HPLC Screening of Bioactives Compounds and Antioxidant Capacity of Different *Hypericum* Species

DANIELA GITEA[1], SIMONA VICAS[2], MANUEL ALEXANDRU GITEA[2]*, SEBASTIAN NEMETH[1], DELIA MIRELA TIT[1]*, BIANCA PASCA[1], LAVINIA PURZA[1], CIPRIAN IOVAN[1]

[1] University of Oradea, Faculty of Medicine and Pharmacy, 29 Nicolae Jiga Str., 410028, Oradea, Romania

[2] University of Oradea, Faculty of Environmental Protection, 26 Gen. Magheru Str., 410048, Oradea, Romania

Our study compares the content in polyphenolic compounds and hypericin, in four species of Hypericum - H. perforatum L., H. maculatum Cr., H. hirsutum L., H. tetrapterum Fr. (syn. Hypericumacutum Mnch.) harvested from spontaneous flora in the north-western area of Transylvania, Romania. These species represent an important source of such compounds with different biological actions. After making the extracts, they were subjected to HPLC-SM analysis. The presence of rutoside in the largest amount (462.82 mg %) in the H. perforatum extract was observed, this containing most of the flavonoid heterosides. For the species H. maculatum, the presence in a much higher amount of the hyperoside (976.36 mg %) is characteristic compared to the other species. Quercetol is the best represented of the flavonoid aglycons, its concentration being the highest in H. hirsutum (659.66 mg %). The hypericin content ranges from 0.2171 g % in the H. tetrapterum extract, to 0.0314 g % in the methanol extract of H. maculatum.The highest antioxidant properties measured by FRAP method were recorded in the case of H. perforatum and H. maculatum.

Keywords: Hypericum sp., antioxidant activity, polyphenolic compounds, hypericin, HPLC-MS

The species of the genus *Hypericum* that grow on the territory of Romania are in number of 11, with 4 varieties and 1 cultivated species [1]. Of these, *H. perforatum* L. is the best known species, being officinal in the Romanian Pharmacopeia the 10th edition and in the European Pharmacopeias [2-4]. For the *Hyperici herba* monograph, only *H. perforatum* is accepted, although that in order to obtain the official product, in practice, other species of *Hypericum* from spontaneous flora are harvested, primarily *H. maculatum* Cr.

The herbal product *Hyperici herba* has a complex chemical composition, the most important compounds being: naphtodianthrones, flavonosides, floroglucinol derivatives, and volatile oil [5]. Naftodianthrons are mainly represented by hypericin and pseudohiphericin and, in a smaller proportion, by protohipericin, protopseudohipericin, cyclopseudohipericin [6]. Flavonosides (4-5%) are represented by rutoside, hyperoside, isoquercitozide, quercitrozide, and by free aglycons in the form of quercetol, kempferol and biflavonoids (I3, II8 biapigenin-0.26%, amentoflavones) [7]. Floroglucinol derivatives have hyperforin as a representant, with a structure similar to that of bitter substances from hops [8]; volatile oil (0.05-0.3%) with similar odor to that of conifers, is composed of monoterpenes (á-pinen, â-pinen, mircen, limonene) and secviterpene (cariophenylene or humulen) [9].

The presence of these bioactive compounds with various biological actions has led to an increased interest in these species for their use in phytotherapy. *H. perforatum* is one of the most studied species, and the research results show its exclusive use in phytotherapy. Studies have demonstrated several pharmacological actions of the St John's wort (common name of *Hypericum perforatum)* extract, the most important being choleretic collagogue, antipruritic, diuretic, antiviral, antimicrobial and healing [10]. In recent decades, it has been discovered that *Hypericum* preparations also have antidepressant action, these being better tolerated by patients than antidepressant synthetic medicines (especially tricyclics). Clinical studies have shown that *Hypericum* preparations have moderate sedative, antidepressant and anxiolytic action, and the anti-inflammatory effect is due to the high content of flavonoids [11]. Moreover, by the antioxidant effect, flavonoids reduce oxidative stress and this action has a decisive role in the prevention and treatment of many diseases [12,13].

