

CHEMICAL COMPOSITION OF WILD APPLE (*Malus sylvestris* (L.) Mill.) LEAF INFUSION

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Wild apples leaves are under-utilized biomaterial, containing various bioactive compounds. The aim of this work was to determine the components and mineral composition of the wild apple leaf infusion. The leaves originated from Svrljig, Nišava District, Southern Serbia. The screening of bioactive components in the infusion was performed by the UHPLC-DAD-MS/MS method, while its mineral content was analyzed by the ICP-OES method. The results indicated the presence of quercetin, isoquercetin, hyperoside, rutin, citric and quinic acids, protocatechuic acid, 3-O-p-coumaroylquinic acid, chlorogenic acid, procyanidin B2, phloretin and phloretin-pentosyl-hexoside, while the most abundant elements were Ca, K, Mg and Na. Therefore, wild apple leaves may be utilized for isolation of polyphenolic antioxidants and minerals and production of extracts with antioxidant activity.

Keywords: Wild apple leaf, infusion, UH-PLC-DAD-MS/MS, ICP-OES

Introduction

From the aspect of ethnopharmacology, various plant species as an attractive source of bioactive ingredients are suitable for valorization and production of herbal products, used in the food and pharmaceutical industry, traditional and official medicine. Wild apples (*Malus sylvestris* (L.) Mill., Rosaceae) are the original apple trees, from which all cultivated apple varieties (*Malus domestica*) originated. Unlike cultivated varieties, wild apples always grow from seeds, and each one is genetically unique. Both the skin and flesh of the wild apple are rich in polyphenols and lipophilic antioxidants such as lupeol and D- α -tocopherol [1]. Agro-industrial byproducts of wild apples can serve as a potential source of valuable functional ingredients in different industries [1,2]. The amount of phenolic compounds in apples is influenced by fruits' variety, climatic conditions, and applied agrotechnological methods. The most commonly identified biologically active phenolic compounds in apples are hydroxycinnamic acid, flavan-3-ols, flavonols, dihydrochalcones, and anthocyanins [2], whose presence determine apple's color shades, vegetative maturity and fruit quality. Phenolic compounds protect DNA, proteins, lipids, and other macromolecular structures from free radical damage [3-8]. Consuming fruits high in phenolic compounds daily lowers the risk of chronic and degenerative diseases [3,4]. The wild apple fruit is especially a good

source of triterpenoids, flavonoids, organic acids, and sterols, compounds demonstrating antioxidant, antimicrobial, and anti-carcinogenic activities [4].

Depending on the polarity of solvents (70% ethanol, 45% propylene glycol, 80% propylene glycol, distilled water, olive or sunflower oil, and 96% ethanol in oil solvent system) and the extraction technique (maceration, percolation, Soxhlet, and ultrasonic-assisted), the obtained wild apple fruit extracts had a wide range of total phenolic, flavonoid and anthocyanin contents [5]. The extraction of flavonoids and tannins from the wild apple fruit originating from Serbia provided better results with virgin olive oil as a non-polar solvent, with higher total phenolic and tannin contents compared to the aqueous extract, thus indicating a high potential of the extracts as a natural source of polyphenols in skin protection products against UV radiation [6]. Emulsions prepared with different aqueous and hydroalcoholic apple fruit extracts presented beneficial dermal effects (increased skin hydration, reduced irritation, post UV radiation exposure protection and potential for skin whitening) [4,5,8].

However, wild apple leaves are under-utilized and cheap biomaterial, containing diverse bioactive compounds. Therefore, this paper aimed to represent valorization of the wild apple leaves from the Southern Serbia for the isolation of bioactive compounds and

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minerals. The main goal was providing information obtained after screening of bioactive components in the infusion by the UHPLC-DAD-MS/MS (ultrahigh performance liquid chromatography coupled with diode array and mass spectrometry detectors) analytical technique, together with the mineral content analyzed by the ICP-OES (inductively coupled plasma - optical emission spectrometry) method. To the best of the authors' knowledge, a detailed analysis of water infusion made from wild apple leaves from the territory of Southern Serbia regarding bioactive compounds and mineral compositions has not been previously reported in the literature. The findings of the study will aid in the advancements and improvements regarding the production and processing of wild apple.

Material and methods

Plant material preparation

"Previously picked and sun-dried wild apple leaves were donated from company D.O.O. "Jeligor" doo, Svrlijig, Serbia (43°24'55.4"N 22°07'12.4"E)." The leaves were preserved in a sealed paper bag.

Chemicals and reagents

The used chemicals were formic acid, purified distilled water and methanol (all HPLC grade, Fisher Chemical) and concentrated nitric acid (65%, p.a, Merck).

Wild Apple Leaves Infusion Preparation

A water infusion was prepared traditionally; 200 mL of boiling water was poured over one tablespoon of dried and chopped leaves, left for 15 minutes, and strained.

