ESSENTIAL OIL CHEMICAL COMPOSITION BIODIVERSITY IN THE *Hypericum* L. SPECIES FROM THE SPONTANEOUS FLORA OF THE REPUBLIC OF MOLDOVA

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Abstract. The *Hypericum* L. species from the spontaneous flora of the Republic of Moldova are distinguished by the content and composition of the essential oil produced through hydrodistillation in Ginsberg's units from the aerial part of the plant (*Hyperici herba)*. *H. perforatum* L. *herba* contains 0.26% (dry matter) of essential oil, *H. elegans* - 0.15% (dry matter), *H. hirsutum* - 0.094% (dry matter), and *H. tetrapterum* - 0.13% (dry matter). GC-MS analysis of the essential oil has demonstrated substantial differences both quantitatively and qualitatively. The number of components identified in the essential oil in different *Hypericum* species is different. Thirty three components were identified in the essential oil isolated from *H. perforatum* L., β - caryophyllene and caryophyllene oxide at concentrations above 12% being the major components followed by α-pinene (8.574%), β-cadinene (4.155%), and β-pinene (3.216%). Eighteen components were identified in the essential oil of *H. elegans*, g-gurjunene, aromadendrene, and undecane being the major components at concentrations of 14.532%, 13.990%, and 10.262 %, respectively. Twenty one components were identified in the essential oil of the *H. tetrapterum* species, copaene (9.271%) and α-longipinene (8.489%) being the major components followed by cadinene (6.423%). Nine components were identified in the essential oil of the *H. hirsutum* species, elevated concentrations being found in caryophyllene oxide (10.435%), phytol (6.056%), α - caryophyllene (5.086%) and undecane (4.279%).

Keywords: *Hypericum* L., species, essential oil, components.

Rezumat. Biodiversitatea compoziţiei chimice a uleiului esenţial la speciile genului *Hypericum* L. din flora spontană a Republicii Moldova. Speciile genului *Hypericum* L. din flora spontană a Republicii Moldova se deosebesc prin conţinutul şi componenţa uleiului esenţial obţinut prin hidrodistilare în aparate Ginsberg din partea aeriană a plantei (*Hyperici herba)*. *H. perforatum* conţine în *herba* 0,26% (s.u.) ulei esenţial, *H. elegans* - 0,15% (s.u.), *H. hirsutum* - 0,094% (s.u.) şi *H. tetrapterum* - 0,13% (s.u.). Analiza GC-MS a uleiului esenţial a demonstrat deosebiri substanţiale atât cantitative cât şi calitative. Numărul componenţilor identificaţi în uleiul esenţial al diferitor specii de *Hypericum* este diferit. În uleiul esenţial separat din *H. perforatum* L. s-au identificaţi 33 componenţi, β-cariofilen şi cariofilen oxid în concentraţii de peste 12% fiind componenţii majori, urmaţi de α-pinen (8,574%), β-cadinen (4,155%) şi β-pinen (3,216%). La *H. elegans* în uleiul esenţial au fost identificaţi 18 componenţi, g-gurjunen, aromadendren şi undecan fiind componenţii majori în concentraţii de 14,532%, 13,990% şi 10,262 %, respectiv. La specia *H. tetrapterum* s-au identificat în uleiul esenţial 21 componenţi, iar componenţii majori sunt copaen (9,271%), α-longipinen (8,489%) urmaţi de cadinen (6,423%). În uleiul esenţial al speciei *H. hirsutum* au fost identificaţi numai 9 componenţi, concentraţii mai ridicate fiind atestate la cariofilen oxid (10,435%), phytol (6,056%), α-cariofilen (5,086%) şi undecan (4,279%).

Cuvinte cheie: *Hypericum* L., speciile, ulei esenţial, componenţi.

INTRODUCTION

The *Hypericum* L. genus of the family Hypericaceae comprises about 460 species that are common for all the continents (MOTAVALIZADEHKAKHKY, 2012). The vital forms include trees, shrubs, and perennial herbs. Five *Hypericum* L. species including *H. perforatum* L., *H. elegans* STEPH., *H. hirsutum* L., *H. tetrapterum* FRIES., and *H. montanum* L. (NEGRU, 2007) have been attested in the Republic of Moldova.

The *H. perforatum* L. is admitted in the European Pharmacopeia 6 and in the Romanian Pharmacopeia X for production of plant pharmaceutic products.

