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COMPOSITION AND ANTI-MICROBIAL ACTIVITY OF ESSENTIAL OIL OF HYPERICI HERB (*HYPERICUM PERFORATUM L.*) FROM VLASINA REGION

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Abstract. *The essential oil from the flowers of Hyperical herb (Hypericum perforatum L.) from the Vlasina region, Serbia, was obtained by steam distillation after drying and grounding of the herbal material. 30 components were resolved in the essential oil by GC-MS analysis, while 21 compounds were identified comparing the recorded mass spectra with the Wiley library of mass spectra. The micro-biological activity of the isolated essential oil was investigated as well. It was found that the essential oil shows micro-biological activity except with Pseudomonas aeruginosa and Candida albicans.*

1. INTRODUCTION

Hyperici herb (*Hypericum perforatum L.*) belongs to the family of CLUSIACEAE (*Hypericaceae*) (1,2) and it is one of the most signitificant species widely spread in Europe, Asia, Nort Africa, New Zeland and America (3,4). Particularly rich deposits of this herbal species are found in Germany, Ukraine and in the Balkan (Serbia, especially its south-eastern part) (5).

Hyperici herb is widely used in folk medicine, and being an important raw material in pharmaceutical industry, it has been extensively studied (6-11). The extracts of floral part of the herb as well as the essential oil obtained by steam distillation were subject of our studies. However, a significant group of compounds present in Hyperici herb was not sufficiently investigated from pharmacological point of view (10). A demand for a thorough study of mutual synergistic action in an organism and anti-microbial activity of the constituents of Hyperici herb is also evident. In order to investigate the composition of the antimicrobal activity of the essential oil, gas chromatography - mass spectrometry

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(GC-MS) was applied.

2. MATERIAL AND METHODS

The herbal material (floral part of the herb) was picked up from Vlasina the region of the south-eastern part of Serbia at the beginning of July 1994, and dried for 7 days in a drafty place, protected from light. The moisture content in dried herbal material was less than 15 %, as required by international pharmacopoeias (12-14). The organoleptic characteristics of the herbal material changed significantly upon drying.

The dried herbal material was ground by an electric mill up to the desired particle size (typically < 1 mm). Some of the relevant parameters of the obtained material are presented in Table 1.

Table 1. Quality parameters of the floral part of Hyperici herb (*Hypericum perforatum L.*)

Appearance	yellowish-green powder
Identification	conforms to the herbal sort
Odour	pleasant, specific
Moisture mass%	9,00
Ash mass%	0,05
Essential oil, mass%	0,32
Microbiological purity	without mold and pathogenic microorganismis

The bright yellow coloured essential oil was obtained by steam distillation ending up with a typical yield of 3.2 cm^3 from 1000 g of grounded, dry herbal material. The composition of the essential oil was analyzed by capillary gas chromatography - mass spectrometry (GC-MS), followed by anti-microbial tests. The essential oil of Hyperici herb was diluted with diethyl ether (1 : 75) prior to GC-MS analysis. The identification of the compounds was performed by comparison of the obtained mass spectra with the mass spectra in the Wiley library.

A HP 5890 series II Plus (Hewlett - Packard) gas chromatograph equipped with a HP 5971 mass spectrometer as a detector and SPB-5 (Supelco) capillary column (30 m x 0.25 mm i.d., 0.25 μm film) was used with hellium as a carrier gas (flow rate: 1 $cm^3 \cdot min^{-1}$). The injector, operated in splitless mode (1 : 50), was held at 250 ^oC. The temperature program started at 100 ^oC, and after 2 min, ramped at 8 $^oC \cdot min^{-1}$ to 300 ^oC. The sample transfer line was heated to 280 ^oC. The scanning rate was 0.8 $scan \cdot sec^{-1}$.

3. RESULTS AND DISCUSSION

A typical GC-MS chromatogram of the Hyperici herb essential oil is shown on Fig. 1, while the results of the quantitative analysis are presented in Table 2.

It should be noted that 30 compounds were detected (see Fig. 1) while 21 hydrocarbons and terpenes were identified (see Table 2) with a typical library search match exceeding 90 %. The most abundant component in the investigated essential oil of

Hyperici herb is cis-caryophyllene (No. 9, table 2). Noticeable amounts of β-farnesene, 2-methyl-dodecane, 1-hexyl-2-propyl-cyclopropane, and naphtalene 1,2,3,4,4a,5,6,8a-octahydro-7-methylene-1-(1-methylethyl) were found as well.