UHPLC-DAD-MS/MS Analysis

The infusion sample (4 μ L) was filtered through 0.45 μ m filter and subjected to the chromatographic separation, performed at 25 °C on Hypersil gold C18 column (50 $\times$ 2.1 mm, 1.9 μ m) using a Dionex Ultimate 3000 UHPLC + system with a diode array detector (DAD) and LCQ Fleet Ion Trap Mass Spectrometer (Thermo Fisher Scientific, USA). The mobile phase consisted of a mixture of two solvents: (A) 0.1% formic acid in water and (B) 0.1% formic acid in methanol, with a gradient mode of operation at an eluent flow rate of 0.25 mL/min. "The linear gradient was 10–30% B for the first two minutes, then 35–40% B from 4 to 5 min, 40–60% B from 5 to 8 min, 60–90% B from 8 to 11 min, followed by an isocratic segment of 90% B from 11 to 14 min, 90 - 10% B from 14 to 14.1 min, ending with an isocratic segment of 10% B until 20 min." Absorption UV–Vis spectra were recorded on DAD (total spectral range λ = 200–800 nm). Mass spectrometric analysis was performed using a 3D-ion trap with electrospray ionization (ESI) in negative ionization mode. The ESI–source parameters were: source voltage 4.5

kV, capillary voltage and temperature -41 V and 350 °C. Xcalibur software (version 2.1) was used for instrument control, data acquisition and processing.

ICP-OES Analysis

The infusion sample was prepared by the wet digestion process. Accurately determined mass/volume of the sample was poured with 2.5 mL of concentrated nitric acid. After that, the mixture was heated in a water bath until the solution became colorless and the steaming stopped. The solution was topped up with distilled water up to the mark in a normal 25 mL vessel. After preparation, the sample was filtered through 0.45 μ m filter and subjected to the quantitative ICP-OES analysis (ARCOS FHE12, SPECTRO, Germany), according to the manufacturer's instructions. Argon 5.0 (purity 99.999%) was used as carrier gas. The instrument conditions and determined parameters were: plasma power 1400 W, coolant and auxiliary gas flow 13 and 0.80 L/min, cross-flow nebulizer with 0.95 L/min flow rate and 30 rpm of pump speed. Multi-standard IV – multi elemental standard solution (Merck), containing Ag, B, Cd, Co, Cr, Cu, Fe, Li, Mn, Na, Ni, Pb, Tl, and Zn in concentrations of 1000 ppm, was used for preparing the calibration solution and obtaining the calibration diagrams within the range of the expected concentrations of the analyzed elements. Ca, K, Mg, P, S, Sr and Si were determined using specific calibration standards (1000 ppm), respectively. Parameters of the calibration lines for the determined elements are presented in Supplement material.

Results and discussion

UHPLC-DAD-MS/MS analysis of the wild apple leaf infusion

UHPLC chromatogram recorded from the MS (A) and DAD-signal at λ =320 nm (B) is shown in Figure 1. Table 1 displays a list of the detected compounds in the sample along with the associated chromatography, UV-Vis, MS/MS, and assignments data. UHPLC-DAD-MS/MS analysis of the extract indicated the presence of flavonoids, phenols, phenolic acids and different natural acids and their derivatives. Some of the main detected compounds are shown in Figures 2-4.

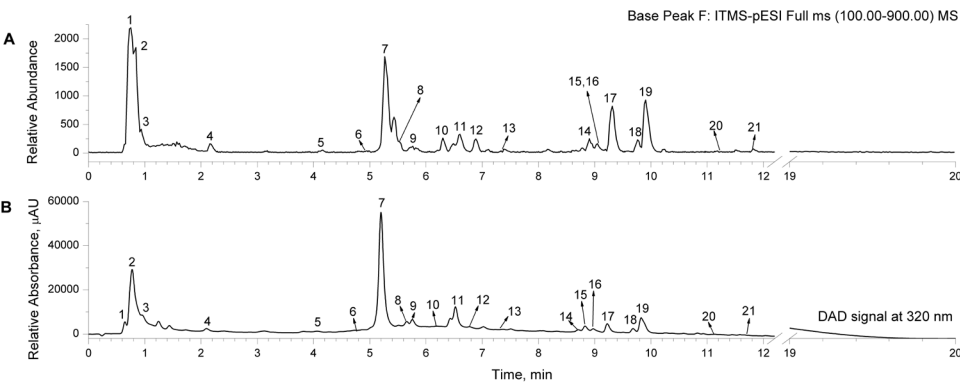


Figure 1. Representative MS (A) and DAD (B) UHPLC chromatograms of the infusion

Table 1. List of detected components in the infusion by using UHPLC-DAD-MS/MS analysis

Peak No.	t _R , min MS	λ _{max} , nm	Molecular ion [M-H] ⁻ m/z	MS/MS fragment ions	Identified compounds (references)
1	0.76	-	191	173,129,117,85(100%), 73,57	Quinic acid [46]
2	0.78	-	195	129(100%)	-
3	0.95	-	191	173,111(100%),99	Citric acid (standard)
4	2.18	220 261 297	153	109(100%)	Protocatechuic acid [47]
5	4.10	305 286sh	337	267,249(100%),163	3-O-p- coumaroylquinic acid [48]
6	4.87	-	515	455,425,353(100%),191	Dicaffeoylquinic acid [48]
7	5.28	325 303sh	353	191(100%)	Chlorogenic acid (standard)
8	5.60	278	577	425,407(100%),289	Procyanidin B2 [47]
9	5.73	325 303sh	353	191(100%),179,173,135	Chlorogenic acid, isomer (standard)
10	6.32	281	289	245(100%),205/203,179 ,161	(-) Epicatechin [49]
11	6.59	313 296sh	337	191,173(100%),163	4-O-p- coumaroylquinic acid [50]
12	6.87	334	563	517(100%)	-
13	7.40	362	483	437(100%),305	-
14	8.93	258 356	463	301(100%)	Hyperoside (quercetin-3-O- galactoside) (standard)
15	9.03	258 356	463	301(100%)	Isoquercitrin (quercetin-3-O- glucoside) (standard)
16	9.07	258 355	609	-	Rutin (quercetin-3-O- rutoside) (standard)
17	9.30	287	567	273(100%)	Phloretinpentosylhex oside [51]
18	9.73	354 258	433	301(100%)	Quercetin pentoside [51]
19	9.90	258 352	447	301(100%)	Quercitrin (quercetin-3-O- rhamnoside) [52]
20	11.17	257 372	301	229,179(100%),151	Quercetin (standard)
21	11.80	-	273	167(100%),123	Phloretin [51]

