30th International Conference Ecological Truth & Environmental Research 2023

Proceedings

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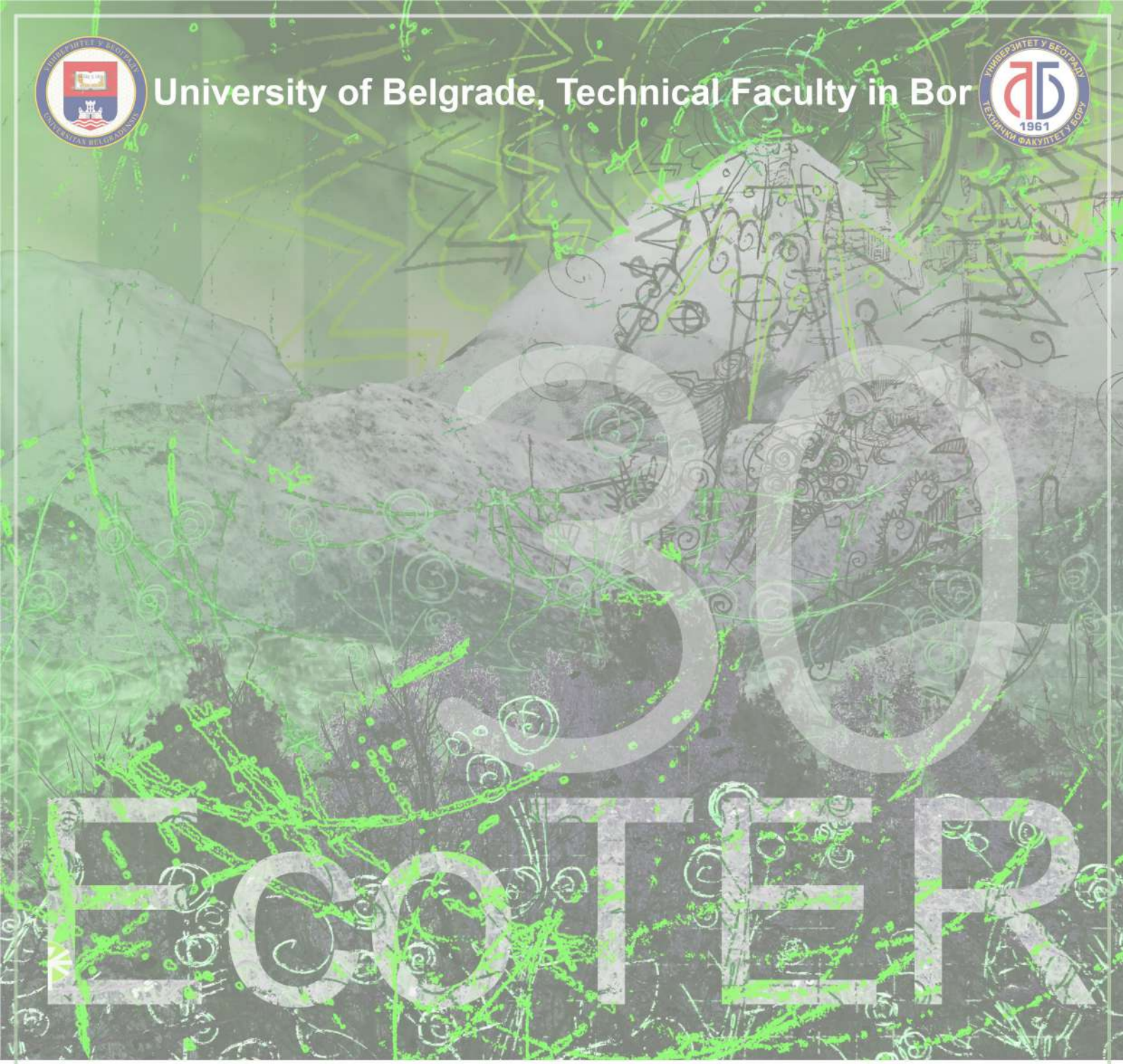

University of Belgrade, Technical Faculty in Bor

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PROCEEDINGS

30th INTERNATIONAL CONFERENCE

ECOLOGICAL TRUTH AND ENVIRONMENTAL RESEARCH – EcoTER'23

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Publisher: University of Belgrade, Technical Faculty in Bor

For the publisher: Prof. Dr Dejan Tanikić, Dean

Printed: University of Belgrade, Technical Faculty in Bor, 100 copies, electronic edition

Year of publication: 2023

ISBN 978-86-6305-137-9

CIP - Каталогизација у публикацији
Народна библиотека Србије, Београд

502/504(082)(0.034.2)
574(082)(0.034.2)