For the determination of the bioactive compounds in plants and their action there is used a large variety of analytical techniques, kinetics [16], chromatographic [17], spectrophotometric [18,19] and combined methods [20, 21]. The HPLC-SM technique has been successfully applied for a rapid separation and identification of active principles from *Hypericum* species. So far, of the 11 species of *Hypericum* from the spontaneous flora of Romania, a total of 7 species have been analyzed: *H. perforatum, H. maculatum, H. hirsutum, H. humifusum, H. elegans, H. umbellatum, H. richeri Ssp. transsilvanicum Celak* [22-24].

Some factors have a major influence when we are talking about the variation of the concentration in bioactive compounds of the plants: environmental conditions, harvesting areas, poor management of waste reaching the water and soil [17,18,20,25-36], soil management practices [35-39]. For a better understanding of the therapeutic potential of Hypericum species that can be found in spontaneous flora of NW part of Transylvania, in this study we intend to analyze the content of flavonosides, hypericin, and the antioxidant action of the extracts obtained from four species of Hypericum as well.

Experimental part

Plant material

The analyzed plant material is represented by the aerial parts of the four *Hypericum* species (*Hypericaceae*) which grow on the territory of the Bihor county (Northwest area of Romania), harvested during the flowering period: *Hypericum perforatum* L.*, Hypericum maculatum* Cr. - variety *typicum* Frohlich,, *Hypericum hirsutum* L.*, H. tetrapterum* Fr. Species identification was based on the morphological characteristics of the aerial parts (stem,

* email : giteamanuel@yahoo.com, Phone: +40745333381;

mirela_tit@yahoo.com; Phone: +40742963812

All the authors had equal contribution at this original article.

leaves and flowers). The harvested material was dried at room temperature and then stored in paper bags, away from light and moisture. All voucher specimens are deposited in the Herbarium of the University of Oradea, Faculty of Medicine and Pharmacy.

Extracts preparation

The preparation of the samples for the identification of polyphenolic compounds and hypericin (from vegetable products) was carried out according to the following technique: 1 g of plant product is extracted on the water bath at 60°C with 100 mL of methanol at reflux, in a reflux condensing flask for 30 min, and then filtered. For the identification of the polyphenolic compounds, two samples of each plant extract were analyzed in parallel, one as such and the other hydrolyzed. The hydrolysis was carried out as follows: one part of the extract was diluted with HCl 2N and kept on the water bath at 80°C, for 60 min. *Hypericum* methanol extracts were used to determine the antioxidant capacity.

HPLC analysis of polyphenols and hypericin

The Agilent 1100 HPLC Series system equipped with UV detector, degasser, binary gradient pump, column thermostat, autosampler was used to assess the hypericin, and for the recognition and quantification of the polyphenolic compounds, mobile phase being the mixture methanol: acetic acid solution 0.1% (V/V). The Agilent 1100 mass spectrometer and the HPLC system were combined (LC/MS). The polyphenolic compounds analysis was realised in conditions that were described before and there were engaged 18 standards: apigenin, caftaric, caffeic, chlorogenic, coumaric, gentisic, ferulic, fisetin, hyperoside, isoquercitrin, p-sinapic acids, rutin, myricetin, quercitrin, quercetin, patuletin, luteolin, kaempferol. The identification of polyphenolic compounds of the studied species was performed based on comparison of their retention times with those of the standards, analyzed under the same experimental conditions. For quantitative determination, the hypericin standard was used. The UV assisted by mass -spectrometry (MS) detection was used for detecting and quantifying the polyphenols and hypericin. The calibration curves for a five point plot were used in the 0.5-50μg/mL range, with good linearity (R^2>0.999), from polyphenolic compounds. The calibration curve of hypericin was performed in the concentration range of 7-175 μg/mL (R^2> 0.999).

