



Quantification of some phenolic acids in the leaves of *Melissa officinalis* L. from Turkey and Bosnia

Ibragić, S.^a, Salihović, M.^a, Tahirović, I.^{a*}, Toromanović, J.^b

^aUniversity of Sarajevo, Faculty of Science, Department of Chemistry, Zmaja od Bosne 33-35, 71000 Sarajevo, Bosnia and Herzegovina

^bUniversity in Bihać, School of Medical Studies, Žegarska aleja bb, 77 000, Bihać, Bosnia and Herzegovina

Article info

Received: 17/04/2014
Accepted: 12/06/2014

Keywords:

Melissa officinalis
caffeic acid
chlorogenic acid
rosmarinic acid
gallic acid
HPLC-ED

*Corresponding author:

E-mail: itah@pmf.unsa.ba
Phone: 00-387-33-279905
Fax: 00-387-33-649359

Abstract: *Melissa officinalis* L. (lemon balm) contains many potentially biologically active compounds, including the caffeic acid (CA), chlorogenic acid (CGA), rosmarinic acid (RA), succinic acid, ursolic acid and thymol. In this study, using the HPLC-ED system, determinations of gallic acid (GA), CGA, RA and CA were performed in hot water extracts of lemon balm. Analyses of GA, CGA, CA and RA were performed in supernatants of lemon balm leaf extracts. The standard solutions of GA, CGA, CA and RA were prepared by dissolving pure substances in the mobile phase. The lemon balm leaves (fresh weight) from Bosnia contained 0.38 mg/g GA, 0.25 mg/g CGA, 0.14 mg/g CA and 5.10 mg/g RA. The lemon balm leaves from Turkey had a higher CA content (0.71 mg/g), while the GA, CGA and RA content was lower (0.22 mg/g, 0.23 mg/g and 0.24 mg/g, respectively). Having many beneficial properties due to the phenolic compounds, lemon should be used as a supplement to a balanced diet.

INTRODUCTION

Melissa officinalis L., commonly known as lemon balm, is a perennial and aromatic herb species of the Lamiaceae family that is native to the Mediterranean region. Due to its ample beneficial properties it is now commercially cultivated worldwide (Shoor, Mondani, Aliverdi et al., 2012). Plants of Lamiaceae family have been used in traditional medicine for treatment of depression, memory enhancement, circulation improvement and indigestion (Shekarchi, Hajimehdipoor, Saeidnia et al., 2012). These plants have shown antioxidant, anti-inflammatory, even anti-carcinogenic properties. Lemon balm is used for several purposes such as an additive in food, a herbal tea, an ingredient in cosmetics, an ornamental and a medicinal plant. It is an aromatic, cooling, sedative herb that lowers fever, improves digestion, relaxes spasms and peripheral blood vessels, and inhibits thyroid activity (Cosge, Ipek and Gurbuz, 2009). In addition, it might present a natural treatment for Alzheimer's disease by amelioration of cognition

(Obulesu and Rao, 2011). Other neurological activities include the inhibition of MAO-A and acetylcholinesterase enzymes and affinity to the GABA A-benzodiazepine receptor (Lopez, Martin, Gomez-Serranillos et al., 2009). Most of its medicinal properties the lemon balm owes to a range of different phenolic compounds. Phenolics are characterized by at least one aromatic ring bearing one or several hydroxyl groups. They are mainly synthesized from cinnamic acid, which is formed from phenylalanine (Michalak, 2006). Phenolic compounds are widely distributed in the plant kingdom and are the most abundant secondary metabolites of plants, with more than 8,000 phenolic structures currently known. They are ubiquitous in all plant organs and are therefore an integral part of the human diet. Plant phenolics include phenolic acids, flavonoids, tannins and the less common stilbenes and lignans. Phenolic acids can be divided into two classes: derivatives of benzoic acid such as gallic acid (GA), and derivatives of cinnamic acid such as caffeic acid (CA) (Dai and Mumper, 2010). Phenolic acids account for

