THE QUALITATIVE ANALYSIS OF THE GREEN TEA EXTRACT USING ESI-MS METHOD

Ivan M. Savić^{1*}, Vesna D. Nikolić¹, Ivana M. Savić¹, Ljubisa B. Nikolić¹,
Marko D. Jović², Maja D. Jović²

¹Faculty of Technology, University of Niš, Leskovac, Serbia

²Faculty of Medicine, University of Niš, Niš, Serbia

(ORIGINAL SCIENTIFIC PAPER)
UDK 663.951:66.061:543.4

The aim of this paper was the identification of the bioactive compounds in the green tea extract enriched with quercetin. The extraction was performed under reflux for 60 min using absolute ethanol at the solid-to-liquid ratio of 1:18 (m/v). The mass spectrometry method with an electrospray interface was applied and tuned with the quercetin standard. Mass spectra were recorded in negative and positive ionization modes with the ion source voltage of 4.95 kV, the source current of 2.61 μ A, the capillary temperature of 275 °C, the capillary voltage of 50 V, the sheath gas pressure of 137 KPa and auxiliary gas pressure of 13.9 KPa. In addition to quercetin, the presence of gallic acid, (-)-gallocatechin, 3-O-caffeoylquinic acid, theobromine, 5-O-galloylquinic acid, (+)-catechin, caffeine, epicatechin-3-gallate, (-)-epigallocatechin-3-gallate, 4-p-coumaroylquinic acid, quercetin-3-O-rutinoside, quercetin-3-galactoside, kampferol-3-O-glucoside, apigenin glycoside, theaflavin-3,3'-digallate, thaflavin-3-gallate and the kaempferol-rhamnose-hexose-rhamnose conjugate was confirmed based on fragmentation patterns available in literature.

Key words: green tea, bioactive compounds, qualitative analysis, ESI-MS method.

Introduction

It is well known that free radicals have a negative impact on a human organism through the membrane destabilization, DNA damage and the cell tissue [1], oxidation of low-density lipoproteins (LDL). The results of their effect are the occurrence of degenerative diseases and different forms of cancer. Strong antioxidants such as vitamin E, vitamin C and β -carotene in the standard form are recommended for the prevention. Recently, it was confirmed that some herbal polyphenols belong in the group of strong antioxidants. Tea (*Camelliae sinensis*) has a high content of polyphenol compounds [2, 3]. There are two kinds of tea, green tea and black tea. The composition of tea depends on climate conditions, soil, season, tea variety and the age of the leaf. Tea contains caffeine with small amounts of other xanthine alkaloids, large amounts of tannins or phenolic substances (5–27 %) consisting of catechins (epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate), proanthocyanidins, flavonols (myricetin, caempherol, quercetin) and gallic acid units. Epigallocatechin gallate is a predominant catechin. Its content varies in the range of 48–55 % of total polyphenols in green tea leaves. Graham has reported the composition of fresh green tea leaf (Table 1), as well as the composition of catechins in the fresh green tea leaf (Table 2) measured in percentage [4].

Table 1. The composition of the fresh green tea leaf

Compounds	Content, %	Compounds	Content, %
Polyphenols	36	Carbohydrates	25
Methyl xanthines	3.5	Protein	15
Amino acids	4	Lignin	6.5
Organic acids	1.5	Lipids	2
Carotenoids	<0.1	Chlorophyll	0.5
Volatiles	<0.1	Ash	5

Table 2. The composition of catechins in the fresh green tea leaf

Compounds	Content, %	Compounds	Content, %
(+)-Catechin	1-2	(+)-Gallocatechin	1-3
(-)-Epicatechin	1-3	(-)-Epigallocatechin	3-6
(-)-Epicatechin gallate	3-6	(-)-Epigallocatechin gallate	7-13

In literature, different methods have described the determination of bioactive compounds in a tea extract. A high performance liquid chromatographic method (HPLC) with UV detection is commonly used for the analysis of tea catechins, caffeine, quercetin and gallic acid [5-7], while Pelillo et al. analyzed tea catechins by using HPLC with UV and MS-electrospray detection [8, 9]. HPLC/MS can be successfully used in the characteriza-

*Author address: Ivan M. Savić, Faculty of Technology, University of Niš,
Bulevar oslobođenja 124, 16000 Leskovac, Serbia.

