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DETERMINATION OF CALCIUM, MAGNESIUM AND POLYPHENOLS IN HAWTHORN FRUITS FROM VULCAN COAL DUMP

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ABSTRACT. The study refers to the content determination of calcium, magnesium and polyphenols in hawthorn fruits collected from Vulcan coal dump. This dump was never covered by fertile soil or fertilizer containing nitrogen, potassium and phosphorus. The hawthorn fruits obtained from dump contains significantly high concentration of calcium and magnesium, compared with the already published data. The polyphenols were evaluated by spectral (UV-Vis spectrophotometry) and chromatographic (TLC and HPLC) methods. The total flavonoids and total polyphenols contents of dump fruits are similar with those obtained in fruits collected from hawthorn culture, being lower than the already published data. In both type of hawthorn fruits could be identified the hyperoside, as the main flavonoid. We found also rutoside, only in traces, in the culture sample.

Keywords: *coal dump, hawthorn fruits, calcium, magnesium, polyphenols.*

INTRODUCTION

Hawthorn (*Crataegus monogyna*) is appearing as a shrub or small tree widespread in Europe, Asia and North Africa. In our country it can be found in lowland and mountain forestry, on the border of meadows and crops and nearby coal dumps, sometimes grown for ornamental purpose. It belongs to the Rosaceae family. It is a shrub with spiny branches and can be identified by the following: ligneous, dark brown branches, having diameters of 1 to 2.5 mm; ovate leaves with pinnate or lobate limb, with a stem up to 2 cm and numerous small, white flowers, disposed in corymb. The flowers have a green-brown tubular calyx consisting in 5 free triangular sepals, a corolla composed of 5 white or yellowish-brown free petals, 15-20 stamens and a monocarpellary

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ovary. The fruit is obovat or globular, with an overall length of 6-10 mm and 4-8 mm wide, red-brown or dark red colored. It has a single oblong brown seed, smooth and glossy. The fruit is a drupe [1-5].

In the last 40 years, hawthorn has become one of the most studied medicinal herbs. Researchers have discovered several active substances in flowers, leaves and fruits. The plant contains triterpens like crategic acid, ursolic acid; flavonoids; purinic bases; phytosterols; volatile oil, tannins, pectin, vitamin C. Flowers and leaves are rich in flavonoids, tannins, triterpene acids and essential oil. The bark contains aesculin, a glycoside from the coumarins group. The fruits are rich in amines, vitamin C, antocyanosides and other polyphenols, carotenoids, sorbitol, glucose and sucrose. These active ingredients are useful in treating cardiovascular diseases and sometimes can substitute the valerian [6-11].

Researches carried out by M. Nicolov have revealed the presence of 17 flavonoidic compounds in hawthorn. The presence of vitamin B and C, carotenoids, sorbitol, glucose, sucrose, chlorogenic and caffeic acid was demonstrated by Cekolinskaia. In order to demonstrate the pharmacological action of the chemical compounds of hawthorn, many experiments on dogs, rats and rabbits had been made. Hawthorn is used in various forms, entering in the soothing tea composition, the anti-asthma and hypertension tea also [6].

As hawthorn is rich in calcium and magnesium, it is recommended in nourishment and heart or brain treatment. The hawthorn preparations are recommended in heart degenerations and coronary sclerosis in the elderly, to treat a heart hypertonia, in myocardial insufficiency after infectious diseases and cardiac arrhythmias. It cause dilatation of blood vessels, in particular coronary blood vessels, reduced peripheral resistance and increased coronary circulation. The blood flow increases, the oxygen consumption is increased too, even efficiency in using oxygen is improved. It acts to eliminate extrasystole of any genesis and to remove any accesses of paroxysmal tachycardia. Crataegus has positive intropic, dromotropic and chronotropic effects, and negative bathmotropic effects. The cardiotropic effect of Crataegus is due by the increased membrane permeability for calcium as well as the inhibition of phosphodiesterase with an increase of intracellular c-AMP concentrations. Inhibitory effects on the sodium/potassium ATPase in vitro were most pronounced with application of Crataegus procyandidins, followed by Crataegus flavonoids, and least with Crataegus extracts [12-20].