about one third of the total dietary intake of polyphenols. Lemon balm is rich in phenolic acids such as GA, chlorogenic acid (CGA), CA and rosmarinic acid (RA). Gallic acid is a trihydroxybenzoic acid, often used as a standard for determining the phenol content by the Folin-Ciocalteu assay where results are being expressed in GA equivalents. Having strong antioxidant properties, GA plays a protective role in oxidative stress. It seems to show anti-viral and anti-fungal effects. It has been shown that aqueous extracts from Lamiaceae can drastically and rapidly reduce the infectivity of HIV-1 at non-cytotoxic concentrations (Geuenich, Goffinet, Venzke et al, 2008). Caffeic acid is often esterified with quinic acid as in CGA, which is the major phenolic compound in coffee. Caffeic acid is known to have a broad spectrum of pharmacological activities including anti-inflammatory, antioxidant, immunomodulatory and antiviral (Pari and Karthikesan, 2007). Besides its well-known antioxidant activity, CA inhibits certain enzyme activities such as lipoxygenases, cyclooxygenase, glutathione S-transferase, and xanthine oxidase. It has also been reported to have antitumor activity, anti-inflammatory properties and to inhibit HIV replication (Chung, Moon, Chang et al, 2004). Both, CA and CGA are antioxidants and might inhibit the formation of mutagenic and carcinogenic N-nitroso compounds. They also inhibit the oxidation of LDL and might therefore contribute to the prevention of cardiovascular disease (Olthof, Hollman and Katan, 2001). Results of *in vivo* microdialysis demonstrated the stress relaxing effect of caffeine and CGA on the serotonin and dopamine levels in the rat hippocampus (Pavlica and Gebhardt, 2005). RA, an ester of CA and 3,4-dihydroxyphenyllactic acid was isolated for the first time from *Rosmarinus officinalis* L. Studies suggest that RA is the phenolic acid that is responsible for most of the antioxidant activities of Lamiaceae species extracts. That activity may be even stronger than that of vitamin E or Trolox. The reason could be in the presence of four hydroxyl groups in the molecule of RA. Other phenolic acids have only one or two hydroxyl groups and are not present in sufficient amounts (Caniova and Brandsteterova, 2001). It was also demonstrated that RA is the major compound that drives anxiolytic and antidepressant - like properties of lemon balm (Taiwo, Leite, Lucena et al, 2012). RA has been reported to exert anti-carcinogenic and anti-allergic activities *in vivo* and *in vitro*. Antiviral and antibacterial properties have been demonstrated *in vitro* (Shekarchi et al., 2012).

EXPERIMENTAL

Chemicals

Methanol (HPLC grade) was purchased from Merck, Germany; acetonitrile and glacial acetic acid from Panreac, Spain; the standards for GA, CGA, CA and RA from Sigma Aldrich, Germany.

Chromatographic system

The liquid chromatographic system consisted of a Shimadzu LC-20AT pump (Kyoto, Japan), BAS Liquid Chromatography CC-5E, LC-4C amperometric detector

with a glassy carbon working electrode (serial), and a reversed-phase column (ODS Hypersil, 5µm 250 x 4,6 mm, Phenomena). The mobile phase had the following composition: methanol + acetonitrile + water + glacial acetic acid (20+10+70+1). The mobile phase was degassed and filtered through 0.45 µm x 47 mm membrane filters before use. The flow rate was maintained at 1 ml/min. The range detector was 50 nA, the applied potential was + 0.84 V versus the Ag/AgCl reference electrode, the injection volume was 20 µL. The analysis was performed at 25 °C.

Sample preparation

Lemon balm leaves were collected in Bosnia and Herzegovina and Turkey and analyzed for content for the following compounds: GA, CGA, CA and RA. Air dried and powdered herbs (1 g) were extracted with hot water (9 ml) at room temperature for 30 minutes. The plant extract was filtered through a gauze for purification purposes. Afterwards, 1 ml of that extract was centrifuged for 20 minutes, at 15,000 rpm and + 4 °C (Micro centrifuge, Hettich; Micro 22R). The obtained supernatants were diluted 1/100 with high-purity water. The supernatants were stored at - 20 °C until analysis. A portion (20 µl) of the resulting prepared sample was applied directly to the HPLC system. The standard solutions for GA, CGA, CA and RA were 0.001 mg/ml, 0.001 mg/ml, 0.003 mg/ml and 0.005 mg/ml, respectively. Their injection volumes were 20 µl.

