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Source / Izvornik: **Ružičkini dani : Međunarodni znanstveno-stručni skup 18. Ružičkini dani „Danas znanost - sutra industrija“ : zbornik radova, 2021, 125 - 131**

Conference paper / Rad u zborniku

Publication status / Verzija rada: **Published version / Objavljena verzija rada (izdavačev PDF)**

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:109:054195

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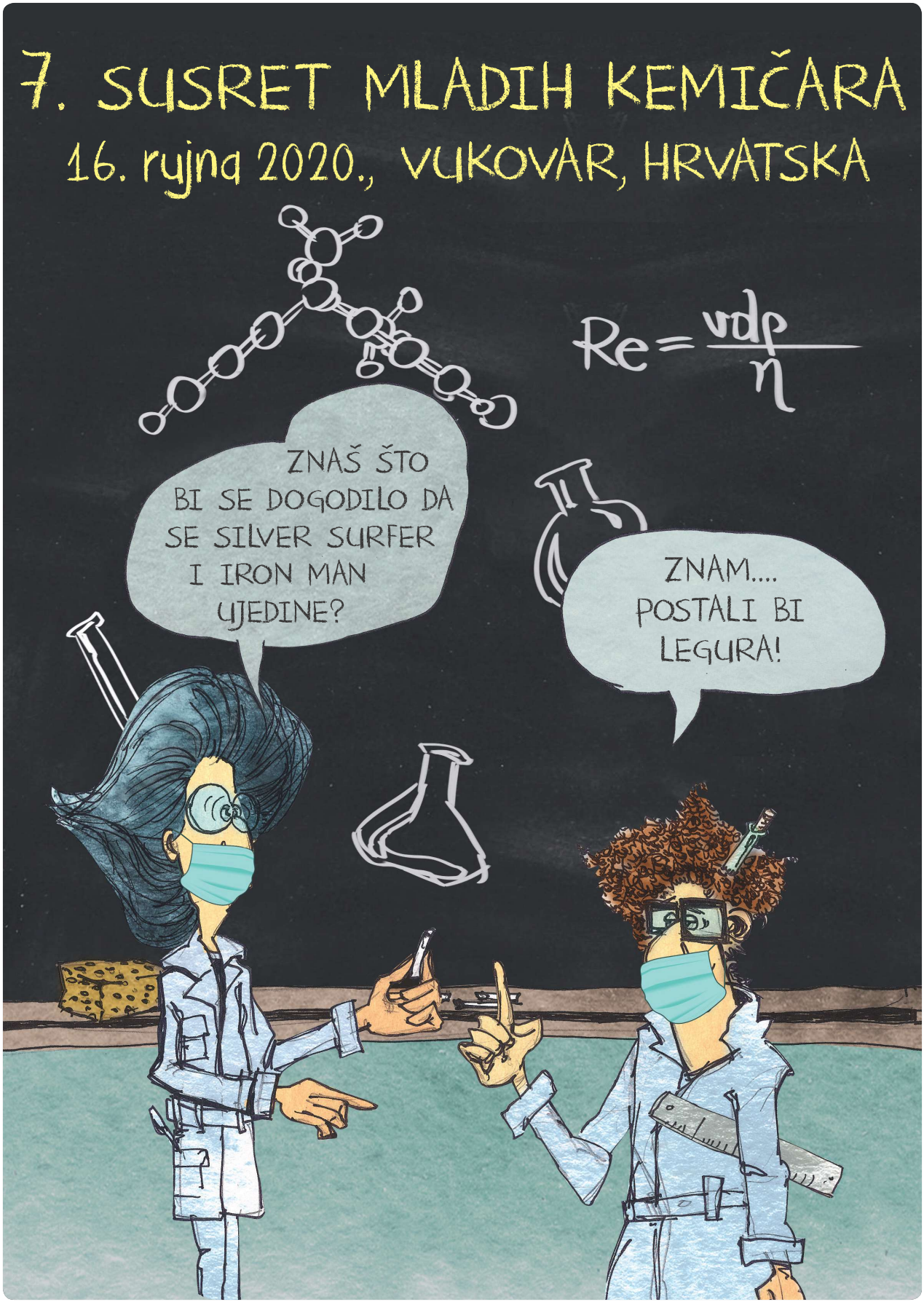
ZBORNIK RADOVA

ISSN 2671-0668 (Online)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Zagreb i Osijek, 2021.

Influence of conventional and innovative extraction techniques on bioactive properties of ground ivy (*Glechoma hederacea* L.)

