DIVERSITY OF THE ESSENTIAL OIL CONTENT AND CHEMICAL COMPOSITION OF *Hyssopus officinalis* L. GENOTYPES

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Abstract. The genotypes of *Hyssopus officinalis* L. *cyaneus*, *ruber* and *albus* forms of Moldova were evaluated. In the drought conditions the indices of quantitative traits which directly influence productivity have the higher value for *f. ruber* and *f. cyaneus*. The content of essential oil is different for the above mentioned genotypes and very high: *f. ruber* - 2.531, *f. cyaneus* - 1.877, and *f. albus* - 1.434% (dry matter). GC-MS analysis of essential oil identified 30-38 compounds for different genotypes. The major compounds are pinocamphone in trans (-) iso and cis forms: for *f. cyaneus*, cis (-) pinocamphone - 51.77%, trans (-) *iso* pinocamphone - 6.70%; for *f. ruber* - 66.94% pinocamphone, 33.31% trans (-) iso pinocamphone and 33.63% –cis(-) pinocamphone; *f.* a*lbus* - 61.1% trans (-) iso- and 2.15% cis (-) pinocamphone, for all genotypes followed by the β-pinene (*cyaneus* form, 8.49%, *albus,* 7.38%, *ruber* , 4.15%) and β-felandren, from 3.64% for *f. ruber* genotype to 6.79% for *f. albus* genotype. The minor compounds of essential oil have different concentration, some of them being present only in one or two genotypes.

Keywords: *Hyssopus officinalis*, genotype, essential oil, composition.

Rezumat. Biodiversitatea genotipurilor de *Hyssopus officinalis* L. Genotipuri de *H. officinalis* L., *f. cyaneus*, *f. ruber* şi *f. albus* din Moldova au fost evaluate. În condiţii de secetă indicii caracterelor cantitative, ce influenţează direct productivitatea au valori mai ridicate la genotipurile ce aparţin *f. ruber* şi *f. cyaneus*. Genotipurile au un conţinut diferit, dar foarte înalt de ulei esenţial: *f. ruber* – 2,531, *f. cyaneus* – 1,877, iar *f. albus* – 1,434% (s.u.). Analiza GC-MS a uleiului esenţial a identificat 30-38 componenţi la diferite genotipuri, componenţii majori fiind pinocamfona în formele trans (-) iso şi cis. La *f. cyaneus* cis (-) pinocamfona conţine 51,77%, trans (-) iso pinocamfonă – 6,70%; la f. *ruber* – 66,94% pinocamfonă, 33,31% trans (-) iso pinocamfonă şi 33,63% –cis (-) pinocamfonă; *f. albus* - 61.1% trans(-) iso - şi 2.15% cis (-) pinocamfonă, urmate la toate genotipurile de β-pinen (*f. cyaneus,* 8,49%, *f. albus,* 7,38%, f. *ruber* , 4,15%) şi β-felandren, de la 3,64% la genotipul f. *ruber* până la 6,79% la genotipul *f. albus*. Componenţii minori au concentraţii diferite, unii fiind prezenţi în uleiul numai al unuia sau a două genotipuri.

Cuvinte cheie: *H. officinalis,* genotip, ulei esential, compoziţie.

INTRODUCTION

Hyssop (*Hyssopus officinalis* L.) is a perennial herb of the Lamiaceae family, an evergreen sub shrub native to countries surrounding the Mediterranean Sea, and can be found in the wild flora in south-eastern Europe and western Asia. Presently, it is grown in Spain, France, Italy, Russia, Ukraine, and Balkans.

The Greek name "Hyssopus" is derived from the Arabic "azzof" (sacred grass). Today it is a well-known species, and used as a medicinal, aromatic spices and honey plant in ancient times (CUCU et al., 1982; GONCEARIUC & ROŞCA,1997; MITIC & DORDEVIC, 2002; VOITKEVICI, 1999; WOLSKI et al., 2006). Hippocrates recommended hyssop for pleurisies, and Dioscorides used it to treat asthma and catarrh (FISCHER-RIZZI, 1990). *H. officinalis* is appreciated for its qualities to fix the eroded land and mobile sands, but also as decorative species. In Moldova hyssop is grown as aromatic plant, and its essential oils are intended for export. This species is important for our country because it is very resistant to drought, frost and winter.