*n.i. – not identified

Quinic acid (with molecular ion m/z 191) as well as its derivatives, 3-O-p-coumaroyl-quinic acid and 4-O-p-coumaroyl-quinic acid (both with molecular ion m/z 337) were detected. 4-O-p-coumaroyl-quinic acid with $[M-H]^-$ at m/z 337 with also intensive ions at m/z 191, 173 (100%), and 163 in the MS/MS spectrum, was detected under the peak No.11 in UHPLC chromatogram (Table 1, Figures 1 and 2); the corresponding UV/Vis spectrum with absorption maximum at $\lambda_{\max} = 313$ nm was shown as inset in Figure 2. These compounds express antifungal, antiviral, anti-neuroinflammatory, antioxidative and radioprotective activities [9-11].

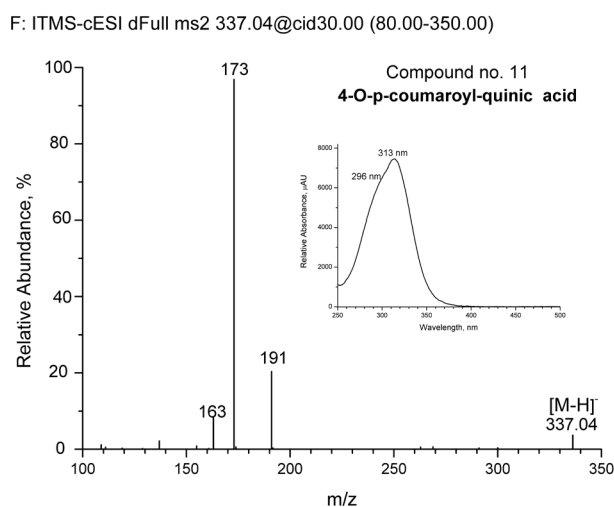


Figure 2. Mass spectrum of 4-O-p-coumaroyl-quinic acid (comp. No. 11, Table 1); the corresponding absorption UV-Vis spectrum was shown as inset in the figure

Chlorogenic acid as a caffeoylquinic acid derivative with molecular ion $[M-H]^-$ at m/z 353 and intensive ions at m/z 191 (100%) in the MS/MS spectrum was detected under the peak No.7 in UHPLC chromatogram (Table 1, Figures 1 and 3). The corresponding UV/Vis spectrum with absorption maximum at $\lambda_{\max} = 325$ nm was also shown as inset in Figure 3. Chlorogenic acid and its isomers (various caffeoylquinic acids such as 3-, 4-, 5-O-caffeoylquinic acids) have been found to exhibit antioxidant, antibacterial, antitumor, anti-inflammatory, antidiabetic, antihypertensive, antidiabetic and neuroprotective effects [23-26].

Dicafeoylquinic acid showed anti-inflammatory, antiviral and antiretroviral effects in biochemical assays [10,12,13]. Citric acid demonstrated anti-inflammatory and antioxidant effect on the brain, as well as hepatoprotective activity [14]. Citric acid is safe at lower concentrations since it is rapidly metabolized in the liver and muscles to bicarbonate. In some patients on hemodialysis, citric acid derivatives are present in the dialysate and are found to have certain beneficial effects, such as improved dialysis performance, fewer episodes of hypotension, lower risk of blood coagulation and better nutri-

tional status [15]. Protocatechuic acid and its derivatives, have shown exceptional importance in the prophylaxis of a large number of diseases associated with oxidative stress, showing various biological activities (antibacterial, antiviral, antitumor, antiulcer, antiaging, antifibrotic, anti-sclerotic, neurological and hepatoprotective) [16-18]. Procyanidin B2 is a proanthocyanidin expressing anti-inflammatory, antioxidative, hepatoprotective and anti-allergy effects [19-22], while epicatechin had anti-aging effects [27].

F: ITMS-cESI dFull ms2 352.95@cid30.00 (85.00-365.00)

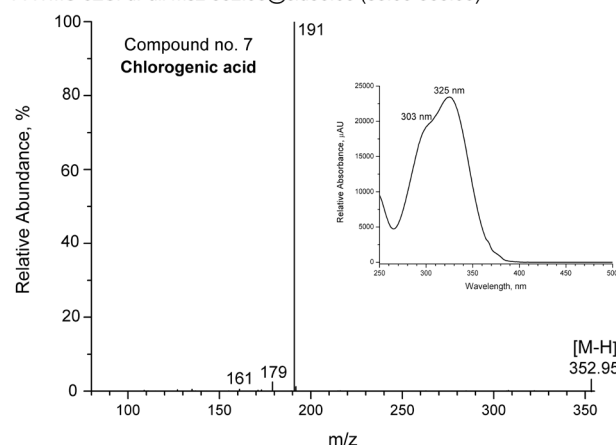


Figure 3. Mass spectrum of chlorogenic acid (comp. No. 7, Table 1); the corresponding absorption UV-Vis spectrum was shown as inset in the figure

