ANTIOXIDANT ACTIVITY, THE CONTENT OF TOTAL PHENOLS AND FLAVONOIDS IN THE ETHANOL EXTRACTS OF Mentha longifolia (L.) HUDSON DRIED BY THE USE OF DIFFERENT TECHNIQUES

In this study, we have examined the yield of extracted substances obtained by means of extraction using 70% ethanol (v/v), the content of total phenols and flavonoids, as well as the antioxidant activity of the extracts obtained from the samples of the herbs dried by means of different techniques. Wild mint Mentha longifolia (L.) Hudson was dried naturally in a laboratory oven at a temperature of 45 °C and in an absorptive low temperature condensation oven at 35 °C. The highest yield of extracts was obtained from the naturally dried herbs and the lowest from the herbs dried in the low temperature condensation drying oven. The content of total phenols and flavonoids was determined by spectrophotometric methods with an FC reagent and by the complexation reaction with aluminium chloride, respectively. The extract of the naturally dried herbs had the highest overall content of phenols (113.8±2.0 mg of gallic acid/g of the dry extract) and flavonoids (106.7±0.3 mg of rutin/g of the dry extract). The highest antioxidant activity determined by the FRAP and DPPH assay was determined in the extracts obtained from naturally dried herbs (2.76±0.15 mmol Fe²⁺/mg of the dry extract and EC₅₀ = 0.022±0.001 mg/ml), while the lowest was obtained from the extracts of herbs dried in the laboratory oven (1.13±0.11 mmol Fe²⁺/mg of the dry extract and EC₅₀ = 0.033±0.001 mg/ml). The HPLC-DAD analysis results showed that the greatest content of phenolic compounds was found in extract obtained from naturally dried plant material. The dominant phenolic component in the all extracts is Kaempferol 3-O-glucoside. The contents of all phenolic compounds strongly depend on the drying conditions.

Keywords: EC₅₀; FRAP; Mentha longifolia (L.) Hudson; drying; total phenols; flavonoids; HPLC-DAD.

Mentha longifolia (L) Hudson (wild mint, English horsemint) belongs to the genus Mentha, Lamiaceae family and is associated with medicinal and aromatic herbs. It is widespread throughout the Mediterranean, Central and Northern Europe, Asia Minor and Africa [1,2], and eleven species can be found in the flora of Serbia [1]. Wild mint is an aromatic and nectariferous plant, and the above-ground plant part possesses a characteristic, aromatic smell. It is used in the pharmaceutical, tobacco and food industry and especially in cosmetology. It shows carminative properties, improves digestion and alleviates the inflammation of respiratory organs. It is used internally in the form of infusers, and externally for baths [3].

Herbal polyphenolic compounds are secondary metabolites with a characteristic aromatic structure, which can be classified into fifteen groups according to the basic part of their molecule, as for example phenols, phenolic acid, flavonoids, anthocyanins, quinones, catechins and tannins, just to name a few [4].

In recent years, many polyphenolic compounds have attracted scientists involved in food medicine
and chemistry, because of their antioxidant, anti-inflammatory, antimutagenic, anticancer, antibacterial, antiviral and antiproliferative properties, as well as their ability to change the function of some basic cell enzymes [5-8]. It has been claimed that polyphenolic compounds show their antioxidant activity in the following ways: by giving out an H-atom, by directly connecting free oxygen and nitrogen radicals, by chelating prooxidant metal ions (Fe or Cu) and by the inhibition of prooxidant enzymes (lipogenesis, myeloperoxidase, xanthine-oxidase, NAD(P)H oxidase, cytochrome enzymes P-450) [9-11]. The antioxidant activity of flavonoids is of extreme importance. These polyphenolic compounds inhibit the oxidation of lipids, inhibit some of the enzyme systems, have an influence on the formation and transformation of peroxyl radicals, etc. [12]. Flavonoids are known as “catchers” of superoxide anions and hydroxyl radicals [13]. Flavonoids show antioxidant activity typical for polyphenolic compounds, thanks to the possibility of donating hydrogen atoms along with the formation of resolutely stable radical, as well as the possibility of the chelation of transition metal ions. Their ability to neutralize free radicals in a water solution is comparable or a few times better than ascorbic acid and tocopherol [14]. The protective effect of flavonoids has been proven in vitro and ex vivo [15].