E-mail: ici_teh@yahoo.com

The manuscript received: April, 30, 2014.

Paper accepted: May, 28, 2014.

tion of flavan-3-ols, flavonoids, quinic esters of caffeine, thearubigins and alkaloids in different kinds of tea [10-12].

The pharmacological activity of polyphenols is important in the inhibition of enzymes (xanthine-oxidase and protein kinase C) responsible for forming superoxide anion radicals, but in the inhibition of cyclooxygenase, lipoxygenase, microsomal monooxygenase and glutathione S-transferase, mitochondrial succinate oxidase, NADH oxidase thus preventing the formation of reactive oxygen species and the development of tumor cells [13]. By blocking the enzyme urokinase, the green tea affects both levels of carcinogenesis, i.e. in the initiation phase and the promotion phase. The consumption of green tea reduces the risk of development of several cancers including colon, breast, ovarian, prostate and lung cancer [14-20].

Also, the polyphenols of green tea reduce the level of lipids, triglycerides and cholesterol in the blood, thereby reducing the risk of circulatory system diseases and atherosclerosis [21]. Tea contains around 5-7 % minerals, mainly potassium, copper, iron and manganese which have an important role in the human body. Tea also acts as a diuretic, helps against the cellulite formation because it limits the absorption of fat and stimulates microcirculation. The green tea extract is used for the skin care, oral cavity, growths on the skin, anti-dandruff in the hair, burns and for the treatment of acne.

One of the most active hydro-soluble flavonoids of green tea is quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-on) [22]. Quercetin has the properties of antihistaminic drugs [23-25] and it alleviates the allergic reactions and asthma attacks. Like most bioflavonoids, quercetin has the impact on the vasodilation of the blood vessels whereby it normalizes the blood pressure [26]. Based on its strong antioxidant [27] and anti-inflammatory [28, 29] activities, it succeeds in protecting the cell structure and the blood vessels from destructive effects of free radicals. In addition to all these activities, it has a strong anticancer activity [29] on different leukemia, breast, ovarian, gastric, liver, respiratory tract and colon tumor cells.

Quercetin can be used for destroying the tumor cells of the skin and prostate in combination with ultrasound without obvious normal cells damage [30]. Also, the treatment of chronic prostatitis and intestinal cystitis can be improved by using quercetin because it is inhibitor of mastocytes. In the market, quercetin is available in the form of tablets and capsules and commonly used in the combination with vitamin C and bromelain, which provide a better effect.

Because of these beneficial effects of quercetin on the human organism, the aim of the study was to obtain the green tea extract enriched with quercetin. In order to use the plant extract for preparing pharmaceutical formulations, it is necessary to investigate the pharmacological activities and it is also important to know the content of the obtained extracts. Thus, the aim of this

paper was also to tune the suitable MS-ESI method for the qualitative analysis of the extract.

Experimental

Reagents

Quercetin (Merck Chemicals Ltd., Notingham, United Kingdom), methanol LC-MS purity (Avantor Performance Materials, Inc., Deventer, Netherlands), formic acid ($\geq 99\%$) (Carlo Erba Reagents, Val de Reuil, France) and absolute ethanol (Alkaloid AD, Skopje, the Republic of Macedonia).

Plant material

Green tea (*Camelliae sinensis*) was purchased from Aleva AD (Novi Kneževac, Serbia). The plant material was dried at room temperature to the moisture content of 6 % in the darkness. After the mechanical disintegration into the laboratory mill, the average particle size of the plant material was 0.4 mm.

Extraction procedure

About 2.0 g of the plant material was transferred into the flask of 100 cm³ and covered with 36 cm³ of absolute ethanol. The extraction was carried out under reflux for 60 min at the boiling point of the extragent (78 °C). The constant temperature of the system was maintained using the water bath. After the extraction process, the solid phase was separated from the liquid phase by vacuum filtration. Then, the extragent was evaporated in the rotary evaporator at 50 °C. After that, the residual solvent was removed by drying the extract in the desiccator to the constant mass. Thus dried extract was further analyzed using the electron spray-mass spectrometry method (ESI-MS) in order to identify the bioactive compounds.

