

Analysis of Macroelements Content of some Medicinal and Aromatic Plants using Flame Atomic Absorption Spectrometry (FAAS)

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Abstract

A large number of medicinal and aromatic plants are used directly from wild, so that accurate identification of species and their chemistry knowledge is essential, preventing from undesirable situations such as intoxication due to confusion with other species, or a buildup of metals over the limit. This paper presents the results of analyzes for 15 samples obtained from eight different species of plants (common elderberry, yellow birdstraw, yarrow, thyme, wild garlic, hawthorn), collected from different areas of Anina Mountains. Were analyzed macroelements Ca, K, Mg using flame atomic absorption spectrophotometry method. Analyzed calcium content recorded values that are within the limits specified in the literature for the same species and was ranged from 5.703 g/kg Ca for *Sambucus nigra* species harvested in the Bozovici and 22.436 g/kg Ca for *Crataegus monogyna* species harvested in Carașova area. The magnesium content of the analyzed plants, was lower for most of the analyzed samples and showed a range from 0.174 g/kg Mg for *Allium ursinum* and 4.749 g/kg Mg for *Sambucus nigra* species, harvested in Staierdorf area. Reduced values are due to antagonism of Ca-Mg, the plants are fixing from soil high amounts of calcium and low of magnesium. In terms of plant potassium content, it varied between 9.6-61.5 g/kg K, the highest value being recorded *Sambucus nigra* species harvested in Carașova area.

Keywords: medicinal plants, macroelements, chemical composition, FASS, *Sambucus nigra*, *Galium verum*, *Achillea setacea*, *Thymus sp.*, *Allium ursinum*, *Crataegus monogyna*

1. Introduction

Herbs are known and used since ancient times, long before their chemical structure and composition was known. Even today, the number of medicinal species used in folk tradition exceeds by far the number of species used by modern phytotherapy, biochemically studied and confirmed by phytopharmaceutical point of view. In rural areas, the locals know a large number of medicinal and aromatic plants they use direct from spontaneous flora. Numerous medicinal species processed and commercialized by various companies specializing in this area, are obtained from direct harvesting of wild flora.

Wild plants are important resources of minerals, which justifies their biochemical investigations and the correlation with therapeutic use of the species. Establishment of various minerals in the plant body is important because when the optimal limits are exceeded, these minerals can become dangerous to humans or even toxic (DJORGEVA & al. [1]). Another factor that can cause toxicity of medicinal plants is environmental pollution, directly by the accumulation of heavy metals in vegetative organs of plants, especially leaves, when it comes to small particles carried in the air, or indirectly through their accumulation in soil (RADULESCU et & al. [2]). The mineral elements are recognized as having important roles in the structure and the functions of plants, animals and humans, significantly influencing

various biochemical processes (SOETAN & al. [3]; SUBRAMANIAN & al. [4]; ŞTEF & al. [5]; LAVATE & al. [6], DAFALLA [7]).

Mineral elements contained in plants is a valuable source in the human diet (MALIK & al. [8]), although they represent only 4-6% of human body weight. Some mineral elements (macroelements) are needed by the body in greater amounts than 100 mg/day and represents 1% of body weight, while microelements are required in smaller quantities of 100 mg/day and represents only 0.01% of body weight (IMELOUANE & al [9]). Excess intake of various minerals can disrupt homeostatic balance leading to the appearance of toxic side effects. Knowledge of nutrient biochemistry have great importance for people with chronic illnesses or those dependent on certain medications that affect the body's ability to use different nutrients. Studies have confirmed the existence of interactions between nutrition and disease, and between nutrition and metabolism of various drugs (SOETAN & al [3]).

Studies of macro and microelements content shows importance in chemotaxonomy, significant differences being found between macro and micronutrients content and botanical family of the analyzed species. It can also be useful to standardize phytopharmaceutical products (ARCEUSZ & al. [10]).

The main purpose of this study is to determine the macroelement content of medicinal species commonly harvested and used in Anina Mountains, in order to determine phytopharmaceutical quality of the herbal biological material, obviating possible unwanted effects.

2. Materials and methods

Plants analyzed were collected from different areas of Anina Mountains, at flowering. Plants were studied in laboratory in order to determine the exact species, using specialized catalogues. Scientific names were updated by Flora Europaea (electronic edition). Precise identification of a species is extremely important, on the one hand avoiding confusion with related species which can even be toxic, and on the other hand provides a correct reporting of the results of biochemical analyzes.