A few new studies of the chemical analysis of the isolators of the herbal species of the Lamiaceae family explain their flavonoid profile. The main components are flavones and their 6-methoxy derivatives, while flavonols are rarely presented [16-18]. Traditional use of most medicinal herbs can refer to the presence of flavonoids, which show a wide range of prophylactic and therapeutic activities [19]. One of the important properties of flavonoids is their antioxidant activity, which can be related to the number and position of the hydroxyl groups in a molecule [20,21]. Flavonoids are exquisite catchers of free radicals, and they possess antibacterial, antifungal, antiviral and anti-inflammatory activities, they have a favorable influence on the cardiovascular system, etc. [22-27]. The additional importance of substances with antioxidant potential comes from their ability to protect other biomolecules, especially fats, from the harmful effects of oxygen. It should be taken into consideration that the reactivity of the sample towards different oxidants and under different experimental conditions (pH, polarity of the environment, emulgators, etc.) has been different and that almost none of the assays completely show the complexity of the influence and the interaction of antioxidants in vivo, which is why it is necessary to apply different methods [28].

Drying is the most effective way of the preservation of raw medicinal herbal materials, since humidity is the main cause of their contamination. The quality of the dried herbal drug depends, to a high extent, on the method of drying. Inadequate drying can cause microbiological impurity, loss of active ingredients, as well as an unwanted change of appearance, which results in a decrease in the quality of the raw materials. Different ways of drying have been determined based on the structure of the herbal material and chemical structure of the active healing ingredients [29].

The process of drying herbal material does not lower only the content of humidity, but also the quantity and the content of volatile compounds [30]. The drying method has a great influence on the qualitative and quantitative content of the essential oil M. longifolia [31].

The aim of this research is the analysis of the influence of the drying method of herbal material on the yield of extracted substances, on the total content of phenols and flavonoids, as well as on the antioxidant activity of extracts determined in vitro by the use of FRAP and DPPH assays.

THE EXPERIMENT

Material

The above-ground parts of the herb in the pheno- logical phase of blooming were gathered in July 2009, from the region of the municipality of Prokuplje, in the town Rastovnica on the mountain Pasjača at an altitude of 400 meters above sea level. The voucher specimens were deposited in the Herbarium of the Faculty of Biology, University of Belgrade, under the accession numbers: BEOU No. 16469.

All of the chemical substances used in the experimental work were of analytical purity. The Folin-Ciocalteu (FC) reagent, DPPH (2,2-diphenyl-1-picrylhydrazil), gallic acid and rutin were obtained from the Sigma Chemical Company, USA. The TPTZ reagent (2,4,6-triprydyl-1,3,5-triazine) was purchased from TCI Europe, Belgium. Sodium acetate trihydrate was produced in the Alkaloid factory, in Skolje, FYR Macedonia. The ethanol was produced in the Zorka-Phar- macy (Šabac, Serbia), the hydrochloric acid, vitamin C, iron(III)chloride, and iron(II)sulfate-7-hydrate were produced in VWR Prolabo (Belgium), and the glacial acetic acid at NRK Engineering, Belgrade, Serbia.

Techniques of drying the herbal material

The herbal material was dried using three different techniques: natural drying (ND) in the shade in a
draughty place for 15 days; in the laboratory oven for fruits, vegetables, medicinal herbs and mushrooms “Stockli”, Switzerland at a temperature of 45 °C (LO) for two days; and by using an LT-CD/60S absor- tional low-temperature condensational oven for fruits, vegetables, medicinal herbs (FREON EKO, Kragujevac) at a temperature of about 35 °C (LTCO) for two days. The content of humidity after drying until reaching constant mass was 11.1% in the case of natural drying, 10.2% in the case of laboratory drying, and in the case of the absorptive low temperature condensation drying oven, 10.7%.

Extraction procedure

A total of 50 g of dried herbal material (the above ground plant parts in bloom) chopped into small pieces were covered with 25 ml of ethanol (70% v/v), left for two hours, after which the mixture was percolated with 70% ethanol at room temperature, according to the Pharmacopoeial procedure for single percolation [32]. The obtained percolate was evaporated on a rotary vacuum-boiler at 50 °C. The dried extracts were chopped into fine powder and dried in the vacuum-dryer at 50 °C until constant mass and kept in well closed glass vessels, in a dry, cold and dark place. The gravimetric analysis defined the yield of the extracted substances.