HPLC analysis of quercetin

The content of quercetin in the green tea extract was obtained by using the developed and validated RP-HPLC method [5]. The elution was isocratic with the flow rate of 1.0 cm³/min. The temperature of the system was maintained at 35 °C. The adequate separation was achieved by using methanol as a suitable mobile phase and ZORBAX Eclipse XDB-C18 column (4.6×250 mm, 5 µm), Agilent Technologies, the USA. Before injecting into the system, the sample and methanol were filtered through a 0.45 µm millipore filter (Econofilters, Agilent Technologies, Germany). The volume of the injected sample was 20 µL, and the wavelength for quercetin detection was 370 nm.

ESI-MS analysis

The scanning was carried out on MS spectrometer with ion trap (Thermo Scientific LTQ XL Linear Ion Trap Mass Spectrometer). By means of this device it was possible to identify the compounds of the green tea extract without their previous HPLC separation based on the literature data about the presence of components

[12]. After the ionization, the molecular ions of desired compounds isolated in the analyzer were treated with the adequate portion of the collision energy in order to obtain their MS² spectra. Mass spectra were recorded in negative and positive ionization modes depending on the nature of the analyzed substance. The applied conditions for the analysis were: the ion source voltage of 4.95 kV, the source current of 2.61 μ A, the capillary temperature of 275 °C, the capillary voltage of 50 V, the sheath gas pressure of 137 KPa and the auxiliary gas pressure of 13.9 KPa. ESI-MS method for the analysis of the green tea extract was tuned using the quercetin standard. Xcalibur software was used for the collection and the analysis of the data. The samples were ionized by using the electrospray (ESI) technique.

Preparation of the sample for MS analysis

The stock solution of quercetin standard and the green tea extract were prepared by dissolving per 25 mg of the samples in 25 cm³ of methanol. After that, the stock solutions were diluted to the concentration of 3 μ g/cm³ with the addition of acid in order to improve the ionization of the analyzed bioactive compounds. Thus obtained solutions were filtered through the filter of 0.45 μ m pore size (Econofilters, Agilent Technologies, Germany) and injected into the MS spectrometer.

Results and discussion

The investigated extract was obtained using absolute ethanol due to better solubility of quercetin in alcoholic solutions [31]. The quercetin content of 1.55 g per 100 g of the dried extract was determined by using RP-HPLC. The ESI-MS method was used for the determination of bioactive compounds in this extract. The electrospray parameter and collision offset were tuned and optimized using quercetin standard in order to favor the generation of the product ion at m/z 301. After optimization, in addition to quercetin, the method was able to confirm the presence of 17 phenol compounds. The presence of these compounds was confirmed based on the comparison of m/z values from the MS² spectra with the literature values for the standards [32]. A positive ionization mode was used for the identification of theobromine and caffeine, while the other compounds were determined in a negative ionization mode. The mass spectrum of quercetin was obtained after the effect of the collision energy of 15 eV and recording in a negative mode (Figure 1a). The peak at m/z 301 is the result of the molecular ion fragmentation. In accordance with literature, the ions at 273, 257, 179, 151 appeared after further fragmentation [33]. The mass spectrum of (+)-catechin is presented in Figure 1b. The peak of deprotonated ion $[M-H]^-$ occurs at m/z 289. The fragmented ions at m/z 271, 245, 205 and 179 were obtained after the effect of the collision energy of 20 eV [32]. It is possible that the ion at m/z 205 was obtained due to the loss of A-ring from the observed flavonoid, while the ion at m/z 245 can be the result of

losing CO₂ group or divalent -CH₂-CHOH- group. The fragments at m/z 271 and at m/z 179 are the results of losing water, i.e. B-ring from the molecule of flavonoid, respectively [34]. The mass spectrum of 5-O-galloylquinic acid was recorded in a negative ionization mode (Figure 1c). The basic deprotonated ion of 5-O-galloylquinic acid occurred at m/z 343, while the fragmented ions can be noticed at m/z 191 and 169. The obtained ions were formed after the effect of the collision energy of 25 eV.

Figure 1. Mass spectrum of: a) quercetin, b) (+)-catechin, c) 5-O-galloylquinic acid (negative ionization mode).