The purpose of this paper is to determin the calcium and magnesium content respectively the polyphenols content in the Crataegus (hawthorn) fruits harvested from a sterile dump in comparison with the fruits collected from culture.

RESULTS AND DISCUSSION

The first step of the study was to determine the soil composition. In order to find out the responsible ions for hawthorn growth, was performed an elemental XRF analysis, which results are in Table 1.

Table 1. XRF elemental analysis results

Element	Si^{4+}	Al^{3+}	K^{+}	Ca^{2+}	Mg^{2+}
wt. %	46.9	20.6	5.08	6.09	1.03

The petrology of the coal dumps refers to clay, marl, shale and clay sandstone. After investigations by X-ray diffraction it could be demonstrated that the dump etiantrosoil is rich in quartz, calcite, feldspar and biotite. The coal dump was never covered by a soil layer, therefore the hawthorn was spread directly on the sterile.

The results presented in Table 1 show that the silicium ions are the most representative ions from soil. These ions are present in all soil types [21-23]. Aluminium and potassium are in the composition of the potassium feldspar and biotite. The latter contains magnesium, which can be released into the soil through alteration of the black mica in the presence of water. Calcium is present in calcite and is released into the soil also by chemical altering.

The main chemical elements necessary for the development of hawthorn are provided in the content within soil minerals. Some minerals could be chemical inactive or on the opposite, could be active in presence of water. Quartz is the main mineral found into the soil sample. Quartz have a hexagonal crystallization and assure a very compact and resistant structure, without cleavage. Featured properties of quartz particles prove that it is chemically inert acting as a neutral component.

Calcite is a typical mineral for sedimentary soils [24,25]. Calcite particles are influenced by water, which could release Ca^{2+} ions in aqueous solution, similarly to the process involved in cave formations. Hawthorn roots could easily assimilate Ca^{2+} ions from dump soil in presence of relative humidity due to larger amount of calcite particles founded there.

The biotite structure features hexagonal crystal planes of SiO_2 tetrahedra (which have a very high mechanical strength) bonded in multi – layers by Mg^{2+}, Al^{3+}, and K^{+} ions which trapped free valences of SiO_2 tetrahedra. The mechanical strength is weaker between the layers than inside of them, the inter–layer distance allows water to penetrate inside of biotite crystal and consequently to release some of the bonding ions. The released ions amount is favored by the presence of small micro – scaled particles [21-23]. The biotite is enough to release a significant amount of Mg^{2+} ions in the presence of water; similar, the amount of potassium feldspar represents an important source of K^{+} ions.

A fertile soil rich in humus can stabilize oligoelements such Ca and Mg as humic acid salts insoluble in water. Furthermore this colloidal suspension is absorbed by plants roots due to the cellular osmotic pressure [9, 10]. The dump soil is characterized by an acute lack of organic material and humus due to the pedogenesis. Furthermore the available minerals are released from parent minerals directly into the water present in dump soil. The hawthorn absorbs this mineralized water in the feeding circuit. There appear a strong interconnection between the Ca and Mg content in the soil samples and the amount in hawthorn.

The results of XRF elemental analysis are in fair agreement with the minerals identified in soil with XRD observation. All identified ions belong to these minerals. In this particular case we could calculate the Ca and Mg amount per solid soil sample as follows:

$$\begin{cases} Ca_{sol} = 6.09 \ \ gr/100gr\ sol \\ Mg_{sol} = 1.03 \ \ gr/100gr\ sol \end{cases} \tag{1}$$

This allows further to calculate the hawthorn fruit extraction coefficient, R, of Ca and Mg according to the relations (2) and (3):

$$R_{Ca} = \frac{Ca_{soil} - Ca_{brier}}{Ca_{soil}} \cdot 100 \ \ [\%] \tag{2}$$

$$R_{Mg} = \frac{Mg_{soil} - Mg_{brier}}{Mg_{soil}} \cdot 100 \ \ [\%] \tag{3}$$

The calcium content was determined based on the following calibration curve: A = 0.011 x C_{Ca} – 0.0004. The calcium content of hawthorn fruits collected from the Vulcan coal dump are ranging between 0.5204 and 0.5358, the average being 0.5258 g Ca/100g solid sample. Reference values are within 0.3046 and 0.4141 g Ca/100g solid sample.

