Milchakova N.A., Ryabogina V.G. Floristic diversity of macrophytes in Kazachya bay (the Crimea, the Black Sea) // Bull. of the State Nikit. Botan. Gard. – 2015. – № 114. – P. 16-22

The article presents an annotated list of macrophytes in Kazachya bay water area, which is going to be included into the state general zoological wildlife reserve "Buhta Kazacha". Species composition of macroalgae and higher plants corresponds to archives (1967 – 1980), published information and results of hydrobotanical surveys (1997-2007), according to actual taxonomic inspection and nomenclatural changes. It was pointed a cozoological importance of macrophyte flora in Kazacha bay. This work contains a checklist of rare macroalgae and discussion relative to increasing the reserve territory due to adjacent water area.

Key words: *macrophytes, species composition, long-term dynamics, rare species, wildlife preserve, Kazachya bay, the Black Sea.*

PLANT BIOCHEMISTRY

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BIOLOGICALLY ACTIVE SUBSTANCES OF CUPRESSUS TORULOSA D. DON

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Introduction

Essential oil is a complicated mixture of terpenoids and other components, isolated out of different parts of a plant; essential oils are used extensively in food, aromatic and pharmaceutical industries. As synthetic chemical substances are quite dangerous for health, usage of natural oils is getting popular that causes further flora investigation [10].

Bhutan Cypress (*Cupressus torulosa* D. Don, sin. *Cupressus tonkinensis* Silba, Himalayan Cypress) is an evergreen tree by height 40 m [8]. Preferable soil is limestone. It is a frost-resistant plant. For the Crimea this species was introduced by seeds from Hamburg in Nikitsky Botanical Gardens (NBG) in 1842. In the end of the XX century it was introduced on the Black sea Coast of the Caucasus. It belongs to category of park trees that`s why it`s mostly spread in the regions with sufficient amount of precipitation: south of France, Portuguese, Spain, north of Italy [3].

The objective of our researches was to study biologically active substances being contained in *Cupressus torulosa* D. Don for further usage in medicine. With this purpose a dynamic of total content of essential oils, phenol compounds and ascorbic acid in needles and cones during annual vegetation cycle was investigated, as well as component composition of essential oil having a maximum content of substances mentioned-above.

Objects and methods of the research

The researche was carried out at the laboratory of Biochemistry, biotechnology and virology of plants in NBG-NSC in 2013. Needles and cones of *Cupressus torulosa* D. Don were chosen as an analyzing material, collected within Arboretum of NBG-NSC.

Determination of an essential oil mass concentration was conducted by its distillation with water vapor out of a row material and further measurement of volume. Content of oil was expressed in volume weight percentage in terms of absolutely dry material [2]. The essential oil composition was determined by chromatograph Agilent Technology 6890 with mass spectrometric sensor 5973. Water heater HP-1 by length 30 m, inside diameter is 0,25 mm. Thermostat temperature was coded in range of 50°C - 250 °C with velocity 4°C /min. Injector

temperature was 250°C. Gas-carrier was helium, its stream velocity was 1 sm³/min Transfer from gas chromatograph to mass spectrometric sensor was conducted at a temperature of 230°C. Source temperature was being kept at 200°C. Electronic ionization was carried out at 70 eV in mass range m/z of 29-450.

Identification was based on comparison analysis of mass-spectra and librarian data NIST05-WILEY (about 500000).

Content of ascorbic acid was determined by iodometric titration [2].

Total phenol compounds was estimated by colorimetric method applying reagent Folin—Ciocalteu (cuvette depth was 10 mm, light filter (560 nm)). Concentration was calculated using graph according to rutin [4].

Results and discussion

It was found out that in spring content of essential oil in needles and cones of *Cupressus torulosa* D. Don is minimal: in June it reaches 0,37% (of dry mass) with further decreasing in July (0,22%). Secondly minimal oil content occurs in autumn: November (0,4%). As to cones, there is only one peak of essential oil content during vegetation, in January (0,1%), while minimum is typical for summer period (0,02%) (Fig.1).