Plants were harvested from soils formed on limestone, specific to the region. The soils are found in a mosaic arrangement, the main soil type being the calcaric cambisols. Humus content is moderate to high (3-6%), total nitrogen content is ranged between 0.13 to 0.30% and the C: N ratio is 12-14; cation exchange capacity is high (30-35 me/100 g soil), and the degree of base saturation varies depending on the depth of hard limestone rock (60-90%), as well as the pH (5.6- 7.2). (IANOŞ & al. [11])

The species analyzed are from the different areas of Anina Mountains as follows: *Sambucus nigra* - Staierdorf (Anina), Bozovici and Caraşova; *Galium verum* – Bozovici, Caraşova and Socolari; *Achillea setacea* – Bozovici, Caraşova, Socolari and Staierdorf (Anina); *Thymus pulegioides* - Bozovici; *Thymus pannonicus* - Caraşova; *Thymus glabrescens* - Staierdorf (Anina); *Allium ursinum* (Anina); *Crataegus monogynya* - Caraşova. Specimen samples were stored after identification in the Herbarium at the Biology Department of our university. Thyme species were treated at gender work because they are difficult to identify by collectors and the product used is a mixture of several species, all of which have medicinal properties.

For **macronutrients determination** 30 g of the sample of the plant are washed for 25 seconds with deionized water and dried in an oven at 103 - 105⁰C for 24 hours. From the dried sample was taken 1-5 g plant, then add 5 ml of 65% concentrated nitric acid. The sample was heated to 80⁰C acid, about 2h to dry. The process is repeated 2 times, then 5 ml of deionized water was added. The suspension was filtered through a filter paper with rapid

filtration and the filtrate was brought to a volume of 50 ml with deionized water. From the filtrate the concentration of macronutrients is read directly by the atomic absorption spectrophotometer, at a wavelength of 766 nm - K, Ca nm- 422.7 and 285 nm – Mg. Chemical analyses were performed in three replications and the mean values are presented. The concentrations of all the measured elements are expressed as g/kg of dry weight. Limit of detection for determined macronutrients was: 0.001 ppm for Ca and Mg and 0.1 ppm for K.

Statistical analysis was made using SPSS.17 Statistics Data Programme.

3. Results and discussions

The data on macronutrient content determined for the analyzed species can be seen in Table 1.

Calcium is a necessary element in plant growth and development. Calcium alongside magnesium enter into the composition middle lamella; has influence on selectivity, absorption and transport of ions through the membrane; plays a role in the organization of chromosomes during mitotic division; enter into the constitution of enzymes and activator acts on others (RUSU et & al. [12]). Calcium is antagonistic action against K^+ ions, NH_4^+ , H^+ , Mg^{2+} , Fe^{3+} , Al^{3+} , removing the harmful action of an excess of these ions (RADULOV [13]).

Table 1. Macroelements content in plant samples

Sample	Identified species	Harvesting place	Macroelements content		
			Ca g/kg	Mg g/kg	K g/kg
Sample 1	<i>Sambucus nigra</i>	Staierdorf	6.515	4.749	55.2
Sample 2	<i>Sambucus nigra</i>	Bozovici	5.703	3.430	50.9
Sample 3	<i>Sambucus nigra</i>	Carasova	8.016	3.230	61.5
Sample 4	<i>Galium verum</i>	Bozovici	12.437	1.288	11.5
Sample 5	<i>Galium verum</i>	Carasova	10.822	0.458	15.7
Sample 6	<i>Galium verum</i>	Socolari	10.628	1.038	12.4
Sample 7	<i>Achillea setacea</i>	Staierdorf	11.825	1.423	15.3
Sample 8	<i>Achillea setacea</i>	Bozovici	10.909	2.151	12.4
Sample 9	<i>Achillea setacea</i>	Carasova	14.854	1.625	17.8
Sample 10	<i>Achillea setacea</i>	Socolari	14.208	1.549	16.8
Sample 11	<i>Thymus glabrescens</i>	Staierdorf	11.707	1.170	14.7
Sample 12	<i>Thymus pulegioides</i>	Bozovici	9.493	1.610	14.4
Sample 13	<i>Thymus pannonicus</i>	Carasova	9.517	1.506	15.5
Sample 14	<i>Allium ursinum</i>	Staierdorf	13.134	0.174	15.7
Sample 15	<i>Crataegus monogyna</i>	Carasova	22.436	4.562	9.6

In the human body calcium, helps alongside phosphorus, the formation and strengthening of bones and teeth. Performs a number of functions among which the muscular contraction, nerve impulse transmission, immunity, promotes the absorption of iron and vitamin B12 (VAN DE GRAFF [14]) is a blood clotting activator of various enzymes (SOETAN & al. [3]).