Antioxidant capacity

For the evaluation of the antioxidant capacity of methanol extracts of *Hypericum*, the FRAP (ferric reducing antioxidant power) method was used [39]. The principle of the assay is based on the reduction of ferric tripyridyltriazine complex [Fe(III)-TPTZ] by an antioxidant to the blue colored complex of [Fe(II)-TPTZ] in acid pH. The reagents used were 300 mM acetate buffer (*p*H 3.6), 20 mM $FeCl_3$. $6H_2O$ solution and 10 mM TPTZ (2,4,6-tripyridyl-s-triazine). The working FRAP solution was freshly prepared by mixing acetate buffer, $FeCl_3 \cdot 6 H_2O$ and TPTZ solution, in the ratio of 10:1:1 (V:V:V). *Hypericum* extracts (100 μL) were dissolved in 2000 μL distillated water and allowed to react with 500 μL of working FRAP solution for 60 min in dark condition, and the absorbance was read at 593 nm (Shimadzu mini UV-Vis spectrophotometer). The calibration curve was made using the aqueous solution of Fe^{2+} in concentrations between 0.1-1 mM (R^2=0.995). The results were given as mmol Fe^{2+}/g.

Results and discussions

Each class of polyphenolic compounds was detected at the wavelength corresponding to the maximum of absorption of the UV spectrum. Thus, the polyphenol-carboxylic acids were detected at a wavelength of 330 nm and the flavonoids and their aglycons at 370 nm. Chromatograms of the unhydrolyzed samples are illustrated in figure 1.a-d.

Using HPLC/MS, were identified and quantified in unhydrolysed and hydrolyzed extracts the free phenylpropanic compounds - p coumaric acid, ferulic acid, glycosidized form of flavonoside-hyperoside, izoquercitrozide, rutoside, quercitrozide and free aglycons - quercetol, kempferol, luteolin and miricetol (table 1).

In the *H. perforatum* extract, the main flavonoid component is the hyperoside (665.38 mg %) followed by isoquercitrozide (569.08 mg %), rutoside (462.82 mg %) and quercitrozide (110.22 mg %). Free flavonoid aglycons are represented by *quercetol* (533.03 mg %) and *kempferol* (11.46 mg %) in a much lower amount. These aglycons also appear in the unhydrolyzed extracts, but in a much smaller amount. The free phenylpropane compounds are represented by gentisic acid, chlorogenic acid, caffeic acid whose presence was confirmed by SM and by p-cumaric acid (9.59 mg %) and ferulic acid (5.62 mg %).

In the extract of *H. maculatum* a very high amount of hyperoside (976.36 mg %) and a very low concentration of

