UDK 633.8:582.929.4:581.559(477.75)

INVESTIGATIONS OF ESSENTIAL OIL COMPONENT COMPOSITION OF *HYSSOPUS OFFICINALIS* L.

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Introduction

Chemical character of essential oils (EO) is quite complex, but in general it presents a mix organic substances such as terpens with acyclic structure (main substances) where this or that component prevails. As a result of long-term essential oils investigations some authors suggested biogenetic schemes which find that essential oil components are formed after consecutive transformations [2, 3, 6, 7, 9]. According to current opinions, each transformation of one terpenic compound into another is under control of a certain gene which codes synthesis of an appropriate ferment. Having a lack of an appropriate ferment sequence of biosynthesis reactions stop and previous ferment is accumulated, that is a key factor which determines essential oil composition of a definite cultivar.

Analyzing open literature sources devoted to essential oil composition of *Hyssopus officinalis* it was revealed that data are quite fragmentary and sometimes even conflicting. Moreoften there is summery data of quantitative content of dominate components sometimes it`s possible to find analysis of component composition of different morphologic forms [1,4, 5, 8, 10, 11]. Data of intraspecific changeability of chemical composition of *Hyssopus officinalis* essential oil, correlations between certain terpenoid compounds, chemical composition of essential oil extracted out of different plant parts are hardly presented. According to mentioned above we carried out investigations aimed at studying the changeability of essential oil compound composition of *Hyssopus officinalis* for further selection.

Objects and research methods

Investigations were being carried out on the South Coast of the Crimea in Nikitsky Botanical Gardens since 2007 till 2012. Plants grown out of seed generation of *Hyssopus officinalis L.* (f.albus, f.cyaneus, f.ruber) were chosen as study material.

Crop inventory was organized during mass blooming. Row material was cut by hand and weighed at ones. Mass concentration of essential oil was determined in field-fresh material by hydrodistillation method applying Clevenger apparatus. Essential oil component composition was investigated using chromatograph Agilent Technology 6890N with mass-spectrometric detector 5973N. Essential oil components were identified according to data of chromatography of mass-spectrum chemical substances included into study mixtures; mass-spectrum database NIST02 (more than 174000 substances) were considered. Indices of component retention were calculated allowing for results of control analysis of essential oils with a set of standard alkanes [17].

Results and discussion

Investigation of EO component composition of *Hyssopus officinalis* seed population revealed intraspecific composition of essential oil isolated out of over-ground material is quite diverse and includes components as follows: pinocamphone, isopinocamphone, α- and β-pinene, sabinene, myrcene, β-phellandrene, linalool, myrtenol, methyleugenol, elemol and etc. EO composition included 60 terpenic compounds, 41 of them were identified. EO analysis (table 1) displayed considerable variations of EO composition. Mainly (70%) plants synthesized pinocamphone, isopinocamphone, β-pinene, sabinene, myrtenol, elemol and immaterial amount of other components.

The second plant group (20%) of *Hyssopus officinalis* accumulated 5 principal terpenoids: pinocamphone (up to 60%), β-pinene (up to 6,2%), β- phellandrene (up to 6,8%), spathulenol (up to 3,5%), myrtenol (up to 6,3%), *caryophyllene (up to 3,5%).*

And the third plant group (10%) of *Hyssopus officinalis* (special group) synthesized: isopinocamphone (up to 61,1%), β-pinene (up to 10,5%), elemol (up to 19%), eudesmol (up to 7,6%). This plant group is special due to biosynthesis of sesquiterpenes (totally up to 25%). EO composition of all study plants mainly corresponds cultivar of *Hyssopus officinalis,* only proportion of the principal components has some variations.

Concentration of independent hydrocarbons in essential oil didn`t exceed 1,5% only maximum content of β-pinene made 10,5%. Standard way of distribution in the range of low values with single-humped curve and minimal values on the track level was typical for all of them.

Plant range by mass concentration of pinocamphone and isopinocamphone, as the main dominants in *Hyssopus officinalis* essential oil should be emphasized. It`s a well-known fact that pinocamphone is a bicyclic terpenic ketone which possesses cis- and trans-forms being in the dynamic balance, that causes constant instability of natural pinocamphone isolated out of *Hyssopus officinalis* essential oil [1].

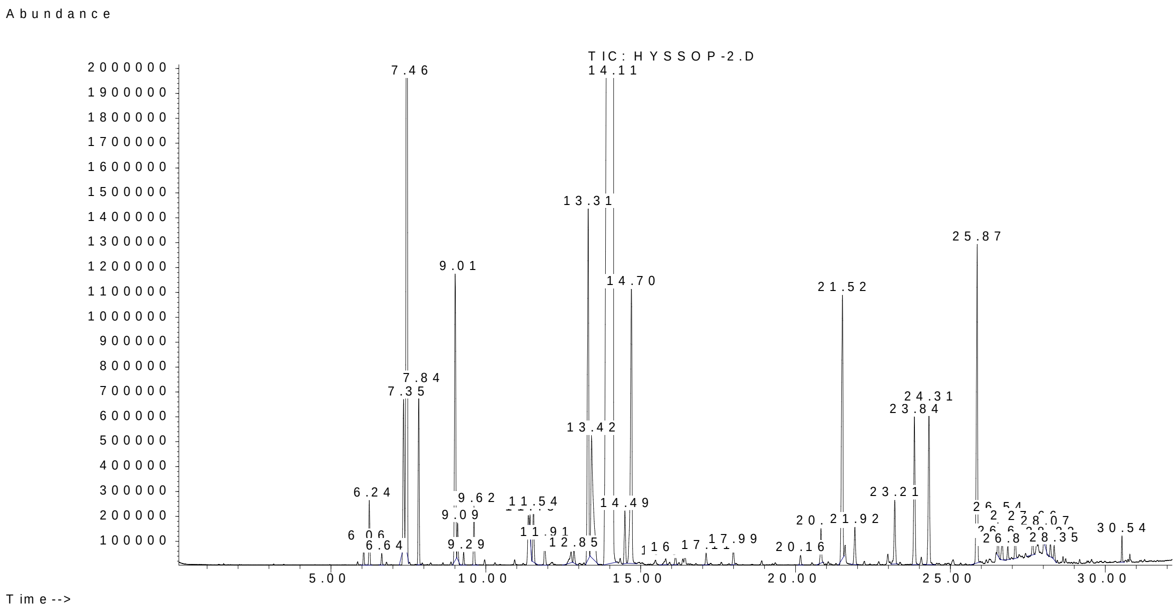
In terms of investigations it was determined that plant distribution by mass concentration of pinocamphone is in range of values from 4,34% up to 60,48% with maximum number of plants (35%) containing pinocamphone (from 10 up to 20%) and the second hump in the range of values is from 30 up to 40%. Other plants are distributed evently with interval up to 10% and from 20 up to 30%. Though, there was revealed a plant with maximum level of biosynthesis of pinocamphone (up to 60,48%) what is a considerable gap in pinocamphone concentration in comparison with other plants. It makes possible to accept two chemotypes of *Hyssopus officinalis:* with middle and high concentration of this terpenoid in the essential oil.