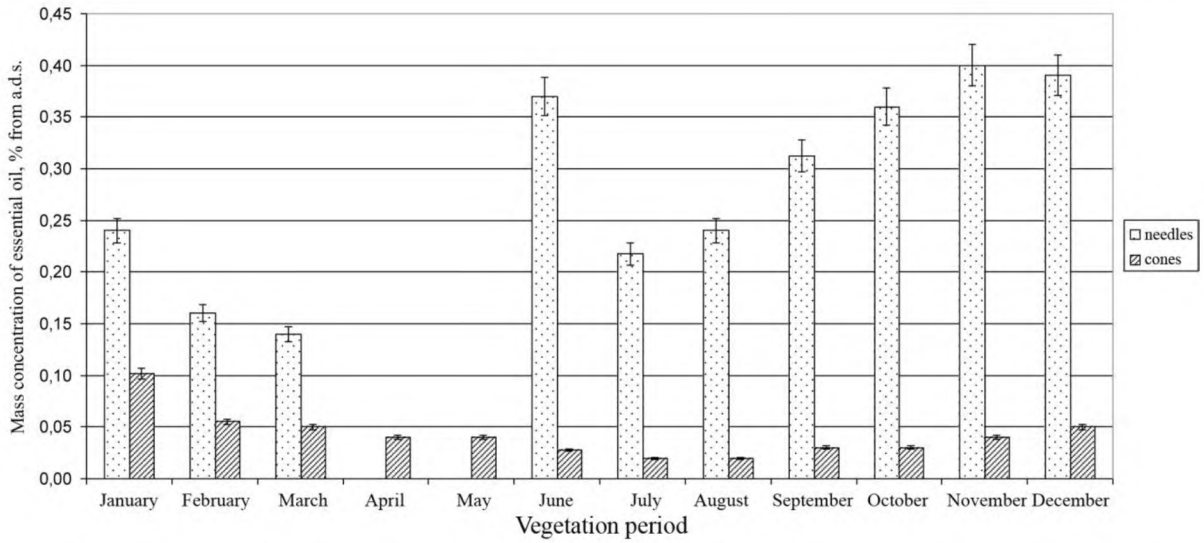

Fig.1 Mass concentration of essential oil in *Cupressus torulosa* D. Don needles needles and cones

Maximum mass concentration of essential oil was obtained out of needles in India – 1,3% [9]. In Vietnam output of essential oil out of air-dry leaves reached 0,13% [14].

During the research composition of individual components in essential oils of *Cupressus torulosa* D. Don needles and cones was studied (fig.2 and 3, table 1). The principal components of essential oil out of needles was sabinene (23,39%), terpinene-4-ol (14,96%), α-pinene (7,99%), γ-terpinene (5,90%).

As to researches in India, the principal components of essential oil out of *Cupressus torulosa* D. Don needles were α-pinene (30,30-34,25%), Δ^3carene (6,52-18,67%), limonene (8,54-23,79%) and sabinene (4,60-19,23%)[9]; in Vietnam: sabinene (29,34%), α-pinene (25,4%), 4-terpineol (13,91%) and γ-terpinene (5,5%) [14]. Essential oil, obtained in Argentina has the most similar quality composition with our results: α-pinene (25,8%), sabinene (22,3%), terpinene-4-ol (9,3%) [11]. Therefore, data obtained in our laboratories coincide with other studies, conducted in different countries, but occurrence of distinctions concerns geographical, population and seasonal variation.