The precursor ion which gives a signal in the spectra at m/z 195 and the fragment ions at m/z 138 and 110 are the result of the fragmentation of caffeine (Figure 2a). The fragmented ion at m/z 138 probably occurs due to the loss of CH₃-N=C=O, where further loss of CO gives the signal at m/z 110 in the mass spectrum [35]. These ions were obtained by using the collision energy of 24 eV and by recording in a positive ionization mode. Mass spectrum of theobromine was also obtained by record-

ing in a positive mode, but after the effect of 16 eV. Its spectrum has a clearly visible parent ion at m/z 181 and the descendant ions at m/z 163, 138 and 108 (Figure 2b). Unlike the spectra of caffeine and theobromine, MS² spectrum of theaflavin-3-gallate was obtained by scanning in negative ionization. The collision energy was 24 eV. The signals of the descendant ions can be noticed at m/z 697, 577, 563, 545 and 407 (Figure 3b).

Figure 2. Mass spectrum of: a) caffeine, b) theobromine (positive ionization mode), c) theaflavin-3-gallate (negative ionization mode).

Gallic acid was successfully identified by scanning in a negative mode. In the mass spectrum, the characteristic signal at m/z 169 originates from $[M-H]^-$ ion (Figure 3a). The signal at m/z 125 is probably the descendant ion which occurred after the loss of CO_2 . In this case, the collision energy was 19 eV, while the mass spectrum of (-)-galocatechin was recorded in a negative mode using the collision energy of 20 eV (Figure 3b). The basic fragmented ion at m/z 305 gives the deprotonated ions at m/z 261, 221, 219, 179 and 165 due to the loss of CO_2 , $C_4H_4O_2$, $C_6H_6O_3$, $C_7H_6O_3$ and $C_7H_8O_3$, respectively [36].

The loss of $C_4H_4O_2$ and $C_4H_6O_2$ is the result of cleaving the A ring of flavan-3-ol. The Retro Diels-Alder rearrangement leads to the loss of $C_7H_6O_3$ and $C_7H_8O_3$, while the rearrangement of the heterocyclic ring leads to the loss of $C_6H_6O_3$ [37]. 3-O-Caffeoylquinic acid has the molecular ion at m/z 353, which gives the fragmented ions at m/z 191, 179, 173 and 135 in the mass spectrum after the effect of 30 eV collision energy (Figure 3c). In the spectrum, the signal at m/z 191 corresponds to quinic acid [38], while the ion at m/z 179 originates from the ion of caffeic acid [39, 40].

Figure 3. Mass spectrum of: a) gallic acid, b) (-)-galocatechin, c) 3-O-caffeoylquinic acid (negative ionization mode).

The mass spectrum of (-)-epigallocatechin-3-gallate is presented in Figure 4a. The peak at m/z 457 corresponds to the parent ion $[M-H]^-$. MS² fragmented ions with the adequate signals at m/z 331, i.e. m/z 305 originate from epigallocatechin, while the signal at m/z 169 originates from gallic acid [41]. 4-*p*-Coumaroylquinic acid gives the peak at m/z 337 after deprotonation (Figure 4b). The descendant ion at m/z 191 originates from

the deprotonated quinic acid, which was formed due to the loss of caffeic acid. The signal at m/z 173 appeared by further losing the water [42]. In addition to the deprotonated ion at m/z 441, the mass spectrum of (-)-epicatechin-3-gallate has the fragmented ion at m/z 289 which is the result of the ester bond cleavage and dissociation of gallic acid (Figure 4c). In the mass spectrum, the peak at m/z 169 is obtained by cleavage of the ester bond and dissociation of (-)-epicatechin units [36].

Figure 4. Mass spectrum of: a) (-)-epigallocatechin-3-gallate, b) 4-p-coumaroylquinic acid, c) epicatechin-3-gallate (negative ionization mode).

The mass spectrum of quercetin-3-O-rutinoside scanned in a negative ionization mode is presented in Figure 5a. The basic ion of quercetin-3-O-rutinoside at m/z 609 gives the signals at m/z 463 after losing rhamnose. The signal at m/z 301 occurs by further removing glucose. MS² spectrum of quercetin-3-O-galactoside was obtained by recording in a negative ionization mode after the effect of 20 eV collision energy (Figure 5b). The basic peak at m/z 463 gives the signal at m/z 301 due to the loss of the galactose molecule. The mass spectrum

of conjugate kaempferol-rhamnose-hexose-rhamnose obtained after recording in a negative mode and using the collision energy of 23 eV is presented in Figure 5c. In addition to the deprotonated ion $[M-H]^-$ at m/z 739, the fragmented ions at m/z 593 ($[M-H]^-$ - rhamnose) and m/z 431 ($[M-H]^-$ - rhamnose-hexose) are also obtained in the mass spectrum. The peak at m/z 285 corresponds to the deprotonated molecule of kaempferol [12].