Hyssop has the antitussive, expectorant, carminative, digestive, anticatarrhal, antispasmodic and sedatives, bronchodilators, diuretics (CUCU et al., 1982), antibacterial (BURT, 2004; KIZIL et al., 2010; MAZZANTI et al., 1998; RENZI et al., 1999), antiviral (CUCU et al., 1982; GONCEARIUC & ROŞCA, 1997), antioxidant (BAJ et al., 2010), antifungal (FRATERNALE et al., 2004; GLAMOCLIJA et al., 2005; LETESSIER et al., 2001) and spasmolytic properties (BAJ et al., 2010; LU et al., 2002) successfully used it to treat various diseases, primarily the respiratory disease, chronic bronchitis, various injuries and ecchymoses (VOITKEVICI, 1999), and also as digestive stimulant due to the bitter substances that it contains (HOFFMAN, 2010). Mostly, these properties are due to the content of highly aromatic essential oil in the leaves, stems and flowers of hyssop (CUCU et al., 1982; HOFFMAN, 2010) in concentrations of 0.3-1.0% (dry matter) (VOITKEVICI, 1999), successfully used in aromatherapy (LIS-BALCHAN, 2006) as pure essential oil or in combination with other essential oil. Essential oil is obtained from the non lignified part of the plant in the flowering stage by the hydrodistillation used the fresh raw material. It is important because in the stage of wilting and drying it is possible to lose up to 40% of the essential oil. Hyssop essential oil is a light yellow liquid with a bitter taste. Technical characteristics are: d20/20: 0917-0965, n20 / D 1473-1486, α20 / D: 6-250. The concentration of 4% in petroleum ether for 48 hours does not cause any skin irritation or sensitizing effect. The phototoxic effect is missing. However, hyssop essential oil should be used with caution and only in recommended doses (VOITKEVICI, 1999).

Various subspecies, varieties of hyssop have been described such as *H. officinalis* subsp. *officinalis* BRIQ; var. *vulgaris* BENTH. - flowers with blue-violet corolla (*f. cyaneus* ALEF. pink, carmine-red color (*f. ruber* (MILL.) ALEF. or white (*f. albus* ALEF.) (CHALCHAT et al., 2001; CUCU et al., 1982; GONCEARIUC & ROŞCA,1997; SHIBKO & AKSSENIV,

2011) var. *decussatus* PERS.; var. *angustifolius* (BIEB.) BENTH. and ssp. *canesscens* (DC.) BRIQ (CUCU et al., 1982). According to some authors the form with blue corolla is richer in essential oil than the form with white or red corolla. According to others, on the contrary, the form with red corolla is richer than the form with blue corolla (CUCU et al., 1982). Our previous research showed that the genotypes with blue corolla (*cyaneus* form) are poorer in essential oil: 0.137-0.680% (dry matter). For more than 66% of the evaluated genotypes with white corolla (*albus* form) the content of essential oil is from 0.600 to 1.161% (dry matter), while those with pink corolla (*ruber* form) accumulated essential oil from 0.345% to 1.101% (dry matter). Thus, the highest content of essential oil was attested in the genotypes with white corolla (GONCEARIUC & ROŞCA, 1997). This paper is intended to study quantitative characters, including essential oil content and quantitative and qualitative analysis of essential oil of *H. officinalis* the *ruber*, *cyaneus* and *albus* form, selected for creation of new varieties.