Many chemical studies on the aerial parts of *H. perforatum* revealed the content of various groups of active biological principles: flavonoids, derivatives of antracen, tannins, essential oil (TĂMAŞ et al., 2001; MENNINI & GOBBI, 2004; GÎTEA et al., 2010). Due to these principles, *Hyperici herba* possesses different pharmacological actions: antioxidant (SILVA et al., 2005) antimicrobial (MOTAVALIZADEHKAKHKY, 2012), antidepressive, anti - inflammatory, antiulcerous, choleretic and cholagogic (GÎTEA et al., 2010).

H. perforatum is characterized by different types of secreting structures: translucide glands or cavities, black nodules, and three types of secretory channels that can be localized in leaves, petals, sepals, and pistils (CICCARELI et al., 2001). The prevalence and diversity of these structures is an evidence of an intense secretory activity of the species. The presence of translucide glands and secretory channels has been also demonstrated in the *H. hirsutum* and *H. tetrapterum* species (GÎTEA et al., 2011). However, there is less literature information on the content and chemical composition of the essential oils isolated from the *H. elegans*, *H. hirsutum*, and *H. tetrapterum* species.

Proceeding from the foregoing, it is timely to analyze the yields and composition of the essential oil extacted from the plant aerial part at the flowering stage in the species of *H. perforatum*, *H. elegans*, *H. hirsutum,* and *H. tetrapterum* from the spontaneous flora of the Republic of Moldova.

MATERIALS AND METHODS

The plant product is represented by the species of *H. perforatum*, *H. hirsutum*, and *H. tetrapterum,* the aerial parts were picked up at the flowering stage of the summer, 2011 from the flora of the Codru Scientific Reservation in the Republic of Moldova, while *H. elegans* was collected in the woods in close vicinity of the village of Târnova, situated in the North of the country. The species were identified in the laboratory of the Codru Scientific Reservation. The plants were dried naturally in a covered, well-airy place.

The volatile oil was produced in Ginsberg units through hydrodistillation for three hours. The data were recalculated for dry weight. The essential oil samples were dehydrated using anhydrous Na_2SO_4 and kept in a freezer. The essential oil was evaluated quantitatively and qualitatively using gas chromatography and mass spectrometry (GC-MS) on an Agilent Technologies type 7890 A GC system, MS Agilent Technologies type 5975 C Mass Selective Detector; Column HP 5MS 30 m x 0.25 mm x 0.25 µm (5 % Phetylmethylsiloxane). The separation was carried out in the following chromatographic conditions - injector temperature of 250°C, detector temperature of 280°C, temperature regime of 250°C (10 degrees/min) up to 280°C (const. 5.5min); the mobile phase - helium 1ml/min; injected volume - 0.1µl of volatile oil. The chromatographic data were analyzed using the SOFTWARE Automated Mass Spectral Deconvolution & Identification System, a NIST product.

RESULTS AND DISCUSSION

These studies have demonstrated that all the *Hypericum* species evaluated synthesize and accumulate essential oil in the aerial part, its content being different in the species at the flowering stage. The highest content of essential oil was isolated in *H. perforatum* making 0.26% (dry matter). *H. elegans herba* contains 0.15% (dry matter). The essential oil content in *H. tetrapterum* makes 0.13% (dry matter), while this index in *H. hirsutum* is the lowest, 0.094% (dry matter).

Seventy four components were discovered in *H. perforatum* essential oil (Fig. 1), of which thirty four were identified and quantified (Table 1). Their contents total 71.64% with the following principle components – caryophyllene (12.155%), α - pinene (8.574%) and caryophyllene oxide (12.119%).