The same situation takes place in plant distribution by isopinocamphone concentration in essential oil. Plant distribution curve by isopinocamphone content in essential oil is bimodal with maximum number of plants (30%) in interval (20-30%) and the second top is in the range of high values of isopinocamphone (from 50-60%) with 35% of plants. Among study plants there is a form with a rather high biosynthesis of isopinocamphone (up to 61,12%) what makes this plant quite appropriate starting material for selection.

Distribution curve by biosynthesis of elemol in the essential oil has a left asymmetry with maximum distribution in interval (2-7%) of average values for this component, only for some plants synthesis of elemol reaches 19%. It should be noted that elemol is monocyclic sesquiterpenic alcohol with a flavor what improves essential oil. Almost all minor components in essential oil of *Hyssopus officinalis* are presented in low concentration with a small interval of variation. In all cases hystograms were unimodal with, there wasn`t the second maximum point in distribution, that is plants belonged to the same chemotype by concentration of terpenoids mentioned above.

Analysis showed the following terpenic compounds were characterized by the highest level of changeability and variation coefficients: β-pinene, sabinene, myrcene, β-phellandrene, caryophyllene.

ISSN 0201-7997. Сборник научных трудов ГНБС. 2014. Том 139



1	6.05	0.144%	α-tuyen	22	16.12	0.094%	neral
2	6.24	0.444%	α-pinene	23	17.11	0.111%	geranial
3	6.64	0.085%	camphene	24	17.99	0.162%	
4	7.34	1.122%	sabinene	25	20.16	0.090%	geranylacetat
5	7.46	9.156%	β-pinene	26	20.82	0.311%	β-burbonene
6	7.83	1.302%	myrcene	27	21.51	2.822%	methyleugenol
7	9.01	2.680%	β-phellandrene	28	21.91	0.382%	caryophyllene
8	9.09	0.244%	1,8-cineol	29	23.20	0.670%	alloaromadendrene
9	9.28	0.097%	trans-ocimene	30	23.84	1.506%	germacrene D
10	9.62	0.472%	cis-cimene	31	24.31	1.664%	bicyclogermacrene
11	11.42	0.132%	terpinolene	32	25.86	3.000%	elemol
12	11.53	0.456%	α-tuyon	33	26.53	0.292%	spathulenol
13	11.90	0.244%	β-tuyon	34	26.67	0.224%	
14	12.75	0.156%	pinocarveol	35	26.85	0.108%	viridiflorod
15	12.84	0.124%	camphora	36	27.09	0.273%	ledol
16	13.30	3.491%		37	27.65	0.219%	γ-eudesmol
17	13.41	2.247%	pinocamphone	38	28.07	0.133%	α-eudesmol
18	14.10	61.122%	isopinocamphone	39	28.23	0.125%	
19	14.49	0.508%	α-terpineol	40	28.35	0.118%	
20	14.70	3.214%	myrtenol	41	30.53	0.169%	rosifoliol
21	15.80	0.087%					

Fig. 1 Chemoform of *Hyssopus officinalis* with the highest concentration of isopinocamphone

Researching the EO composition of *Hyssopus officinalis* isolated out of seed population, unique chemotypes were found out. In this way we marked out methyleugenol chemotype which contains 51,32% of methyleugenol, up to 13,1% of isopinocamphone, till 6,77% of elemol. At the same time linalool chemotype was revealed that has the following EO composition: pinocamphone – 2,94%, isopinocamphone – 33,38%, linalool – 34,88%; and uncommon chemotype was found out in terms of this research: pinocamphone – 4,34%, isopinocamphone – 7,77%, methyleugenol – 2,25%, elemol – 10,49%, manool – 21,7%, viridiflorol – 7,51% and others.

Such a pronounced polychemism and a weak connection of some EO components give a great opportunity for an individual plant selection out of *Hyssopus officinalis* seed population. This plant is characterized by high level of plant population heterogeneity. This type of changeability and correlation of biosynthesis processes of some terpenoids is kept in the seed generation that proves cultivar genetic stability and permits to mark out it as an independent taxonomic unit of *Hissopus* genus.

As a study cultivar *H. officinalis* has three plant forms with white, blue and pink flowers, we carried out investigation of EO component composition of these forms. Comparison study of EO composition revealed there is no a considerable difference by hydrocarbon content between f.albus (9,59%) and f.ruber (10,31) forms. While f.cyaneus form contains hydrocarbon twice less (4,4%). Heighten concentration of alcohols was marked for f.albus (till 8,69%), a bit less than for f.cyaneus (till 5,73%) and f.ruber form had the least value (till 4,61%). As to aldehydes and ketones, basic difference wasn`t noted. F.cyaneus and f.ruber have the same concentration (59,8% each) and f.albus contains a bit more (till 62,17%).

