.6±1.0 mg GAE/g in ultrasound-assisted extraction. Nevertheless, in the case of the ultrasound probe, the duration of extraction was 5 min in comparison to 60 min of maceration, thus ultrasound-assisted extraction was favored due to a view of industrial requirements (a higher extraction yield for shorter extraction time). The mechanical and thermal effects presented in ultrasound-assisted extraction cause the degradation

of cell walls, the release of cell contents, higher penetration of extraction medium into the herbal matrix, the increase in mass transfer, and therefore the enhancement of polyphenols yield (Deng et al., 2015; Horžić et al., 2012).

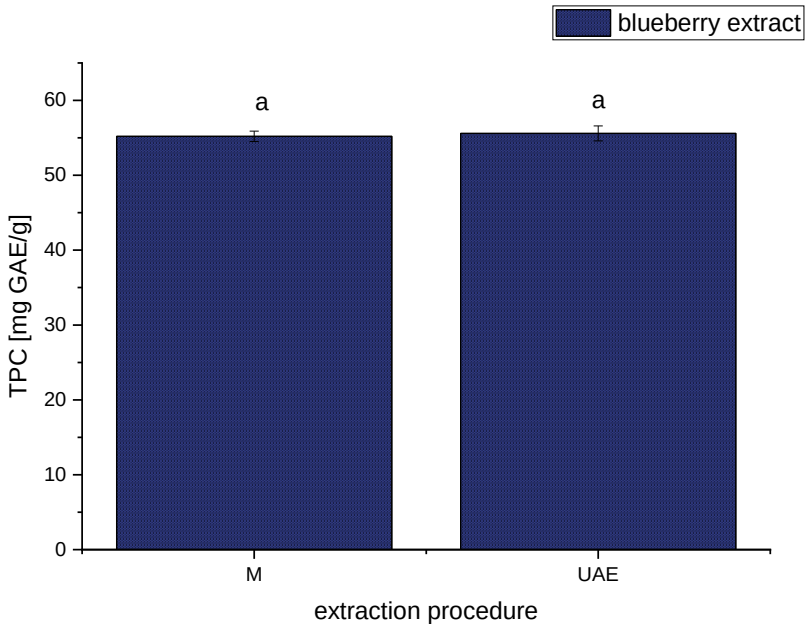
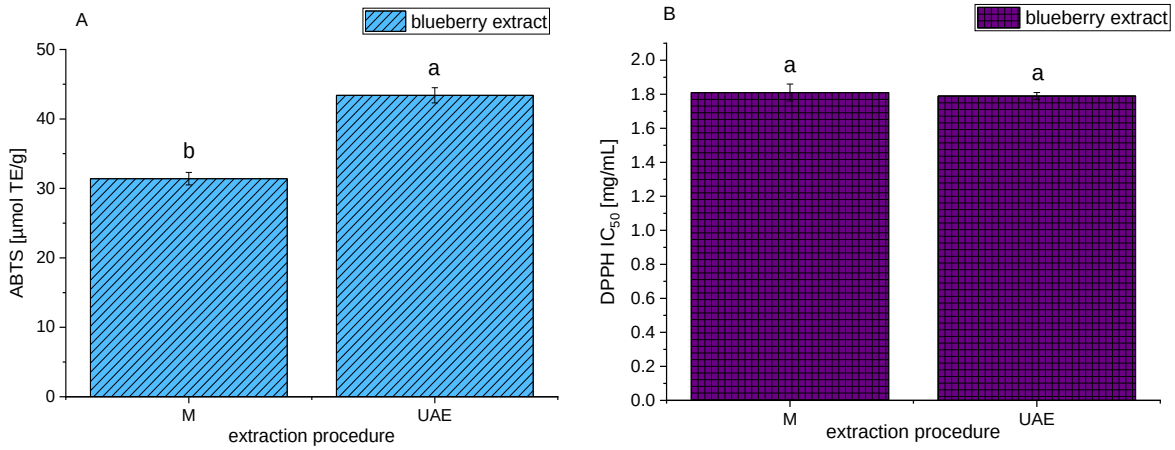


Figure 1. Total polyphenol content (TPC) of ethanol blueberry *extracts prepared using maceration and ultrasound-assisted extraction, UAE; gallic acid equivalent, GAE; the letters above bars showed statistically significant differences (p<0.05; n=3; analysis of variance, Duncan's post-hoc test).*

On the other hand, ABTS radical scavenging potential was significantly different between the extracts prepared using maceration and ultrasound-assisted extraction (31.4±0.9 μmol TE/g and 43.4±1.1 μmol TE/g, respectively, Figure 2A). ABTS antioxidant activity of ethanol blueberry extracts did not follow the trend of polyphenol concentration and the extract obtained in ultrasound-assisted extraction possessed a higher activity. According to the literature data, non-phenolic components, including ascorbic acid, β-carotene, uric acid, triterpenoid saponins, and thiols, as well as synergism of active molecules have an important role in the antioxidant capacity of the extracts (Bi et al., 2012; Foti and Amorati, 2009).