The determination of total phenol contents

Total phenol content in the extracts was determined according to the Folin-Ciocalteu reagent [33], using gallic acid as the standard. The extract solution in 70% ethanol (0.2 ml, 1 mg ml⁻¹) was mixed with the FC reagent (1 ml) and an aqueous solution of Na₂CO₃ (0.8 ml, 7.5%). After 30 min of incubation at room temperature, the absorbance of the reaction compound at 765 nm was measured by a Varian Cary-100 spectrophotometer. The overall phenol content was expressed as mg of gallic acid/g of dry extract, and calculated using the equation of the standard curve:

\[ A(765 \text{ nm}) = 12.722c_{\text{gallic \ acid \ (µg \ ml}^{-1}) + 0.0034, \quad R^2 = 0.9994 \]

The determination of total flavonoid contents

The content of the total flavonoids in the extracts were determined by the spectrophotometrical method, which is based on the production of complex compounds of flavonoids with aluminium chloride [36]. Each plant extract (2.0 ml, 1.0 mg ml⁻¹) in 70% ethanol was mixed with 0.10 ml of 10% aluminum chloride, 0.10 ml of (1.0 M) potassium acetate and 2.8 ml of distilled water. After 30 min of incubation at room temperature, the absorbance of the reaction mixture was measured at 415 nm in relation to distilled water. Rutin was chosen as the standard and the total flavonoid content was expressed as mg of rutin/g of dry extract, and counted using the equation of the standard curve:

\[ A(415 \text{ nm}) = 7.2328c_{\text{rutin \ (µg \ ml}^{-1}) - 0.2286, \quad R^2 = 0.9919 \]

Determination of the antioxidant activity of the extracts

FRAP Assay

The antioxidant activity of the extracts was determined by the FRAP assay [36,37]. The calibration curve for the determination of the antioxidant capacity was obtained by measuring the absorption of the series of standard water solutions of Fe²⁺ in the concentration range from 0.20 to 1.00 mmol Fe²⁺/dm³. A total of 0.100 ml of extract in 70% ethanol of 0.1 mg/ml concentration and 3 ml of freshly prepared FRAP reagent (acetate buffer + TPTZ reagent + FeCl₃·6H₂O in the ratio 10:1:1) were added in the test tube. After incubation at a temperature of 37 °C, the absorption was measured at 593 nm in relation to a random attempt. FRAP values were expressed as mmol Fe²⁺/mg of the dry extract.

DPPH Assay

The antioxidant activity of the extracts was determined by the DPPH assay [38-41]. A series of extract solutions were made in 70% ethanol in the range of concentrations from 0.01 to 1.0 mg/ml, and DPPH radicals in the concentration of 0.3 mM in 70% ethanol for the purpose of the determination of antioxidant activity. A total of 1 ml of the DPPH radical solution and 2.5 ml of extract solutions of different concentrations were mixed together. After 30 minutes of incubation at room temperature in the dark, the absorbance was measured at 517 nm by the Varian Cary-100 spectrophotometer.

The capacity for neutralizing free radicals was calculated according to the following equation:

\[ \text{The capacity for neutralizing DPPH radicals, (\%)} = 100 - \left[ \frac{(A_s - A_b)}{A_c} \right] \times 100/A_c \]

where \( A_s \) is the absorbance in the presence of the plant extract in the DPPH solution, \( A_c \) is the absorbance of the control solution (containing only DPPH) and \( A_b \) is the absorbance of the sample extract solution without DPPH. The \( EC_{50} \) value was calculated according to the experimental data by the use of the sigmoidal non-curve method and SigmaPlot 2000 trial software. The \( EC_{50} \) values for vitamin C, which were used for comparison, were obtained in the same
way as for the herbal extracts in the range of concentrations from 0.001 to 0.1 mg/ml [41].

HPLC Analysis

The HPLC analyses were performed on an Agilent 1100 Series HPLC-DAD system (Agilent Technologies) consisting of micro vacuum degasser, binary pump, thermostated column compartment and variable wavelength detector. The column was an Agilent Eclipse XDB-C18 4.6 mm ID×150 mm (5 μm). The mobile phase was composed of solvent (A) 0.15% (w/v) phosphoric acid in H₂O:MeOH (77:23, v/v, pH 2) and solvent (B) methanol as follows: isocratic 0-3.6 min 100% A + 0% B; linear gradient in 24 min 80.5% A + 19.5% B; isocratic with 80.5% A + 19.5% B up to 30 min; linear gradient in 60 min 51.8% A + 48.2% B; linear gradient in 67.2 min 0% A + 100% B; followed by isocratic elution with 100% B for last 5 min. Flow rate was 1 ml min⁻¹, temperature 15 °C. The dosing volume of extracts concentration of 10 mg ml⁻¹, was 15 μl. Peak detection in UV region at 350 nm was used [42,43].