MATERIALS AND METHODS

In this study, the biological material is represent by the three genotypes of *H. officinalis*, is about *f. cyaneus* with blue-violet corolla, *f. ruber* with pink corolla and *f. albus* with white flowers, selected by the higher content of essential oil in the previous research. Biometric evaluations of the quantitative characters that influence the content and production of essential oil were performed in accordance with the existing methods. In order to determine the content of essential oil, the samples of fresh herbs in the flowering stage were collected in the morning hours. The essential oil was separated by hydrodistillation for 60 minutes, using the Ginsberg apparatus: 100g of fresh aerial part into 200 ml of water. The content of essential oil was recalculated per dry matter. After distillation essential oil was dried over Na_2SO_4 and kept in the freezer. Qualitative and quantitative analysis of the essential oil was determined by Gas Chromatography coupled with Mass Spectrometry (GC-MS): gas chromatograph - Agilent Technologies 7890; mass selective detector 5975C Agilent Technologies with quadruple, capillary column (30m x 0.25mm i.d., film thickness 0.25 μм) with HP-5ms non-polar stationary phase. The injector and detector temperature were 250°C and 280°C respectively, using a temperature gradient from T_1 = 70°C (2 min), T_2 = 200°C (5°C/min) to T_3 = 300°C (20°C/min, 5 min). Mobile phase: helium 1ml/min, injected volume of essential oil - 0.03 µl, split rate - 1:100. The identification of chromatographic peaks was performed using the software package AMDIS ™, coupled with NIST database.

RESULTS AND DISCUSSIONS

The evaluation of three genotypes belonging to three different forms of *H. officinalis* L. - *f. cyaneus* with blue corolla (Fig. 1), *f. ruber* with pink corolla (Fig. 2) and *f. albus* with white corolla (Fig. 3), in the drought and heat conditions in 2012 demonstrated that the first and second forms are more vigorous; the plant size (height) is 75-80 cm and we noticed a higher number of floral stems - 79-82 per plant (Table 1). The ear of inflorescence consists of a relatively larger number of verticiles, respectively, flowers especially for *ruber* form. It is known that the *H. officinalis* flowers are richer in essential oil than leaves or stems (CUCU et al., 1982). This fact was confirmed by our evaluation. Thus, plants genotypes belonging to *cyaneus* and *ruber* forms have the highest number of flower stems with longer inflorescences, which formed a larger number of verticiles and flowers. For these forms the content of essential oil is higher - 1.877% (dry matter) for *cyaneus* form, but the highest – 2.531% (dry matter) was attested to *f. ruber* (Table 1). For *f. albus*, characterized by small plant size (63.0 cm), lower number of flower stems per plant (68.0), shorter inflorescences and lower verticiles (respectively, flowers) on the spike-like inflorescence, the content of essential oil was the lowest - 1.434% (dry matter).

Figure 1. *H. officinalis, f. cyaneus* (original).

Figure 2. *H. officinalis, f. ruber* (original).

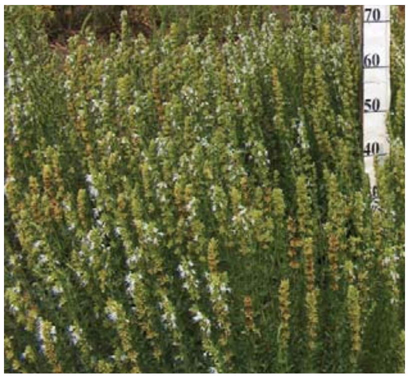

Figure 3. *H. officinalis, f. albus* (original).

Comparing the results obtained by other researchers who described genotypes with low content of essential oil 0.5-0.75% (CHALCHAT et al., 2001; GORINOVIC et al., 1995; MITIC & DORDEVIC, 2002) or 0.18% (dry matter) (BAJ et al., 2010; GARG et al., 1999) to the genotypes evaluated by us, it was noticed a higher content of essential oil. The cause of this difference may be the separation of essential oil from dried plants that can lead to the loss of more essential oil, especially during the drying period, mentioned in some scientific papers (CHALCHAT et al., 2001; GORINOVIC et al., 1995; MITIC & DORDEVIC, 2002).

Table 1. The indices of some quantitative traits in the genotypes of *H. officinalis*, 2012.

Genotype, Form	Plant height, cm	Inflorescence length, cm	flower stems per plant	Verticiles on the inflorescence	Content of essential oil, % (dry matter)
cyaneus	75.0	25.1	82.0	11.8	1.877
ruber	80.0	24.9	79.0	12.7	2.531
albus	63.0	23.6	68.0	11.0	1.434

Another reason is the growth and development in the drought conditions of 2012, year when the content of essential oil was the highest in recent years. In our opinion, the high content of essential oil is due to breeding and selection of drought resistant genotypes with high content of essential oil. This fact was confirmed by the comparison of this index while the breeding work started in 1997, when the content of hyssop essential oil ranged from 0.137-0.680 % (dry matter) for *f. cyaneus* and over 0.600-1.161% (dry matter) for *f. ruber* and *f. albus*. In those studies it was found that the highest content of essential oil is characteristic for *f. albus*, followed by the *f. ruber*, and the lowest is for *f. cyaneus* (GONCEARIUC & ROŞCA, 1997) that differs from the results presented in this paper, the highest content of essential oil being registered by *f. ruber*.