Comparison study of EO composition for some terpenoid compounds revealed basic difference for some specimens. In this way, the largest mass fraction of β-pinene was registered for f.albus form (till 10,50%), a little bit less for f.cyaneus, f.ruber forms (till 9,12%). Though it`s necessary to note that distribution curve by this character for all plant forms has left-side distribution with maximum number of plants containing β-pinene with interval of 3-5%.

Basic differences in EO of f.albus, f.cyaneus, f.ruber forms by mass fraction of β-phellandren, spathulenol, myrtenol, caryophillen, ledol and other terpenic compounds weren`t registered. All of them had a single-humped curve in the range of low values.

Comparison study of plant distribution by biosynthesis of pinocamphone and isopinocamphone in essential oil of different forms presented the followings: maximum level of pinocamphone mass fraction in essential oil was marked for plants of f.ruber form - till 60,48%, f.cyaneus form – till 36,37%, f.albus – till 35,24%. Quite different situation takes place in biosynthesis of isopinocamphone. Maximum mass fraction of isopinocamphone occurred in essential oil of f.albus plants (till 61,12%, see fig.1), a bit less – for f.cyaneus (till 57,93%) and the minimum concentration was registered for f.ruber plants – till 38,14% (table 1).

Table 1

EO component composition of different *H. officinalis* morphological forms

Component	Variations of component mass fraction, %		
	f.albus	f.cyaneus	f.ruber
sabinene	0,19 – 0,42	0,38 – 1,53	0,19 – 0,69
β-pinene	0,41 – 2,89	0,42 – 3,16	0,88 – 4,35
myrcene	0,26 – 0,46	0,25 – 1,35	0,15 – 0,71
β-phellandrene	0,19 – 1,11	0,22 – 2,12	0,32 – 0,82
linalool	0,13 – 34,88	0,22 – 0,93	0,12 – 0,91
α-tuyon	0,17 – 1,09	0,30 – 1,02	0,75 – 1,40
pinocamphone	1,12 – 35,24	12,59 – 36,37	11,54 – 60,48
isopinocamphone	34,65 – 61,12	34,67 – 57,93	4,44 – 38,14
α-terpineol	0,25 – 0,73	0,30 – 0,32	0,29 – 1,43
myrtenol	3,63 – 6,31	1,21 – 3,43	1,68 – 4,17
methyleugenol	0,52 – 37,80	0,23 – 0,73	0,32 – 1,70
caryophillene	1,82 – 3,77	2,12 – 7,70	1,40 – 5,17
elemol	0,33 – 2,81	0,63 – 1,49	0,66 – 3,21
ledol	0,21 – 0,92	0,26 – 0,88	0,11 – 0,52
spathulenol	1,39 – 3,52	0,83 – 3,35	0,48 – 2,39
viridiflorol	0,23 – 2,15	0,10 – 2,16	0,10 – 7,24
manool	0,36 – 5,85	0,10 – 5,76	0,19 – 20,16

Investigation of EO component composition of *H. officinalis* made it possible to mark out of the f.albus plants a valuable chemoform with biosynthesis of a rare terpenoid – methyleugenol (till 37,80%). That`s why we can say with certainly about two chemotypes of *H. officinalis:* with high and low concentration of this terpenoid in essential oil. It`s worth to note this chemotype is characterized by low level of pinocamphone biosynthesis (till 2,2%). Besides among f.albus plants there was found out a chemotype which synthesizes mostly linalool (34,88%). One more peculiarity of this chemotype is a low mass fraction of pinocamphone (till 2,24%). So, talking about revealed chemotypes we have inhibition of pinocamphone biosynthesis while linalool biogenesis takes place.

Essential oil is a complex chemical substance, capable to be effected by a number of factors. Terpenoid composition of essential oil depends upon plant development phase, hydrothermal factors which make a background for plant growth and development, and even the terms of yielding. According to literature data yielding time of flower row material is of great importance as essential oils have considerable changes under influence of daily and seasonal variations [13, 16, 18, 19]. Studies in variation of some EO terpenoids mass fraction of *H. officinalis* during a day aren`t covered in open literature sources in comparison with determination of EO component composition of *H. officinalis* different cultivars which is presented in a lot of scientific works.

That`s why one of the principal tasks of our research was to trace the variations of EO component composition during a day. As a study case we chose *H. officinalis* plants of f.albus form, which EO component composition was determined. Over-ground mass of row material was taken for analysis at 5.00, 8.00, 13.00 and 18.00 o`clock. Data of dynamics of EO component composition of *H. officinalis* during the day are presented in table 5.3.

As to table 2, dominant components of *H. officinalis* essential oil are cys- and trans- forms of pinocamphone, which are in dynamical balance. According to analysis at 5.00 o`clock pinocamphone mass fraction made 38,48%, but isopinocamphone is much lower – 21,03%. Then at 8.00 o`clock pinocamphone biosynthesis reduced till 22,94%, while biosynthesis of isopinocamphone increased till 33,38% that is 1,5 times as much.