Statistical analyses

All of the measurements were performed in triplicate and the results were shown as the arithmetic mean (±standard deviation). The experimental data were analyzed using Microsoft Excel 2000 and Origin 7 trial. A Newman-Keuls multiple comparison test, using a WINKS SDA software package (TexasSoft, 6th Edition, Cedar Hill, Texas, 2007 trial version) was performed to determine if there were significant differences in the DPPH scavenging activities, total content of phenolic and flavonoid compounds in extracts from plants dried by different techniques. The values were considered statistically significant at p < 0.05.

RESULTS AND DISCUSSION

The yield of extracts, the content of total phenols and flavonoids in the extracts

The results show that the drying process has an influence not only on the yield of total extracted substances from Mentha longifolia, but also on the content of total phenols and flavonoids obtained from the extracts (Table 1). The highest yield of the extracted substances, 11.6 g/100 g of plant material was obtained by the extraction of naturally dried plant material, while drying in the laboratory oven and low-temperature condensational oven produced 9.4 and 8.9 g of the extracted substances per 100 g of the herb, respectively.

The results show that natural drying is the best way of drying from the aspect of the content of phenol and flavonoids in the extracts as well as the quantity of the extracted phenols and flavonoids according to the mass of herbs. The total quantity of phenols extracted from the naturally dried herb is two times larger than in the case of herbs dried in ovens, while the quantity of the extracted flavonoids is 70% higher in the case of the naturally dried herbs than the herbs dried in an oven.

The antioxidant activity of the extracts

The antioxidant activities of the extracts obtained from the herbs dried in different ways are shown in Figure 1 and Table 2. The extracts show the characteristic change of the degree of neutralization with an increase in the concentration of the dry substance in the sample.

The highest value of antioxidant activity (determined by the use of the FRAP and DPPH assays) was found in the extract obtained from the naturally dried herbs, while the extracts obtained from the herbs dried in the laboratory oven and low-temperature condensational oven show considerably lower antioxidant activity.

The standardized value of the antioxidant capacity (determined by the use of FRAP assay) of the extracts (in relation to 1 g of the starting herbs) is three times higher compared to the values obtained from the herbs dried in the oven. The EC₅₀ value for the extracts obtained from herbs dried in the oven is 50% higher than in the case of the naturally dried herbs. These values lead to the conclusion that natural drying leads, only in small amounts, to the degradation of the polyphenolic structure of compounds, which is related to the antioxidant activity [19].

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Table 1. The yield of extracted substances, total phenols and flavonoids in the Mentha longifolia extracts and herbs

<table>
<thead>
<tr>
<th>The drying method of the herbal material</th>
<th>The yield of extracted substances</th>
<th>Total phenols</th>
<th>Total flavonoids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/100 g of fresh herb</td>
<td>mg GAE/g of dry extract</td>
<td>mg GAE/g of fresh herb</td>
</tr>
<tr>
<td>Natural drying</td>
<td>5.7</td>
<td>113.8±2.0</td>
<td>6.52±0.11</td>
</tr>
<tr>
<td>Laboratory oven</td>
<td>4.6</td>
<td>85.1±1.5a</td>
<td>3.96±0.07b</td>
</tr>
<tr>
<td>Low temperature condensational oven</td>
<td>4.4</td>
<td>83.7±1.2a</td>
<td>3.68±0.05b</td>
</tr>
</tbody>
</table>

a Differences between values for parameters designated with the same letters were not statistically significant with a 95 % confidence interval
The antioxidant activity of vitamin C ($EC_{50} = 0.011 \pm 0.0001$ mg/ml) is two times higher compared to the extract with the highest antioxidant activity.

The good correlation between $EC_{50}$ and the content of phenols ($R^2 = 0.969$) and flavonoids ($R^2 = 0.999$) can be seen in Figure 2, which confirms that phenols and flavonoids are responsible for the antioxidant activity of the extracts.