Quantitative and qualitative analysis showed that the essential oil of the evaluated genotypes contains a different number of components, and their concentration is also different. Thus, in the essential oil of *f. cyaneus* there were identified 34 components, *f. ruber* - 38 and *f. albus* - 30 components that represent 97.94%, 98.31% and 97.14% respectively of the total weight (Table 2).

Table 2. Composition of the essential oil from the genotypes of *H. officinalis*, 2012.

№	Compound / concentration	Genotypes		
		f. cyaneus	***f. ruber***	***f. albus***
1	o-xylene	0.16	0.12	0.12
2	α –pinene	0.36	0.17	0.29
3	camphene	-	*0.05*	*0.06*
4	sabinene	1.31	0.97	1.45
5	β -pinene	**8.49**	**4.15**	**7.38**
6	β - thujone	1.48	1.08	1.73
7	P- ocimene	-	0.11	0.07
8	β -felandren	**4.83**	**3.64**	**6.79**
9	1.8-cineole	0.22	0.27	0.24
10	α -terpinene	-	0.06	-
11	trans- β -ocimene	0.17	0.09	-
12	*cis*- β -ocimene	0.60	0.19	-
13	γ-terpinene	0.11	0.38	0.31
14	δ-terpinene	-	0.14	0.08
15	(+)-4-caren	-	0.08	0.06
16	Δ 3-caren	**3.20**	**1.27**	**1.51**
17	β – thujone	0.16	0.10	-
18	α – thujone	0.10	0.24	0.35
19	mirtenat de myrtenil	**2.33**	**3.17**	**2.59**
20	Trans (-) iso pinocamphone	**6.70**	**33.31**	**61.1**
21	Cis (-)pinocamphone	**51.77**	**33.63**	**2.15**
22	α-terpineol	0.46	0.54	0.43
23	(+) pinocampheol	**1.52**	**2.48**	**2.62**
24	geraniol	0.51	0.04	0.07
25	borneol	0.09	-	-
26	(z)-citral	0.42	-	-
27	mirtenol	-	0.76	0.27
28	α E- citral	0.63	0.06	0.09
29	Carvacrol	**0.34**	**3.31**	**5.49**
30	myrtenil acetate	**1.24**	0.15	0.08
31	neryl acetate	0.17	0.06	-
32	geranyl acetate	1.18	0.05	-
33	(+)β-Burbonen	0.72	0.51	0.59
34	(-)α-gurjunen	0.16	0.17	0.07
35	β cariophilene	0.75	0.67	0.83
36	Alo-aromadendrene	0.63	0.62	0.23
37	D-germacren	**2.55**	**2.88**	**1.80**
38	β-Elemene	1.02	0.51	-
39	caryophyllene oxide	0.13	0.13	-
40	byciclogermacrene	**3.43**	**2.20**	0.64
No. of identified components		34	38	30
Total identified components, %		**97.94**	**98.31**	**97.14**

In the essential oil that was separated from the evaluated hyssop genotypes, monoterpene ketones are the major components: pinocamphone in the form trans (-) iso and cis (Table 2; Figs. 4; 5; 6). For the genotype with blue corolla belonging to the *f. cyaneus*, the major component of the essential oil is cis (-) pinocamphone, with concentration of 51.77% and trans (-) iso pinocamphone with 6.70%.