Quercetin and its derivatives, isoquercitrin (quercetin-3-O-glucoside), rutin (quercetin-3-O-rutinoside), quercetin pentoside, quercitrin (quercetin-3-O-rhamnoside) have been shown to have excellent bioactivities such as antioxidant, anti-inflammatory, anti-obesity and anti-cancer [28]. Apart from its anti-inflammatory effect, quercetin is also capable of modulating the immune system and stabilizing the mast cells, whereas it expresses cytoprotective activity in the gastrointestinal system [29]. Quercetin is also known to have beneficial effects on patients with different autoimmune diseases. In vivo studies on mice showed that quercetin improves symptoms in rheumatoid arthritis and reduces arthritic scores by reducing blood levels of proinflammatory cytokines and inhibiting the migration of neutrophils. In vitro studies showed that quercetin reduces levels of peripheral blood cells in multiple sclerosis, and modulates levels of circulating cytokines. Quercetin was also shown to express immunomodulatory activity in atopic dermatitis and systemic lupus erythematosus [30]. In addition, quercetin has anticancer properties by inhibiting cell proliferation, promoting apoptosis and altering different parts of the cell cycle, which was found in both in vivo and in vitro studies [31]. Quercetin can also be used in patients with asthma, rhinitis and atopic dermatitis due to its anti-allergic properties [32]. Quercetin has also been used alone

and together with vitamin C for both the prophylaxis and treatment of COVID-19 infection, and it was shown it has beneficial effects on the outcome of the infection, even when administered in critically ill patients [33].

Isoquercitrin is a glycoside and a conjugated form of quercetin. It expresses anti-inflammatory, antidiabetic, neuroprotective, anticancer and antioxidant activity, but its poor solubility in water and other hydrophilic media reduces its bioavailability. Recently, isoquercitrin has been enzymatically modified with far greater bioavailability, reducing systolic blood pressure and expressing atheroprotective effects in rodents [34], while aiding in muscle mass increase [35]. Hyperoside (quercetin-3-O-galactoside) with molecular ion $[M-H]^-$ at m/z 463 and intensive ions at m/z 301 (100%) in the MS/MS spectrum was detected under the peak No.14 in UHPLC chromatogram (Table 1, Figures 1 and 4), with maximum at $\lambda_{max} = 258$ nm and 356 nm in the corresponding UV/Vis spectrum (Figure 4). Hyperoside possesses antioxidant, anti-inflammatory, hepatoprotective, and anti-arthritis activities [36-38].

F: ITMS-cESI dFull ms2 463.09@cid30.00 (115.00-475.00)

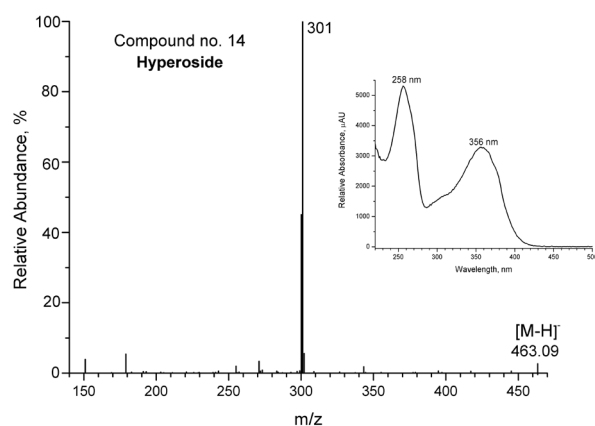


Figure 4. Mass spectrum of hyperoside (comp. No. 14, Table 1); the corresponding absorption UV-Vis spectrum was shown as inset in the figure

Rutin, a polyphenolic bioflavonoid, expressed anti-inflammatory, antioxidant, anticancer, antidiabetic, neuroprotective, cardio-protective and antidepressant activities [39-42]. Phloretin is a dihydrochalcone, expressing antioxidant, anti-inflammatory, antidiabetic, antiproliferative, antimetastatic and antiangiogenic activities [43-45].

Minerals Obtained from the Wild Apple Leaf

Table 2. Concentrations of minerals in the infusion

Element	Extract (µg/mL)	Element	Extract (µg/mL)	Element	Extract (µg/mL)	Element	Extract (µg/mL)
K	34.43	Mg	5.32	Cu	0.13	Bi, Cd	0
P	22.66	Si	0.73	Sr	0.11	Co, Cr	0
Ca	18.55	Zn	0.26	Pb	0.1	Li, Mn	0
Na	16.8	Ag	0.14	As	0	Ni, Ti	0
S	9.56	Ba	0.13	B	0	Hg, Ce	0

K was detected in the highest concentration of all analyzed macroelements, while Zn was detected in the highest concentration of the group of microelements. The infusion is also rich in Ca, Mg, Na, P, and S (Table 2). Ca, found in the concentration of 18.55 µg/mL, is necessary for the proper development of bones and teeth, maintaining muscle strength, exchanging nerve messages between the brain and the body [53]. Ca was also reported to reduce levels of LDL (low density lipids) and increase levels of HDL (high density lipids), reduce the number of hypertensive disorders in pregnancy, lower blood pressure among younger people and reduce the risk of colorectal adenomas [54]. K, detected in concentration of 34.43 µg/mL, is important for the proper function of the kidneys, heart, and muscles and in the transmission of messages through the nervous system [55]. Proper intake of K protects against hypertension and could even be beneficial in improving bone health [56]. Mg, found in concentration of 5.32 µg/mL, is necessary for the normal functioning of the body, in terms of regulating blood pressure and heart rhythm [57]. Na (16.8 µg/mL), is one of the most important electrolytes necessary for blood pressure regulation and most important ion in the extracellular fluid [58], while P (22.66 µg/mL) is included in different metabolic processes in human organism [59]. S is present in this extract in concentration of 9.56 µg/mL. This mineral is extremely important for the normal functioning of numerous processes in the organism, such as repairing and building DNA and protecting cells from damage [60]. Among analyzed heavy metals (As, Cd, Hg, Pb), only Pb was detected in negligible amount.