The antioxidant activity of the extracts expressed as mmol Fe$^{2+}$/g of the extract (Figure 3) is in good

![Figure 1. Antioxidant activity of the Mentha longifolia extracts.](image1)

![Figure 2. The correlation between the $EC_{50}$ and the total content of phenols and flavonoids for the Mentha longifolia extracts.](image2)

**Table 2. Antioxidant activity of the Mentha longifolia extracts**

<table>
<thead>
<tr>
<th>The drying method of the herbal material</th>
<th>mmol Fe$^{2+}$/g of dry extract</th>
<th>mmol Fe$^{2+}$/g of fresh plant</th>
<th>$EC_{50}$ / mg ml$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural drying</td>
<td>2.76±0.15</td>
<td>0.157±0.008</td>
<td>0.021±0.002</td>
</tr>
<tr>
<td>Laboratory oven</td>
<td>1.13±0.21$a$</td>
<td>0.052±0.009$b$</td>
<td>0.036±0.004$c$</td>
</tr>
<tr>
<td>Low temperature condensational oven</td>
<td>1.22±0.15$a$</td>
<td>0.054±0.007$b$</td>
<td>0.033±0.001$c$</td>
</tr>
</tbody>
</table>

$^a$Differences between values for parameters designated with the same letters were not statistically significant with a 95 % confidence interval.
correlation with the content of phenols ($R^2 = 0.973$) and flavonoids ($R^2 = 0.998$), which indicates the importance of the way of drying for the preservation of the antioxidant potential of herbal material.

**HPLC Analysis**

HPLC chromatograms of analysed extract as well amount of identified phenolic compound in extracts are shown in Figures 4-6 and in Table 3, respectively.

Phenolic compounds content (Table 2) presented in examined extracts are calculated using chlorogenic acid as a reference compound.

The HPLC-DAD analysis results show that the greatest content of phenolic compounds was found in extract obtained from naturally dried plant material.

![Figure 3](image3.png)

*Figure 3. The correlation between the FRAP and the total content of phenols and flavonoids for the Mentha longifolia extracts.*

![Figure 4](image4.png)

*Figure 4. HPLC chromatogram of Mentha longifolia extracts obtained from plant material dried in low temperature condensational oven.*
Figure 5. HPLC chromatogram of Mentha longifolia extracts obtained from plant material dried naturally.

Figure 6. HPLC chromatogram of Mentha longifolia extracts obtained from plant material dried in laboratory oven.

The dominant phenolic component in all extracts is Kaempferol 3-O-glucoside and their contents strongly depend on the drying conditions. Generally, the contents of phenolic compounds in extracts decrease with rising drying temperature, which is in correlation with the determined antioxidative activity and phenolic and flavonoid contents.

CONCLUSION

The herbal material drying process has a significant influence on the yield of extracted substances obtained by the extraction process, as well as on the content of phenols and flavonoids in the obtained extracts. The greatest quantity of the extracted substances (11.6 g/100 g of dry herbal extract) was obtained...
from herb dried naturally in the shade in a draughty place. This extract had the highest content of total phenols (113.8±2.0 GAE/g of dry herbal extract) and flavonoid compounds (106.7±0.3 RE/g of dry herbal extract) and showed a better antioxidant potential in relation to the extracts obtained from the herbs dried in ovens. Recounted on the starting material, the highest content of phenols, flavonoids and antioxidant potential were found in the herbs dried naturally in the shade in a draughty place.

Vitamin C with an EC50 value of 0.011 mg/ml had an antioxidant activity two times higher than the extract obtained from naturally dried herbs.

**Nomenclature**

FRAP - Ferric reducing ability of the plasma
FC reagent - Folin-Ciocalteu reagent
DPPH - 2,2-diphenyl-1-picrylhydrazil
EC50 - effective concentration of the extract necessary to decrease the DPPH radical concentration by 50% mg GAE/g of the dry herbal extract - mg of gallic acid/g of dry extract
mg RE/g of the dry herbal extract - mg of rutin/g of dry extract
TPTZ - 2,4,6-tripyridyl-1,3,5-triazine
ND - natural drying
LTCO - low temperature condensational oven
LO - laboratory oven

### Acknowledgements

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### REFERENCES


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**Table 3. Phenolic compound of the Mentha longifolia extracts**