The calcium content studied of medicinal plants varies in the range of 6 g/kg and 22 g/kg. In the case of plants collected from Staierdorf, the determined values of the calcium content are ranged between 6.514 and 13.134 g/kg. The highest content was determined for *Allium ursinum* species, followed by *Achillea setacea* species (11.825 g/kg).

Plant samples collected from Bozovici, is characterized by a low calcium content compared to the same species analyzed in the neighboring areas. The exception is *Galium verum* species which records the highest value in the mentioned area (12.437 g/kg).

In the Caraşova area, harvested plant samples had a calcium content ranged between 8.016 and 22.436 g/kg. After analysis the greatest amount of calcium was determined for *Crataegus monogyna* species. *Sambucus nigra* species proved to be the poorest in this macronutrient from all the plant samples harvested in the area of the village Caraşova.

The highest value of calcium content in the harvested plants from Socolari area was determined in case of *Achillea setacea* species (14.208 g/kg Ca). Different amounts of calcium accumulation can be attributed to different species ability to assimilate this macroelement, type of soil and the presence of other ions in the soil solution which may have a synergistic or antagonistic effect on calcium absorption.

In case of *Sambucus nigra* species, the calcium content in inflorescences has the following values: 6.515 g/kg for specimens collected from Staierdorf area, 8.016 g/kg in inflorescences harvested from Caraşova and 5.703 g/kg in the case of specimens collected from Bozovici area. Data from the literature indicate values of calcium content ranged between 1.99 and 5.15 g/kg in specimens collected from eastern Poland (KOŁODZIEJ & al. [15]). Plants from our samples have a higher quantity of this macronutrient, for all three samples.

The calcium content of *Galium verum* species is ranged between 10.628 g/kg and 12.437 g/kg. The highest calcium content was determined for plants harvested in the surroundings of Bozovici area.

Achillea setacea plants have calcium content ranged between 5.703 g/kg and 14.208 g/kg, the highest value being determined in samples collected in the Bozovici area, and the lowest in samples harvested in Caraşova area.

ŞTEF & al. [5] determined for the species *Galium mollugo* a quantity of 18.67 g/kg Ca, and for the *Achillea millefolium* species a value of 17.31 g/kg Ca, but cannot be specified the area where the plant samples were collected, because they came from local markets.

The calcium content of the *Thymus* genus plants is relatively close, even if they are different species, determined values ranging from 9.493 g/kg in the Bozovici area and 11.707 g/kg in the Staierdorf area. Calcium content in the analyzed species is comparable with those determined by KUCUKBAY & al. [15] for other species of the genus *Thymus*, adhere to the *Th. kotschyanus*, *Th. syriacus*, *Th. sipyleus*, *Th. eigii*, *Th. fallax*, *Th. revolutus*, whose calcium content in leaves varied between 8.38 - 25.57 mg/g. The highest value were determined for the first three species, and range between 16-26 mg/g.

In plants, magnesium performs many essential functions: in the process of photosynthesis; in carbohydrate metabolism; in protein synthesis of nucleic acids as a component of the ribosomes; in the regulation of cell permeability; transport and storage of backup substances; in the process of germination; in the regulation of cell's osmotic pressure and pH. Excess magnesium disrupts plant absorption of K^+ , Ca^{2+} and Mn^{2+} , leading to the emergence of specific deficiencies of these nutrients (RADULOV [13]).

In the human body magnesium has an important role in the formation of teeth and bones, in thermoregulation, regulates calcium balance and is an activator of numerous enzyme systems (DINU & al [17]).

The magnesium content of the analyzed plants is relatively low compared to the data presented in the literature, the values are ranged between 0.2 and 4.7 g/kg Mg. In the Staierdorf area, magnesium content of the analyzed plants is between 0.174 g/kg (*Allium ursinum*) and 4.749 g/kg (*Sambucus nigra*).

Plant samples collected in the Bozovici area contain different amounts of magnesium, after analysis we determined a minimum value of 1.288 g/kg Mg and a maximum value of 3.430 g/kg Mg. The highest Mg content was determined for *Sambucus nigra* species. *Achillea*

setacea and *Thymus pulegioides* species show lower amounts of magnesium compared with *Sambucus nigra* species. The lowest value was obtained for species *Galium verum*, harvested in Bozovici area, where a high concentration of Ca was determined.