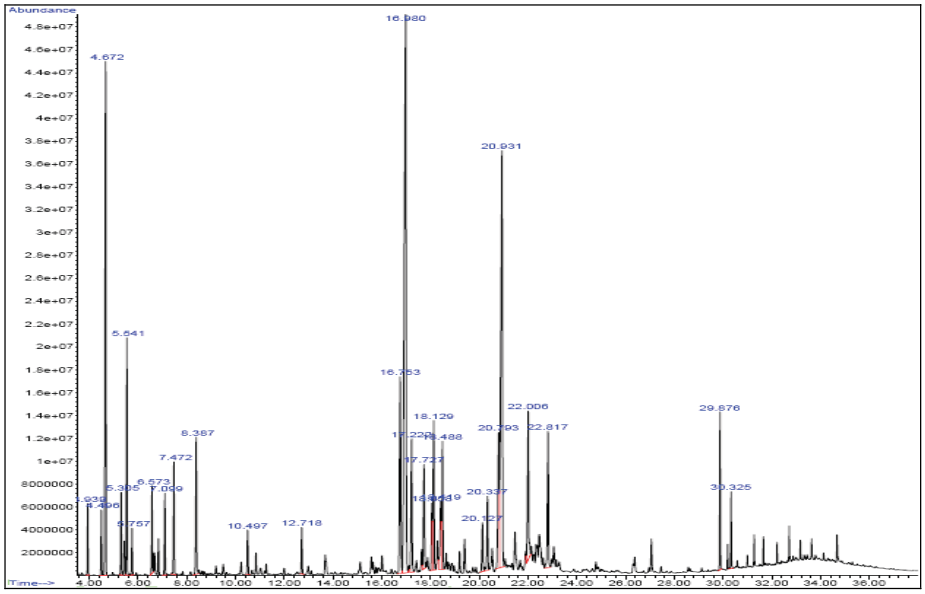

Figure 1. Gas-cromatogram of essential oil from aerial parts of *H. perforatum* L.

The previous studies have demonstrated that the essential oil content in the aerial part in the *H. perforatum* plants collected in Turkey (ERKEN et al., 2001), Greece (PAVLOVIĆ et al., 2006), Serbia (SAROGLOU et al., 2007), Italy, and France (SCHWOB et al., 2002) varies considerably. The quantity and composition of the essential oil isolated from *Hypericum* species vary not only with the phenological development phase, but depends on the prevalence areal. For example, the yields of the essential oil isolated from the aerial part of the *H. perforatum* L. species picked up in different localities of southeastern France is 0.03%-0.12% (SCHWOB et al., 2002), while in the Tadjikistan flora it makes 0.1-0.4% (SHARAPOV et al., 2010).

The major components of the volatile oil isolated from the *H. perforatum* plants harvested in South-Eastern France include caryophyllene oxide, β-caryophyllene, spathulenol, β-funebrene, γ-muurolene (E)-b-farnesene, and caryophylladienol (SHARAPOV et al., 2010). The volatile oil yields from the *H. perforatum* species collected in Tadjukistan are 01.%-0.4%, the major components including germacrene D (13.7%), α-pinene (5.1%), (E)-caryophyllene (4.7%), n-dodecanol (4.5%), cariofilen oxid (4.2%), biciclogermacren (3.8%), and spathulenol (3.4%) (SHARAPOV et. al., 2010). The same species from the Central Italy flora contain 0.07% of essential oil with the major components as follows: (E)-caryophyllene (21.6-23.0%) and

germacrene D (19.5-20.8%) (BERTOLI et al., 2011), while the aerial part of the *H. perforatum* plants harvested in South-Estern France at different developmental phases, the volatile oil content varies from 0.070% to 0.058%, reaching its maximum at the full floweing stage – 0.092% (SCHWOB et al., 2004).

Table 1. Chemical composition of the essential oil from the *Hypericum* L. species of the flora in the Republic of Moldova, %.

RI	Components	*H. perforatum*	*H. elegans*	*H. tetrapterum*	*H. hirsutum*
3.941	nonane	0.782	0.574	1.036	2.743
4.496	α-phellandrene	0.81			
4.672	α-pinene	**8.574**	**4.779**	0.232	
5.305	3-methylnonane	1.055			
5.440	sabinene	0.443			
5.541	β-pinen	**3.216**	0.378		
5.757	β-myrcene	0.589			
6.573	p-cymene	1.218			
6.676	limonene	0.389			
7.097	2-methyildecane	1.576	0.837		
7.744	cis-linalool oxid			0.193	
8.387	undecane	2.096	**10.262**	**3.157**	**4.279**
10.497	terpinen-4-ol	0.675			
10.839	α-terpineol	0.319			
11.192	decanal			1.213	
12.718	nonanoic acid	0.722			
12.981	1-decanol	0.19		1.207	
13.536	2-undecanone			0.282	
13.682	tridecane	0.513			
15.116	α-longipinene	0.316		**8.489**	
15.137	1-undecanol			1.574	
15.755	copaene		1.21	**9.271**	
16.499	dodecanal			0.412	
16.753	β-cedren	**4.155**			
16.989	B-caryophyllene	**12.175**	1.294	1.351	
17.635	(+)-longiciclene	0.407		2.912	
17.727	α-caryophyllene	2.09		0.820	**5.086**
17.881	hexadecane	0.242	0.642		
18.129	1-dodecanol	2.735	1.472		
18.488	aromadendrene	2.158	**13.99**	0.718	
18.808	g-gurjunene		**14.532**		
19.396	δ-cadinene	0.651	3.047	**6.423**	1.492
20.337	nerolidol	1.378	0.840		
20.931	caryophyllene oxide	**12.119**		1.676	**10.435**
23.051	α -bisabolol	0.428		1.036	
24.786	benzyl benzoate	0.137			
27.059	1-tetradecanol	0.569	1.072		
29.876	thunbergol	1.762	**3.101**	0.433	2.409
30.186	heneicosane	0.266	0.528		0.762
30.325	phytol	0.842	2.593	**4.501**	**6.056**
32.213	tetracosane		0.436	0.318	0.702
Components identifiend, %		**71.277**	**61.587**	**47.224**	**33.964**
The number of identified compounds		**33**	**18**	**21**	**9**