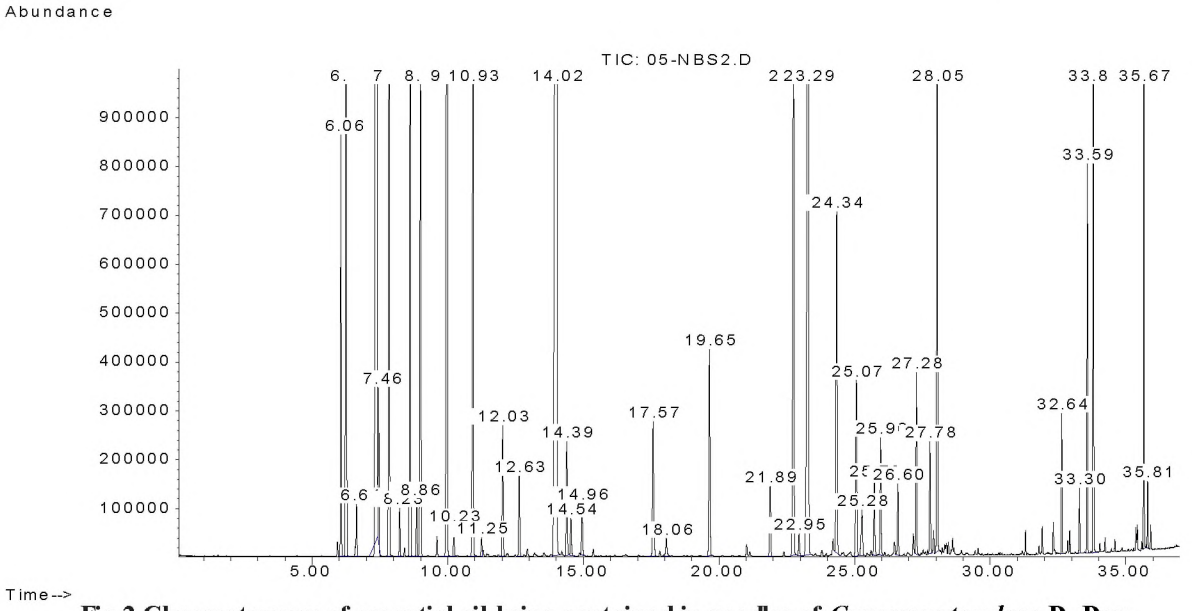

Fig.2 Chromatogram of essential oil being contained in needles of *Cupressus torulosa* D. Don

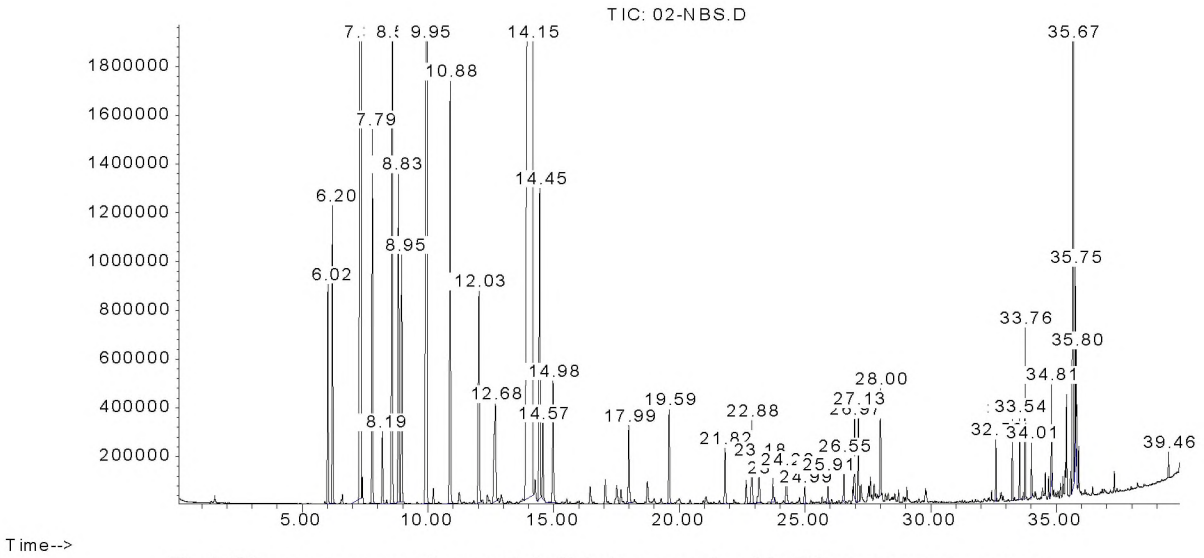

Fig.3 Chromatogram of essential oil being contained in Bhutan cypress cones

Composition of essential oil extracted out of cones mostly contains terpenine-4-ol (41,95%), sabinene (11,08%) and γ-terpenine (8,12%). In spite of the low mass concentration, cone essential oil is valuable by high content of terpenine-4-ol. Considered it is a component that gives antiseptic action to essential oil of a tea tree [6]. Terpenine-4-ol possesses antivirus, antibacterial, antifungal, insecticidal, antioxidant, antineoplastic and anti-inflammatory action [7]. That`s why essential oil of *Cupressus torulosa* D. Don cones is perspective material in the field of medicine, in a case of minimal tree damage.

Our data are corroborated by scientists from India [12, 13], where the main component of the cone essential oil was terpenine-4-ol as well (till 26%). In Indian tests against Gram-positive microorganisms (*Bacillus subtilis*, *Staphylococcus aureus*, *Bacillus megaterium*, *Bacillus coagulans*), Gram-negative microorganisms (*Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella typhii*), in antifungal investigations (*Candida albicans*, *Aspergillus flavus*, *Trichoderma lignorum*, *Cryptococcus neoformans*) there was noted a higher antifungal activity than antibacterial one. Therefore crème contained some essential oil

of *Cupressus torulosa* D. Don cones was suggested as an antimicrobial agent for skin diseases treatment [13].