Conclusion

Wild apple leaves infusion contained a variety of bioactive compounds such as quercetin, isoquercetin, hyperoside, quercetin pentoside, rutin, citric acid, quinic acid, protocatechuic acid, 3-O-p-coumaroylquinic acid, chlorogenic acid, procyanidin B2, phloretin and phloretin-pentosyl-hexoside. Also, this infusion is a good source of K, P, Ca and Na, while toxic elements are not found, or their concentrations are negligible. According to the obtained results, it can be concluded that wild apple leaves have the potential to be used as a raw material for extraction of bioactives and minerals. Therefore, further detailed experiments and analyses must be conducted, in order to ensure the most optimal valorization

of this under-utilized biomaterial.

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References

- [1] Zhang C, Chen X, Song H, Liang Y, Zhao C, Li H. Volatile compound profiles of *Malus baccata* and *Malus prunifolia* wild apple fruit. *Journal of the American Society for Horticultural Science*. 2017, 142(2), 126-134. <https://doi.org/10.21273/JASHS03968-16>
- [2] Mihailović NR, Mihailović VB, Kreft S, Ćirić AR, Joksović LG, Đurđević PT. Analysis of phenolics in the peel and pulp of wild apples (*Malus sylvestris* (L.) Mill.). *Journal of Food Composition and Analysis*. 2018, 67, 1-9. <https://doi.org/10.1016/j.jfca.2017.11.007>
- [3] Mendoza-Wilson AM, Castro-Arredondo SI, Espinosa-Plascencia A, Robles-Burgueño Mdel R, Balandrán-Quintana RR, Bermúdez-Almada Mdel C. Chemical composition and antioxidant-prooxidant potential of a polyphenolic extract and a proanthocyanidin-rich fraction of apple skin. *Heliyon*. 2016, 2(2), e00073. <https://doi.org/10.1016/j.heliyon.2016.e00073>
- [4] Kolarević A, Stojiljković D, Dinić S, Nešić I. Effect of application of emulsions with standardized extract of wild apple fruit (*Malus sylvestris* (L.) Mill., Rosaceae) on biophysical skin parameters: an in vivo study. *Acta Medica Mediana*. 2020, 48-55. <https://doi.org/10.5633/amm.2020.0307>
- [5] Stojiljković D, Tadić V, Stanković M, Roganović S, Arsić I. Standardized extract of wild apple fruit in alkyl-polyglucoside-based cosmetic cream - estimation of stability, safety, antioxidant activity and efficiency. *International Journal of Cosmetic Science*. 2018, 40, 285-294. <https://doi.org/10.1111/ics.12462>
- [6] Nešić I, Stojiljković D, Tadić V. Skin photoprotection products with wild apple fruit extracts as a source of bioactive polyphenols: a comparison of polar and non-polar solvents. In: *Book of Abstracts International Conference on Radiation Applications* (RAP 2019). Belgrade, Serbia, 2019. p. 137. https://www.rap-conference.org/19/RAP_2019_Book_of_Abstracts.pdf
- [7] Petkova N, Ognyanov M, Inyutin B, Zhelev P, Denev P. Phytochemical composition and antioxidant activity of *Malus baccata* (L.) Borkh. fruits. *Food Science and Applied Biotechnology*. 2020, 3, 47-55. <https://doi.org/10.30721/fsab2020.v3.i1.46>
- [8] Opris O, Lung I, Soran ML, Stegarescu A, Cesco T, Ghendov-Mosanu A, Podea P, Sturza R. Efficient extraction of total polyphenols from apple and investigation of its SPF properties. *Molecules*. 2022, 27(5), 1679. <https://doi.org/10.3390/molecules27051679>
- [9] Ma J.-N, Ma C.-M. Chapter 71 - Antifungal inhibitory activities of caffeic and quinic acid derivatives. In: *Coffee in Health and Disease Prevention*. Preedy VR, ed., Academic Press, 2015, 635-641. <https://doi.org/10.1016/B978-0-12-409517-5.00071-1>
- [10] Zanello PR, Koishi AC, Rezende Júnior C.de O, Oliveira LA, Pereira AA, de Almeida MV, dos Santos CND, Bordignon J. Quinic acid derivatives inhibit dengue virus replication in vitro. *Virology Journal*. 2015, 12, 223. <https://doi.org/10.1186/s12985-015-0443-9>
- [11] Jang SA, Park DW, Kwon JE, Song HS, Park B, Jeon H, Sohn EH, Koo HJ, Kang SC. Quinic acid inhibits vascular inflammation in TNF- α -stimulated vascular smooth muscle cells. *Biomedicine & Pharmacotherapy*. 2017, 96, 563-571. <https://doi.org/10.1016/j.biopha.2017.10.021>
- [12] McDougall B, King PJ, Wu BW, Hostomsky Z, Reinecke MG, Robinson WE Jr. Dicafeoylquinic and dicafeoyltartaric acids are selective inhibitors of human immunodeficiency virus type 1 integrase. *Antimicrobial Agents and Chemotherapy*. 1998, 42(1), 140-146. <https://doi.org/10.1128/AAC.42.1.140>
- [13] Jang G, Lee S, Hong J, Park B, Kim D, Kim C. Anti-inflammatory effect of 4,5-dicafeoylquinic acid on RAW264.7 cells and a rat model of inflammation. *Nutrients*. 2021, 13(10), 3537. <https://doi.org/10.3390/nu13103537>
- [14] Abdel-Salam OME, Youness ER, Mohammed NA, Youssef Morsy SM, Omara EA, Sleem AA. Citric acid effects on brain and liver oxidative stress in lipopolysaccharide-treated mice. *Journal of Medicinal Food*. 2014, 17(5), 588-598. <http://doi.org/10.1089/jmf.2013.0065>
- [15] Ureña-Torres P, Bieber B, Guebre-Egziabher F, Ossman R, Jadoul M, Inaba M, Robinson BM, Port F, Jacquelinet C, Combe C. Citric acid-containing dialysate and survival rate in the dialysis outcomes and practice patterns study. *Kidney360*. 2021, 2(4), 666-673. <http://doi.org/10.34067/KID.0006182020>
- [16] Kakkar S, Bais S. A review on protocatechuic acid and its pharmacological potential. *ISRN Pharmacology*. 2014, 2014, 952943. <http://dx.doi.org/10.1155/2014/952943>
- [17] Wang Q, Ren X, Wu J, Li H, Yang L, Zhang Y, Wang X, Li Z. Protocatechuic acid protects mice from influenza A virus infection. *European Journal of Clinical Microbiology & Infectious Diseases*. 2022, 41(4), 589-596. <http://doi.org/10.1007/s10096-022-04401-y>
- [18] Lee WJ, Lee SH. Protocatechuic acid protects hepatocytes against hydrogen peroxide-induced oxidative stress. *Current Research in Food Science*. 2022, 5, 222-227. <https://doi.org/10.1016/j.crf.2022.01.006>
- [19] Cai W, Zhang Y, Jin W, Wei S, Chen J, Zhong C, Zhong Y, Tu C, Peng H. Procyanidin B2 ameliorates the progression of osteoarthritis: An in vitro and in vivo study. *International Immunopharmacology*. 2022, 113, 109336. <https://doi.org/10.1016/j.intimp.2022.109336>
- [20] Feng J, Wang C, Liu T, Li J, Wu L, Yu Q, Li S, Zhou Y, Zhang J, Chen J, Ji J, Chen K, Mao Y, Wang F, Dai W, Fan X, Wu J, Guo C. Procyanidin B2 inhibits the activation of hepatic stellate cells and angiogenesis via the Hedgehog pathway during liver fibrosis. *Journal of Cellular and Molecular Medicine*. 2019, 23(9), 6479-6493. <https://doi.org/10.1111/jcmm.14543>
- [21] Liang Y, Chen G, Zhang F, Yang X, Chen Y, Duan Y, Yu M, Zhang S, Han J. Procyanidin B2 reduces vascular calcification through inactivation of ERK1/2-RUNX2 pathway. *Antioxidants*. 2021; 10(6), 916. <https://doi.org/10.3390/antiox10060916>
- [22] Razif D, Norazmir MN, Zolkapli E, Siti SMM, Nur RS, Habsah B. A review of procyanidin: updates on current