Figure 5. Mass spectrum of: a) quercetin-3-O-rutinoside, b) quercetin-3-O-galactoside, c) conjugate kaempferol-rhamnose-hexosa-rhamnose (negative ionization mode).

The mass spectrum of kaempferol-3-O-glucoside obtained using the collision energy of 23 eV is presented in Figure 6a. The parent ion gives the signal at m/z 447, and the fragmented ion gives the signal at m/z 285 due to the loss of glucose. Apigenin and luteolin present the important aglycones which are the main part of most glycosylflavonoids. Based on the obtained fragmentation in the mass spectrum, it can be concluded that apigenin glycoside is present in the investigated extract (Figure 6b).

The molecule of apigenin glycoside has the molecular ion at m/z 563 and the fragmented ions at m/z 503, 473, 443 and 353. The ions at m/z 443, 473 and 503 especially indicate the presence of substituted pentose [41]. The mass spectrum of theaflavin-3,3'-digallate was obtained by scanning in a negative ionization mode and using the collision energy of 20 eV (Figure 6c). Theaflavin-3,3'-digallate gives the deprotonated ion at m/z 867 and the fragmented ions with the characteristic signals at m/z 823, 715, 697, 545 and 527 in the mass spectrum.

Figure 6. Mass spectrum of: a) kaempferol-3-O-glucoside, b) apigenin glycoside, c) theaflavin-3,3'-digallate (negative ionization mode).

Conclusion

In this paper, the ESI-MS method was successfully tuned for the qualitative analysis of the green tea extract enriched with quercetin. The following bioactive compounds: gallic acid, (-)-gallocatechin, 3-O-caffeoylquinic acid, theobromine, 5-O-galloylquinic acid, (+)-catechin, caffeine, epicatechin-3-gallate, (-)-epigallocatechin-3-gallate,

4-p-coumaroylquinic acid, quercetin-3-O-rutinoside, quercetin-3-galactoside, kampferol-3-O-glucoside, apigenin glycoside, theaflavin-3,3'-digallate, theaflavin-3-gallate and the kaempferol-rhamnose-hexose-rhamnose conjugates were identified in the plant extract. Due to the presence of different polyphenolic compounds, the obtained extract can be used for preparing the formulations with the expressed pharmacological activity. Thus, the aim of further investigation will be the implementation of pharmacological tests in order to define the pharmaceutical activities of the green tea extract.

Acknowledgments

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia under the project TR-34012.

References

- [1] J. Moskovitz, K. A. Yim, P. B. Chock, Free radicals and disease, *Archives of Biochemistry and Biophysics*, 397(2) (2002) 354-359.
- [2] I. M. Savić, V. D. Nikolić, I. M. Savić, Lj. B. Nikolić, M. Z. Stanković, K. Moder, Optimization of total flavonoid compound extraction from *Camellia sinensis* using the artificial neural network and response surface methodology, *Chemical Industry*, 67(2) (2013) 249-259.
- [3] C. Cabrera, R. Artacho, R. Giménez, Beneficial effects of green tea – A review, *Journal of the American College of Nutrition*, 25(2) (2006) 79-99.
- [4] H. N. Graham, Green tea composition, consumption, and polyphenol chemistry, *Preventive Medicine*, 21(3) (1992) 334-350.
- [5] I. M. Savic, V. D. Nikolic, I. M. Savic, Lj. B. Nikolic, M. Z. Stankovic, Development and validation of a new RP-HPLC method for determination of quercetin in green tea, *Journal of Analytical Chemistry*, 68(10) (2013) 906-911.
- [6] H. Wang, K. Helliwell, X. You, Isocratic elution system for the determination of catechins, caffeine and gallic acid in green tea using HPLC, *Food Chemistry*, 68(1) (2000) 115-121.
- [7] Y. Zuo, H. Chen, Y. Deng, Simultaneous determination of catechins, caffeine and gallic acids in green, Oolong, black and pu-erh teas using HPLC with a photodiode array detector, *Talanta*, 57(2) (2002) 307-316.
- [8] M. Pelillo, B. Biguzzi, A. Bendini, T.G. Toschi, M. Vanzini, G. Lercker, Preliminary investigation into development of HPLC with UV and MS-electrospray detection for the analysis of tea catechins, *Food Chemistry*, 78(3) (2002) 369-374.
- [9] M. Pelillo, M. Bonoli, B. Biguzzi, A. Bendini, T. G. Toschi, G. Lercker, An investigation in the use of HPLC with UV and MS-electrospray detection for the quantification of tea catechins, *Food Chemistry*, 87(3) (2004) 465-470.
- [10] G. Beretta, S. Furlanetto, L. Regazzoni, M. Zarrella, R. M. Facino, Quenching of α , β -unsaturated aldehydes by green tea polyphenols: HPLC-ESI-MS/MS studies, *Journal of Pharmaceutical and Biomedical Analysis*, 48(3) (2008) 606-611.
- [11] M. N. Clifford, S. Stoupi, N. Kuhnert, Profiling and Characterization by LC-MS n of the Galloylquinic Acids