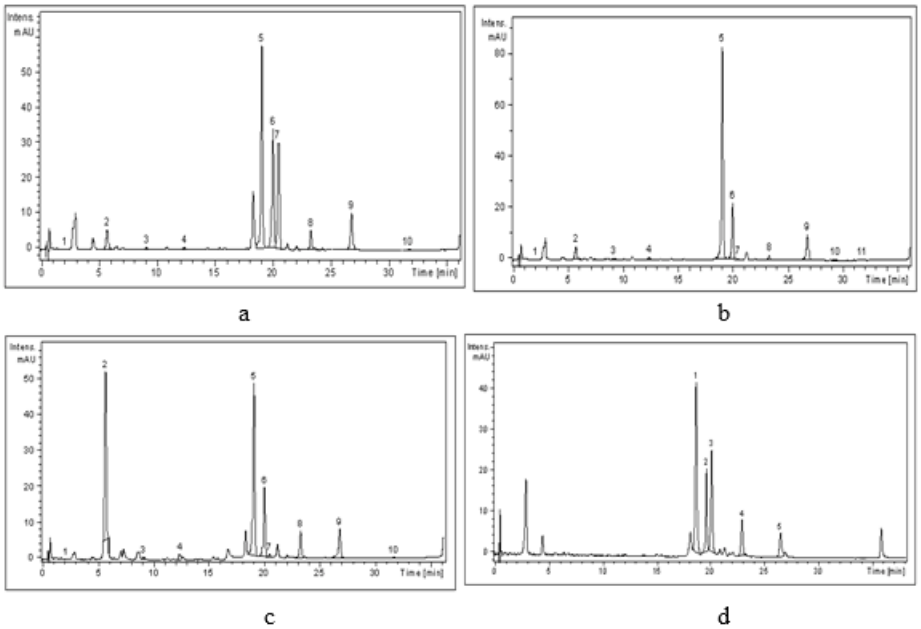

Fig.1. Chromatograms of unhydrolysed extracts of *H. perforatum* (a), *H. maculatum* (b), *H. tetrapterum* (c), *H. hirsutum* (d), UV

Table 1
QUANTITATIVE DETERMINATIONS OF POLYPHENOLS BY HPLC (µg/100 g NP) IN *HYPERICUM* SP. METHANOL EXTRACT

Sample / Compound	R_T min	*H. perforatum* L.		*H. maculatum* Crantz *ssp. typicum*		*H. tetrapterum* Fries		*H. hirsutum* L.	
		N	NH	N	NH	N	NH	N	NH
p- coumaric acid	9.20	4.29	9.59	2.72	5.19	3.15	7.29	-	6.82
Ferulic acid	12.4	4.10	5.62	5.87	8.65	9.11	17.30	-	5.57
Hyperoside	19.00	665.38	-	976.36	-	545.14	-	470.50	-
Izoquercitrozide	19.90	569.08	-	370.28	-	319.42	-	328.67	-
Rutoside	20.4	462.82	-	6.49	-	10.50	-	382.66	-
Quercitrozide	23.30	110.22	-	39.17	-	160.69	-	194.35	-
Quercetol	26.80	77.71	533.03	73.86	435.58	65.60	181.77	44.68	659.66
Kaempferol	31.70	3.36	11.46	2.90	4.42	3.70	7.21	-	12.12
Luteolin	29.2	-	-	2.05	2.19	-	-	-	-
Miricetol	21.10	-	2.24	-	0.71	-	1.71	-	5.15

N - unhydrolysed extract, NH - hydrolysed extract, R_T - retention time

rutoside (6.49 mg %) are observed. Free flavonoid aglycons are *quercetol* with a higher concentration in the hydrolysed extract (435.58 mg %), *kempferol* with a much lower concentration of 6.35 mg % and *miricetol* (0.71 mg %). *Luteolin* was highlighted in the hydrolysed extract of *H. maculatum* in a concentration of 2.19 mg%. The free phenylpropane compounds are represented by gentisic acid, chlorogenic acid, caffeic acid whose presence was confirmed by SM and of p-cumaric acid (5.73 mg %) and ferulic acid (9.06 mg %).

In the *H. tetrapterum* extract, the main flavonic component is represented by *hyperoside* (545.14 mg %) followed by *isoquercitrin* (319.42 mg %), *quercitrozide* (160.69 mg %) and in a much smaller amount *rutoside* (10.5 mg %). Free flavonoid aglycons are represented by *quercetol* (181.77 mg %) and *kempferol* (7.21 mg %) in a much lower amount. These aglycons also appear in unhydrolysed extracts but in a much smaller quantity. The free phenylpropanic compounds are represented by gentisic acid, chlorogenic acid, caffeic acid whose presence was confirmed by SM and by p-cumaric acid (7.24 mg %) and ferulic acid (17.3 mg %).

In the extract of *H. hirsutum*, the main flavonoid component is represented by *hyperazide* (470.5 mg %), followed by *rutoside* (382.66 mg %) and *isoquercitrozide* (328.67 mg %), and in a smaller amount *quercitrin* (194.35 mg) %) and *quercetol* (44.68 mg %). Free flavonoid aglycons are represented by *quercetol* (659.66 mg %) and *kempferol* (12.12 mg %) in a much smaller amount. These aglycons also appear in unhydrolysed extracts, but in a much smaller amount. The free phenylpropanic compounds are represented by gentisic acid, chlorogenic acid, caffeic acid, whose presence was confirmed by SM and p-cumaric acid (6.82 mg %) and ferulic acid (5.57 mg %).