RESULTS

The quantification was done using LabSolution software of Shimadzu (Kyoto, Japan). Concentrations of GA, CGA, CA and RA were calculated using the equation:

$$\text{Compound}_{(\text{mg/ml})} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \cdot \gamma_{(\text{mg/ml})}$$

A_{sample} - peak area for plant sample with the same retention time as standard

A_{standard} - peak area of the standard

γ - concentration of the standard

The qualitative determination of the listed compounds in samples was based on the comparison of retention times obtained from different extracts of examined plants with the retention time of the corresponding standard. According to the acquired chromatograms, the retention times of GA, CA, CGA, and RA were 3.5 min, 6.9 min, 9.6 min, and 23.6 min, respectively. The list of compounds that were analyzed in plants extracts is shown in Table 1.

Table 1: Content of GA, CGA, CA and RA (mg/g of fresh weight) in lemon balm leaves.

Country /Content (mg/g)	GA	CGA	CA	RA
Bosnia and Herzegovina	0.38	0.25	0.14	5.10
Turkey	0.22	0.23	0.71	0.24

Comparison of phenolic acids content (mg/g of fresh weight) found in lemon balm samples collected in Bosnia and Herzegovina and Turkey is shown in Figure 1. Representative chromatograms of water extracts of

lemon balm from Bosnia and Herzegovina and Turkey are shown in Figure 2 and 3.

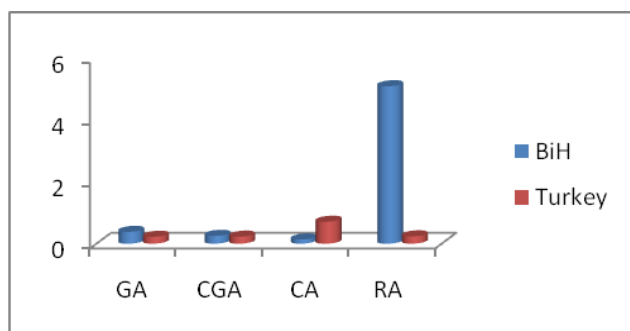


Figure 1: Comparison of phenolic acids content (mg/g of fresh weight) found in lemon balm samples collected in Bosnia and Herzegovina and Turkey.

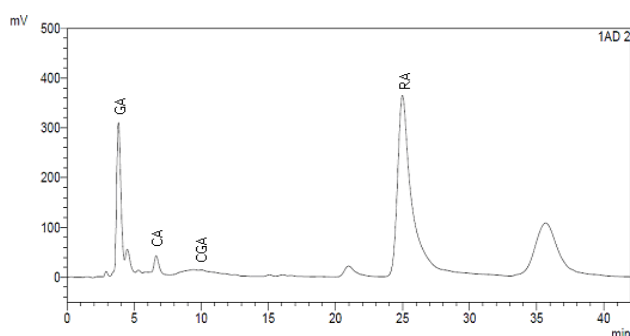


Figure 2: Chromatogram of a lemon balm extract sample from Bosnia and Herzegovina.

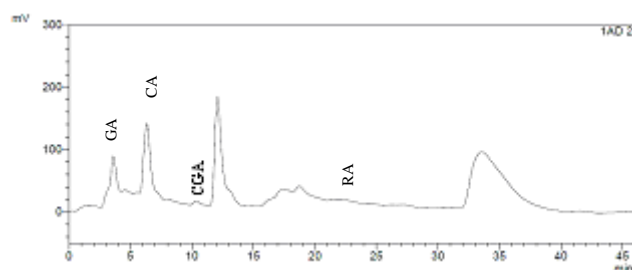


Figure 3: Chromatogram of a lemon balm extract sample from Turkey.