- of Green Tea, Tara Tannin, and Tannic Acid, *Journal of Agricultural and Food Chemistry*, 55(8) (2007) 2797-2807.
- [12] D. Del Rio, A. J. Stewart, W. Mullen, J. Burns, M. E. Lean, F. Brighenti, A. Crozier, HPLC-MSn analysis of phenolic compounds and purine alkaloids in green and black tea, *Journal of Agricultural and Food Chemistry*, 52(10) (2004) 2807-2815.
- [13] V. E. Steele, G. J. Kelloff, D. Balentine, C. W. Boone, R. Mehta, D. Bagheri, C. C. Sigman, S. Zhu, S. Sharma, Comparative chemopreventive mechanisms of green tea, black tea and selected polyphenol extracts measured by in vitro bioassays, *Carcinogenesis*, 21(1) (2000) 63-67.
- [14] X. Zhang, D. Albanes, W. L. Beeson, P. A. van den Brandt, J. E. Buring, A. Flood, J. L. Freudenheim, E. L. Giovannucci, R. A. Goldbohm, K. Jaceldo-Siegl, E. J. Jacobs, V. Krogh, S. C. Larsson, J. R. Marshall, M. L. McCullough, A. B. Miller, K. Robien, T. E. Rohan, A. Schatzkin, S. Sieri, D. Spiegelman, J. Virtamo, A. Wolk, W. C. Willett, S. M. Zhang, S. A. Smith-Warner, Risk of colon cancer and coffee, tea, and sugar-sweetened soft drink intake: Pooled analysis of prospective cohort studies, *Journal of the National Cancer Institute*, 102(11) (2010) 771-783.
- [15] M. J. Shrubsole, W. Lu, Z. Chen, X. O. Shu, Y. Zheng, Q. Dai, Q. Cai, K. Gu, Z. X. Ruan, Y. T. Gao, W. Zheng, Drinking green tea modestly reduces breast cancer risk, *Journal of Nutrition*, 139(2) (2009) 310-316.
- [16] A. A. Ogunleye, F. Xue, K. B. Michels, Green tea consumption and breast cancer risk or recurrence: A meta-analysis, *Breast Cancer Research and Treatment*, 119(2) (2010) 477-484.
- [17] Q. Dai, X. O. Shu, H. Li, G. Yang, M. J. Shrubsole, H. Cai, B. Ji, W. Wen, A. Franke, Y. T. Gao, W. Zheng, Is green tea drinking associated with a later onset of breast cancer?, *Annals of Epidemiology*, 20(1) (2010) 74-81.
- [18] C. M. Nagle, C. M. Olsen, C. J. Bain, D. C. Whiteman, A. C. Green, P. M. Webb, Tea consumption and risk of ovarian cancer, *Cancer Causes and Control*, 21(9) (2010) 1485-1491.
- [19] N. Kurahashi, S. Sasazuki, M. Iwasaki, M. Inoue, S. Tsugane, Green tea consumption and prostate cancer risk in Japanese men: a prospective study, *American Journal of Epidemiology*, 167(1) (2008) 71-77.
- [20] N. Tang, Y. Wu, B. Zhou, B. Wang, R. Yu, Green tea, black tea consumption and risk of lung cancer: a meta-analysis, *Lung Cancer*, 65(3) (2009) 274-283.
- [21] A. Larson, D. Symons, T. Jalili, Quercetin: A Treatment for Hypertension? – A Review of Efficacy and Mechanisms, *Pharmaceuticals*, 3(1) (2010) 237-250.
- [22] T. Z. Nurulain, Green tea and its polyphenolic catechins: Medicinal uses in cancer and noncancer applications, *Life Sciences*, 78 (2006) 2073-2080.
- [23] D. Kempuraj, B. Madhappan, S. Christodoulou, W. Boucher, J. Cao, N. Papadopoulou, C. L. Cetrulo, T. C. Theoharides, Flavonols inhibit proinflammatory mediator release, intracellular calcium ion levels and protein kinase C theta phosphorylation in human mast cells, *British Journal of Pharmacology*, 145(7) (2005) 934-944.
- [24] Y. D. Min, C. H. Choi, H. Bark, H. Y. Son, H. H. Park, S. Lee, J. W. Park, E. K. Park, H. I. Shin, S. H. Kim, Quercetin inhibits expression of inflammatory cytokines through attenuation of NF- κ B and p38 MAPK in HMC-1 human mast cell line, *Inflammation Research*, 56 (2007) 210-215.