The results of the HPLC/SM analyzes bring details both of the identity and of the content in flavonoid substances. The presence of a much lower concentration of rutoside in the species of *H. maculatum* and *H. tetrapterum* is confirmed as compared to the amount of *H. perforatum* and *H. hirsutum*. Thus, the presence of rutoside can be used as a differentiation criterion of the species.

For the *H. maculatum species*, the presence of a much higher amount of the hyperozide compared to the other species (545.14 mg/100 g np) is characteristic, by analyzing the vegetable product harvested from different areas of Transylvania, Romania [22,23].

In the case of aglycons, miricetol, kaempferol and quercetol were determined in all the hydrolysed samples from all *Hypericum* species taken into work in different concentrations and luteolin was determined only in the extract of *H. maculatum* both in the unhydrolysed and hydrolysed samples. In all the cases, the concentration of flavonoid aglycons increases after hydrolysis, which is explained by the presence of flavonoid O-glycosides, which, after hydrolysis, releases aglycons. This aspect is supported by several recent studies, showing differences in the concentrations of the analyzed principles, due to the influence of harvesting conditions, of preservation and obtaining of the extracts and the quality of the analyzed plant material [40]. Quercetol is the best represented of the flavonoid aglycons, its concentration being the highest in *H. hirsutum* (659.66 mg %). These values indicate that the *Hypericum* species contain an increased amount of glycosides derived from this aglycane.

Using HPLC analysis combined with mass spectrometry for extracts obtained from *Hypericum* species, results a wide variation of the hypericin concentration - from 0.2171 g % in the *H. tetrapterum* extract to 0.0314 g in the methanol extract of *H. maculatum* (table 2). Through this method, the precise separation and dosing of hypericin from the total of the existing hypericins in the H*ypericum* species has been achieved (fig. 2. a-d).

Species	Hypericin [g %]
H. perforatum	0.1645
H. maculatum	0.0314
H. tetrapterum	0.2171
H. hirsutum	0.0543

Table 2
QUANTITATIVE DETERMINATIONS OF HYPERICIN BY HPLC IN *HYPERICUM SP.* METHANOL EXTRACT

Studies conducted so far show that for *H. perforatum* the concentration of hypericin varies from 1.4-0.15%, differences occur depending on the harvested plant material, for example flowers or vegetative parts of the plant [40]. In the European Pharmacopoeia at St. John's wort monograph it is specified exactly the hypericin content of the vegetable product, namely at least 0.08% total hypericin expressed as hypericin ($C_{30}H_{16}O_8$, M_r= 504.4). Considering this, all sudied species of *Hypericum* fall within the limit imposed by the European Pharmacopoeia [2,3].

Antioxidant capacity

The antioxidant capacity of *Hypericum* extracts measured by the FRAP method is shown in figure 3.

The results were processed by one-way analysis of variance (ANOVA). Mean value differences were analyzed with Tukey's test (p=0.05) [40]. The highest antioxidant properties measured by FRAP method were recorded in