The magnesium content was determined based on the following calibration curve: A = 0.573 x C_{Mg} – 0.0001. The magnesium content vary between 0.3421 and 0.3622, and the average is 0.3555 g Mg/100g solid sample. The reference value for the magnesium content ranges between 0.1502 and 0.1565 g solid sample mg/100g.

Finally results that the Ca extraction coefficient is 91% and Mg extraction coefficient is 65% for the hawthorn fruits growth on the dump soil. The calcite content is significantly increased, compared to biotite. The lesser amount of biotite conducts to an enhanced extraction coefficient for Mg. This proves that dump soil is able to assure an optimal amount of Ca and Mg for the hawthorn fruits situated far over the standard range. The lack of heavy

metals in the dump area is a favorable assumption for human use of hawthorn. This is a sufficient condition for a random and wild crop, suitable only for home application (brier marmalade, depurative and refreshing tea etc.).

The polyphenols were investigated by TLC (Figure1). The compounds separated from samples – hawthorn fruit extracts from coal dump respectively from culture were compared with the used standards: rutoside, hyperoside, chlorogenic acid and caffeic acid. In the used visualization condition the flavonoids show yellow to orange bands and the other polyphenols have blue to bluish-green bands.

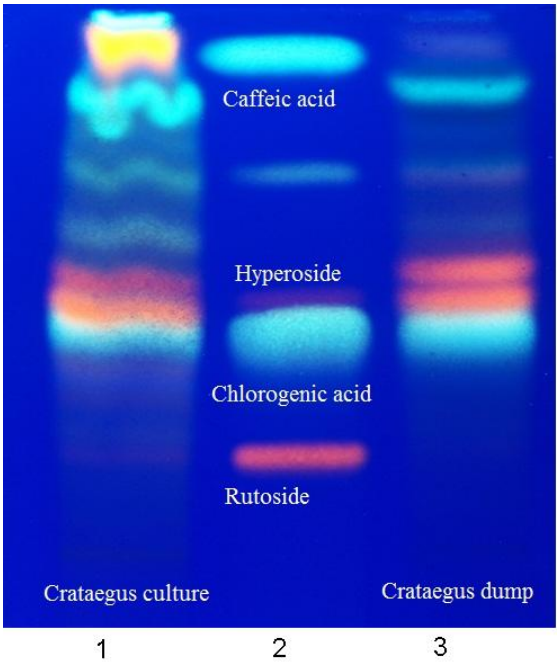

Figure 1. Polyphenols TLC chromatogram

The sample from culture contains trace of rutoside, that can not be identified in the coal dump sample. In both samples were identified based on the shape, position and color of bands the chlorogenic acid and the hyperoside. From TLC chromatogram can be observed that the culture sample is more rich in flavonoids that the coal dump sample, due by the presents of more blue, bluish-green and yellow-orange bands.