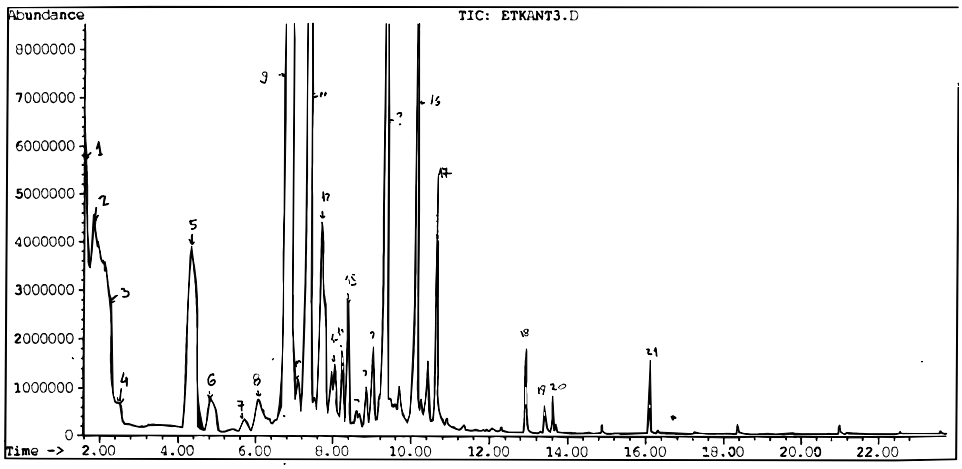

Fig. 1. A typical GC-MS chromatogram of the Hyperici herb (*Hypericum perforatum L.*) essential oil

Table 2. Survey of the identified components in the essential oil of Hyperici herb (*H. perforatum L.*)

No.	Component	Content in %
1.	α - pinene	
2.	β - pinene	3,257
3.	2-methyl-decane	
4.	undecane	
5.	2-methyl-dodecane	5,698
6.	tridecane	0,889
7.	longipinene	
8.	α - copaene	1,130
9.	cis-caryophyllene	48,495
10.	3,7-guaiadiene	0,759
11.	β - farnesene	12,115
12.	naphtalene 1,2,3,4,4a,5,6,8a-octahydro-7-methylene-1-(1-methylethyl)	3,919
13.	β - chimachalene	0,762
14.	BHT (from diethyl ether used as solvent)	0,737
15.	cadinene	1,070
16.	1-hexyl-2-propyl-cyclopropane	4,973
17.	cyclododecane	1,762
18.	6,10,14-trimethyl-2-pentadecanon	0,498
19.	ciclotetradecane	0,196
20.	nonadecane	0,164
21.	eicosane	0,349

The amount of the components 1-4 (see Table 2) is given in the form of sum, because their chromatographic separation was not satisfactory. The amount of longipinene is also not listed since the peak of this component is distinctly small.

The data in the available literature about the composition of the essential oil in Hyperici herb vary: up to 0,01% (15), 0,1-0,3% (16), 0,1-0,5% (17) to max. 1% (18).

As the composition of the essential oil is concerned, in the updated literature the identification of some components has been also quoted, but quantitative data mainly refer to the composition of the group of organic compounds, but not to the individual compounds.

Therefore, our results are not only in the compliance with literary data but they also give siguificant contribution to better knowledge of the content as well as the composition of the essential oil of Hyperici herb.

The anti-microbial activity of the essential oil of Hyperici herb was tested in vitro using the method of diffusion on disc (Ph. Jug. IV) with the following microorganisms:

Staphylococcus aureus 6538	+
Escherichia coli 95	+
Bacillus subtilis "S"	+
Pseudomonas aeruginosa	-
Sarcina lutea 9341	+
Aspergillus niger	+
Candida albicans	-
Bacillus subtilis 841	+
Salmonella enteritidis	+
Klebsiella pneumoniae	+

It was found that the essential oil of Hyperici herb (*Hypericum perforatum L.*) shows micro-biological activity with tested microorganisms, except with Pseudomonas aeruginosa and Candida albicans.

The obtained results for the composition of the essential oil of Hyperici herb and its microbial activity, could be a useful starting point for the investigation of the course of biosynthesis of some components of the essential oil. This will be the subject of our further investigation.

4. CONCLUSION

The composition of the essential oil of Hyperici herb (*Hypericum perforatum L.*) from the Vlasina region, Serbia, was investigated by using an advanced analytical technique, GC-MS. 30 components were resolved in the essential oil by GC-MS analysis, and 21 hydrocarbons and terpens were identified with a typical library search match exeeding 90 %.

The micro-biological activity of the essential oil of Hyperical herb was tested using the method of diffusion on disc. As expected, the isolated essential oil shows micro-biological activity with the tested microorganisms (Staphylococcus aureus 6538, Escherichia coli 95, Bacillus subtilis "S", Sarcina lutea 9341, Aspergillus niger, Candida albicans, Bacillus subtilis 841, Salmonella enteritidis, Klebsiella pneumoniae).

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SASTAV I ANTI-MIKROBIOLOŠKA AKTIVNOST ETARSKOG ULJA BILJKE KANTARION (*HYPERICUM PERFORATUM L.*) SA PODRUČJA VLASINE

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Etarsko ulje iz osušenog i samlevenog cvetnog dela biljke kantariona (Hypericum perforatum L.) sa područja Vlasine (Srbija) izdvojeno je destilacijom pomoću vodene pare. Metodom GC-MS analize u ulju je otkriveno 30 komponenata od kojih je 21. komponenta identifikovana poređenjem sa masenim spektrima biblioteke Wiley. Ispitana je i mikrobiološka aktivnost izolovanog etarskog ulja. Utvrđeno je da, sem na Pseudomonas aeruginosa i Candida albicans, etarsko ulje pokazuje mikrobiološku aktivnost.