INTERNATIONAL Conference Ecological Truth & Environmental Research (30 ; 2023)
Proceedings [Elektronski izvor] / 30th International Conference Ecological Truth & Environmental Research - EcoTER'23, 20-23 June 2023, Serbia ; organized by University of Belgrade, Technical faculty in Bor (Serbia) ; co-organizers University of Banja Luka, Faculty of Technology – Banja Luka (B&H) ... [et al.] ; [editor Snežana Šerbula]. - Bor : University of Belgrade, Technical faculty, 2023 (Bor : University of Belgrade, Technical faculty). - 1 elektronski optički disk (CD-ROM) ; 12 cm

Sistemski zahtevi: Nisu navedeni. - Nasl. sa naslovne strane dokumenta. - Preface / Snežana Šerbula. - Tiraž 100. - Bibliografija uz svaki rad.

ISBN 978-86-6305-137-9

а) Животна средина -- Зборници б) Екологија – Зборници

COBISS.SR-ID 118723849

30th International Conference
Ecological Truth and Environmental Research – EcoTER'23

is organized by:

UNIVERSITY OF BELGRADE
TECHNICAL FACULTY IN BOR (SERBIA)

Co-organizers of the Conference:

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Vaccinium myrtillus LEAF WASTE EXTRACTS WITH NATURAL DEEP EUTECTIC SOLVENT

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Abstract

Vaccinium myrtillus leaf waste extracts were prepared using lactic acid+amonium acetate and maceration, heat- and ultrasound-assisted extractions (HAE and UAE, respectively). The obtained extracts were characterized via analysis of total polyphenol content (TPC), pH, zeta potential, conductivity, density, surface tension, viscosity, and antioxidant potential. The TPC was the highest in the extract prepared in HAE (53.0±0.9 mg GAE/g), whereas the extracts from maceration and UAE possessed significantly lower TPC (50.4±0.7 and 49.5±0.4 mg GAE/g). The ABTS radical scavenging potential was the highest in the extract prepared in HAE (38.4±1.1 µmol TE/g), followed by the extract obtained using UAE (33.7±1.5 µmol TE/g) and maceration (30.2±1.7 µmol TE/g). The DPPH antioxidant capacity followed the same trend: HAE>UAE>maceration. The zeta potential was low in all extracts (2.66±0.48 mV for macerate, 2.57±0.20 mV for HAE, and 3.17±0.13 mV for UAE), while the conductivity was in the range of 1.87±0.13 and 1.84±0.11 mS/cm (maceration and HAE) to 2.10±0.11 mS/cm (UAE). The density varied from 1.105±0.009 g/mL for macerate to 1.122±0.006 and 1.117±0.001 g/mL for HAE and UAE extracts. There were no statistically significant differences in the surface tension and viscosity (~23.5 mN/m and ~5.5 mPa·s). The highest TPC and antioxidant potential were measured in the extract obtained using HAE, whereas the extract prepared by ultrasound waves possessed the highest zeta potential and conductivity. Therefore, the extraction technique should be chosen depending on the future application of V. myrtillus extract.

Keywords: extract, natural deep eutectic solvent, *Vaccinium myrtillus*, waste.

INTRODUCTION

Vaccinium myrtillus L. (Ericaceae) possesses significant economic importance due to the application of its fruits and sometimes leaves in different food, functional food, pharmaceutical, cosmetic, and health-care formulations [1]. The plant contains anthocyanins, phenolic acids, fatty acids, stilbenes, iridoid glycosides, dietary fibers, vitamins, and minerals, while leaf extracts have astringent, antibacterial, antioxidant, anti-inflammatory, lipid-

lowering, hypolipidemic, and hypoglycemic activities [1–3]. Additionally, the extraction of the mentioned bioactive compounds from different plant parts can be a good alternative to the valorization of plant waste [4]. Maceration, as a traditional extraction method, is adequate for the extraction of thermosensitive compounds and does not require a complicated and expensive device. However, it has a lower extraction yield, prolonged extraction time, and a large quantity of plant material and extraction solvents causing an environmentally negative impact [5,6]. Therefore, the implementation of novel extraction techniques, such as heat- and ultrasound-assisted extractions (HAE and UAE, respectively) has been established. Their advantages include a lower solvent consumption, shorter extraction time, and a high extraction yield while supporting the concept of a "green" solvent with the aim to minimize a negative impact on the environment [5]. According to the literature, natural deep eutectic solvent (NADES) is used to enhance the recovery of polyphenol compounds from the herbal matrix and overcome the limitations of conventional and toxic organic extraction mediums [7,8]. NADES is environmentally friendly, relatively safe, less hazardous, and biodegradable due to its composition containing primary metabolites, including sugars, organic bases, plant and amino acids [7,9,10].