In the Caraşova area, magnesium content of the plant samples is ranged between 0.458 and 3.230 g/kg. Values above 1 g/kg Mg were determined for *Achillea setacea* species (1.625 g/kg Mg) and *Thymus panonicus* (1.506 g/kg Mg). *Galium verum* species contains the lowest amount of magnesium.

In the Socolari area, magnesium content of the analyzed plants is lower, the maximum being determined in case of *Achillea setacea* species, 1.549 g/kg Mg, and the minimum for *Galium verum* species, 1.038 g/kg Mg.

The magnesium value of the *Sambucus nigra* species is ranged between 3.230 and 4.749g/kg. The highest value was determined in plants harvested in the Staierdorf area. According to the literature, the magnesium content of this species is lower than the values determined for *Sambucus nigra* plants harvested from Poland witch range between 4.15-8.83 g/kg (KOLODZIEJ & al. [15]).

The plants of *Galium verum* have a magnesium content ranged between 0.458 and 1.288g/kg. The highest magnesium content was determined for plant samples collected from the Bozovici area. The lower magnesium content of this species was determined for samples collected in the Caraşova area. *Galium verum* plants harvested in the Caraşova have a high content of calcium and magnesium content at lower, likely due to the antagonism of the two cations.

Magnesium content of *Achillea setacea* species is ranged between 1.423 and 2.151g/kg. Samples harvested from the Bozovici area are characterized by the highest magnesium content. In this case, as for the *Galium verum* species, the Ca-Mg antagonism occurs, samples harvested in the Bozovici area having the highest content of magnesium and lowest of calcium from all *Achillea setacea* plants studied.

Magnesium content values of the *Thymus* genus varies widely between 1.170 g/kg to 1.610 g/kg. The samples collected in the Bozovici area have the highest value of magnesium content, calcium is a minority component in these samples. The samples collected from Staierdorf area have the lower Mg content, and the highest content of calcium. KUCUKBAY & al. [16] determined in leaves of different species of the genus *Thymus* spontaneous, in Turkey, a much higher magnesium content, content that varied between 2.69 - 9.45 mg/g.

The results concerning the macronutrients content of the genus *Thymus* are comparable with data from the literature, the limit values are set for family Lamiaceae. Thus, ARCEUSZ et al. [10] determined for the Lamiaceae family, after analyzing several different genera of this family, a calcium content ranged between 12.1-34.3 mg/g, a magnesium content ranged between 1.16-7.78 mg/g and a content of K ranged between 3.57-35.2 mg/g.

Potassium is an essential element for plant life. It has a multiple role, influenced by the interaction between plant, soil and climatic conditions, which makes it difficult to easily decipher the specific mechanism of action. Potassium is found in plants almost exclusively in the form of ion (RADULOV & GOIAN [18]).

The average content of potassium in plants is 10 mg/g. Potassium content depends on the species, age, plant's organ and environmental factors (pH, light intensity, the absence or presence of other ions, water). In the leaves of superior plants, the average content of potassium is between 1.66 to 2.75% K dry matter, which may be up to 8%. Potassium distribution in various plant organs, compared to other cations, is (values average): 82% fruit, 62% in the roots, leaves 35% and 49% in strain (BORLAN & al. [19]).

Potassium is an essential element not only for plants but also for animals and humans.

The role of K in the human body is complex intervening in the forming proteins, maintaining cellular balance, acid-base balance, transport of oxygen and carbon dioxide in the blood, nerve impulse management (LUX-SPARSCHUH [20]), muscle contraction in cardiac muscle specuila, glycogenesis.

Potassium content of studied medicinal plants is ranged between 9 and 62 g/kg. In all species studied, the highest values of potassium content were determined in Caraşova area, and lowest in the Bozovici area.

The highest values of potassium contents were determined in *Sambucus nigra* species, the determined values being approximately 5 times higher than in other species. The potassium content determined for *Sambucus nigra*, corresponds to the values found in the literature (KOŁODZIEJ & al. [15]). The maximum amount was determined for the samples taken from the Caraşova area, i.e. 61.5 g/kg. The lowest amount of the potassium was found for the samples harvested in Bozovici area, 50.9 g/kg, while the samples in the area around Staierdorf containing 55.2 g/kg.

In the particular case of *Galium verum*, the potassium content is ranged between 11.5 and 15.7 g/kg. Similar research on the content of potassium but in *Galium mollugo*, indicates an average value of this macronutrient of 10.59 g/kg (ŞTEF & al. [5]). As in case of *Sambucus nigra* species, the maximum potassium content was determined for samples harvested in Caraşova area, and the minimum at Bozovici area.