Danijela Šeremet[1], Ana Mandura[1], Aleksandra Vojvodić Cebin[1], Stela Jokić[2], Draženka Komes[1*]

[1]*Faculty of Food Technology and Biotechnology, University in Zagreb, Pierottijeva 6, 10 000 Zagreb, Croatia*
[2]*Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology, Franje Kuhača 18, 31 000 Osijek, Croatia*
[*]*Corresponding author: dkomes@pbf.hr*

Summary

Ground ivy (*Glechoma hederacea* L.) is a perennial plant commonly grown in Europe, Asia and America and used for generations in folk medicine. The aim of this study was to investigate the bioactive potential of *Glechoma hederacea* L. extracts prepared by conventional (infusion, decoction and maceration) and innovative (high hydrostatic pressure extraction, ultrasound assisted extraction and subcritical water extraction) techniques of extraction. The obtained water extracts were evaluated spectrophotometrically for total phenols and hydroxycinnamic acid derivatives, as well as antioxidant capacity (DPPH and ABTS assays). Individual phenolic compounds were determined using HPLC-DAD. The extract prepared by decoction (100 °C, 20 min) resulted in the highest total phenolic content (37.39 mg GAE/g dmb), antioxidant capacity (162.53 and 177.24 mmol Trolox/g dmb) and content of hydroxycinnamic acid derivatives (15.90 mg caffeic acid/g dmb), with rosmarinic acid as predominant (2.00 mg/g dmb). Generally, conventional techniques of extraction resulted in a higher extraction efficiency than innovative ones.

Keywords: *Glechoma hederacea* L., ground ivy, innovative extraction techniques, phenolic acids

Introduction

People looked for medicines in nature since ancient times and evidence of the use of medicinal herbs dates back even 5000 years ago (Petrovska, 2012). Even today it is estimated that approximately 80 % of the global population still relies on the use of herbal medicine as primary health care (WHO, 2019). Therefore, natural products and/or structures still have a significant role in the discovery of different drugs (Newman et al., 2012).
Ground ivy (*Glechoma hederacea* L.) is a perennial herb common to temperate Asia, Europe and America where it can be found in shady places, dry ditches, waste grounds and on the sides of moist meadows. Although ground ivy has been used for generations in folk medicine for its cardiotonic, astringent, diuretic, pectoral, stimulant and tonic properties (Kumarasamy et al., 2002), its chemical and bioactive content is still not well investigated. The beneficial effect of medicinal herbs arises from their bioactive composition. Nowadays, many innovative extraction techniques, such as enzyme-, ultrasound- and microwave-assisted extraction, pulsed electric field extraction, subcritical water extraction, etc., have been implemented in

order to eliminate the degradative effect of conventional techniques, namely the high temperature, on polyphenols' stability during extraction (Azmir et al., 2013). Ultrasound-assisted extraction has been investigated for many years and has found industrial application in the form of ultrasonic reactors for the extraction and preparation of tinctures from different herbs (Vinatoru et al., 2017). Subcritical water extraction is based on improving the physical and chemical properties of water as a solvent by combining high pressures and temperatures above the boiling point to maintaining water in liquid state (Zakaria and Kamal, 2016). High hydrostatic pressure extraction utilizes high fluid pressure at room temperature, between 100 and 1000 MPa, which leads to the disruption of the sample at the cellular level resulting in the fast permeation of solvent into cells and efficient mass transfers and dissolution (Huang et al., 2013).

The aim of this study was to evaluate the impact of conventional (infusion, decoction and maceration) and innovative (subcritical water extraction, ultrasound assisted extraction and high hydrostatic pressure extraction) extraction techniques on the bioactive content of ground ivy. The obtained extracts were characterised for total phenolic and hydroxycinnamic acid derivatives contents, as well as antioxidant capacity (DPPH and ABTS assay) using spectrophotometric methods. Individual phenolic acids were determined using HPLC-DAD.

Materials and Methods

Materials

Ground ivy was bought from a local supplier. Hydrochloric acid and Folin–Ciocalteu's reagent were supplied from Kemika (Zagreb, Croatia). HPLC standards of rosmarinic acid, caffeic acid, chlorogenic acid and *p*-coumaric acid, (S)-6-Methoxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,20-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and gallic acid were purchased from Sigma-Aldrich (St. Louis, USA). Sodium hydroxide, sodium nitrite and sodium molybdate were purchased from Gram-mol d.o.o. (Zagreb, Croatia). Methanol was supplied from Panreac (Barcelona, Spain). Ethanol, acetonitrile and formic acid were bought from Carlo Erba (Val de Reuil, France). All chemicals used for experimental procedures were of analytical or HPLC grade.