- bioactivities and potential health benefits. *Biointerface Research in Applied Chemistry*. 2022, 12, 5918-5940. <https://doi.org/10.33263/BRIAC125.59185940>
- [23] Xu J-G, Hu Q-P, Liu Y. Antioxidant and DNA-protective activities of chlorogenic acid isomers. *Journal of Agricultural and Food Chemistry*. 2012, 60(46), 11625-11630. <https://doi.org/10.1021/jf303771s>
- [24] Santana-Gálvez J, Cisneros-Zevallos L, Jacobo-Velázquez DA. Chlorogenic acid: Recent advances on its dual role as a food additive and a nutraceutical against metabolic syndrome. *Molecules*. 2017, 22(3), 358. <https://doi.org/10.3390/molecules22030358>
- [25] Hayakawa S, Ohishi T, Miyoshi N, Oishi Y, Nakamura Y, Isemura M. Anti-cancer effects of green tea epigallocatechin-3-gallate and coffee chlorogenic acid. *Molecules*. 2020, 25(19), 4553. <https://doi.org/10.3390/molecules25194553>
- [26] Liu D, Wang H, Zhang Y, Zhang Z. Protective effects of chlorogenic acid on cerebral ischemia/reperfusion injury rats by regulating oxidative stress-related Nrf2 pathway. *Drug Design, Development and Therapy*. 2020, 14, 51-60. <https://doi.org/10.2147/DDDT.S228751>
- [27] Si H, Lai C-Q, Liu D. Dietary epicatechin, a novel anti-aging bioactive small molecule. *Current Medicinal Chemistry*. 2021, 28(1), 3-18. <https://dx.doi.org/10.2174/0929867327666191230104958>
- [28] Hai Y, Zhang Y, Liang Y, Xiaoyu M, Xiao Q, Jianbo X, Weiming X, Yane L, Tianli Y. Advance on the absorption, metabolism and efficacy exertion of quercetin and its important derivatives. *Food Frontiers*. 2020, 1, 420-434. <https://doi.org/10.1002/fft2.50>
- [29] Li Y, Yao J, Han C, Yang J, Chaudhry MT, Wang S, Liu H, Yin Y. Quercetin, inflammation and immunity. *Nutrients*. 2016, 8(3), 167. <https://doi.org/10.3390/nu8030167>
- [30] Shen P, Lin W, Deng X, Xin B, Liang H, Zhe Ch, Kai Q, Ying H, Shenghao T. Potential implications of quercetin in autoimmune diseases. *Frontiers in Immunology*. 2021, 12, 689044. <https://doi.org/10.3389/fimmu.2021.689044>
- [31] Tang SM, Deng XT, Zhou J, Li QP, Ge XX, Miao L. Pharmacological basis and new insights of quercetin action in respect to its anti-cancer effects. *Biomedicine & Pharmacotherapy*. 2020, 121, 109604. <https://doi.org/10.1016/j.biopha.2019.109604>
- [32] Jafarinia M, Sadat Hosseini M, Kasiri N, Fazel N, Fathi F, Hakemi MG, Eskandari N. Quercetin with the potential effect on allergic diseases. *Allergy, Asthma & Clinical Immunology*. 2020, 16, 36. <https://doi.org/10.1186/s13223-020-00434-0>
- [33] Colunga Biancatelli RML, Berrill M, Catravas JD, Marik PE. Quercetin and vitamin C: An experimental, synergistic therapy for the prevention and treatment of SARS-CoV-2 related disease (COVID-19). *Frontiers in Immunology*. 2020, 11, 1451. <https://doi.org/10.3389/fimmu.2020.01451>
- [34] Owczarek-Januszkiewicz A, Magiera A, Olszewska MA. Enzymatically modified isoquercitrin: Production, metabolism, bioavailability, toxicity, pharmacology, and related molecular mechanisms. *International Journal of Molecular Sciences*. 2022, 23(23), 14784. <https://doi.org/10.3390/ijms232314784>
- [35] Omi N, Shiba H, Nishimura E, Tsukamoto S, Maruki-Uchida H, Oda M, Morita M. Effects of enzymatically modified isoquercitrin in supplementary protein powder on athlete body composition: A randomized, placebo-controlled, double-blind trial. *Journal of the International Society of Sports Nutrition*. 2019, 16(1), 39. <https://doi.org/10.1186/s12970-019-0303-x>
- [36] Wang G, Wu S, Wu C, Zhang Q, Wu F, Yu B, Zhang S, Wu S, Wu G, Zhong Y. Association between non-alcoholic fatty liver disease with the susceptibility and outcome of COVID-19: A retrospective study. *Journal of Cellular and Molecular Medicine*. 2021, 25, 11212-11220. <https://doi.org/10.1111/jcmm.17042>
- [37] Qiu J, Zhang T, Zhu X, Yang C, Wang Y, Zhou N, Ju B, Zhou T, Deng G, Qiu C. Hyperoside induces breast cancer cells apoptosis via ROS-mediated NF- κ B signaling pathway. *International Journal of Molecular Sciences*. 2020, 21(1), 131. <https://doi.org/10.3390/ijms21010131>
- [38] Sun K, Luo J, Jing X, Xiang W, Guo J, Yao X, Liang S, Guo F, Xu T. Hyperoside ameliorates the progression of osteoarthritis: An in vitro and in vivo study. *Phytomedicine*. 2021, 80, 153387. <https://doi.org/10.1016/j.phymed.2020.153387>
- [39] Sun XY, Li LJ, Dong QX, Zhu J, Huang YR, Hou SJ, Yu XL, Liu RT. Rutin prevents tau pathology and neuroinflammation in a mouse model of Alzheimer's disease. *Journal of Neuroinflammation*. 2021, 18, 131. <https://doi.org/10.1186/s12974-021-02182-3>
- [40] Enogieru AB, Haylett W, Hiss DC, Bardien S, Ekpo OE. Rutin as a potent antioxidant: Implications for neurodegenerative disorders. *Oxidative Medicine and Cellular Longevity*. 2018, 2018, 6241017. <https://doi.org/10.1155/2018/6241017>
- [41] Ma Y, Yang L, Ma J, Lu L, Wang X, Ren J, Yang J. Rutin attenuates doxorubicin-induced cardiotoxicity via regulating autophagy and apoptosis. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*. 2017, 1863(8):1904-1911. <https://doi.org/10.1016/j.bbadis.2016.12.021>
- [42] Foudah AI, Alqarni MH, Alam A, Devi S, Salkini MA, Alam P. Rutin improves anxiety and reserpine-induced depression in rats. *Molecules*. 2022, 27(21), 7313. <https://doi.org/10.3390/molecules27217313>
- [43] Nakhate KT, Badwaik H, Choudhary R, Sakure K, Agrawal YO, Sharma C, Ojha S, Goyal SN. Therapeutic potential and pharmaceutical development of a multitargeted flavonoid phloretin. *Nutrients*. 2022; 14(17), 3638. <https://doi.org/10.3390/nu14173638>
- [44] Tuli HS, Rath P, Chauhan A, Ramniwas S, Vashishth K, Varol M, Jaswal VS, Haque S, Sak K. Phloretin, as a potent anticancer compound: from chemistry to cellular interactions. *Molecules*. 2022, 27(24), 8819. <https://doi.org/10.3390/molecules27248819>
- [45] Yang C-H, Ou Y-C, Lin C-C, Lin Y-S, Tung M-C, Yu C-C, Lin J-T, Wen C-Y. Phloretin in benign prostate hyperplasia and prostate cancer: a contemporary systematic review. *Life*. 2022, 12(7), 1029. <https://doi.org/10.3390/life12071029>
- [46] Masiike K, Mhlongo MI, Mudau SP, Nobela O, EN Ncube, Tugizimana F, George MJ, Madala NE. Highlighting mass spectrometric fragmentation differences and similarities between hydroxycinnamoyl-quinic acids and hydroxycinnamoyl-isocitric acids. *Chemistry Central Journal*. 2017, 11, 29. <https://doi.org/10.1186/s13065-017-0262-8>
- [47] Cuadrado-Silva CT, Pozo-Bayón MA, Osorio C. Targeted metabolomic analysis of polyphenols with antioxidant activity in sour guava (*Psidium friedrichsthalianum* Nied.) fruit. *Molecules*. 2017, 22(1), 11. <https://doi.org/10.3390/molecules22010011>