Table 2

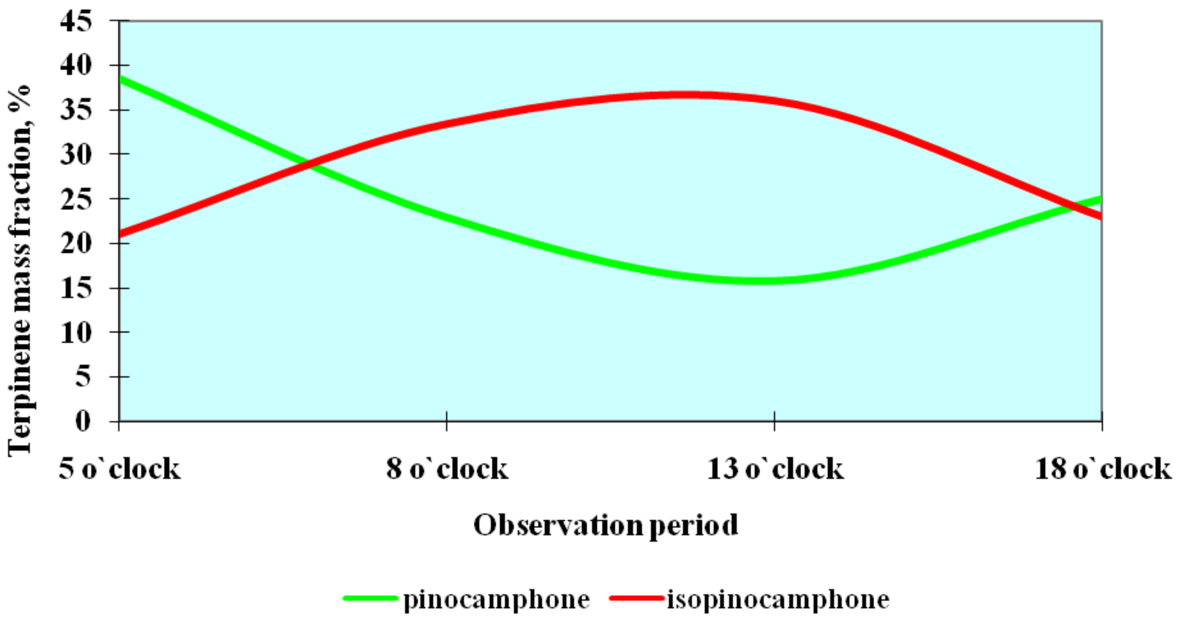
Variations of EO component composition of *H. officinalis* during a day

Component	Time of component occurance, min	Component mass fraction, % for analyzing time			
		5 o`clock	8 o`clock	13 o`clock	18 o`clock
sabinene	7,33-7,37	0,680	0,166	–	–
β-pinene	7,43-7,50	4,769	1,389	3,193	1,372
myrcene	7,83-7,85	0,579	0,333	–	–
β-phellandrene	9,02-9,03	1,231	1,578	0,366	0,225
linalool	11,64-11,81	0,935	4,883	0,708	1,090
pinocamphone	13,45-13,84	38,486	22,940	15,806	25,007
isopinocamphone	14,12-14,37	21,033	33,377	35,968	22,993
myrtenol	14,74-14.93	5,481	3,607	4,895	5,549
methyleugenol	21,50-21,53	0,630	0,351	1,589	2,257
caryophyllene	21,94-21,97	1,207	1,063	1,427	1,654
germacrene-D	23,89-23,93	3,081	4,284	1,806	1,594
elemol	25,85-25,95	4,140	0,309	5,582	8,328
spathylenol	26,56-26,59	–	1,188	2,881	2,110
cayophyllenoxid	26,62-26,66	1,707	0,682	1,748	2,013
viridiflorol	26,86-26,90	0,433	0,236	0,465	0,813
epimanool	33,34	0,319	0,237	3,446	2,366
phytol	33,73	0,657	0,627	1,367	2,148

Reduction of pinocamphone biosynthesis continues by 13 o`clock (till 15,81%), and mass fraction of isopinocamphone slightly increases (till 35,97%). At 18 o`clock pinocamphone biosynthesis intensifies 1,7 times as much till 25,00%, while isopinocamphone biosynthesis decreases 1,6 times less till 22,99%. Thereby, during a day reduction of pinocamphone and increasing of isopinocamphone have the same values. According to figure 2, biosynthesis of pinocamphone and isopinocamphone are in antiphase or dynamical balance. As to dynamics of myrtenol and elemol biosynthesises we have the following data: at 8 o`clock there is a sharp reduction of elemol mass fraction in comparison with its concentration at 5 o`clock, then at 13 o`clock and 18 o`clock its biosynthesis intensifies. The same situation is registered for biosynthesis of myrtenol. Since 5 till 8 o`clock certain reduction of myrtenol mass fraction occurs, but after that we have increasing of myrtenol biosynthesis.

Biosynthesis of β-pinene acts in another way. Its maximum concentration in essential oil is registered at 5 o`clock (4,78%), but then a sharp reduction till 1,39% happens by 8 o`clock. Its biosynthesis increases up to 3,1%, that is 2,3 times as much at 13 o`clock, but by 18 o`clock it sharply reduces till 1,37%, again 2,3 times less.

As to concentration of other components of *H. officinalis* essential oil during a day, their mass fraction variates in terms of mistake and sharp changes are not registered. Anatomico-morphological study of plants found out that *H. officinalis* has essential oil glandulas on all organs that`s why all organs are supposed to contain essential oil. Literature sources [10, 11,1] point only at essential oil availability in over-ground row material. We attempted to isolate essential oil out of stems, leaves, inflorescences, over-ground mass of flower material, seeds and determine its component composition.



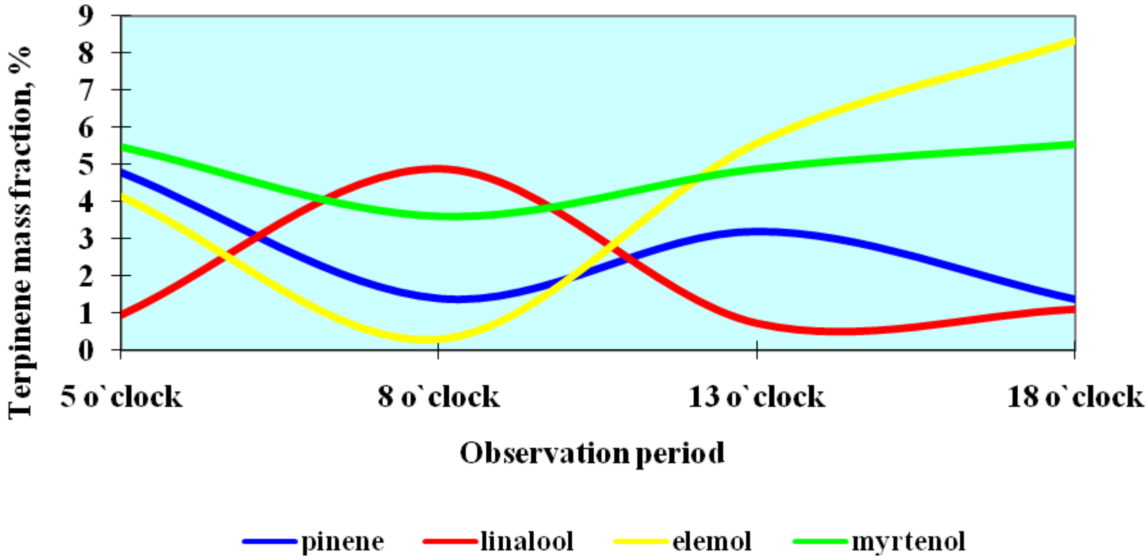


Fig.2 Peculiarities of biosynthesis variations of the principal terpenoids in *H. officinalis* essential oil during day