The essential oil separated from the genotype with pink corolla of *ruber* form contains 66.94% of pinocamphone: 33.31% - trans (-) iso pinocamphone and 33.63% -cis (-) pinocamphone, and in the separated essential oil from the genotype with white corolla, the *albus* form, the main component is trans (-) iso pinocamphone-61.1%, the concentration of cis (-) pinocamphone was only 2.15%. Thus, the richest in iso-and cis- pinocamphone is the genotype with white corolla. In all three genotypes pinocamphone is followed by β-pinene, being the highest concentration of this component in the essential oil of *cyaneus* form - 8.49% and *albus* form - 7.38%. The *ruber* form contains 4.15% of β-pinene. The concentrations of the third component - β-felandren is from 3.64% for *ruber* form to 6.79% for *albus* form. These components are followed by the carvacrol in different concentrations (3.31%, 5.49%) in the genotypes with pink (*ruber* form) and white (*albus* form) corolla, but in case of the genotype with blue corolla (*cyaneus* form) by the 2.55% d-germacren, and 3.43% byciclogermacrene. *H. officinalis* genotypes that were selected and described above are distinguished both by the essential oil content and composition of the hyssop and they were studied by other authors in other countries. It is known that the essential oil that accumulates in the herba of *H. officinalis* var. *decumbens* is distinguished by a lower content of ketones. The major component of this variety is not pinocamphone but linalool, 1.8-cineole, limonene, or other components. For example, the hyssop from the spontaneous flora of Montenegro has a lower content of essential oil and the number of identified components in the oil is high – 68, with the major components methyl eugenol (38.3%), limonene (37.4%) and β-pinene (9.6%) (GORUNOVIC et al., 1995).

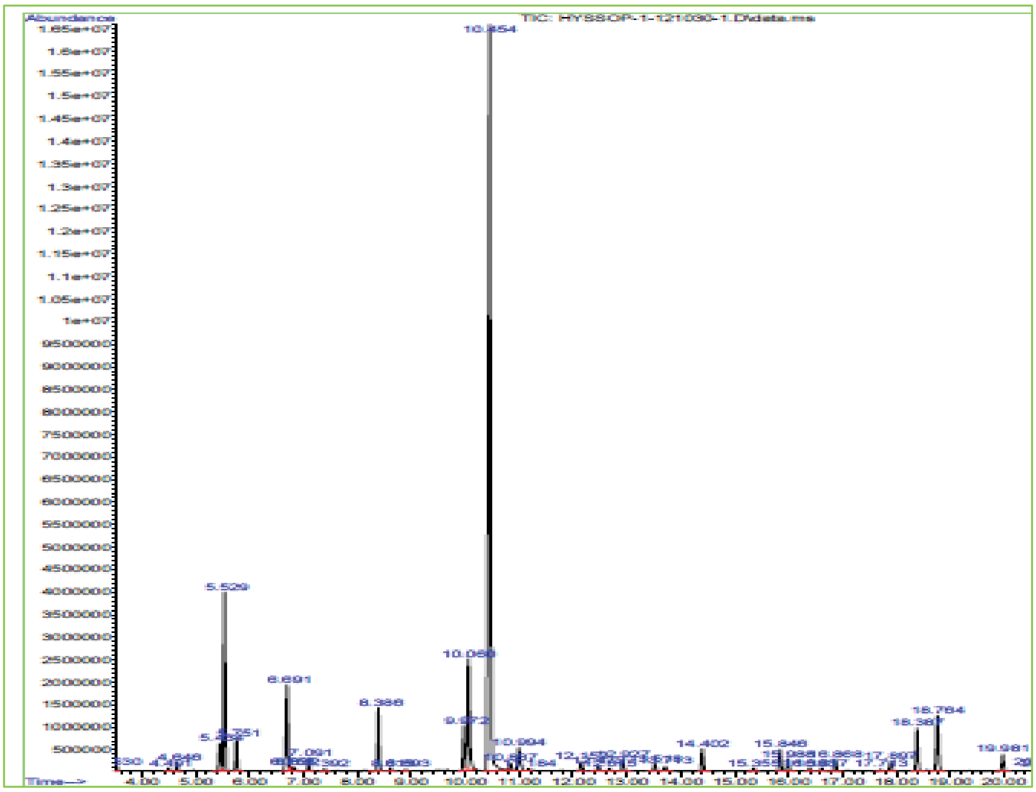

Figure 4. Chromatography of *H. officinalis*, *f. cyaneus* essential oil.