These studies have shown that forty nine components are present in *H. elegans* essential oil (Fig. 2). Of them eighteen were identified with the following major components - g-gurjunene (14.532%), aromadendrene (13.99%), undecane (10.262%), and α-pinene (4.779%) (Table 1). The *H. elegans* plants picked up from the spontaneous flora in South-Eastern Serbia at the flowering stage contain 0.08% of essential oil with the major components - undecane (31.9%), α-pinene (16.7%), and nonane (6.1%) (RADULOVIĆ et al., 2010).

We found fifty six components in the essential oil of *H. tetrapterum* (Fig. 3). Twenty two of them were identified, with such major components as copaene (9.271%), α-longipinene (8.489%), and δ-cadinene (6.423%) (Table 1). The same major components were identified in the *H. tetrapterum* essential oil originated from Greece α-copaene (11.3%) and α-longipinene (9.7%), as well as caryophyllene oxide, 8.9% and n-undecane, 7.4%. *H. tetrapterum* oil coming from Italy has also the same two major components - α-copaen (12.7%) and α-longipinen (8.1%) (BERTOLI et al., 2011; PAVLOVIĆ et al., 2006). The essential oil content in Greek *H. tetrapterum* constitutes 0.2%, while that from Central Italy is of 0.1%. The essential oil content of the Greek *H. tetrapterum* species is higher (0.2%), while that from Central Italy (0.01%) is lower than in Moldova.

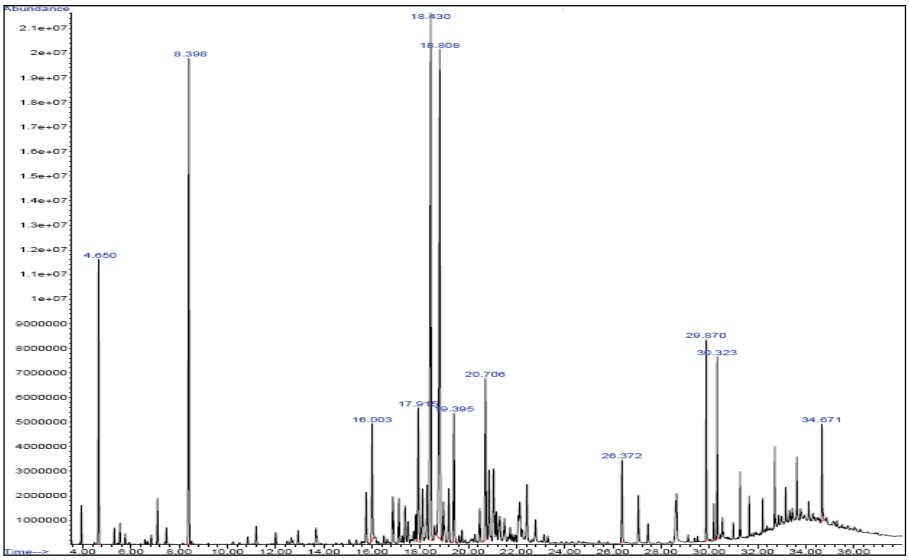

Figure 2. Gas-cromatogram of essential oil from aerial parts of *Hypericum elegans* STEPH.