Potassium content of *Achillea setacea* species is slightly higher than for *Galium verum* species, determined values after samples analysis were ranged between 12.4 and 17.8 g/kg. Samples collected from the village Caraşova is characterized by the highest content of potassium, followed by those harvested from Socolari area, 16.8 g/ka, and Staierdorf area, 15.3 g/kg. Samples in the Bozovici area have the lowest potassium content.

Potassium content values of the genus *Thymus sp.* are very similar for all three samples analyzed respectively for all three species analyzed. Samples collected from localities Staierdorf and Bozovici containing 14.7 g/kg and 14.4 g/kg. A slightly higher value was determined for samples collected at Caraşova, i.e. 15.5 g/kg.

For hawthorn (*Crataegus monogyna*), calcium values (22.436 g/kg) and potassium (9.6 g/kg) were close to the values determined for the same species by ŞTEF & al. [5], Ca - 21.41 g/kg, K - 9.8 g/kg. An exception is magnesium content, which in investigated area was almost twice higher than the values specified by literature (4.562 g/kg compared to 2.48 g/kg).

Recent research conducted by ANTAL & al. [21] in the Anina Mountains, aimed at determining the cadmium content for a total of 29 species of medicinal plants and drew attention to the fact that a large number of medicinal species, used for medicinal proposes in the studied area, have a cadmium content above the allowed limit. Thus, *Viola tricolor*, *Hypericum perforatum*, *Agrimonia Eupatoria*, *Thymus pulegioides*, *Betula pendula*, *Urtica dioica*, *Plantago lanceolata*, *Achillea millefolium* are the main species that accumulate in tissues this heavy metal. For this reason, we recommend that genus *Thymus* and *Achillea*, within the plants studied by us, may not be used, because macronutrients and trace elements have different degrees of solubility and are found in infusion (QUERALT & al. [22], ANTAL & al. [21]), reaching the human body.

In first pair case, there is strong evidence ($t = 8.674$, $p = 0.000$) that calcium is absorbed in studied medicinal plants in higher quantities than magnesium. In this data set, calcium content is higher, on average, by approximately 4.7 units. The 95% Confidence Interval (95% CI) is from 7.13 to 11.8. This confirms that, although the difference in macronutrient quantities is statistically significant, it is actually relatively small.

Table 2. Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Ca	11.4803	15	3.98204	1.02816
	Mg	1.9975	15	1.37861	0.35595
Pair 2	K	22.6267	15	17.44868	4.50523
	Ca	11.4803	15	3.98204	1.02816
Pair 3	Mg	22.6267	15	17.44868	4.50523
	K	1.9975	15	1.37861	0.35595

In second pair case ($t = 2.138$, $p = 0.051$) we have the evidence that potassium is absorbed in higher quantities than calcium, potassium content being higher by 11.1 units (95% CI = 0 – 22.3). The difference between absorbed potassium and calcium is statistically significant and relatively high. (Table 3)

Table 3. Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Ca - Mg	9.48273	4.23410	1.09324	7.13796	11.82750	8.674	14	0.000
Pair 2	K - Ca	11.14640	20.19121	5.21335	-.03512	22.32792	2.138	14	0.051
Pair 3	Mg - K	20.62913	16.64145	4.29680	11.41340	29.84486	4.801	14	0.000

In the last pair case, paired samples test shows that potassium is predominantly absorbed in studied medicinal plants compared with magnesium ($t = 4.801$, $p = 0.000$). The 95% Confidence Interval (95% CI) is from 11.4 to 29.8. The difference between quantities of these 2 macronutrients is statistically significant and high. (Table 4)

Table 4. Correlations between macronutrients content in studied medicinal plants

		Ca	Mg	K
Ca	Pearson Correlation	1	-0.016	-0.629*
	Sig. (2-tailed)		0.956	0.012
	N	15	15	15
Mg	Pearson Correlation	-0.016	1	0.611*
	Sig. (2-tailed)	0.956		0.015
	N	15	15	15
	Pearson Correlation	-0.629*	0.611*	1
	Sig. (2-tailed)	0.012	0.015	
	N	15	15	15

*Correlation is significant at the 0.05 level (2-tailed)

Values of Pearson coefficient indicates significant correlations between macronutrients content in studied medicinal plants at the 0.05 level.