The quantitative determination of flavonoids (Table 2) is determined by calculating the content of total flavonoids expressed in rutoside in dry product, which according to Petricici and Servis must be between 1.1 – 2.28 % [25] and after laboratory investigations we obtained a total flavonoids content expressed

in rutoside of 0.31 mg % in the sterile dump sample and 0.38 mg % in the culture sample. The quantitative determination of polyphenols shown in Table 2, is determined by calculating the polyphenols content expressed in caffeic acid. For example, Tadić et al. have obtained 3.54 % polyphenols in hawthorn fruits [20], and after laboratory investigations, we obtained a content of total polyphenols expressed in caffeic acid of 0.22 mg % in the sterile dump sample and 0.23 mg % in the culture sample. The calculation of total flavonoids respectively polyphenols content were performed using the following calibration curves:

- Total flavonoids expressed in rutoside:
  - $A = 31.77 \times C_{rutoside} + 0.002$
- Polyphenols expressed in caffeic acid:
  - $A = 4.092 \times C_{caffeic\ acid} + 0.119$

Table 2. Total flavonoids content expressed in rutoside and polyphenols content expressed in caffeic acid

The determinations	Sterile dump	Culture	Reference value
Total flavonoids content, expressed in rutoside [mg%]	0.31	0.38	1.1 – 2.28
Polyphenols content, expressed in caffeic acid [mg%]	0.22	0.23	3.54

In Figure 2 are presented the HPLC chromatograms.

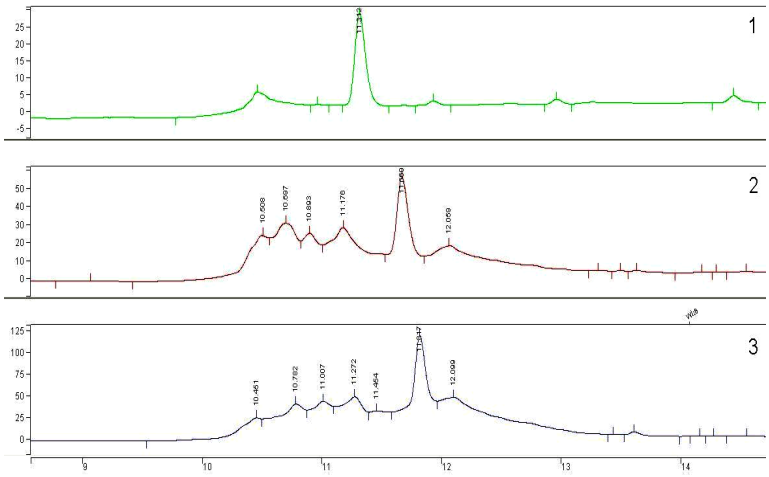

Figure 2. HPLC chromatogram of hyperoside (1) and Crataegus fruits from culture (2) and from sterile dump (3)

In Figure 3 is highlight the UV-Vis spectrum obtained during the HPLC analysis for: a) standard hyperoside; b) hyperoside from culture sample and c) hyperoside from sterile dump sample.

Comparing the HPLC chromatograms (the same retention time) and the obtained UV-Vis spectra (the same allure and absorbtion maximum) presented above we can conclude that both Crataegus fruits samples contains hyperoside.

Other therapeutically important polyphenols from hawthorn fruits are the procyanidines that were determined by calculating the content of procyanidine expressed in cyanidine chloride, according to European Pharmacopoeia and must be at least 1% [1]. According to our laboratory investigations was obtained a content of procyanidine of 0.98% for both samples, less than the prevision of Pharmacopoeia.

