TOTAL LIPIDS, PROTEINS, MINERALS, ESSENTIAL OILS AND ANTIOXIDANT ACTIVITY OF THE ORGANIC EXTRACTS OF *MENTHA LONGIFOLIA* (L.) GROWING WILD IN KOSOVO

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The soil and climatic conditions are conducive for the growth of a lot of medical herbs in Kosovo. *Mentha longifolia* (L.) is native to Europe, Central Asia and Australia. Lipids, proteins, minerals and essential oils were quantitatively determined from the *Mentha longifolia* (L.) growing wild in Germia Park (located in the northeast of Pristina). Total proteins were analyzed, by Kjeldahl method. The total amount of proteins in the *Mentha longifolia* (L.) is 8.227 %. Lipids are analyzed by Soxhlet extraction. The total amount of lipids in the *Mentha longifolia* (L.) are 5.804 %. Essential oils were isolated using steam distillation. The total amount of essential oils in the *Mentha longifolia* (L.) are 0.589 %. The mineral content was studied and analyzed by flame atomic absorption spectrometry. Six elements, sodium, potassium, calcium, zinc, iron and copper were determined in the *Mentha longifolia* (L.). The mean levels of sodium, potassium, calcium, zinc, iron and copper are 509.7 mg kg^{-1}, 4055 mg kg^{-1}, 9097 mg kg^{-1}, 256.7 mg kg^{-1}, 11841 mg kg^{-1}, 82.83 mg kg^{-1} in the *Mentha longifolia* (L.), respectively. Iron and calcium are present in large amounts in the *Mentha longifolia* (L.). The antioxidant activity of essential oil was evaluated by means of the 2,2-diphenyl-1-picrylhydrazil (DPPH) radical scavenging method. During the analysis of antioxidant activity of the extracts of *Mentha longifolia* (L) it has been observed that only H_2O extract shows strong antioxidant activity than BHT and BHA.

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Introduction

For primary health care needs, 80% of world population relies mainly on plant based traditional medicines because according to them, medicinal plants are natural or near to nature and are always safe. The important utilities of many plants have long been published but a large number of them remain unexplored up to now. So there is a necessity to explore their uses and to conduct pharmacognostic and pharmacological studies to ascertain their therapeutic properties.[1-3] Plants' diversity has a considerable importance as a source of pharmaceutically active substances.[4-7]

Mentha longifolia (L.) is perennial herb 40-120 cm high with musty scent. Stem white or grey-villous, sometimes sparsely hairy. Leaves are sessile or shortly petiolate usually oblong elliptical, hairs simple. Extremely variable in height, leaf size and shape, indumentum and inflorescence and complicated by the occurrence of hybrids.[8] *Mentha* species are widely used in conventional medicine, for their antispasmodic, antiseptic and emmenagogue effects. Moreover, their essential oils are used in chewing gums, alcoholic beverages, cosmetics, perfumes, toothpastes and mouthwashes.[9] The plant is mainly used as salad, spice and for tea besides mint herbage used for wool dyeing.[10]

Various biological activities have been reported for some species of *Mentha*, as antibacterial,[11-13] antifungal,[6] and antioxidant activity.[14-16]

Our research group is interested to analyze the chemical profile of different medicinal plants which are growing wild in the region of Kosova and Albania.[17-24] The aim of this research was to determine the quantity of proteins, lipids, minerals, essential oil and antioxidant activity in the *Mentha longifolia* (L.), growing in Germia Park. Germia is a regional Park located in the north-east of Pristina, capital city of Kosovo, and it covers an area of 62 km^2.

Experimental

Plant materials

The leaves of *Mentha longifolia* (L.), growing wild in Germia Park (located in the north-east of Pristina), was collected in July 2013. We took four samples from *Mentha longifolia* (L.). Voucher specimens were deposited in the Herbarium of the Department of Veterinary, University of Prishtina. The plants were dried at room temperature (22 °C).

Extraction of essential oils

Hydrodistillation was conducted by a standard procedure[25] (Clevenger apparatus) with dried *Mentha longifolia* (L.) leaves which had previously been chopped in a domestic blender. The isolation experiment was carried out continuously on a heating mantle at 60-80 ^{0}C until no further oil was extracted. The essential oil was dried over anhydrous Na_2SO_4 and after filtration stored in a dark bottle at 4 ^{0}C until tested and analyzed.