In terms of investigations it was found out that all parts of *H. officinalis* contained essential oil. Study of EO component composition permitted to identify 41 compounds. Essential oil includes hydrocarbons, alcohols, aldehydes, ketones and esters a bit. Hydrocarbons are presented by sabinene, myrcene, α- and β-pinene, β-phellandren.

Analyzing EO component composition of the over-ground plant mass it occurred that dominant compounds are pinocamphone and isopinocamphone with mass fraction in total ranges from 48,0% up to 77,0 %, then β-pinene takes place (till 10,50%) while some plants had it till 19,51%. Mass fraction of myrtenol ranges from 3,61-5,55%, elemol – 3,13-14,80% (some chemotypes reach 19,04%), methyleugenol – from 0,63% till 4,1% and linalool till 1,50% (table 3).

EO composition isolated out of leaves have some differences from over-ground EO.

Table 3

EO component composition isolated out of *H. officinalis* over-ground mass

Component	Parameters			
	X±Sx	V,%	min	max
sabinene	0,316±0,1227	78,6	0,17	0,68
β-pinene	2,680±0,8169	61,0	1,37	4,77
myrcene	0,505±0,0627	24,9	0,33	0,61
β-phellandrene	0,850±0,3297	77,6	0,22	1,58
linalool	1,055±0,1617	30,7	0,71	1,48
α-tuyon	0,383±0,0661	34,6	0,21	0,52
pinocamphone	20,563±7,4962	72,9	2,94	38,49
Isopinocamphone	28,343±3,7156	26,2	21,03	35,97
α-terpineol	0,708±0,0312	8,8	0,65	0,79
Myrtenol	4,883±0,4492	18,4	3,61	5,55
Methyleugenol	1,208±0,4399	72,9	0,35	2,26
Caryophyllen	1,338±0,1289	19,3	1,06	1,65
Elemol	5,340±1,1014	41,3	0,31	8,33
Ledol	0,660±0,1047	31,7	0,37	0,87
Spathylenol	1,825±0,4177	45,8	1,12	2,88
Viridiflorol	0,485±0,1187	49,0	0,24	0,81

Manool	1,590±0,7902	99,4	0,23	3,44
caryophyllen oxide	1,538±0,2934	38,2	0,68	2,01

The principal component is isopinocamphone with mass fraction ranging from 34 till 52%. At the same time pinocamphone concentration was much less – 9,36 – 42,97%. Less concentration was registered and for β-pinene in comparison with over-ground mass – 0,42-4,35%. Mass fraction of myrtenol and elemol had the same variations as it was in over-ground mass EO. Distinctive feature of EO component composition isolated out of leaves is a high concentration of sesquiterpenes and especially such terpenic compounds as: viridiflorol (till 7,24%), manool (till 20,16%) and also phytol (till 6,75%) and oktakzan (till 22,61%) which are not typical for essential oil isolated out of over-ground mass (table 4).

EO component composition isolated out of stems was of great interest for science, as nevertheless stems are ballast in row material and contains a minimal EO concentration, it influences on EO quality in general. Analysis of stem component composition revealed that a dominant component of this EO is isopinocamphone with mass fraction of 56,7% for some specimens. At the same time a low level of pinocamphone biosynthesis took place, maximum 6,35%. In comparison with other plant organs stem EO is characterized by high biosynthesis of such components as elemol (till 10,39%), viridiflorol (till 7,51%) and maximum level of manool biosynthesis (till 21,7%). Methyleugenol, revealed in EO of one of specimens in maximum concentration (51,32%) is of great importance (table 4).