The *H. hyrsutum* species contains twenty four components in its volatile oil (Fig. 4), of which nine were identified constituing 33.964% (Table 1). The major constituents attested in the essential oil of this species collected in Moldova are caryophyllene oxide (10.435%), phytol (6.056%), and α-caryophyllene (5.086%). In France spontaneous flora, this species synthesizes and accumulates other major components in the essential oil, such as n-nonane (52%) and n-undecane 30%, while the plants harvested in Serbia have three major components in essential oil n-undecan (32.2%), patchoulene (11.8%), and cariofilen oxide (9.3%) (GUDŽIC et al.,007), the concentration of the latest being similar to that we have attested in *H. hyrsutum* from Moldova. In the samples from Central Italy, the major components of this species include (E,E)-α–farnesene (7.0–13.8%) and E-β-farnesene (7.2–9.4%) (BERTOLI et al., 2011), that we have not found in *H. hyrsutum* from Moldova.

A comparison of the quantitative and qualitative data for the essential oil isolated from the *H. perforatum, H. elegans, H. tetrapterum,* and *H. hyrsutum* species of the spontaneous flora in the Republic of Moldova has led us to a conclusion that all these species contain nonane, undecane, δ-cadinene, thunbergol, and phytol, but at different concentrations. The species also differ in their major constituents of the essential oil. Thus, in *H. perforatum* essential oil, the major components, as mentioned above, include β-caryophyllene, caryophyllene oxide, and α-pinene. Significant concentrations of caryophyllene oxide (10.435%) are contained only in *H. hyrsutum* essential oil, the other species differ from *H. perforatum* in both major components and the number and concentration of minor components. Caryophyllene oxide, β-caryophyllene, and α-pinene are the compounds that are common for the essential oil of *H. perforatum* plants harvested at the flowering stage from Moldova, Tadjukistan, Uzbekistan, Turkey, Serbia, France, and Greece (SHARAPOV et. al., 2010; BASER et al., 2002; SAROGLOU et. al., 2007; SCHWOB et. al., 2002; PAVLOVIĆ et. al., 2006).

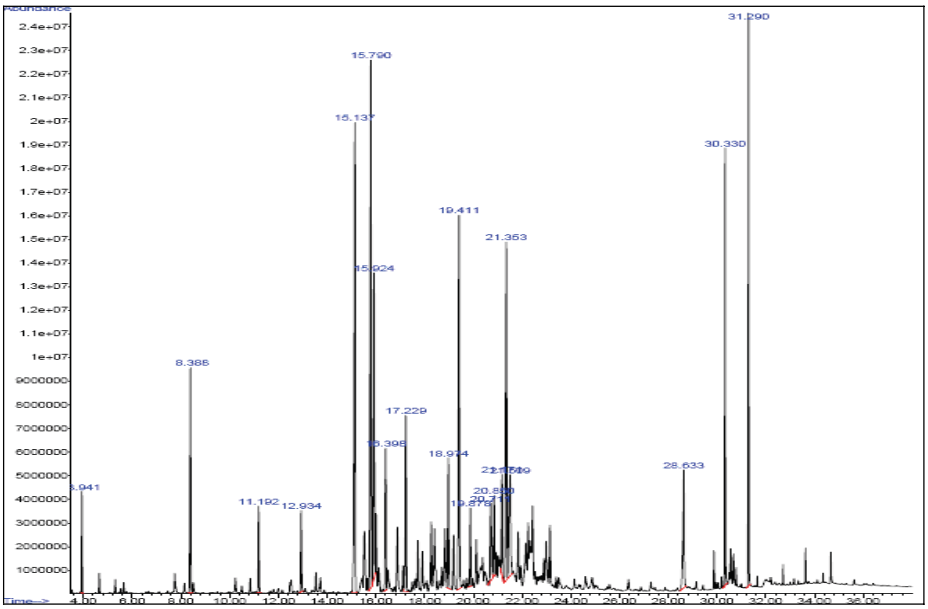

Figure 3. Gas-cromatogram of essential oil from aerial parts of *H. tetrapterum* FRIES.