In the hyssop essential oil, *cyaneus, ruber* and *whites* forms from the Yugoslavian spontaneous flora the main component is cis-and trans-pinocamphone (CHALCHAT et al., 2001), as well as the genotypes evaluated by us, but only the major component in these genotypes is followed by the pinocarvone and not by β-pinene as in case of the Moldavian genotypes. In other forms of hyssop, coming from Italy, the essential oil, pinocamphone is followed by the β-pinene in concentrations higher than in our genotypes, the difference being the significant content of linalool and camphor (FRATERNALE et al., 2004) that in the essential oil content of our genotypes was not identified.

Obviously, the genotype of hyssop with white corolla (Table 2) selected by us, and *H. officinalis* from Turkey are comparable displaying similar concentrations of iso-pinocamphone (57.27%) and β-pinene (7.23%) in the essential oil (KIZIL et al., 2010). The difference is given by the concentration of the other components except β-pinene, difference highlighted by the chromatogram.

In the essential oil separated from the hyssop coming from the Turkish flora, the first two major components are followed by terpinen-4-ol and trans-pinocarvone (KIZIL et al., 2010), but in case of the Moldavian genotype with white corolla, by the β-felandren and carvacrol. There are significant differences between qualitative and quantitative composition of the essential oil separately from *H. officinalis* L. ssp. *officinalis* from Lublin, Poland and our genotypes, although the major component is the same - cis-pinocamphone (BAJ et al., 2010) as well as *f. cyaneus* from Moldova.