Methods

Conventional extraction techniques

Infusion (INF), decoction (DEC) and maceration (MAC) were performed using distilled water as solvent and sample to solvent ratio of 1:25, w/v. The parameters for infusion, decoction and maceration were as follows: 80 °C for 30 min, 100 °C for 20 min and room temperature for 48 h, respectively. The extracts were separated by centrifugation (9500 rpm, 20 min) and filtration (Whatman® filter papers 4) and stored at +4 °C until further analyses.

Innovative extraction techniques

All innovative techniques of extraction were performed using distilled water as solvent and sample to solvent ratio of 1:25, w/v. Subcritical water extraction (SWE) was performed in specially designed equipment, as previously described by Jokić et al. (2018). SWE was performed for 5 min at temperature of 150 °C with pressure of 30 bar. Ultrasound-assisted extraction was performed in an ultrasound bath (Elmasonic 2 120, Elma, Singen, Germany) operating at nominal power of 200 W and frequency of 37 kHz. The extraction was carried out for 30 (U30) and 60 (U60) min. High hydrostatic pressure extraction (HHPE) was performed in specialized apparatus (Stansted Fluid Power, Harlow, Great Britain) by varying pressure (200 and 400 MPa) and pressure holding time (10 and 20 min). The obtained extracts from all extractions were separated by centrifugation (9500 rpm, 20 min) and filtration (Whatman® filter papers 4) and stored at +4 °C until further analyses.

Determination of total phenolic content (TPC), antioxidant capacity and hydroxycinnamic acid derivatives

TPC was determined spectrophotometrically (Genesys 10S UV-VIS Spectrophotometer, Thermo Fisher Scientific, Waltham, MA, USA), according to the method of Singleton and Rossi (1965). The calibration curve was constructed on gallic acid standard and the results were expressed as gallic acid equivalents on dry matter basis (mg GAE/g dmb). Antioxidant capacity was determined using DPPH and ABTS radical cation decolourization assays by Brand-Williams et al. (1965) and Re et al. (1999), respectively. Trolox was used for the external standard calibration curve, and the results were expressed as Trolox equivalent per dry matter basis of the sample (mg Trolox/g dmb). Content of hydroxycinnamic acid derivatives was performed following method described by Matkowski et al. (2008) and the results were expressed as mg caffeic acid/g dmb. All measurements were performed in triplicate.

HPLC determination of individual phenolic acids

The HPLC analysis was performed on an Agilent Series 1200 chromatographic system (Agilent Technologies, Santa Clara, CA, USA) using a Zorbax Extend C18 (4.6 × 250 mm, 5 µm i.d.) chromatographic column (Agilent Technologies, USA) and coupled with a Photodiode Array Detector (Agilent Technologies, Santa Clara, CA, USA). The elution was performed in a gradient with a two-component mobile phase consisting of (A) 1 % (v/v) formic acid solution in water and (B) 1 % (v/v) formic acid solution in acetonitrile, according to the following regimen: 0 min – 93 % A, 7 % B; 5 min – 93 % A, 7 % B; 45 min – 60 % A, 40 % B; 47 min – 30 % A, 70 % B; 52 min – 30 % A, 70 % B. The flow was 1 mL/min, the injection volume 5 µL and the column temperature 25 °C. The chromatograms were recorded at 320 nm. Identification of phenolic acids was conducted comparing the retention times and characteristic absorption spectrums (190–400 nm) with commercially available standards. Quantification was enabled by establishing calibration curves (20–100 µg/mL). The analysis was performed in a triplicate. All samples were filtered through a 0.45 µm membrane filter (Nylon Membranes, Supelco, Bellefonte, PA, USA) prior to the analysis.

Results and Discussion

Total phenolic content (TPC) and content of hydroxycinnamic acid derivatives

TPC and content of hydroxycinnamic acid derivatives of differently prepared ground ivy extracts are presented on Figs. 1 and 2, respectively.