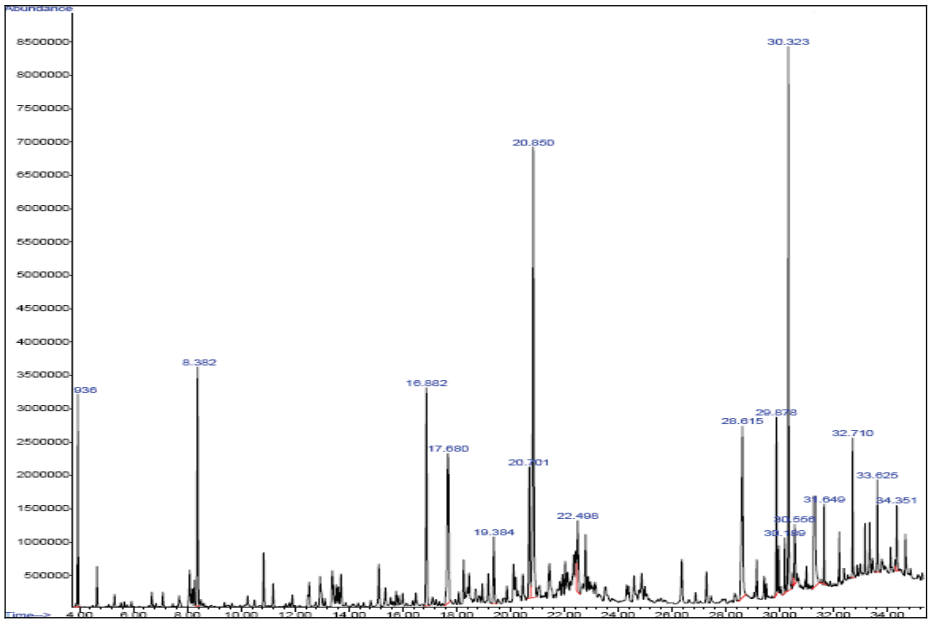

Figure 4. Gas-cromatogram of essential oil from aerial parts of *H. hirsutum* L.

CONCLUSIONS

1. The content and quantitative chemical composition of the esssential oil isolated from the *H. perforatum, H. elegans, H. hirsutum*, and *H. tetrapterum* species of the spontaneous flora in the Republic of Moldova have been estimated for the first time.

2. The *H. perforatum* species is characterized by a maximum content of essential oil reachig 0.26% (dry matter). The *H. elegans* and *H. tetrapterum* species contain 0.15% (dry matter), 0.13% (dry matter) of essential oil, respectively, while *H. hirsutum* is distinguished by a minimum content of essential oil - 0.094% (dry matter).

3. The major components in *Hypericum* essential oil differ with the species: β-cariofilen (12.175%), caryophyllene oxide (12.119%), and α-pinene (8.574%) in *H. perforatum*; g-gurjunene (13.99%), aromadendrene (13.99%), and undecane (10.262%) in *H. elegans*; dodecanal (9.271%) and α-longipinene (8.489%) in *H. tetrapterum*; caryophyllene oxide (10.435%) and phytol (6.056%) in *H. hirsutum*.

4. Nonane, undecane, cadinene, thunbergol, and phytol are the components that are common for *H. perforatum, H. elegans, H. hirsutum*, and *H. tetrapterum*, their concentration being different.

REFERENCES

BERTOLI ALESSANDRA, CUNEIT Ç., TEIXEIRA DA SILVA J. A. 2011. *Hypericum Species as Sources of Valuable Essential Oils*. Medicinal and Aromatic Plant Science and Biotechnology. Global Science Books. Ltd. London. **5**: 29-47.

BASER K. H. C., OZEK T., NURIDDINOV H. R., DEMIRCI A. B. 2002. *Essential oils of two Hypericum species from Uzbekistan*. Chemistry of Natural Compounds. Springer. New York. **38**: 54-57.

CICCARELI DANIELA, ANDREUCCI ANDREA, PAGNI ANNA MARIA. 2001. *Translucent Glands and Secretory Canals in Hypericum perforatum L. (Hypericaceae*): *Morphological, and Histochemical Studies During the Course of Ontogenesis*. Annals of Botany. Available online at http://www. idealibrary.com on Ideal. **88**: 637-644. (Accesed: July 3, 2001).

ERKEN S., MALYER H., DEMIRCI F., DEMIRCI B., BASER K. H. C. 2001. *Chemical investigations on some Hypericum species growing in Turkey*. Chemistry of Natural Compounds. Springer, New York. **37**(5): 434-438.