In the present study, *V. myrtillus* leaf waste extracts were prepared using lactic acid+amonium acetate, as NADES, and maceration, HAE, and UAE. The extracts were characterized in terms of analysis of total polyphenol content (TPC), zeta potential, conductivity, pH, density, surface tension, viscosity, and antioxidant capacity.

MATERIALS AND METHODS

Plant material and reagents

V. myrtillus leaf waste was herbal dust, the particle size of 0.3 mm resulting from the grinding of the initial plant material in the Institute for Medicinal Plants Research ”Dr Josif Pančić”, Serbia. Folin-Ciocalteu reagent and gallic acid (Merck, Germany), sodium carbonate (Fisher Science, UK), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) or ABTS, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid or Trolox, and 2,2-diphenyl-1-picrylhydrazyl or DPPH (Sigma-Aldrich, Germany), lactic acid and ammonium acetate (Fisher Bioreagents, Belgium) were used.

Preparation of natural deep eutectic solvent

The mixture of lactic acid and ammonium acetate (3:1) with water was prepared at 60°C with constant mixing for approximately 20 min until a stable transparent liquid was formed. In order to evaporate water from the mixture and to create NADES, the mixture was placed in a rotary evaporator, Heizbad Hei-VAP (Heidolph, Germany) at 60°C, pressure of 50 mbar and rotation speed of 200 rpm for 2 h. Subsequently, NADES was diluted using water (1:1).

Extraction procedures

Maceration

Maceration was performed at 25°C using the incubator shaker KS 4000i control (IKA, Germany) at a solid-to-solvent ratio of 1:30 g/mL, lactic acid+amonium acetate with 50% of water for 60 min.

Heat-assisted extraction

HAE was performed at 80°C using the incubator shaker KS 4000i control (IKA, Germany) at a solid-to-solvent ratio of 1:30 g/mL, lactic acid+amonium acetate with 50% of water for 30 min.

Ultrasound-assisted extraction

UAE was performed using an ultrasound bath, Sonopuls (Bandelin, Germany) at a solid-to-solvent ratio of 1:30 g/mL, lactic acid+amonium acetate with 50% of water for 20 min.

All extracts were prepared in the Erlenmeyer flasks covered by aluminium foil to avoid light exposure and evaporation of water. After the extraction, the samples were filtered using filter paper and stored at 4°C until further analyses.

Determination of total polyphenol content

The total polyphenol content (TPC) was determined spectrophotometrically at 765 nm using the modified Folin-Ciocalteu method [11]. The results are expressed as milligrams of gallic acid equivalents per gram of plant material (mg GAE/g).

Determination of antioxidant capacity (ABTS and DPPH tests)

The ABTS assay was based on the procedure described by Re *et al*. [12] 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). The DPPH assay was based on the procedure described by Horžić *et al*. [13] 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 DPPH free radicals.

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

Determination of pH, zeta potential, and conductivity

pH value of the extracts was determined using pH meter HI 2211 (Hanna Instruments, USA). Each sample was measured three times at room temperature.

The measurements of zeta potential and conductivity were performed using photon correlation spectroscopy in Zetasizer Nano Series, Nano ZS (Malvern Instruments Ltd., UK). Each extract was measured three times at room temperature.

Measurement of density, surface tension, and viscosity

The density and surface tension of the extracts were determined using Force Tensiometer K20 (Kruss, Germany). Each extract (20 mL) was examined three times at room temperature.

The viscosity of the extracts was examined using Rotavisc lo-vi device (IKA, Germany). Each extract (6.7 mL) was examined three times at room temperature.

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 impact of three extraction procedures (maceration, HAE, and UAE) on TPC, pH, zeta potential, conductivity, density, surface tension, viscosity, and antioxidant capacity of *V. myrtillus* leaf waste extracts prepared using NADES was examined and the results are shown in Table 1.

Table 1 *Total polyphenol content (TPC), antioxidant capacity (ABTS and DPPH tests), pH, zeta potential (ζ), conductivity (G), density (ρ), surface tension (γ), and viscosity (η) of Vaccinium myrtillus leaf waste extracts prepared using maceration, heat- and ultrasound-assisted extractions (HAE and UAE, respectively), and lactic acid+amonium acetate*

Variables	Maceration	HAE	UAE
TPC [mg GAE/g][x]	50.4±0.7[b*]	53.0±0.9[a]	49.5±0.4[b]
ABTS [mmol TE/g][§]	30.2±1.7[c]	38.4±1.1[a]	33.7±1.5[b]
DPPH IC_{50} [mg/mL][£]	6.15±0.06[c]	5.55±0.25[a]	5.99±0.05[b]
pH	1.59±0.03[a]	1.63±0.02[a]	1.66±0.04[a]
ζ [mV]	2.66±0.48[b]	2.57±0.20[b]	3.17±0.13[a]
G [mS/cm]	1.87±0.13[b]	1.84±0.11[b]	2.10±0.11[a]
ρ [g/mL]	1.105±0.009[b]	1.122±0.006[a]	1.117±0.001[a]
γ [mN/m]	23.2±0.8[a]	23.7±0.5[a]	23.5±0.7[a]
η [mPa·s]	5.52±0.10[a]	5.54±0.12[a]	5.50±0.07[a]

[x]GAE, gallic acid equivalents; [§]TE, Trolox equivalents; [£]IC_{50}, concentration required to neutralize 50% of DPPH radicals; [*]values with different letters (a-b) in each row showed statistically significant differences ($p<0.05$; n=3; analysis of variance, Duncan's *post-hoc* test).