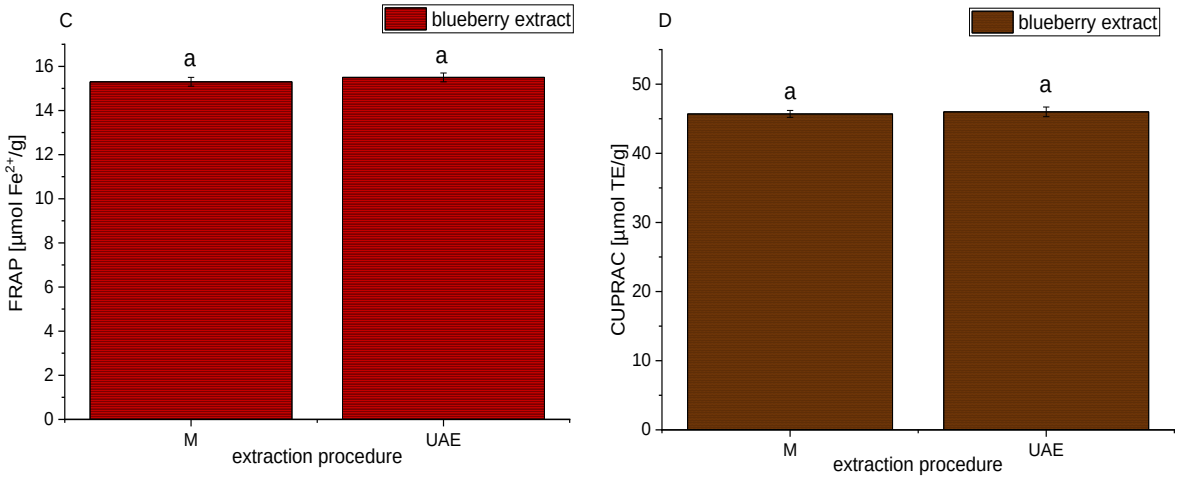


Figure 2. ABTS (A) and DPPH (B) radical scavenging activity, ferric reducing antioxidant potential (FRAP, C), and cupric ion-reducing antioxidant capacity (CUPRAC, D) of ethanol blueberry extracts prepared using maceration and ultrasound-assisted extraction, UAE; Trolox equivalent, TE, the concentration of the extract required to scavenge 50% of DPPH free radicals, IC_{50}; the letters above bars showed statistically significant differences ($p<0.05$; n=3; analysis of variance, Duncan's post-hoc test).

DPPH radical scavenging activity of ethanol *V. myrtillus* extracts was the same after maceration and ultrasound-assisted extraction (1.81±0.05 and 1.79±0.02 mg/mL, respectively, Figure 2B), as in the case of TPC (Figure 1). According to the literature data, the DPPH scavenging activity of extracts was correlated with the concentration of flavonoids, as a large group of polyphenols (Hirano et al., 2001).

Also, no significant differences in ferric and cupric-ion reducing antioxidant potential were observed between the extracts prepared using different extraction procedures (15.3±0.2 and 15.5±0.2 µmol Fe^{2+}/g and 45.7±0.5 and 46.0±0.7 µmol TE/g, respectively, Figures 2C and 2D). Apak et al. (2006) reported that there was a correlation between the TPC determined in the Folin-Ciocalteu method and the antioxidant activity shown in the CUPRAC test. Additionally, the polyphenols that showed the highest cupric-ion-reducing antioxidant potential are catechin, epicatechin, epicatechin gallate, epigallocatechin, epigallocatechin gallate, quercetin, rutin, caffeic, gallic, and chlorogenic acids (Apak et al., 2007). Furthermore, apart from polyphenol compounds, thiols, D-ascorbic acid, mannitol, and glucose can reduce the cupric ions (Özyürek et al., 2011).

Conclusions

The aim of the study was the investigation of the influence of two extraction procedures (traditional, i.e. maceration, and novel, i.e. ultrasound-assisted extraction) on polyphenol yield and antioxidant potential of ethanol blueberry extracts. There was no statistically significant difference between the TPC of the extracts prepared using different extraction procedures but ultrasound-assisted extraction was favored due to a view of industrial requirements (a higher extraction yield for shorter extraction time). ABTS antioxidant activity did not follow the trend of polyphenol concentration and the extract obtained in ultrasound-assisted extraction possessed a higher activity. DPPH radical scavenging and ferric and cupric ion-reducing antioxidant potential were the same after maceration and ultrasound-assisted extraction. Due to a higher ABTS antioxidant capacity and significantly shorter extraction time, blueberry extract prepared using an ultrasound probe was favored. The present research was an initial step in the preparation of blueberry extracts which can be potentially implemented in food, pharmaceutical, and cosmetic products.