DISCUSSION

Plants are valuable sources of medicinal compounds that contain a broad spectrum of biological activities. Approximately 25 - 50% of current pharmaceuticals are derived from plants and show lesser side effects than the synthetic drugs. The therapeutic properties of lemon balm are mainly ascribed to its content of phenolic compounds. Having antioxidant activities, phenolic compounds show protective effects in terms of cardiovascular, neurodegenerative diseases and even cancer as all these states are linked to oxidative stress. Furthermore, phenolics were found to modulate the activity of a wide range of enzymes and cell receptors. Only recently, the health effects of dietary polyphenols have come to the attention of nutritionists (Dai and Mumper, 2010). Phenolics can be extracted from fresh, frozen or dried plant samples. Usually before extraction plant samples are treated by milling, grinding and homogenization, which may be preceded by air-drying

or freeze-drying. It is generally known that the yield of chemical extraction depends on the type of solvents with varying polarities. Methanol has been generally found to be more efficient in extraction of lower molecular weight polyphenols while the higher molecular weight flavanols are better extracted with acetone. Even though extraction temperature can promote the extraction rate, long extraction times and high temperature increase the chance of oxidation of phenolics which decrease the yield of phenolics in the extracts. Specifically, heating tends to hydrolyse RA, which produces CA and results in substantial losses (Caniova and Brandsteterova, 2001). HPLC currently represents the most popular and reliable technique for analysis of phenolic compounds. Acetonitrile and methanol are the most commonly used organic modifiers (Dai and Mumper, 2010). In this study, the mobile phase was acidified with glacial acetic acid to minimize peak tailing. The GA, CGA, CA and RA content in lemon balm samples collected in Bosnia and Herzegovina were 0.38, 0.25, 0.14 and 5.10 mg/g, respectively. The lemon balm leaves from Turkey had a higher CA content (0.71 mg/g), while the GA, CGA and RA content was lower (0.22, 0.23 and 0.24 mg/g, respectively). Overall, plant samples collected in Bosnia and Herzegovina proved to be richer in the phenolic acids content. The lemon balm leaves from Turkey exceeded in the CA content, only. Lemon balm from Bosnia and Herzegovina may exert therapeutic activities more intensively than lemon balm from Turkey. Among the phenolic compounds of interest, RA content was the highest. Being particularly important in preventing oxidative stress and associated diseases, lemon balm with high RA content should be considered a part of regular diet. It should be stressed that comparisons of same plant species from different regions may result in imprecision as the composition of a plant is known to depend considerably on extrinsic and intrinsic factors including soil and climatic conditions, plant ontogenesis phases, harvest and plant storage (Shekarchi et al., 2012). In addition, Rusaczonok, Świdorski and Waszkiewicz-Robak (2010) have previously concluded that it would be difficult to compare results obtained by different studies as there are different approaches in extraction procedures, analytical methods and mathematical calculations.

CONCLUSIONS

The obtained results indicate that hot water extracts of lemon balm leaves collected in Turkey and Bosnia and Herzegovina show different contents of phenolic acids analyzed in this study. Overall, samples collected in Bosnia and Herzegovina had a higher content of gallic acid, chlorogenic and rosmarinic acid. In different regions, lemon balm varies in its phenolic content, yet it remains a global natural remedy for infections, indigestion, depression and anxiety, spasms and diseases related to oxidative stress and a source of novel therapeutical tools.