ISSN 0201-7997. Сборник научных трудов ГНБС. 2014. Том 139

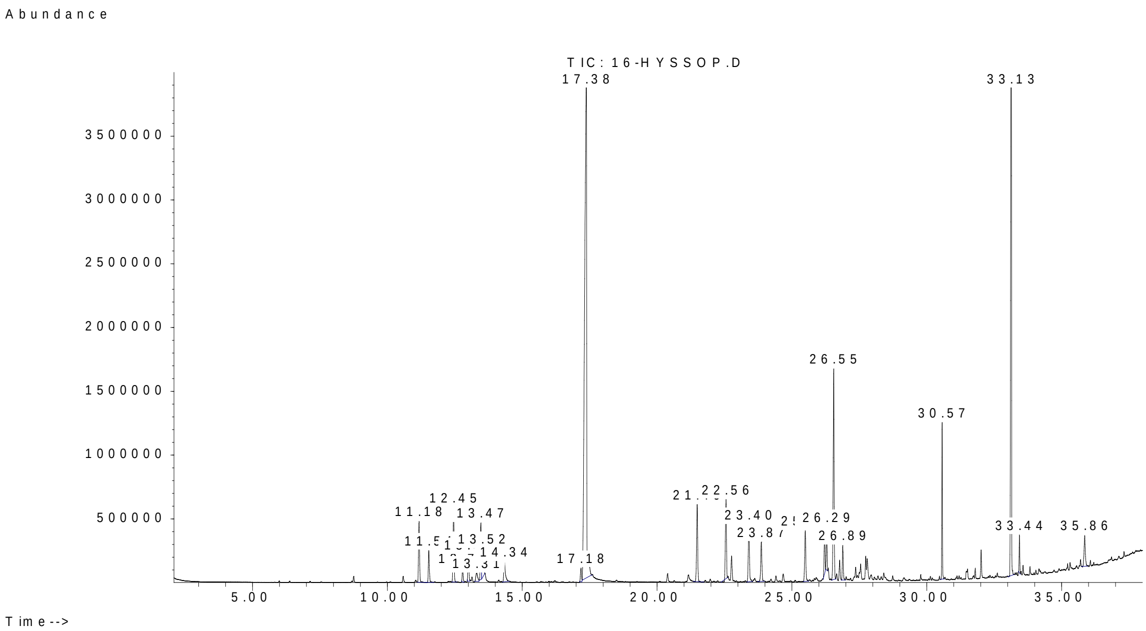
Table 4

Variations of EO component composition isolated out of different *H. officinalis* plant organs

Component	**Mass fraction of component in essential oil of the following morphologic forms**								
	Leaf			Inflorescence			Stem		
	f.albus	f.cyaneus	f.ruber	f.albus	f.cyaneus	f.ruber	f.albus	f.cyaneus	f.ruber
1	2	3	4	5	6	7	8	9	10
sabinene	0,19-0,42	0,38-0,82	0,24-0,69	0,34-0,39	0,57-1,53	0,,19-0,63	tracks	tracks	tracks
β-pinene	0,41-1,39	0,42-3,16	1,88-4,35	2,30-2,89	0,92-2,31	0,88-3,25	0,34	0,21	1,17
myrcene	0.27-0,36	0,25-0,82	0,20-0,40	0,26-0,46	0,64-1,35	0,15-0,71	tracks	tracks	tracks
β-phellandren	0,19-0,20	0,42-2,12	0,49-0,78	0,47-1,11	0,22-1,42	0,32-0,82	tracks	tracks	Tracks
linalool	0,71-0,81	0,22-0,87	0,69-0,91	0,13-0,89	0,26-0,93	0,72-0,88	0,21	0,28	34,8
α-tuyon	0,17-1,09	0,87-1,02	1,05-1,40	0,25-0,88	0,30-0,90	0,75-0,83	0,30	0,50	0,10
pinocamphone	1,12-9,36	13,81-22,87	11,45-42,97	11,23-13,82	12,59-17,65	40,47-60,48	0,30	6,35	4,34
isopinocamphon	34,65-	34,67-46,73	4,44-24,28	51,52-60,98	57,11-57,93	14,98-38,14	13,10	56,70	7,77
A-terpineol	0,43-0.73	0,31-0,32	0,39-1,43	0,25-0,37	0,30-0,31	0,29-0,33	tracks	tracks	tracks
myrtenol	5,35-5,75	1,42-3,43	1,83-4,17	3,63-6,31	1,21-2,70	1,68-2,70	1,27	4,39	0,56
methyleugenol	0,55-3,60	0,28-0,73	0,49-1,70	0,52-2,46	0,23-0,41	0,32-0,86	51,32	0,47	2,25
elemol	2,64-2,73	4,02-7,79	2,03-5,17	1,82-3,77	2,12-4,39	1,40-3,06	6,77	3,05	10,39
caryophyllene	0,33-2,81	0,63-1,49	1,29-3,21	0,24-0,52	0,70-1,09	0,66-0,80	1,22	1,37	3,67
ledol	0,21-0,92	0,51-0,88	0,20-0,52	0,51-0,55	0,26-0,48	0,11-0,35	0,68	0,54	tracks
spathylenol	1,39-2,89	1,82-3,35	1,05-2,39	2,33-3,52	0,83-2,29	0,48-1,68	0,61	0,91	3,55
Caryophyllene	0,35-1,36	0,94-1,11	0,59-1,91	0,29-0,66	0,30-0,50	0,33-0,46	tracks	0,69	2,02
viridiflorol	1,36-2,15	0,91-2,16	1,19-7,24	0,23-0,82	0,10-1,10	1,10-0,18	tracks	2,56	7,51
manool	5,55-5,85	1,05-5,76	1,41-20,16	0,36-1,82	0,10-1,56	0,19-0,32	2,06	8,29	21,70
Octadecene-1	-	-	8,02	-	-	-	-	-	-
phytol	-	-	6,75	-	-	-	-	-	-
oktakozan	22,61	-	-	-	-	-	-	-	-

Anlysis of the EO component composition of *H. officinalis* isolated out of generative organs (inflorescences) revealed certain differences in component concentration in comparison with EO out of other plant parts. EO isolated out of inflorescences is characterized by the highest level of pinocamphone biosynthesis (till 60,48%) and isopinocamphone (till 61%). Though mass fraction of other components ranges in the same way in comparison with EO out of other plant parts. Biosynthesis of such components as methyleugenol, viridiflorol and especially manool is a little bit lower.

As literature sources don`t give component composition of *H. officinalis* essential oil , isolation of EO out of seeds and study its component composition in comparison with EO out of other plant parts was of great importance for us (Fig.3).



1.	11.17	2.22% α-tuyon	13.	22.55	2.92% humulene
2.	11.53	1.18% β-toyon	14.	23.40	2.36% germacrene D
3.	12.45	2.73% camphora	15.	23.87	1.63% bycyclogermacrene
4.	12.79	0,49% menthone	16.	25.49	2.07% elemol
5.	13.00	0.95% pinocamphone	17.	26.21	1.40% spathylenol
6.	13.30	0.53% borneol	18.	26.29	1.24% caryophylleneoxide
7.	13.46	2.00% isopinocamphone	19.	26.55	7.54% viridiflorol
8.	13.52	1.56% menthol	20.	26.88	1.09% hhumulenoxide
9.	14.33	1.14% methyl chavicol	21.	30.57	3.52% hydrofarnesylacetate
10.	17.18	0,48% α-phenchylacetate	22.	33.13	19.57% manool
11.	17.37	38.00% anethole	24.	35.85	1.39% squalene
12.	21.48	3.08% caryophyllene			