- [48] Clifford MN, Johnston KL, Knight S, Kuhnert N. Hierarchical scheme for LC-MSn identification of chlorogenic acids. *Journal of Agricultural and Food Chemistry*. 2003, 51, 2900-2911. <http://dx.doi.org/10.1021/jf026187q>
- [49] Stöggli WM, Huck CW, Bonn GK. Structural elucidation of catechin and epicatechin in sorrel leaf extracts using liquid-chromatography coupled to diode array-, fluorescence-, and mass spectrometric detection. *Journal of Separation Science*. 2004, 27, 524-528. <https://doi.org/10.1002/jssc.200301694>
- [50] Clifford MN, Zheng W, Kuhnert N. Profiling the chlorogenic acids of aster by HPLC-MSn. *Phytochemical Analysis*. 2006, 17, 384-393. <http://dx.doi.org/10.1002/pca.935>
- [51] Petkovska A, Gjamovski G, Petreska Stanoeva J, Stefova M. Characterization of the polyphenolic profiles of peel, flesh and leaves of *Malus domestica* cultivars using UHPLC-DAD-HESI-MSn. *Natural Product Communications*. 2017, 12(1), 35-42. <https://doi.org/10.1177/1934578X1701200111>
- [52] Silva BA, Ferreres F, Malva JO, Dias ACP. Phytochemical and antioxidant characterization of *Hypericum perforatum* alcoholic extracts. *Food Chemistry*. 2005, 90, 157-167. <https://doi.org/10.1016/j.foodchem.2004.03.049>
- [53] Li K, Wang XF, Li DY, Chen YC, Zhao LJ, Liu XG, Guo YF, Shen J, Lin X, Deng J, Zhou R, Deng HW. The good, the bad, and the ugly of calcium supplementation: a review of calcium intake on human health. *Clinical Interventions in Aging*. 2018, 13, 2443-2452. <https://doi.org/10.2147/CIA.S157523>
- [54] Cormick G, Belizán JM. Calcium intake and health. *Nutrients*. 2019, 11(7), 1606. <https://doi.org/10.3390/nu11071606>
- [55] John SK, Rangan Y, Block CA, Koff MD. Life-threatening hyperkalemia from nutritional supplements: uncommon or undiagnosed? *The American Journal of Emergency Medicine*. 2011, 29, 1237.e1-1237.e2. <https://doi.org/10.1016/j.ajem.2010.08.029>
- [56] Weaver CM. Potassium and health. *Advances in Nutrition*. 2013, 4(3), 368S-77S. <https://doi.org/10.3945/an.112.003533>
- [57] Gröber U, Schmidt J, Kisters K. Magnesium in prevention and therapy. *Nutrients*. 2015, 7(9), 8199-8226. <https://doi.org/10.3390/nu7095388>
- [58] Logan AG. Dietary sodium intake and its relation to human health: a summary of the evidence. *Journal of the American College of Nutrition*. 2006, 25, 165-169. <https://doi.org/10.1080/07315724.2006.10719528>
- [59] Chang AR, Anderson C. Dietary phosphorus intake and the kidney. *Annual Review of Nutrition*. 2017, 37, 321-346. <https://doi.org/10.1146/annurev-nutr-071816-064607>
- [60] Fuss JO, Tsai CL, Ishida JP, Tainer JA. Emerging critical roles of Fe-S clusters in DNA replication and repair. *Biochimica et Biophysica Acta-Molecular Cell Reserch*. 2015, 1853, 1253-1271. <https://doi.org/10.1016/j.bbamcr.2015.01.018>