Determination of Mineral Content

An analysis of the collected leaves samples, by the method of Taleisnik et al,[26] showed the presence of sodium, potassium, calcium, zinc, iron and copper. Therefore, the leaves were washed with distillated water to remove any dust and were dried in an oven at 105 °C for 48 h. The dried

samples were pounded in a porcelain mortar until they formed a powder. Then, a 2 g sample was calcified in an oven at 300-400 °C. The ashes were placed into 100 cm^3 of normal flask. Next, 10 mL of 1 mol dm^{-3} nitric acid was added to each flask, homogenized, and then shaken for 20 min in a shaker. The homogenized samples were filtered and filled till 100 mL with 1 mol dm^{-3} HNO_3. Minerals like sodium, potassium, calcium, zinc, iron and copper were analyzed Atomic Absorption Spectrometry (Buc Scientific Model 200A).

Determination of protein content

The Kjeldahl method[27] was used for determination of proteins in which digestions, distillation and titration of the sample was done. The value of nitrogen was converted to protein by multiplying to a factor of 6.25.

Determination of lipid content

The solvent extraction method[28,29] was used for the determination of thelipid content of the samples. Diethyl ether was used as a solvent.

Antioxidant activity- DPPH assay

The hydrogen atom or electron donation abilities of the corresponding extracts and some pure compounds were measured from the bleaching of the purple-colored methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). This spectrophotometric assay was done using the stable radical DPPH as a reagent according to the method of Burits and Bucar.[30] Briefly, 50 µL of the extracts (various concentrations) were added to 5 ml of the DPPH solution (0.004% methanol solution). After 30 min incubation at room temperature, the absorbance was read against pure methanol at 517 nm. The radical scavenging activities of the samples were calculated as percentages of inhibition according to the following equation:

$$I(\%) = 100 \frac{A_{blank} - A_{sample}}{A_{blank}} \qquad (1)$$

where

A_{blank} is the absorbance of the control (containing all reagents except the test compound), and

A_{sample} is the absorbance of the test compound.

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the plot of inhibition percentages against extract concentration using PHARM/ PCS-version 4. All tests were done in triplicate.

Results and Discussion

The chemical aspects of *Mentha longifolia* (L.) were analyzed with the goal to determine the chemical nature. We have quantitatively analyzed the primary and secondary metabolites. The amount of lipids, proteins and essential oil of *Mentha longifolia* (L.) is given in Table 1.

From experimental data (Table 1) we can see that the amounts of lipids were 5.804 %, proteins 8.227 % and essential oil 0.589 % in the *Mentha longifolia* (L.). Figure 1 show the diagrams for the lipids, proteins and essential oil amounts to *Mentha longifolia* (L.) giving in percentage. On Figure 1 we can see that the amounts of proteins, lipids and essential oils in *Mentha longifolia* (L.) are as follows: proteins > lipids > essential oil.

Table 1. The amounts of lipids, proteins and essential oil of the *Mentha longifolia* (L.).

Components	Mean value (%)	Standard deviation
Lipids	5.804	0.21
Proteins	8.227	0.17
Essential oil	0.589	0.01

The amount of essential oil (0.589 %) of *Mentha longifolia* (L.) growing in Kosovo is almost same as that of *Mentha piperita* growing in Libya[15] (0.64 %). However, it is lower than the amount of essential oil of *Mentha longifolia* (L.) growing in Serbia[31] (0.9 %). The amount of proteins (8.227 %) of *Mentha longifolia* (L.) growing in Kosovo is comparable to that of *Mentha longifolia* (L.) growing in Pakistan[32] (7.491 %). The amount of lipids (5.804%) in *Mentha longifolia* (L.) growing in Kosovo is higher than that in *Mentha longifolia* (L.) growing in Pakistan[32] (2.34 %).

We determined the quantity of the minerals like sodium, potassium, calcium, zinc, iron and copper. The amount of minerals of *Mentha longifolia* (L.) are given in Table 2.

Table 2 shows that the average values in mg kg^{-1} of sodium, potassium, calcium, zinc, iron and copper in the sample are 509.7, 4055, 9097, 256.7, 11841 and 82.83 mg kg^{-1}. The plant contains large amounts of calcium (9097 mg kg^{-1}) and iron (11841 mg kg^{-1}).

Table 2. Quantity of minerals of the *Mentha longifolia* (L.) giving in mg kg^{-1}.