Fig.3 Chromatogram of *H. officinalis* EO isolated out of seeds

In terms of investigations it was revealed that component composition of *H. officinalis* EO isolated out of seeds dramatically differs from EO composition isolated out of other plant parts. 24 components were identified in EO composition. The principal component are anethol ($C_{10}H_{12}O$), a component characterized by anise flavor and sweet anise taste, its mass fraction in EO makes 38,0%. EO of *H. officinalis* isolated out of other plant parts doesn`t include this component. Besides anethol there are also α-tuyon (2.22%), β-tuyon (1.18%), camphora (2.73%), menthone (1.56%), methylchavicol (1.14%), squalene (1.39% and a quite high mass fraction of manool (19.57%). At the same time it has to be noted, those components which prevail in EO composition isolated out of over-ground row material, make low concentration in EO out of seeds. So, mass fraction of pinocamphone made 0,95% and isopinocamphone – 2,00%.

Analysis of correlations between certain components of *H. officinalis* essential oil revealed both positive and negative connections between biosynthesis of terpenic compounds. Determined conjugacy in biosynthesis of *H. officinalis* EO components has rather low correlation coefficients in many cases (table 5). Such correlation takes place for pinocamphone and myrtenol (R= - 0,19), pinocamphone and β-pinene (R= - 0,20), pinocamphone and linalool (R= - 0,29). There is only negative correlation between pinocamphone and other components. Though isopinocamphone and other components makes both positive and negative correlations.

So, positive correlation between isopinocamphone and methyleugenol (R= - 0,45) makes it possible to select both components simultaneously, while negative correlation between pinocamphone and methyleugenol (R= - 0,40) permits to select one compound with a high concentration, as they are inversely oriented.

Table 5

Correlations between components of *H. officinalis* essential oil

Components	Correlation coefficient, R	Components	Correlation coefficient, R
myrtenol and elemol	- 0,05	pinocamphone and isopinocamphone	- 0,87
myrtenol and β-pinene	- 0,11	pinocamphone and myrtenol	- 0,19
myrtenol and methyleugenol	- 0,14	pinocamphone and elemol	- 0,42
myrtenol and β-phellandrene	- 0,06	pinocamphone and β-pinene	- 0,20
myrtenol and linalool	0,22	pinocamphone and methyleugenol	- 0,40
elemol and β-pinene	- 0,56	pinocamphone and linalool	- 0,29
elemol and methyleugenol	- 0,13	isopinocamphone and myrtenol	0,12
elemol and linalool	0,37	isopinocamphone and elemol	0,22
elemol and β-phellandrene	0,05	isopinocamphone and β-pinene	- 0,34
β-pinene and methyleugenol	0,36	isopinocamphone and methyleugenol	0,45
β-pinene and β-phellandrrene	- 0,54	isopinocamphone and β-phellandrene	0,18
β-pinene and linalool	- 0,08	isopinocamphone and linalool	0,09
methyleugenol β-phellandrene	0,35	β-phellandrene and linalool	0,29
methyleuhenol and linalool	- 0,09		

In marked out chemotypes with a high fraction of pinocamphone (60,48%) it was registered a low concentration of methyleugenol (0,87%) and vice versa having mass fraction of methyleugenol 51,32% level of pinocamphone concentration reduces till 0,30%.

Such correlations indicate biosynthesis conjugacy of some terpenic compounds of *H. officinalis* and strengthen dependence in concentration of certain EO compounds. High negative correlation coefficient (R= - 0,87) between dominant components of essential oil (pinocamphone and isopinocamphone) limits selection of both terpenic compounds simultaneously among seed generation of *H. officinalis*, increasing of one compound concentration causes reduction of another. Though conjugacy in biosynthesis of minor EO compounds for plants from artificial *H. officinalis* population is not so large, as coefficients of their conjugate correlation mainly have low values what indicates a weak mutuality of their biosynthesises and dominance of fluctuate changeability, caused by intraspecific heterogeneity. *H. officinalis* possesses biochemical heterogeneity with methyleugenol and linalool chemotypes, as well as low and high level of correlation between EO components concentration. Such a type of changeability, conjugacy degree and mutuality of biosynthesis processes of certain terpenic compounds keeps in seed generation what indicates genetic species resistance and permits to classify it as a separate taxonomic unit from *Hyssopus* L. genus.

Determined regularities present wide opportunities for intraspecific selection of perspective for industry and breeding work *H. officinalis* chemotypes as well as new starting material for breeding of new cultivars.

Conclusions

Thereby in terms of this research it was determined that *H. officinalis* accumulates essential oil in all plant organs (stem, leaf, inflorescence, seeds). Chemical analysis of essential oil isolated out of plants from Piedmont Crimea revealed this essential oil is good quality and contains hydrocarbons, alcohols, ketones, phenols and other compounds. *H. officinalis* EO isolated out of over-ground mass includes 60 compounds, only 41 were identified. Intraspecific changeability of EO component composition, caused by seed propagation, was determined as well.

The following chemotypes were found out, not presented in scientific literature before: methyleugenol chemotype, chemotypes with a high concentration of oktakozan, elemol, manool, viridiflorol. It`s possible to come across information about such cultivar of *H. officinalis* as *H. officinalis* var. *decumbens*, which is characterized by lower concentration of pinocamphone and isopinocamphone. Its principal components are linalool (49,6%), 1,8-cyneole (13,3%) and limonene (5,4%) [18, 19]. Besides there is no any descriptions about their morphological characteristics, which could distinguish *H. officinalis* var. *decumbens* and *H. officinalis*. In seed generation of *H. officinalis* we could find out a chemotype with the following EO component composition: pinocamphone – 2,94%, isopinocamphone – 33,38%, linalool – 34,88%. If *H. officinalis* var. *decumbens* was classified as a separate taxonomic unit on the ground of higher linalool concentration in EO component composition, our researches dispute that fact.