Izvod

HEMIJSKI SASTAV INFUZIJE LISTA DIVLJE JABUKE (*Malus sylvestris* (L.) Mill.)

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Listovi divlje jabuke predstavljaju nedovoljno iskorišćen biomaterijal, koji sadrže različita bioaktivna jedinjenja. Cilj ovog rada je utvrđivanje komponenti i mineralnog sastava infuzije listova divlje jabuke. Listovi su poreklom iz Svrlijga, okruga Nišava, Južna Srbija. Skrining bioaktivnih komponenti u infuziji urađen je UHPLC-DAD-MS/MS metodom, dok je mineralni sadržaj analiziran ICP-OES metodom. Analiza infuzije je pokazala prisustvo kvercetina, izokvercetina, hiperozida, rutina, limunske kiseline, kininske kiseline, protokatehinske kiseline, 3-O-p-kumaroilkininske kiseline, hlorogenske kiseline, procijanidina B2, floretina i floretin-pentosil-heksozida, dok su Ca, K, Mg i Na bili elementi prisutni u najvećoj koncentraciji. Stoga se listovi divlje jabuke mogu iskoristiti za izolaciju polifenolnih antioksidanata i minerala, kao i za proizvodnju ekstrakata sa antioksidativnom aktivnošću.

Ključne reči: list divlje jabuke, infuzija, UHPLC-DAD-MS/MS, ICP-OES