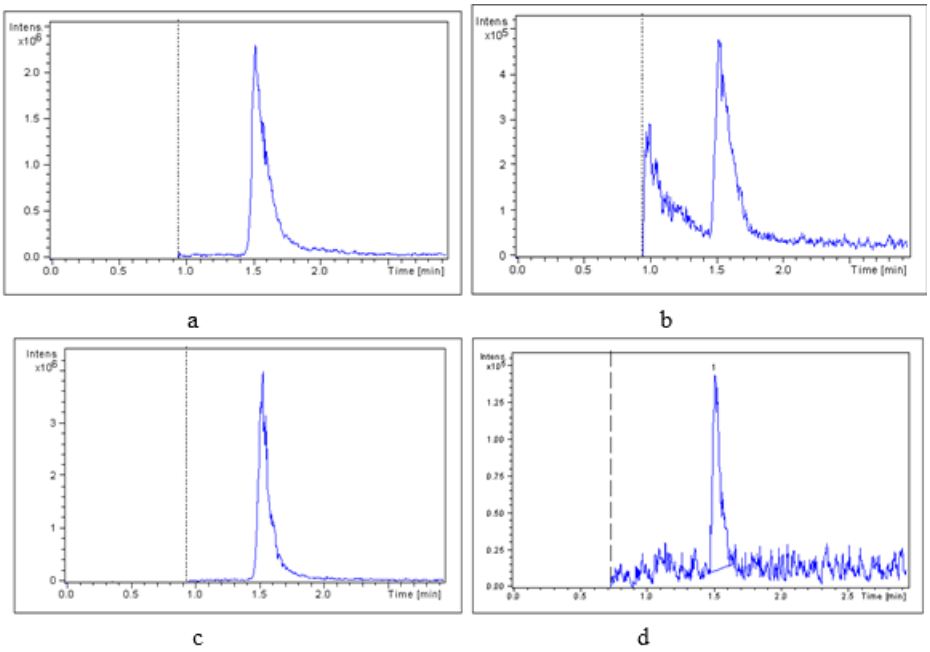

Fig.2. Chromatograms of hypericin from the extract of *H. perforatum* L. (a), *H . maculatum* Cr. *ssp. typicum* (b), *H. tetrapterum* Fr. (c), *H. hirsutum* L. (d)

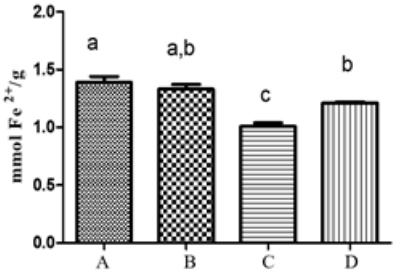

Fig.3. Antioxidant capacity of *Hypericum* extracts determined by FRAP assay (A - *H. perforatum* L., B - *H. maculatum,* C - *H. tetrapterum,* D - *H. hirsutum* L.; a, b, c - describe significant statistical differences between the *Hypericum* extracts)

the case of *H. perforatum* and *H. maculatum*. From the point of view of the antioxidant capacity there are statistically significant differences between the extracts of *H. perforatum*, *H. tetrapterum* and *H. hirsutum*. The antioxidant capacity of the extract of *H. maculatum* differs statistically significant only from the H. *tetrapterum* extract.

The antioxidant capacity of *H. performatum* is due to the presence of phenolic acids, especially caffeic and p-coumaric acid content. The antioxidant capacity of the extract was determined by FRAP method, resulting a value of 420±5.89 µM Trolox/100 g dw. The p-coumaric acid was identified in our extracts, *H. perforatum* recording the highest level in this bioactive compound. Some authors evaluated the antioxidant potential of *H. perforatum* and have shown that flavonoid glycosides and phenolic acids (chlorogenic acid) are responsible for this biological activity [9, 41].

Conclusions

HPLC/MS analysis allowed a qualitative and quantitative detailed analysis of the flavonoid components and hypericin. For each analyzed species the number of separated fractions and their identity were identified, some of them were only confirmed by MS. Following the HPLC-SM analysis of the unhydrolyzed samples of the four *Hypericum* species, we found that the hyperoside is representative for all the analyzed species with the highest value in *H. maculatum*. Rutoside has been identified in all the analyzed *Hypericum* species, but in *H. perforatum* its concentration is about 40 times higher than in *H. maculatum* and *H. tetrapterum*.

From the point of view of the hypericin content, all analyzed species respect the condition imposed by the European Pharmacopoeia.

The officinal species *H. perforatum* has the best antioxidant action due to its high content in polyphenolic compounds.