- [25] D. Kempuraj, M. L. Castellani, C. Petrarca, S. Frydas, P. Conti, T. C. Theoharides, J. Vecchiet, Inhibitory effect of quercetin on tryptase and interleukin-6 release, and histidine decarboxylase mRNA transcription by human mast cell-1 cell line, *Clinical and Experimental Medicine*, 6(4) (2006) 150-156.
- [26] F. Pérez-Vizcaino, M. Ibarra, A. L. Cogolludo, J. Duarte, F. Zaragoza-Arnáez, L. Moreno, G. López-López, J. Tamargo, Endothelium-independent vasodilator effects of the flavonoid quercetin and its methylated metabolites in rat conductance and resistance arteries, *Journal of Pharmacology and Experimental Therapeutics*, 302(1) (2002) 66-72.
- [27] M. Zhang, S. G. Swarts, L. Yin, C. Liu, Y. Tian, Y. Cao, M. Swarts, S. Yang, S. B. Zhang, K. Zhang, S. Ju, D. J. Olek, L. Schwartz, P. C. Keng, R. Howell, L. Zhang, P. Okunieff, Antioxidant properties of quercetin, *Advances in Experimental Medicine and Biology*, 701 (2011) 283-289.
- [28] A. W. Boots, L. C. Wilms, E. L. Swennen, J. Kleinjans, A. Bast, G. R. Haenen, In vitro and ex vivo anti-inflammatory activity of quercetin in healthy volunteers, *Nutrition*, 24(7) (2008) 703-710.
- [29] U. J. Joshi, A. S. Gadge, P. D'Mello, R. Sinha, S. Srivastava, G. Govil, Anti-inflammatory, antioxidant and anticancer activity of quercetin and its analogues, *International Journal of Research in Pharmaceutical and Biomedical Sciences*, 2(4) (2011) 1757-1766.
- [30] S. Paliwal, J. Sundaram, S. Mitragotri, Induction of cancer-specific cytotoxicity towards human prostate and skin cells using quercetin and ultrasound, *British Journal of Cancer*, 92(3) (2005) 499-502.
- [31] R. S. Razmara, A. Daneshfar, R. Sahraei, Solubility of quercetin in water+ methanol and water+ ethanol from (292.8 to 333.8) K, *Journal of Chemical and Engineering Data*, 55(9), (2010) 3934-3936.
- [32] M. N. Bravo S. Silva, A. V. Coelho, L. V. Boas, M. R. Bronze, Analysis of phenolic compounds in Muscatel wines produced in Portugal, *Analytica Chimica Acta*, 563(1) (2006) 84-92.
- [33] W. Mullen, T. Yokota, M. E. Lean, A. Crozier, Analysis of ellagitannins and conjugates of ellagic acid and quercetin in raspberry fruits by LC-MSn, *Phytochemistry*, 64(2) (2003) 617-624.
- [34] W. M. Stöggli, C. W. Huck, G. K. Bonn, 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*, 27(7-8) (2004) 524-528.
- [35] B. A. Collings, M. A. Romaschin, MS/MS of Ions in a low pressure linear ion trap using a pulsed gas, *Journal of the American Society for Mass Spectrometry*, 20(9) (2009) 1714-1717.
- [36] J. Sun, F. Liang, Y. Bin, P. Li, C. Duan, Screening non-colored phenolics in red wines using liquid chromatography/ultraviolet and mass spectrometry/mass spectrometry libraries, *Molecules*, 12(3) (2007) 679-693.
- [37] M. Monagas, R. Suárez, C. Gómez-Cordovés, B. Bartolomé, Simultaneous determination of nonanthocyanins in phenolic compounds in wines by HPLC-DAD/ESI-MS, *American Journal of Enology and Viticulture*, 56(2) (2005) 139-147.
- [38] L. Barros, M. Dueñas, A. M. Carvalho, I. C. Ferreira, C. Santos-Buelga, Characterization of phenolic compounds in flowers of wild medicinal plants from Northeastern Portugal, *Food and Chemical Toxicology*, 50(5) (2012)