<table>
<thead>
<tr>
<th>Component</th>
<th>Peak no.</th>
<th>Natural drying</th>
<th>Low temperature condensational oven</th>
<th>Laboratory oven</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercetin 3,7-O-diglucoside</td>
<td>1</td>
<td>0.52</td>
<td>0.35</td>
<td>0</td>
</tr>
<tr>
<td>Kaempferol 3-O-(6&quot;-O-malonylglucoside)-7-O-glucoside</td>
<td>2</td>
<td>0.71</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Phenolic &quot;P5&quot;</td>
<td>3</td>
<td>2.08</td>
<td>2.66</td>
<td>0.45</td>
</tr>
<tr>
<td>Phenolic &quot;P6&quot;</td>
<td>4</td>
<td>0.58</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Kaempferol 3-O-sophoroside</td>
<td>5</td>
<td>1.46</td>
<td>0.76</td>
<td>0</td>
</tr>
<tr>
<td>Luteolin 5-O-glucoside</td>
<td>6</td>
<td>1.69</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Kaempferol 3-O-(6&quot;-O-malonylglucoside)-7-O-rhamnoside</td>
<td>7</td>
<td>17.20</td>
<td>1.24</td>
<td>1.48</td>
</tr>
<tr>
<td>Apigenin 5-O-glucoside</td>
<td>8</td>
<td>7.53</td>
<td>3.18</td>
<td>1.65</td>
</tr>
<tr>
<td>Apigenin 4'-O-glucoside</td>
<td>9</td>
<td>0.81</td>
<td>0</td>
<td>2.03</td>
</tr>
<tr>
<td>Kaempferol 3-O-glucoside</td>
<td>10</td>
<td>61.36</td>
<td>40.30</td>
<td>37.10</td>
</tr>
<tr>
<td>Genkwanin 5-O-glucoside</td>
<td>11</td>
<td>0.56</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Kaempferol 3-O-(6&quot;-O-malonylglucoside)</td>
<td>12</td>
<td>0.85</td>
<td>0.47</td>
<td>0.72</td>
</tr>
<tr>
<td>Kaempferol 3-O-rhamnoside</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>5.38</td>
</tr>
<tr>
<td>Genkwanin 5-O-(6&quot;-O-malonylglucoside)</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>1.95</td>
</tr>
<tr>
<td>Kaempferol 7-O-rhamnoside</td>
<td>15</td>
<td>1.73</td>
<td>0.83</td>
<td>0.91</td>
</tr>
<tr>
<td>Genkwanin 4'-O-glucoside</td>
<td>16</td>
<td>0.52</td>
<td>0.52</td>
<td>0.57</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>-</td>
<td>97.60</td>
<td>50.30</td>
<td>52.14</td>
</tr>
</tbody>
</table>
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ANTIOKSIDATIVNA AKTIVNOST, SADRŽAJ UKUPNIH FENOLA I FLAVONOIDA ETANOLNIH EKSTRAKATA *Mentha longifolia* (L.) HUDSON OSUŠENE RAZLIČITIM TEHNIKAMA

U radu je ispitana prisutnost ekstraktivnih materija dobijenih ekstrakcijom sa 70% etanolom (v/v), sadržaj fenola i flavonoida, kao i antioksidativna aktivnost ekstrakata dobijenih iz uzoraka herbe osušene različitim tehnikama. Divljana *Mentha longifolia* (L.) Hudson, sušena je prirodnim putem, u laboratorijskoj sušnici na temperaturi 45 °C i absorbcionoj niskotemperaturnoj kondenzacionoj sušari na 35 °C. Najveći prinos ekstrakta dobijen je iz herbe sušene prirodnim putem, a najmanji iz herbe sušene u niskotemperaturnoj kondenzacionoj sušari. Sadržaj ukupnih fenola i flavonoida određen je spektrofotometrijskim metodama sa Folin-Ciocalteu reagensom, odnosno kompleksiranjem sa aluminijum-bloridom, respektivno. Ekstrakt herbe osušene prirodnim putem ima najveći sadržaj ukupnih fenola (113,8±2,0 mg galne kiseline/g suvog ekstrakta) i flavonoida (106,7±0,3 mg rutina/g suvog ekstrakta). Najveću antioksidativnu aktivnost određenu FRAP i DPPH testom pokazali su ekstrakti dobijeni iz herbe sušene prirodnim putem (2,76 mmol Fe²⁺/g suvog ekstrakta i EC₅₀ = 0,021±0,002 mg/ml), a najmanju sušene u laboratorijskoj sušnici (1,13±0,21 mmol Fe²⁺/g suvog ekstrakta i EC₅₀ = 0,036±0,004 mg/ml). Rezultati HPLC-DAD analize pokazuju da je najveći sadržaj fenolnih jedinjenja prisutan u ekstraktu dobijenom iz prirodno sušenog biljneg materijala. Dominantna fenolna komponenta je kaempferol 3-O-glukozid. Sadržaj fenolnih jedinjenja jako zavisi od uslova sušenja.

**Ključne reči:** EC₅₀; FRAP; *Mentha longifolia* (L.) Hudson; sušenje; ukupni fenoli; flavonoidi; HPLC-DAD.