References

1.SARBU, I., STEFAN, N., OPREA A., Vascular Plants of Romania, illustrated land determinator (Plante vasculare din Romania, determinator ilustrat de teren), Victor B. Victor Ed., Bucharest, 2013, pp. 448-452.
2.***, European Pharmacopoeia VIth Ed., 2 Vol., Council of Europe Strasbourg, 2008, pp. 2958-2959.
3.*** European Pharmacopoeia, Suppl., Council of Europe Strasbourg, 2001, pp. 972-973.
4.*** Romanian Pharmacopoeia (Farmacopeea Romana), Xth Edition, Medical Ed., Bucharest, 1993, pp. 483-484.
5.BARNES, J., ANDERSON, L.A., PHILLIPSON, J.D., J. Pharmacol., **53**, nr. 5, 2001, p.583.
6.EDZARD, E., Hypericum. The genus Hypericum, Taylor & Francis Inc., London, 2003, pp. 77-94.
7.UMEK, A., KREFT, S., KARTNIG, T., HEYDEL, B., Planta Med., **65**, nr. 4, 1999, p. 388.
8.SCHWOB, I, BESSIERE, J.M., VIANO, J., C. R. Biol., **325**, 2002, p. 781.
9.ORCIC, D.Z., MIMICA-DUKIC, N.M., FRANCISKOVIC, M.M., PETROVIC, S.S., JOVIN, E.D., Chem. Central J., **5**, nr. 34, 2011, p. 1.
10.ISTUDOR, V., Pharmacognosy, Phytochemistry and Phytotherapy (Farmacognozie, Fitochimie si Fitoterapie), Ist Vol., Medical Ed., Bucharest, 1998, pp. 233-235.
11.BUTTERWERCK, V., JURGENLIEMK, G., NAHRSTEDT, A., WINTERHOFF, H., Planta Med., **66**, 2000, p. 3.
12.ABU SHOUK, A., ABDO SALEM, A.M., ABDEL-DAIM, M.M., Iran. J. Basic Med. Sci., **20**, 2017, p. 503.
13.ABU SHOUK, A.I., NEGIDA, A., AHMED, H., ABDEL-DAIM, M.M., Biomed. Pharmacother., **85**, 2017, p. 635.
14.ABDEL-DAIM, M.M., TAHA, R., GHAZY, E.W., EL-SAYED, Y.S., Can. J. Physiol. Pharmacol., **94**, 2016, p. 81.
15.AL-SAYED, E., MARTISKAINEN, O., SEIF EL-DIN, S.H., SABRA, A.N., HAMMAM, O.A., EL-LAKKANY, N.M., ABDEL-DAIM, M.M., Biomed. Res. Int., **2014**, 2014, p. 9.
16.BUNGAU, S., BALDEA, I., COPOLOVICI, L., Rev. Chim.(Bucharest), **54**, no. 3., 2003, p. 213.
17.COPOLOVICI, D., BUNGAU, S., BOSCENCU, R, TIT, D.M., COPOLOVICI, L., Rev. Chim.(Bucharest), **68**, no. 3, 2017, p. 530.