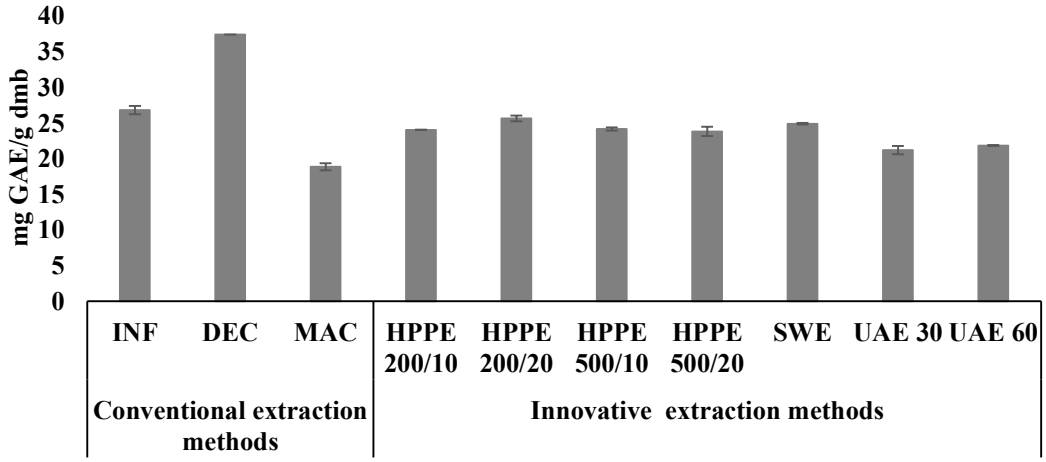

Figure 1. Total phenolic content of differently prepared extracts of ground ivy

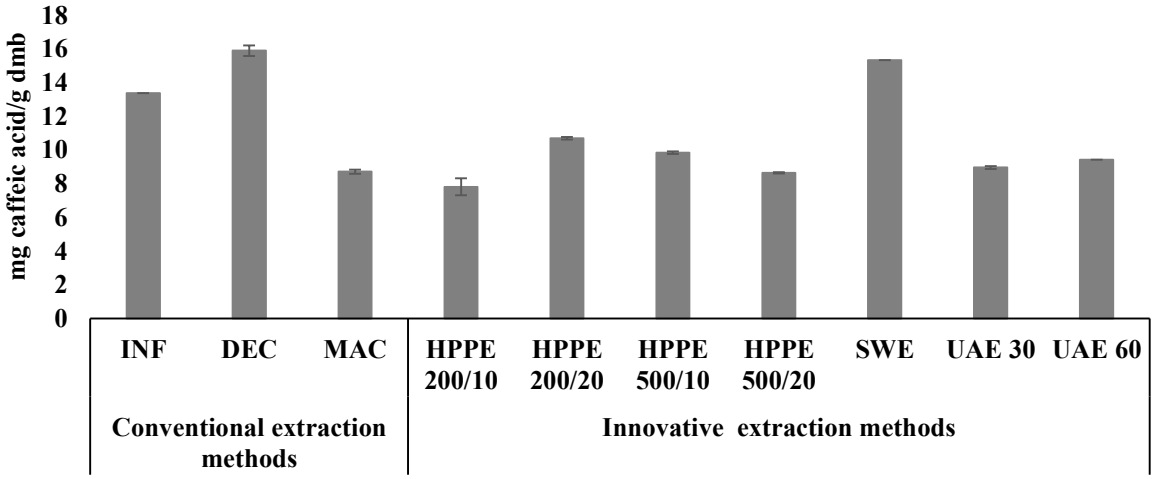

Figure 2. Content of hydroxycinnamic acid derivatives of differently prepared extracts of ground ivy

Conventional technique of extraction DEC resulted with the highest TPC (37.39 mg GAE/g dmb) and hydroxycinnamic acid derivatives content (15.9 mg caffeic acid/g dmb). Among the applied innovative techniques of extraction, HHPE 200/20 was the most successful in the extraction of total phenolics (26.20 mg GAE/g dmb), while SWE in the extraction of hydroxycinnamic acid derivatives (15.34 mg caffeic acid/g dmb).

Content of individual phenolic acids

Content of individual phenolic acids (rosmarinic acid, caffeic acid, chlorogenic acid and *p*-coumaric acid) is shown in Table 1.

Table 1. Content (μg/g dmb) of individual phenolic acids of differently prepared extracts of ground ivy

SAMPLE	Rosmarinic acid	Caffeic acid	Chlorogenic acid	*p*–coumaric acid
INF	1200.9	189.8	411.7	/
DEC	2001.0	198.6	512.1	/
MAC	20.1	15.8	27.4	46.9
SWE	144.6	299.5	183.5	46.5
U30	483.7	374.0	59.3	103.6
U60	499.5	393.7	58.8	97.6
HPPE 200/10	687.7	404.9	79.4	112.1
HPPE 200/20	652.9	479.8	99.3	106.3
HPPE 400/10	507.1	377.8	62.4	86.8
HPPE 400/20	506.8	381.7	57.4	77.5