- 1576-1582.
- [39] M. N. Clifford, K. L. Johnston, S. Knight, N. Kuhnert, Hierarchical scheme for LC–MSn identification of chlorogenic acids, *Journal of Agricultural and Food Chemistry*, 51(10) (2003) 2900-2911.
- [40] M. N. Clifford, S. Knight, N. Kuhnert, Discriminating between the six isomers of dicaffeoylquinic acid by LC–MSn, *Journal of Agricultural and Food Chemistry*, 53(10), 2005, 3821-3832.
- [41] Y. Kim, K. L. Goodner, J. D. Park, J. Choi, S. T. Talcott, Changes in antioxidant phytochemicals and volatile composition of *Camellia sinensis* by oxidation during tea fermentation, *Food Chemistry*, 129(4) (2011) 1331-1342.
- [42] A. M. Nandutu, M. Clifford, N. K. Howell, Analysis of phenolic compounds in Ugandan sweet potato varieties (NSP, SPK AND TZ), *African Journal of Biochemistry Research*, 29-36.

Izvod

KVALITATIVNA ANALIZA EKSTRAKTA ZELENOG ČAJA PRIMENOM ESI-MS METODE

Ivan M. Savić¹, Vesna D. Nikolić¹, Ivana M. Savić¹, Ljubiša B. Nikolić¹, Marko D. Jović², Maja D. Jović²

(ORIGINALNI NAUČNI RAD)
UDK 663.951:66.061:543.4

¹Tehnološki fakultet, Univerzitet u Nišu, Leskovac, Srbija

²Medicinski fakultet, Univerzitet u Nišu, Niš, Srbija

Cilj ovog rada bio je da se identifikuju bioaktivna jedinjenja u ekstraktu zelenog čaja obogaćenog kvercetinom. Ekstrakcija je vršena pod refluks u toku 60 min primenom apsolutnog etanola pri odnosu biljne sirovine i rastvarača 1:18 (m/v). Za identifikaciju komponenata primenjena je metoda masene spektrometrije sa elektron sprej interfejsom, koja je podešena u odnosu na standard kvercetina. Maseni spektri snimani su u negativnom i pozitivnom jonizacionom modu sa naponom jonskog izvora 4,95 kV, jonskom strujom 2,61 μ A, temperaturom kapilare 275 °C, naponom kapilare 50 V, pritiskom gasa u omotaču 137 KPa i pritiskom pomoćnog gasa 13,9 KPa. Pored kvercetina u ekstraktu je identifikovano prisustvo galne kiseline, (-)-galokatehina, 3-O-kofeoilhininske kiseline, teobromina, 5-O-galoilhininske kiseline, (+)-katehina, kofeina, epikatehin-3-galata, (-)-epigalokatehin-3-galata, 4-p-kumaroilhininske kiseline, kvercetin-3-O-rutinozida, kvercetin-3-O-galaktozida, kamferol-3-O-glukozida, apigenin glikozida, teaflavin-3,3'-digalata, teaflavin-3-galata, konjugata kemferol-ramnoze-heksoze-ramnoze na osnovu literaturno dostupnih fragmentacionih šema.

Ključne reči: zeleni čaj, bioaktivna jedinjenja, kvalitativna analiza, ESI-MS metod.