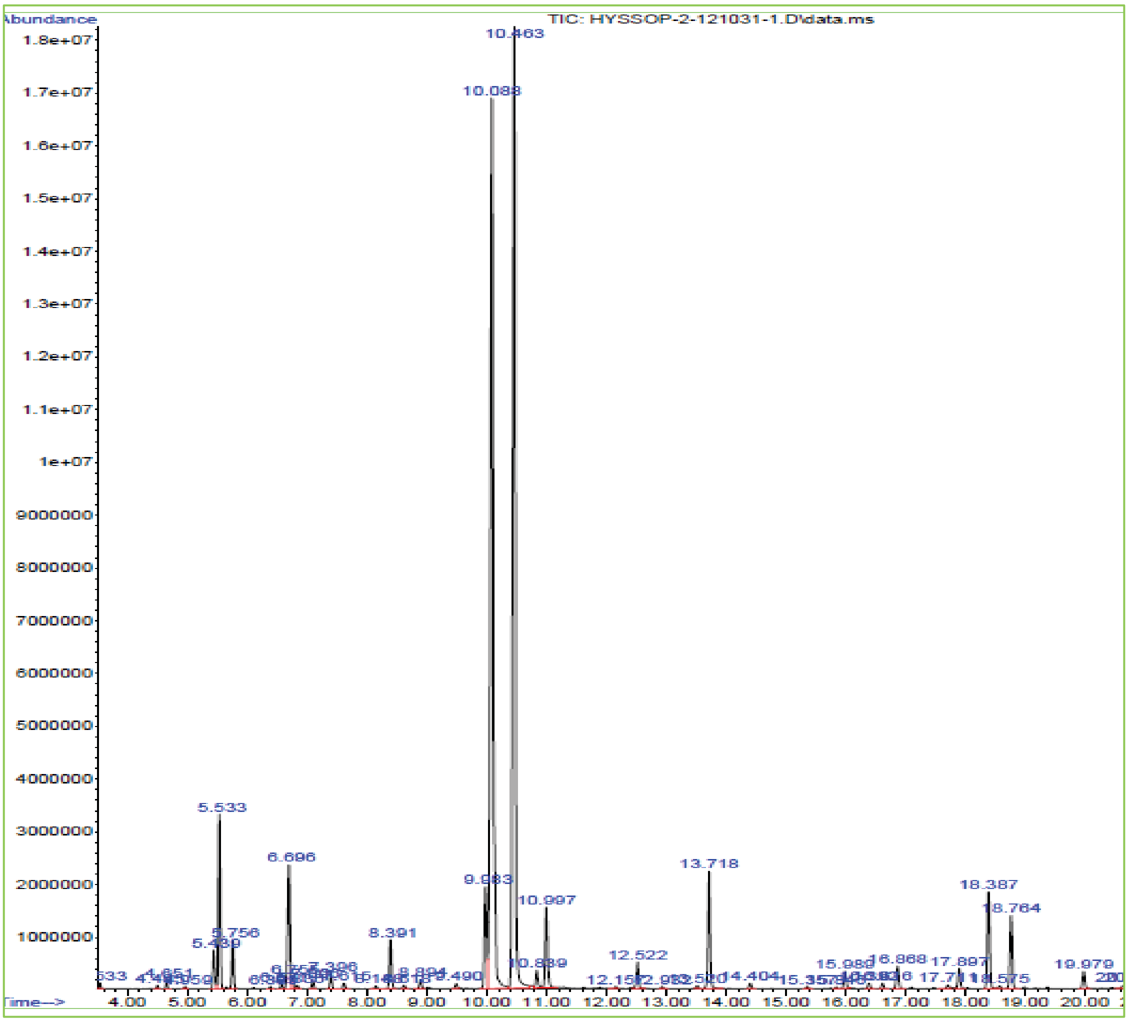

Figure 5. Chromatography of *H. officinalis, f. ruber* essential oil.

The chemotypes described by other researchers also differ in terms of concentrations of linalool, 1, 8-cineole, methyl eugenol, limonene, β-pinene, cis and trans pinocamphone, β-cariophilene, d-germacren etc. (CHALCHAT et al., 2001; GORUNOVIC et al., 1995; OZER et al., 2005; SALVATORE et al., 1998).

It is obvious that the *H. officinalis* genotypes the *f. cyaneus, f. ruber* and *f. albus* selected by us are distinguished from other genotypes, forms, varieties by the qualitative and quantitative chemical composition of the essential oil. Qualitative and quantitative chemical composition of hyssop essential oil could be strictly related to its antibacterial and healing characteristics. Thus, KIZIL et al. (2010) showed that oil rich in iso-pinocamphone has antimicrobial action against *S. aureus, C. albicans* and *E. coli* but has no similar action on *P. aeruginosa*.

The different individual antimicrobial, antifungal action depending on the concentration of certain components, that is also reported by several researchers (FRATERNALE et al., 1982; LETESSIER et al., 2001; MAZZANTI et al., 1998; RENZI et al., 1999. Chemical composition and, especially, the concentration of cis-pinocamphone depends on the property of hyssop essential oil to be used to treat epilepsy, especially for children (BAJ et al., 2010), other characteristics and properties (antioxidant, relaxing cytotoxic, etc.) (BAJ et al., 2010; KIZIL et al., 2010; LU et al., 2002; SALVATORE et al., 1998).