REFERENCES

- Caniova, A., Brandsteterova, E. (2001). HPLC analysis of phenolic acids in *Melissa officinalis*. *J Liq Chrom & Rel Technol*, 24 (17), 2647–2659.
- Chung, T. W., Moon, S. K., Chang, Y. C., Cho, G., Kim, S. H., Kim, J. G., Kim, C. H. (2004). Novel and therapeutic effect of caffeic acid and caffeic acid phenyl ester on hepatocarcinoma cells: complete regression of hepatoma growth and metastasis by dual mechanism. *FASEB J Research Communication*, 18 (14), 1670-1681.
- Cosge, B., Ipek, A., Gurbuz, B. (2009). GC/MS Analysis of Herbage Essential Oil from Lemon Balms (*Melissa officinalis* L.) Grown in Turkey. *J Appl Biol Sci*, 3 (2), 136-139.
- Dai, J., Mumper, R. J. (2010). Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. *Molecules*, 15, 7313-7352.
- Geuenich, S., Goffinet, C., Venzke, S., Nolkemper, S., Baumann, I., Plinkert, P., Reichling, J., Keppler, O. T. (2008). Aqueous extracts from peppermint, sage and lemon balm leaves display potent anti-HIV-1 activity by increasing the virion density. *Retrovirology*, (5) 27, 1-16.
- Lopez, V., Martin, S., Gomez-Serranillos, M. P., Carretero, M. E., Jager, A. K., Calvo, M. I. (2009). Neuroprotective and Neurological Properties of *Melissa officinalis*. *Neurochem Res*, 34, 1955–1961.
- Michalak, A. (2006). Phenolic Compounds and Their Antioxidant Activity in Plants Growing under Heavy Metal Stress. *Polish J of Environ Stud*, 15 (4), 523-530.
- Obulesu, M., Rao, D. M. (2011). Neuroprotective Effect of Traditional Japanese-Chinese, Korean, and European Plant Extracts: An insight into therapeutic avenues. *J Neurosci Rural Pract*, 2: 56-61.
- Olthof, M. R., Hollman, P. C. H., Katan, M. B. (2001) Chlorogenic Acid and Caffeic Acid Are Absorbed in Humans. *J Nutr*, 131: 66–71.
- Pari, L., Karthikesan, K. (2007). Protective role of caffeic acid against alcohol-induced biochemical changes in rats. *Fundam Clin Pharmacol*, 21, 355–361.
- Pavlica, S., Gebhardt, R. (2005). Protective effects of ellagic and chlorogenic acids against oxidative stress in PC12 cells. *Free Rad Res*, 39 (12), 1377–1390.
- Rusaczonok, A., Świdorski, F., Waszkiewicz-Robak, B. (2010) Antioxidant properties of tea and herbal infusions – a short report. *Pol J Food Nutr Sci*, 60 (1): 33-35.
- Shekarchi, M., Hajimehdipoor, H., Saeidnia, S., Gohari, A. R. (2012). Morteza Pirali Hamedani. Comparative study of rosmarinic acid content in some plants of Labiatae family. *Pharmacogn Mag*, (8) 29, 37-41.
- Shoor, M., Mondani, F., Aliverdi, A., Golzardi, F. (2012). Interaction Effect of CO₂ Enrichment and Nutritional Conditions on Physiological Characteristics. *Nat Sci Biol*, 4 (1), 121-130.
- Taiwo, A. E., Leite, F. B., Lucena, G. M., Barros, M., Silveira, D., Silva, M. V., Ferreira, V. M. (2012). Anxiolytic and antidepressant-like effects of *Melissa officinalis* (lemon balm) extract in rats: Influence of administration and gender. *Indian J Pharmacol*, 44 (2), 189-192.

Summary/Sažetak

Melissa officinalis L. (matičnjak) sadrži mnoge potencijalno biološki aktivne spojeve uključujući kafeinsku kiselinu (CA), hlorogensku (CGA), ružmarinsku (RA), sukcininsku, ursoličnu kiselinu i timol. U ovom radu upotrebom HPLC-ED sistema određivan je sadržaj galne kiseline (GA), CGA, CA i RA u supernatantima ekstrakta listova matičnjaka. Standardne otopine GA, CGA, CA i RA pripremljene su otapanjem čistih supstanci u mobilnoj fazi. Listovi matičnjaka iz Bosne su sadržavali 0.382 mg/g GA, 0.254 mg/g CGA, 0.136 mg/g CA i 5.1 mg/g RA. Listovi matičnjaka iz Turske su imali viši sadržaj CA (0.71 mg/g), dok je sadržaj GA, CGA i RA bio niži (0.22 mg/g, 0.23 mg/g i 0.24 mg/g, respektivno). Sadržaji kiselina su izraženi po masi svježeg lista.