Elements	Mean value (%)	Standard deviation
Sodium	509.7	0.55
Potassium	4055	0.19
Calcium	9097	0.035
Zinc	256.7	0.21
Iron	11841	0.032
Copper	82.83	0.029

The result of the following study agrees with earlier study of elemental distribution in medicinal plant species as reported by Kim et al.[33] The result of the present study shows a high level of macro elements accumulation in the sampled plants. It is important to note that benefits accorded to human health by some plants, used in homeopathic system, have been traced to presence of Ca, Cr, Fe, Mn, Ca, K and Zn in those plants.[34] These elements equally contribute to neurochemical transmission and are food constituents of biological molecules. The mineral composition results of the *Mentha longifolia* (L.) show that this plant contains rich source of mineral elements, this result assumes importance. when the usefulness of mineral like Ca, Mg, K and Na in the body are considered.

The amount of sodium (509.7 mg kg^{-1}), iron (11841 mg kg^{-1}) and copper (82.83 mg kg^{-1}) in *Mentha longifolia* (L.) growing in Kosovo is higher than those in *Mentha longifolia* (L.) growing in Pakistan (sodium 29.9 mg kg^{-1}, iron 53.4 mg kg^{-1} and copper 6 mg kg^{-1}). [32]

The amount of iron (11841 mg kg^{-1}), zinc (256.7 mg kg^{-1}) and copper (82.83 mg kg^{-1}) in *Mentha longifolia* (L.) growing in Kosovo is higher than those in of *Mentha piperita* (iron 531.5 mg kg^{-1}, zinc 12.64 mg kg^{-1} and copper 11.52 mg kg^{-1}) and *Mentha spicata* (iron trace, zinc 4.654 mg kg^{-1} and copper 1.757 mg kg^{-1}) growing in Turkey.[16] The amount of calcium (4055 mg kg^{-1}) in *Mentha longifolia* (L.) growing in Kosovo is lower than that in *Mentha piperita* (12150 mg kg^{-1}) and *Mentha spicata* (4396 mg kg^{-1}) growing in Turkey.[16]

The DPPH radical scavenging method was used to evaluate the antioxidant properties of *Mentha longifolia* (L.) in comparison with those BHT and BHA. Table 3 shows antioxidant activity of extracted oil of *Mentha longifolia* (L.).

Table 3. Antioxidant activity of extracted oil of *Mentha longifolia* (L.).

Samples	IC_{50} (μg mL^{-1})
Diethyl ether	29.10
Chloroform	28.86
Ethyl acetate	18.98
1-Butanol	17.81
Water	7.89
Butylatedhydroxytoluene (BHT)	13.43
Butylatedhydroxyanisole (BHA)	11.55

All extracts of *Mentha longifolia* (L.) (water, n-butanol, ethyl acetate, chloroform and diethyl ether) have been able to reduce DPPH stable radical from DPPH-H violet color to DPPH-H with yellow color. Comparison of the neutralizing activity of the extracts of *Mentha longifolia* (L.) with that of BHT (13.43 μg mL^{-1}) and BHA (11.55 μg mL^{-1}) shows that only the aqueous extract shows stronger antioxidant activity. Hence, one can conclude that only the aqueous extract has a stronger antioxidant activity as compared those of BHT and BHA. According to the results recorded in Table 3, the highest radical scavenging activity was observed in the extracts of solvents in the following order: H_2O > BHA > BHT > n-BuOH >EtOAc > $CHCl_3$ > Et_2O.

Conclusion

In recent years, use of medicinal plants and their conservation has been accorded great importance. They are used globally by the indigenous and marginal communities for curing various diseases. These medicinal plant species are mostly used as food supplement along with their oral decoctions.

Mint species are used widely throughout the world as an important medicinal plant. The aim of this research was to determine the quantity of primary metabolites (lipids and proteins) and to analyze minerals as sodium, potassium, calcium, zinc, iron and copper. The important results of this study can be summarized as follows.

The total amount of proteins, lipids and essential oils in the *Mentha longifolia* (L.) is 8.227 %, 0.804 % and 0.589 %, respectively. The macro-elements present in *Mentha longifolia* (L.) occur in the order Fe > Ca > K > Na > Zn > Cu. Our current study on nutritional evaluation of *Mentha longifolia* (L.) has revealed that this plant is a good source of nutrients (proteins, lipids and minerals) and can be used as substitutes for food deficient in any of these nutrients. Further, the aqueous extract of the plant possessed a strong antioxidant activity. Antioxidant properties of the essential oils and various extracts from plants are of great interest in both fundamental science and the food industry, since their possible use as natural additives emerged from a growing tendency to replace synthetic antioxidants by natural ones.

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