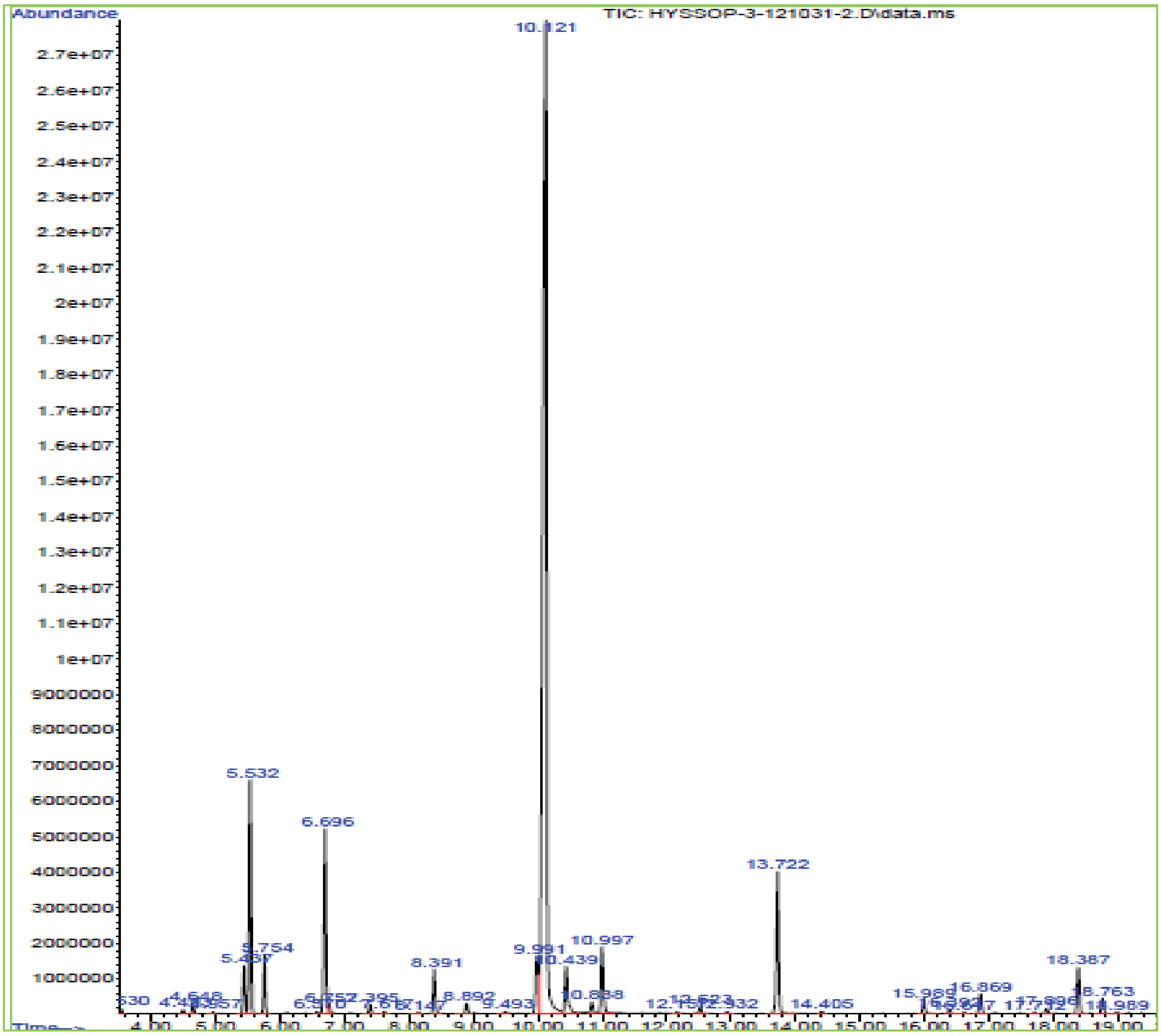

Figure 6. Chromatography of *H. officinalis, f. albus*, essential oil.

CONCLUSIONS

H. officinalis genotypes with pink (*ruber* form), white (*albus* form), blue (*cyaneus* form) corolla were evaluated and it was demonstrated that in drought conditions the indices of important quantitative characters (plant height, number of the flower stems per plant, inflorescence length, number of verticiles per ear inflorescence) are higher in the genotypes with pink and blue corolla.

Hyssop genotypes have different content of essential oil, but very high: *ruber* form - 2.531% (dry matter), *cyaneus* form - 1.877% (dry matter) and *albus* form - 1.434% (dry matter).

The GC-MS analysis allowed the identification of the components in the essential oil: *cyaneus* form 34 components, *ruber* form - 38 and *albus* form - 30 components, in the rate of 97.14 - 98.31%.

For all genotypes the major components of the essential oil are monoterpene ketones: pinocamphone the form trans (-)-cis iso.

For the *cyaneus* form, the major component of the essential oil is cis (-) pinocamphone, with concentration of 51.77% and trans (-) iso pinocamphone with 6.70%; *ruber* form contains 66.94% pinocamphone: 33.31% - trans (-) iso pinocamphone and 33.63% -cis(-) pinocamphone; the *albus* form is the richest in trans (-) iso pinocamphone-61.1%, and only 2.15% is cis (-) pinocamphone.

In all genotypes pinocamphone in the essential oil is followed by β-pinene (*cyaneus* form - 8.49%, *albus* form - 7.38% and *ruber* form contains 4.15%) and by β-felandren in the concentration of 3.64% for *ruber* form to 6.79% for *albus* form.

The concentration of minor components in the essential oil is 1-3% (mirtenat of myrtenil, d-germacren, byciclogermacrene, pinocampheol, Δ3-caren, β-thujone, sabinene, myrtenil acetate) and other components that have different concentrations in different genotypes, but very low, some of whom are present in the essential oil only in one or two genotypes.

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