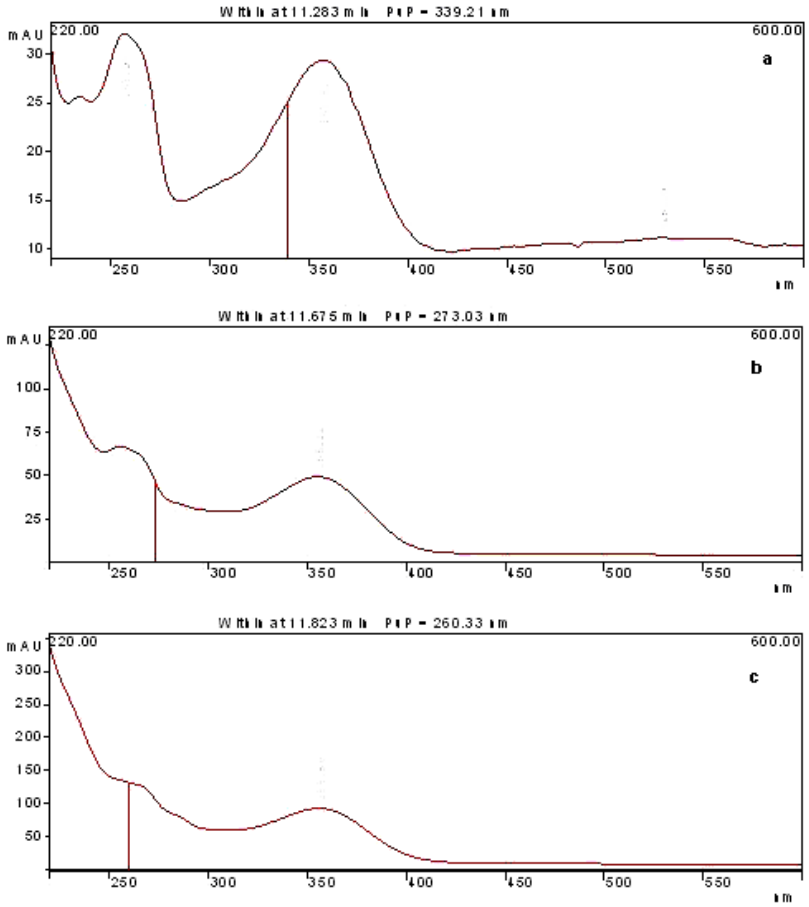

Figure 3. Hyperoside UV-Vis spectra – standard (a) and Crataegus samples (b – culture sample, c – dump sample)

Comparing the obtained results with those already published we can conclude that the calcium and magnesium contents of the dump hawthorn fruits contain 20% more calcium than those obtained by Boudraa et al. [26] and about 50% more calcium and twice more magnesium than those determined by Ozcan [11].

The results for total flavonoids obtained for the culture and dump sample are similar, but much lower than the reference data values. In case of polyphenols we obtained also similar values between the two samples and again, much lower than the reference value. Probably the content in polyphenols and flavonoids of the hawthorn fruits are influenced not just by the soil type, but also by the humidity during a year and the geographical position of the growing area. The culture and the sterile dump being situated in proximity the climatic influence was similar in both areas. This can be the explanation of the similar values obtained for total flavonoids and polyphenols content.

CONCLUSIONS

We can conclude that the hawthorn (Crataegus) fruits obtained from the culture and from the dump are similar, both containing smaller amounts of flavonoids and polyphenols, than the already published data. From the class of flavonoids can be identify the hyperoside in both samples, but the rutoside is present just in trace in culture sample. The calcium and the magnesium concentration are higher than usual in the dump sample, being more proper to be used in nourishment and heart or brain treatments. Based on these studies can be conclude that a soil more rich in calcium and magnesium minerals should be more proper for culture of hawthorn to obtain fruits more rich in these therapeutically important ions.

EXPERIMENTAL SECTION

Vegetal materials

The hawthorn fruits from the sterile dump were collected in the first half of September from the Vulcan coal dump (Petroşani Basin, Hunedoara County, Romania) and they were preserved according to the standard procedures SR 1631-1:2003.

The hawthorn fruits from the culture were collected in September.

The soil from sterile dump was collected in the same time with the fruits.

The soil analysis

The samples were dried at 80 ºC for 12 h, powdered (325 mesh) and mixed with boric acid in a 1:4 ratio (100 mg of sample and 400 mg of H_3BO_4). The mixture was pressed at 203 MPa for 10 minutes, obtaining 2,5 cm diameter pellets of 100 mg/cm^2 surface density.

The elemental soil analysis was performed according to the standard sampling and operating procedures using a S_4 Pioneer X-ray fluorescence spectrophotometer (XRF) in order to measure the main elements from beryllium to uranium. The results were expressed in percentage from soil.