The highest content of rosmarinic (2001.0 μg/g dmb) and chlorogenic (512.1 μg/g dmb) acid was determined in DEC extract, characterized also with the highest TPC and hydroxycinnamic acids derivatives content. However, it is interesting to point out that *p*-coumaric acid was not identified in the DEC extract, either in INF. HHPE yielded extracts the richest in *p*-coumaric acid (200 MPa, 10 min) and caffeic acid (200 MPa, 20 min).
High content of rosmarinic acid is characteristic for species within *Lamiaceae* family and the obtained results are in accordance with previous studies (Belščak-Cvitanović et al., 2014). The content of rosmarinic, caffeic and chlorogenic acids in different parts of ground ivy was investigated by Döring and Petersen (2014). They reported the highest content of rosmarinic acid in flowers (12.53 %), then in roots (1.31 %), while significantly lower contents were quantified in leaves and stems - 0.88 and 0.64 %, respectively. Further, the results of the present study are in accordance with the study by Chou et al. (2019) who also reported the presence of chlorogenic, caffeic and ferulic acids in water extract of ground ivy, with rosmarinic acid as predominant (1397.90 mg/100 g). The same authors also reported the presence of other phenolic compounds in ground ivy water extract, such as daidzin, rutin, genistin and genistein.

Antioxidant capacity

Antioxidant capacity of obtained extracts, determined by ABTS and DPPH assays, are presented on Fig. 3.

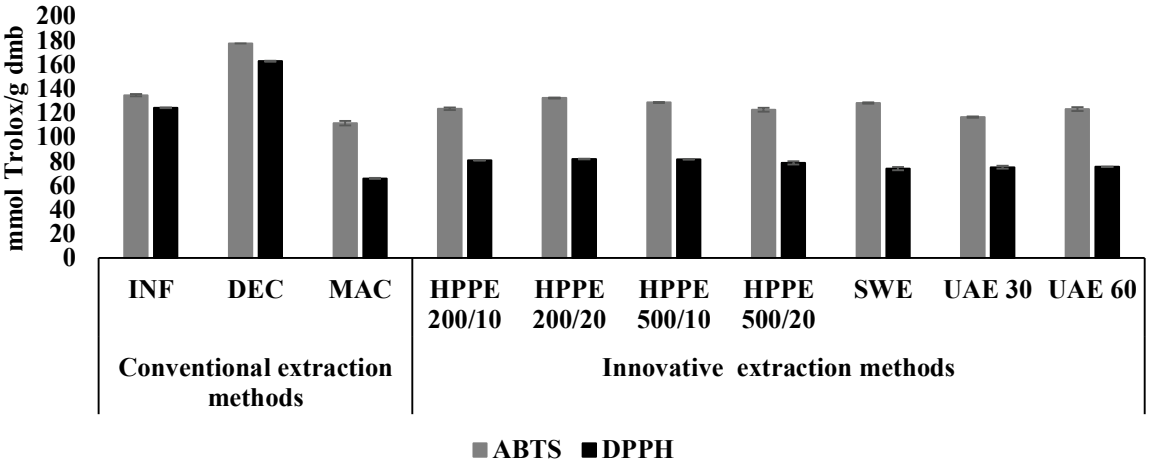

Figure 3. Antioxidant capacity of differently prepared extracts of ground ivy determined by ABTS and DPPH assays

Conventional technique of extraction DEC resulted in the extract with the highest antioxidant capacity (162.53 and 177.24 mmol Trolox/g dmb). High correlations between TPC and antioxidant capacity were observed, as well as with the content of hydroxycinnamic acids derivatives and individual phenolic acids. Among conventional extraction techniques it is evident that temperature was the main parameter to impact the extraction efficiency and antioxidant capacity. Higher extraction temperatures were in favour of higher contents of hydroxycinnamic acids in the extracts and their resulting antioxidant capacity.

Conclusions

Conventional extraction techniques, especially decoction, yielded extracts richer in total phenolic and hydroxycinnamic acid derivatives content, as well as in rosmarinic and chlorogenic acid, while high hydrostatic pressure was the most sufficient in the extraction of caffeic and *p*-coumaric acid.
Ground ivy (*Glechoma hederacea* L.) proved to be a valuable source of natural bioactive compounds, especially phenolic acids.

Acknowledgement

This study was conducted in the framework of research project "*Formulating encapsulated systems of bioactive ingredients from traditional plants: mountain germander and ground ivy for the development of innovative functional food products*,, (IP-2019-045879) funded by the Croatian Science Foundation.

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