Acknowledgments

The authors acknowledge their gratitude to the Ministry of Education, Science and Technological Development of Serbia, contract numbers 451-03-47/2023-01/200003, 451-03-47/2023-01/200135, 451-03-47/2023-01/200026, and 451-03-47/2023-01/200019.

Conflict of interest

The authors declare that they have no financial and commercial conflicts of interest.

References

Apak R., Güçlü K., Özyürek M., Esin Karademir S. & Erçağ E. (2006) The cupric ion reducing antioxidant capacity and polyphenolic content of some herbal teas. *International Journal of Food Sciences and Nutrition, 57*, 292-304. https://doi.org/10.1080/09637480600798132

Bi L., Tian X., Dou F., Hong L., Tang H. & Wang S. (2012) New antioxidant and antiglycation active triterpenoid saponins from the root bark of *Aralia taibaiensis*. *Fitoterapia, 83*(1), 234-240.

Deng Y., Zhao Y., Padilla-Zakour O. & Yang G. (2015) Polyphenols, antioxidant and antimicrobial activities of leaf and bark extracts of *Solidago canadensis* L. *Industrial Crops and Products, 74*, 803-809.

Čutović N., Batinić P., Marković A., Radanović D., Marinković A., Bugarski B. & Jovanović A.A. (2022) Physicochemical properties of winter savory extracts prepared using ultrasound-assisted extraction. *Lekovite sirovine, 42*, 34-32. http://dx.doi.org/10.5937/leksir2242034C

Foti M.C. & Amorati R. (2010) Non-phenolic radical-trapping antioxidants. *Journal of Pharmacy and Pharmacology, 61*(11), 1435-1448.

Galván d'Alessandro L.G., Kriaa K., Nikov I. & Dimitrov K. (2012). Ultrasound assisted extraction of polyphenols from black chokeberry. *Separation and Purification Technology, 93*, 42-47. https://doi.org/10.1016/j.seppur.2012.03.024

Guo C., Yang J., Wei J., Li Y., Xu J. & Jiang Y. (2003) Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. *Nutrition Research, 23*(12), 1719-1726. https://doi.org/10.1016/j.nutres.2003.08.005

Hirano R., Sasamoto W., Matsumoto A., Itakura H., Igarashi O. & Kondo K. (2001) Antioxidant ability of various flavonoids against DPPH radicals and LDL oxidation. *Journal of Nutritional Science and Vitaminology, 47*(5), 357-362. http://dx.doi.org/10.3177/jnsv.47.357

Horžić D., Režek Jambrak A., Belščak-Cvitanović A., Komes D. & Lelas V. (2012) Comparison of conventional and ultrasound assisted extraction techniques of yellow tea and bioactive composition of obtained extracts. *Food and Bioprocess Technology, 5*, 2858-2870.

Jensen H.D., Krogfelt K.A., Cornett C., Hansen S.H. & Christensen S.B. (2002) Hydrophilic carboxylic acids and iridoid glycosides in the juice of American and European cranberries (*Vaccinium macrocarpon* and *V. oxycoccos*), lingonberries (*V. vitis-idaea*), and blueberries (*V. myrtillus*). *Journal of Agricultural and Food Chemistry, 50*, 6871-6874.

Jovanović A., Petrović P., Đordjević V., Zdunić G., Šavikin K. & Bugarski B. (2017) Polyphenols extraction from plant sources. *Lekovite sirovine, 37*, 45-49. https://doi.org/10.5937/leksir1737045j

Khan I.A. & Abourashed E.A. (2011) Leung's encyclopedia of common natural ingredients: used in food, drugs and cosmetics. *John Wiley & Sons*, New York, USA.

Özyürek M., Güçlü K., Tütem E., Sözgen Başkan K., Erçağ E., Esin Çelik S., Baki S., Yildiz L., Karamanc S. & Apak R. (2011) A comprehensive review of CUPRAC methodology. *Analytical Methods, 11*(2), 2439-2453. https://doi.org/10.1039/C1AY05320E

Petrović P., Ivanović K., Jovanović A., Simović M., Milutinović V., Kozarski M., Petković M.,

Cvetković A., Klaus A. & Bugarski B. (2019) The impact of puffball autolysis on selected chemical and biological properties: puffball extracts as potential ingredients of skin-care products. *Archives of Biological Sciences, 71*(4), 721-733. https://doi.org/10.2298/ABS190725055P

Re R., Pellegrini N., Proteggente 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. https://doi.org/10.1016/S0891-5849(98)00315-3

Zheng W. & Wang S. (2001) Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agricultural and Food Chemistry, 49*, 5165-5170. https://doi.org/10.1021/jf010697n.