The results are read with Spectra Plus software and the determination of elements is done using Dyna Match international database. Measurements were made according to EN ISO 9001:2000.

Three replicates were made for each sample.

The determination of calcium and magnesium

The vegetal material sample was mineralized using a Berghof microwave MWS_2 oven. The dry mineralization was achieved in three stages: first stage (T_1) at 145°C, for 10 minutes, the second stage (T_2) at 160°C, for 10 minutes and the third stage (T_3) at 190°C, for 20 minutes, using 80% of power. After mineralization the white ash was treated with 1% HNO_3 and diluted to 10 ml with deionized water. The obtained solution was used for the determinations of calcium and magnesium in the hawthorn fruit.

In order to establish the content of calcium and magnesium in the hawthorn fruits it was used atomic absorption spectrometry, AAS. It was used a Shimadzu atomic absorption spectrometer, AA 6300. The determinations were carried out according to EN 1134: 1994 respectively ISO 5725:1998, ISO 3696: 1987, ISO 3696: 1995. The AAS spectrophotometer uses calcium lamp, at 10 mA respectively a magnesium lamp, at 8 mA.

The determination of calcium was performed at 422.7 nm, using background compensation with deuterium lamp. The calibration curve was made between 0 - 2.5 ppm.

The determination of magnesium was performed at 285.2 nm, using background compensation with deuterium lamp. The calibration curve was made between 0 - 0.5 ppm.

Three replicates were made for each sample.

The TLC determination of polyphenols

The flavonoides and other polyphenols were determined by thin layer chromatography using a silica chromatographic plate with fluorescence indicator at 254 nm. The mobile phase was formic acid (Merck) – water – ethyl-methyl-cetone (Merck) - ethyl acetate (Merck), in proportion of 10:10:30:50 v/v.

The used standards were chlorogenic acid, hyperoside, caffeic acid and rutoside, each having 1 mg/mL in methanol. It was applied 30 µL from the samples and 10 µL from each standard.

After drying, the plate was sprayed with diphenyboriloxiethylamine (10 g/L) in methanol and then with polyethylenglycol 400 (50 mL/L) in ethanol. After 30 minutes the chromathogram was observed in fluorescence at 365 nm [27].

The HPLC determination of hyperoside

The determination was carried out by high performance liquid chromatography using a Varian Star HPLC system.

As standard was used hyperoside, 0.1 mg/mL in methanol.

The analysis conditions were: silica C18 column (Phenomenex, Luna C18, 150 x 4.6 mm, 5 μm); like mobile phase was used a binary gradient prepared from 0.1% (v/v) trifluoracetic acid (Merck) in water and acetonitril (Merck). The elution started with a linear gradient, beginning with isocratic elution followed for the next 15 minutes with 95 % trifluoracetic acid, then for 5 minutes with 5 % trifluoracetic acid and at the end for the 10 minutes with 95 % trifluoracetic acid. The flow rate was 1 mL/min. The DAD detector was operated at 270 nm and the injection volume was 10 μL.

The spectral determination of polyphenols

The polyphenols were determined using phosphotungstenic reagent, at 715 nm, according to Romanian Pharmacopoeia [28]. As standard was used the caffeic acid, 1 mg/mL in methanol. There was build a calibration curve from 0.002 to 0.013 mg/ml range.

The total flavonoids were determined using aluminium chloride 2.5 %, at 430 nm, according to Romanian Pharmacopoeia [28]. As standard was used the rutoside, 0.1 mg/ml in methanol. There was build a calibration curve from 0.004 to 0.016 mg/ml range.

The procyanidines were determined according to European Pharmacopoeia, using extraction in buthanol, at 545 nm. The results were expressed in cyanidine chloride [1].