Besides for the first time comparison analysis of *H. officinalis* essential oil isolated out of different plant parts was carried out in terms of this research. It made possible to reveal quantitative differences in composition of terpenic compounds. Qualitative analysis of essential oil from *H. officinalis* seeds was conducted for the first time as well. 24 components with dominance of anethole were identified in its composition.

References

1. *Aksyonova L.V., Khlypenko L.A., Bakova N.N.* K introduktsii issopa v usloviyakh Yuzhnogo berega Kryma // Materialy VIII mezhdunarodnoy konferentsii “ Sovremenniye issledovaniya v sadovodstve” Yalta. – 2000, - CH. 3. – S. 11-14.
2. *Varentsev V.I.* O teorii evolutsii efirnykh masel // Masloboino-zhyrovoye delo. – 1931. - №2/3. – S. 32-36.
3. *Goryayev M.I., Pliva I.* Metody issledovaniya efirnykh masel. – Alma-Ata: Izd-vo AN Kazakhskoy SSR, 1969. – 752 s.
4. *Mashanov V.I., Andreyeva N.F., Mashanova N.S., Logvinenko I.E.* Noviye efiromaslichniye kultury. – Simferopol: Tavriya, 1988. – S. 34-38.
5. *Nikitina A.S.* Pharmakognosticheskoye izucheniye zmeyegolovnika moldavskogo (*Dracocephalum moldavica* L.) i issopa lekarstvennogo (*Hyssopus officinalis* L.) s tselyu

obosnovaniya primeneniya v pharmatsii i meditsine: avtoref. dis. na soiskaniye uchen.stepeni kand.pharm.nauk.: spets. 15.00.02. – Pyatigorsk, 2008. – 22 s.

6. *Nikolayev A.G.* Biologicheskaya rol komponentov efirnykh masel. – Kishinev: Shtiintse, 1972. – S. 9-28.

7. *Nilov V.I., Vilyams V.V., Mykhelson L.A.* O prevrashchenii efirnykh masel v rasteniyakh // Zapiski Nikitskogo botanicheskogo sada. – 1929. – vyp.3. – S. 3-36.

8. *Rabotyagov V.D., Svidenko L.V., Derevyanko V.N., Boiko M.F.* Efirnomaslichniye i lekarstvenniye rasteniya, introdutsirovanniye v Khersonskoy oblasti – Kherson: Ailant, 2003. – S. 161-165.

9. *Tanasiyenko F.S., Kasimovskaya N.N.* Ob izmenenii sootnosheniya komponentov efirnyh masel v rasteniyah. // Phiziologiya i biokhimiya kulturnykh rastenij. – M., 1981. – Vyp. 13, № 4. – S. 406-410.

10. *Tymchuk K.S., Chelovskaya L.N.* Biokhimicheskaya kharakteristika issopa lekarstvennogo // Voprosy intensifikatsii efiromaslichnogo proizvodstva v Moldavskoj SSR. – Kishinev, 1982 – S. 189-200.

11. *Khlypenko L.A., Bakova N.N., Rabotyagov V.D., Shcherbakova Yu.P., Vinogradov B.A.* Izucheniye roda *Hyssopus* L. V usloviyakh Yuzhnogo berega Kryma // Bull. Nikit.botan.sada, 2004. – Vyp.0. – S. 59-63.

12. Sharygina I.S. Nekotoriye voprosy biologii i biokhimii *Origanum vulgare* L. *Hyssopus officinalis* L. // Bot.journ. – 1959. – T. 44, № 8. – S. 1124-1128.

13. *Chang X., Alderson P.G., Wright Ch.J.* Variation in the Essential Oils in Different Leaves of *Basil* (*Ocimum basilicum* L.) at Day Time // The Open Horticulture Journal, 2009. – Vol. 2. - P. 13-16.

14. *De Filipps. Hissopus* L. // Flora Europaea. Cambridge, 1972. – Vol. 3. – P. 169 –170.

15. *Mazzanti G., Battinelli L.* Antimicrobial properties of the linalool-rich essential oil of *Hyssopus officinalis* L. var decumbens (Lamiaceae). – Flavour and Fragrance Journale. – Roma: 1998. – 13(5). − P. 289-294.

16. *Miguel M.G., Duarte F., Venâncio F., Tavares R.* Chemical composition of the essential oils from *Thymus mastichina* over a day period // World Conference on Medicinal and Aromatic Plants, Budapeste, Hungria, 2001. – P. 8-11.

17. *Jennings W., Shibamoto T.* Qualitative analysis of Flavor and Volatiles by Glass Capillary Gas Chromatography. – Academic Press rapid Manuscript Reproduction, 1980. -472p.

18. *Tonger O., Karaman S., Kizil S., Diraz E.* Changes in Essential Oil Composition of *Oregano* (*Origanum onites* L.) due to Diurnal Variations at Different Development Stages // Not. Bot. Hort. Agrobot. Cluj, 2009. – Vol. 37, № 2. – P. 177-181.

19. *Yaldiz G.* Seasonal and diurnal variability of essential oil and its components in *Origanum onites* L. grown in the ecological conditions of Cukurova / G. Yaldiz, N. Sekeroglu, M. Ozguven, M. Kirpik // Grasas y Aceites, 2005. - Vol. 56, Fasc. 4. – P. 254-258.

Rabotyagov V.D., Shibko A.N. Investigations of essential oil component composition of *Hyssopus officinalis* L. // Works of the State Nikit. Botan. Gard. – 2014. – V. 139 – P. 88 – 100.

The article covers data about content and essential oil component composition of three forms of *Hyssopus officinalis* cultivar growing under conditions of the pied-mont area of the Crimea. This data were presented for the first time. 41 terpenic compounds were identified. The main ones are pinocamphone (up to 60%) and izopinocamphone (up to 61%). *H. officinalis specimen* with content of linalool 34.88%, izopinocamphone 33.38% and pinocamphone 2.94% was marked out for further selection..

Key words: *Hyssopus officinalis, pinocamphone, izopinocamphone, component, changeability, plant top.*