Except for the medicinal plant species, calcium absorption by plants is influenced by many other factors like: soil pH, soil CEC, high sodium content of soil, parental material and interactions with other cations. Dependence between calcium content and species of medicinal plants is positive, but relatively moderate, coefficient r is equal to 0.845. (Figure 1)

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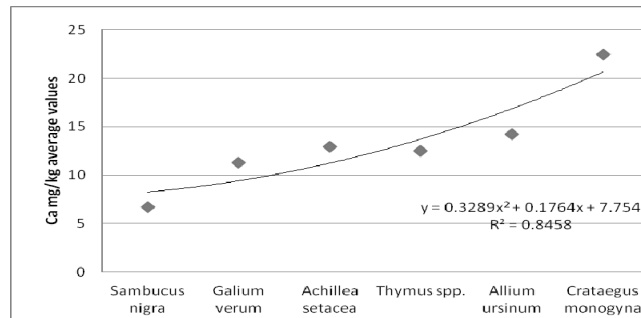


Figure 1. Correlation between calcium content (average values) and studied medicinal plant species

Correlation between magnesium content and studied medicinal plant species is medium, $r = 0.792$. Soil with high levels of K or Ca will typically provide less Mg to the plants. Potassium is a stronger competitor with Mg than calcium. When the soil K level is higher than desired, plant Mg levels are low. (Figure 2)

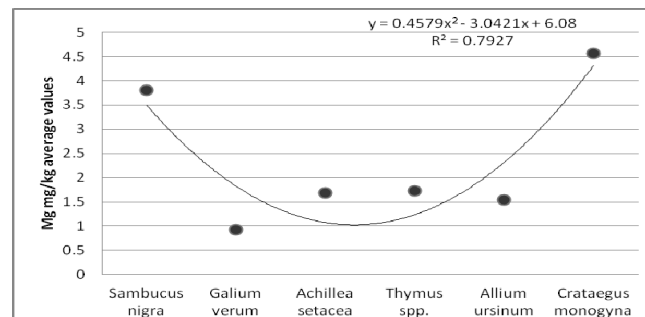


Figure 2. Correlation between magnesium content (average values) and studied medicinal plant species

Between plant species and content of absorbed potassium exists the strong correlation, $r = 0.727$. Unlike *Galium verum*, *Achillea setacea*, *Thymus sp.*, *Allium ursinum* and *Crattaegus monogyna* species that have approximately the same content of potassium, *Sambucus nigra* species contain double amount of this macroelement. (Figure 3)

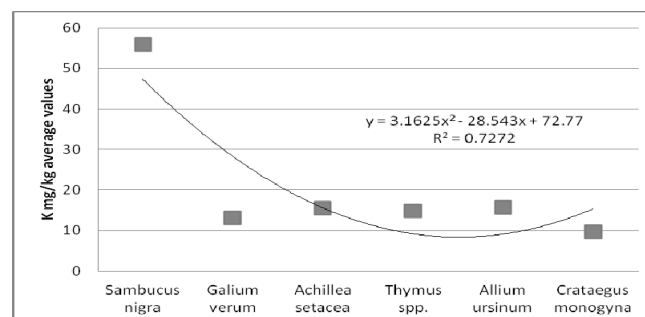


Figure 3. Correlation between potassium content (average values) and studied medicinal plant species

4. Conclusions

The calcium content of studied medicinal plants recorded values that are within the limits specified in the literature.

The magnesium content of the analyzed plants is relatively low compared to the data presented in the literature. The highest content was recorded for elderberry harvested in the Bozovici area, lower than the values for the same species found in the literature. It can be seen from these data, the pronounced manifestation of Ca-Mg antagonism. Due to rendzic limestone soil, the plants in this area, fix high calcium quantities and small amounts of magnesium or potassium.

Potassium content of medicinal plants studied is between 10 and 62 g/kg. In all species studied, the highest values of potassium contents were determined in the Caraşova area, and lowest in the Bozovici area. Depending on the species analyzed, the highest values were recorded for elder (*Sambucus nigra*), values comparable with data from the literature.

A high content of exchangeable calcium and a large amount of the K⁺ cations in the soil's colloidal complex leads to the appearance of the magnesium deficiency in plants and grass tetany (hypomagnesaemia) in animals.

Correlations between the average content of macronutrients and medicinal herb species shows that the highest rate of absorption is in potassium case, followed by calcium, and the lowest is in magnesium case. All studied medicinal herb species contain important quantities of potassium, calcium and magnesium and should be used in human nutrition except *Thymus* sp. and *Achillea* sp. for the reasons mentioned in the paper.

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