18.PALLAG, A., BUNGAU, S.G., TIT, D.M., JURCA, T., SIRBU, V., HONIGES, A., HORHOGEA, C., Rev. Chim.(Bucharest), **67**, no. 3, 2016, p. 530.
19.BUNGAU, S.G., VONHAZ G., TIT, D.M., COPOLOVICI, L., Biharean Biol., **9**, nr. 1, 2015, p. 55.
20.BUNGAU, S., FODOR, A., SZABO, I., MUTIU, G., Arch. Balk. Med. Un., **46**, nr. 1, 2011, p. 77.
21.CARAC, A., BOSCENCU, R., DEDIU, A. V., BUNGAU S. G., DINICA R. M., Rev. Chim.(Bucharest), **68**, no. 7, 2017, p. 1423
22.TAMAS, M., DRAGULESCU, C., ONIGA, I., GLIGA, F., Acta oecologica, **8**, nr. 1-2, 2001, p. 25.
23.GITEA, D., Species of Hypericum in Phytotherapy (Specii de Hypericum in fitoterapie), University of Oradea Ed., Oradea, 2015, p. 98-105.
24.COSTE, A., HALMAGYI, A., BUTIUC-KEUL, A.L., DELIU, C., COLDEA, G., HURDU, B., J. Plant. Biotechn., **110**, nr. 2, 2012, p. 213.
25.POPA, M., POPA, D., Int. J. Agric. Inform. Environ. Syst., **8**, nr. 1, 2017, p. 63.
26.POPA, M., LATO, A., CORCHES, M., RADULOV, I., BERBECEA, A., CRISTA, F., NITA, L., LATO, K.I., POPA, D., Agrolife Sci. J., **5**, nr. 1, 2016, p. 174.
27.POPESCU, D.E., BUNGAU, C., PRADA, M., DOMUTA, C., BUNGAU, S., TIT, D.M., J. Environ. Prot. Ecol., **17**, nr. 3, 2016, p. 1011.
28.DOMUTA, C., SANDOR, M., CIOBANU, G., DOMUTA, C., BORZA, I., BREJEA, R., VUSCAN, A., J. Environ. Prot. Ecol., **13**, nr. 1, 2012, p. 135.
29.DOMUTA, C., SANDOR, M., CIOBANU, G., SAMUEL, A.D., CIOBANU, C., DOMUTA, A., BORZA, I., DOMUTA, C., BREJEA, R., GITEA, M., J. Environ. Prot. Ecol., **13**, nr. 2, 2012, p. 736.
30.SAMUEL, A.D., DOMUTA, C., SANDOR, M., VUSCAN A., BREJEA, R., Rom. Agric. Res., 28, 2011, p. 157.
31.SAMUEL, A.D., DOMUTA., C., CIOBANU, C., SANDOR, M., Rom. Agric. Res., **25**, 2008, p. 61.
32.TIT, D.M., BUNGAU, S., NISTOR CSEPPENTO, C., COPOLOVICI, D.M., BUHAS, C., J Environ. Prot. Ecol., **17,** nr. 4, 2016, p. 1425.
33.BUNGAU, S., BUNGAU, C., TIT, D.M., J. Environ. Prot. Ecol., **16**, nr. 1, 2015, p. 56.
34.BUNGAU, S, SUCIU, R., BUMBU, A., CIOCA, G., TIT, D.M., J. Environ. Prot. Ecol., **16**, nr. 3, 2015, p. 980.
35.COPOLOVICI, L., TIMIS, D., TASCHINA, M., COPOLOVICI, D., CIOCA, G., BUNGAU, S., Rev.Chim.(Bucharest), **68**, no. 9, 2017, p. 2076.
36.TASCHINA, M., COPOLOVICI, D. M., BUNGAU, S., LUPITU, A. I., COPOLOVICI, L., IOVAN, C., Farmacia, **65**, nr. 5, 2017, p. 709.
37.SAMUEL, A.D., BREJEA, R., DOMUTA, C., BUNGAU S., CENUSA, N., TIT, D.M, J. Environ. Prot. Ecol., **18**, nr. 3, 2017, p. 871.
38.SAMUEL, A.D., TIT, D.M., MELINTE FRUNZULICA, C.E., IOVAN, C., PURZA, L., GITEA, M., BUNGAU, S., Rev.Chim.(Bucharest), **68**, no. 10, 2017, p. 2243.
39.VICAS, S., PROKISCH, J., RUGINA, D., SOCACIU, C., Notulae Bot., **37**, nr. 2, 2009, p. 112.
40. SMELCEROVIC, A., VERMA, V., SPITELLER, M., AHMAD, S.M., PURI S.C., QAZI, G.N., Phytochem., **67**, nr. 2, 2006, p. 171.
WOJDYLO, A., OSZMIANSKI, J., CZEMERYS, R.,Food Chem., **105**, 2007, p. 940

Manuscript received: 21.10. 2017