REFERENCES

1. ***, “European Pharmacopoeia”, Ed. VII, Medpharm Scientific Publisher, Stuttgart, **2011**.
2. I. Hodişan, I. Pop, „Botanică sistematică”, Editura Didactică şi Pedagogică, Bucharest, **1976**, 324.
3. I. Pop, I. Hodisan, D. Mitotelu, L. Lungu, I. Cristurean, G. Mihai, “Botanică sistematică”, Didactică şi Pedagogică Press, Bucharest, **1983**, 301-306.
4. G. Racz, A. Racz, E. Cociu, “Plante medicinale şi aromatice”, Ceres Publishing House, Bucharest, **1970**, 172-174.
5. N. Ştefan, A. Oprea, „Botanică sistematică”, Alexandru Ioan Cuza University Press, Iaşi, **2007**, 552.

6. I. Ciulei, E. Grigorescu, U. Stănescu, „Plante medicinale, fitochimie şi fitoterapie – Tratat de farmacogzonie", Vol. I, Editura Medicală, Bucharest, **1993**, 452-458.
7. C. Pârvu, "Universul plantelor – Mică enciclopedie", Enciclopedică Press, Bucharest, **1997**, 471-472.
8. C.Pârvu, "Enciclopedia plantelor, Plante din flora României", Vol 3, Tehnică Press, Bucharest, **2004**, 678-681.
9. A. Radu, "Botanică farmaceutică", Didactică and Pedagogică Press, Bucharest, **1974**, 404-405.
10. I. Ciulei, L. Sommer, V. Istudor, „Farmacognozie", vol. I, IMF Press, Bucharest, **1979**.
11. M. Ozcan, H. Haciseferogullari, T. Maracoglu, D. Aslan, *Journal of Food Engineering*, **2005**, *68(4)*, 409-413.
12. G. Mohan, "Plante medicinale fitoterapie", All Publishing House, Bucharest, **1998**, 235-236.
13. G. Siegel, U. Gasper, F. Schnalke et al., *Phytother Res*, **1996**, *10*, S195-S198.
14. Anon, *Altern Med Rev*, **1998**, *3(2),* 138-139.
15. M. Schussler, J. Holzl, U. Fricke, *Arzneimittelforschung,* **1995**, *45(8),* 842-845.
16. M. Schussler et al., *Planta Med*, **1993**, *59(7)*, 88.
17. C. Mang, V. Herrmann, R. Butzer et al., *Eur J Clin Pharmacol*, **1997**, *52*, A59.
18. V.H.P.T. Ammon, R. Kaul, *Dtsch Apoth Z*, **1994**, *26*, 33-36, *27*, 21-35, *28,* 35-42.
19. G. Rohr, G. Meier, *Dtsch Apoth Z*, **1997**, *137(42),*104-116.
20. V.M. Tadić, S. Dobrić, G.M. Marković, S.M. Dordević, I.A. Arsić, N.R. Menković, T. Stević, *J Agric Food Chem*, **2008**, *56(17)*, 7700-9.
21. A. Brasovan, V. Codrea, V. Mândroc, R. Campean, N. Olah, *Analele Universitatii din Oradea, Fascicula Biologie*, **2009**, *XVI/2*, 40-42.
22. A. Brasovan, R.F. Campean, G. Arghir, V. Codrea, *Metalurgia International*, **2010**, *XV(7)*, 40-43.
23. A. Braşovan, V. Mândroc, R. Câmpean, I. Petean, V. Codrea, G. Arghir, *Analele Universităţii din Oradea, Fascicula Biologie*, **2011**, *XVIII/1*, 5-9.
24. M. Surpăţeanu, „Elemente de chimia mediului", Matrix Rom Press, Bucharest, **2004**, 176-215.
25. I. Petricici, L. Servis, *Acta Pharm. Jugosl.*, **1997**, *27*, 75.
26. S. Boudraa, L. Hambaba, S. Zidani, H. Boudraa, *Fruits Journal*, **2010**, *65 (2)*, 75-84.
27. ***, "Homoopathisches Arzneibuch", Deutscher Apotheker Verlag, Stuttgart, **2011**.
28. ***, "Farmacopeea Română", Ed. X, Editura Medicală, Bucharest, **1993**.