As can be seen from Table 1, the TPC was the highest in the extract prepared in HAE (53.0±0.9 mg GAE/g), while the extracts obtained in maceration and UAE possessed significantly lower TPC (50.4±0.7 and 49.5±0.4 mg GAE/g). Therefore, the extraction technique had a statically significant influence on the polyphenol yield that is in agreement with the literature data [5]. Namely, the application of a higher temperature provides better extraction efficiency by disruption of cellular structures, increment of cell membrane permeability, and breakdown of polyphenols-lipoproteins interactions that result in the increase in the solubility and mass transfer of polyphenol compounds [14]. On the other hand, in UAE, there is a potential degradation of polyphenols through the production of free radicals by ultrasound waves. Additionally, the presence of a higher amount of plant particles contributes to the ultrasound waves attenuation and the restriction of their active part [14].

The ABTS radical scavenging capacity was the highest in the extract prepared in HAE (38.4±1.1 μmol TE/g), followed by the extract obtained using UAE (33.7±1.5 μmol TE/g) and maceration (30.2±1.7 μmol TE/g). The DPPH antioxidant potential followed the same trend: HAE>UAE>maceration (5.55±0.25, 5.99±0.05, and 6.15±0.06 mg/mL, respectively). The results of the antioxidant potential of the extracts are in correlation with TFC values which is in agreement with the literature data where the concentration of flavonoids (as a

large group of polyphenols) significantly influenced free radical scavenging of the extracts [15].

pH ranged from 1.59 in macerate to 1.63 and 1.66 in HAE and UAE extracts. The zeta potential was low in all *V. myrtillus* extracts (2.66±0.48 mV for macerate, 2.57±0.20 mV for HAE, and 3.17±0.13 mV for UAE), while the conductivity was in the range of 1.87±0.13 and 1.84±0.11 mS/cm (maceration and HAE) to 2.10±0.11 mS/cm (UAE). Measurement of the zeta potential of herbal extracts is important from the aspect of their further applications, including encapsulation into various carriers and coagulation or flocculation in the treatment of drinking water or wastewater. According to the literature data, plant extracts with a higher conductivity have the better antioxidant potential [16]. However, it was not the case with *V. myrtillus* extracts because ions in the extracts originated from NADES and plant matrix can impact the conductivity without improving their antioxidant potential. Thus, the performing of antioxidant assays is necessary in the case of *V. myrtillus* leaf waste extracts.

The density varied from 1.105±0.009 g/mL for macerate to 1.122±0.006 and 1.117±0.001 g/mL for HAE and UAE extracts. Florindo *et al*. study [17] reported that the density of eutectic solvent strongly depended on its composition and temperature. Therefore, it can explain minor differences between the samples because all extracts contain the same extraction medium and all measurements were performed at room temperature. As can be seen from Table 1, there were no statistically significant differences in the surface tension and viscosity of the extracts (~23.5 mN/m and ~5.5 mPa·s, respectively). Since the used NADES contained 50% of water, all extracts possessed a higher surface tension. Namely, it can be explained by a relatively high interaction of water molecules through hydrogen bonds. The viscosity of eutectic solvents was in the range from 0.05 to 50 mPa·s, whereas their composition and temperature significantly impact the viscosity of the extracts [17,18]. The obtained results of the lower viscosity of *V. myrtillus* leaf waste extracts are expected due to higher water content in NADES because the extract's viscosity is affected by the strength of the hydrogen bonding and van der Walls interactions.

CONCLUSION

The aim of the present study was the physicochemical characterization and investigation of the antioxidant capacity of *V. myrtillus* leaf waste extracts prepared using NADES, as an extraction medium, and maceration, HAE, and UAE. The extract obtained in HAE possessed the highest polyphenol yield and radical scavenging capacity. On the other hand, the extract prepared by ultrasound waves possessed the highest zeta potential (as a predictor of the potential application in water treatment) and conductivity (as a potential predictor of antioxidant capacity). Thus, the extraction technique should be chosen depending on the future application of *V. myrtillus* leaf waste extract.

ACKNOWLEDGEMENT

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

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