GÎTEA DANIELA, ŞIPOŞ MONICA, TĂMAŞ M., PAŞCA BIANCA. 2010. *The analysis of alcoolic extracts of Hypericum species by UV/VIS spectrophotometry.* Analele Universităţii din Oradea - Fascicula Biologie. **17**(1): 111-115.

GÎTEA DANIELA, ŞIPOŞ MONICA, TĂMAŞ M., PAŞCA BIANCA. 2011. *Secretory structures at Species of Hypericum Genera from Bihor Country, Romania.* Note I. Vegetative organs. Farmacia. Edit. Medicală, Bucureşti: **59**(3): 424-430.

GUDŽĆI B., ŠMELCEROVIĆ A., ĐORĐEVIĆ S., MIMICA-DUKIĆ NEDA, RISTĆ M. 2007. *Essential oil composition of Hypericum hirsutum L.* Flavour and Fragrance Journal. John Wiley & Sons Ltd. UK. **22**(1): 42-43.

MENNINI TIZIANA & GOBBI M. 2004. *The antidepressant mechanism of Hypericum perforatum*. Life Sciences. Elsevier science publishing Compani, Inc. New York. **75**: 1021-1027.

MOTAVALIZADEHKAKHKY ALIREZA. 2012. *Antimicrobial activity and chemical composition of essential oils of four Hypericum from Khorasan, Iran*. Journal of Medicinal Plants Research. Academic journals. Lagos. **6**(12): 2478-2487.

NEGRU A. 2007. *Determinator de plante din flora Republicii Moldova*. Edit. „Universul". Chişinău. 83 pp.

PAVLOVIĆ MILICA, TZAKOU OLGA, PETRAKIS P. V., COULADI MARIA. 2006. *The essential oil of Hypericum perforatum L., Hypericum tetrapterum FRIES and Hypericum olympicum L. growing in Greece.* Flavour and Fragrance Journal. John Wiley & Sons Ltd. London. **21**(1): 84-87.

RADULOVIĆ N., ĐORĐEVIĆ ALLEKSANDRA, PALIĆ R. 2010. *The Intrasectional Chemotaxonomic Placement of Hypericum elegans Stephanex WILLD*. Chemistry & Biodiversity. Verlag Helvetica Chimica Acta AG. Zurich. Switzerland. **7**(4): 943-952.

SAROGLOU VASILIKI, MARIN P. D., RANICIC ANA, VELJIC M., SKALTSA HELEN. 2007. *Composition and antimicrobial activity of the essential oil of six Hypericum species from Serbia*. Biochemica Systematics and Ecology. Elsevier. Amsterdam. **35**(3): 146-1520.

SCHWOB ISABELLE, BESSIERE J. M., VIANO JOSETTE. 2002. *Composition of the essential oils of Hypericum perforatum L. from southeastern France*. Comptes Rendus Biologies. Elsevier Masson SAS. France. **325**: 781-785.

SCHWOB ISABELLE, BESSIERE J. M., MASOTTI VERONIQUE, VIANO JOSETTE. 2004. *Changes in essential oil composition in Saint John's worth (Hypericum perforatum L.) aerial parts during its phenological cycle*. Biochemical Systematic and Ecology. Elsevier. Amsterdam. **32**(8): 735-745.

SHARAPOV F., GULMURODOV I., SETYER W. 2010. *Essential oil composition of Hypericum perforatum L. and Hypericum scabrum L. growing wild in Tajikistan.* Journal of Chemical and Pharmaceutical Research. U.S. Government Printing Office: Washington, DC. **2**(6): 284-290.

SILVA B. A., FERRERES F., MALVA J. O., DIAS A. C. P. 2005. *Phytochemical and antioxidant characterization of Hypericum perforatum L. alcoholic extracts.* Food Chemistry. Elsevier Science Publishers Ltd. Barking. **90**: 157-167.

TĂMAŞ M., DRAGULESCU C., ONIGA ILIOARA, GLIGA FLORINA. 2001. *Comparative phytochimical research on some species of Hypericum and populations of H. perforatum L. (Hypericaceae) in Romania.* Acta oecologica. Edit. Universităţii „Lucian Blaga". Sibiu. **8**(1-2): 25-31.

***. European Pharmacopoeia 6. 2007. Supp. Council of Europe. Strausburg. **2**: 2958-2959.

***. Farmacopeia Română. 1993. Ediţia a X-a. Medicală Press. Bucharest: 483-484.

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