Table 1

Component composition of *Cupressus torulosa* D. Don essential oil

№	Holding time	Component	Mass concentration in needles essential oil, %	Mass concentration in cone essential oil, %
1	2	3	4	5
1	6.06	α-thujene	1,71	1,29
2	6.26	α-pinene	7,99	1,75
3	6.64	camphene	0,25	
4	7.39	sabinene	23,39	11,08
5	7.45	β-pinene	0,48	
6	7.84	myrcene	3,90	2,25
7	8.22	α-phellandrene	0,21	0,45
8	8.62	α-terpinene	3,58	4,50
9	8.85	*n*-cymene	0,27	2,14
10	8.99	limonene	2,69	1,79
11	9.97	γ-terpinene	5,90	8,12
12	10.22	*Trans*-sabinenehydrat	0,15	
13	10.92	terpinolene	2,76	3,03
14	11.24	*cis*- sabinenehydrat	0,09	
15	12.02	*trans*-p-ment-2-en-1-ol	0,68	1,88
16	12.63	*cis*-*n*-ment-2-en-1-ol	0,44	1,34
17	14.01	terpinene-4-ol	14,96	41,95
18	14.39	α- terpineol	0,60	2,48
19	14.54	*cis*- piperitol	0,21	0,50
20	14.95	*trans*-piperitol	0,27	0,97
21	17.57	bornyl acetate	0,82	
22	18.06	terpinene-4-ol acetate	0,13	0,61
23	19.65	α- terpinyl acetate	1,14	0,69
24	21.88	caryophyllene	0,41	0,43
25	22.95	humulene	0,13	0,64
26	23.29	epi-bicyclosesquiphellandrene	6,76	0,38
27	23.72	hermakren D		0,18
28	24.25	isoledene		0,29
29	24.34	episonarene	1,99	
30	24.99	δ-cadinene		0,13
31	25.06	*cis*-calamene	1,11	
32	26.59	caryophyllene oxide	0,35	0,29
33	26.97	α-cedrol		0,46
34	27.12	humulene oxide		0,56
35	27.28	Epi-cubenol	0,79	
36	27.77	γ-cadinole	0,58	
37	28.05	α- cadinole	3,16	0,59
38	32.59	Manoil oxide		0,29
39	32.64	Epi-manoil oxide	0,48	
40	33.25	Abieta-8(14),9(11),12-trien		0,37
41	33.29	abietan	0,23	
42	33.58	phyllocladene	1,27	
43	33.81	13(16),14-labdien-8-ol	3,45	0,75
44	34.01	8-β-oxi-sandaracopimarene (nesukol)		0,24
45	35.67	totarol	1,80	0,39
46	35.67	Totarol acetate		5,44

47	35.81	Ferrugenol	0,22	0,36
Total indentified components			**95,31**	**98,58**

Concentration of ascorbic acid in *Cupressus torulosa* D. Don needles made 66-95mg/100g, in cones – 40-48 mg/100g. The highest vitamin C content in needles was marked in winter (January), in summer it was lower (July), but in autumn it got increased. Production of material with ascorbic acid contain out of *Cupressus torulosa* D. Don needles and cones is not efficient, as needles of Scotch pine (Pinus *sylvestris*) contains much more vitamin C (374-506 mg%) [1].

Concentration of phenol substances in *Cupressus torulosa* D. Don needles ranged 3000-3600 mg/100 g of dry material, less in cones 2800-3400 mg/100 g. Its maximum concentration was marked in winter (January) and summer (July), minimum – spring (May).

In comparison with other coniferous plants (Scotch pine – 1800 mg/100 g of dry substance, Siberian cedar– 2000 mg/100 g [5]) content of phenol substances in needles of *Cupressus torulosa* D. Don exceeds 1,5-2 times. That`s why needles of *Cupressus torulosa* D. Don can be considered a real source of phenol compounds. But for a lack of details about their qualitative composition there is a necessity to carry out additional investigations in this direction.

Conclusions

During research a composition of essential oil extracted out of needles and cones of *Cupressus torulosa* D. Don has been studied. The main components of needles essential oil were sabinene (23%), terpinene-4-ol (15%), α-pinene (8%). Cone essential oil mostly contained terpinene-4-ol (42%), sabinene (11%) and γ-terpinene (8%). In spite of a low mass concentration (max 0,1%) cone essential oil is more promising in the field of medicine.

Extracts out of *Cupressus torulosa* D. Don needles and cones are perspective sources of phenol compounds, but there is a necessity to investigate their qualitative composition more thoroughly.

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Marchuk N.Yu., Paliy A.E. The biological active substances of *Cupressus torulosa* D. Don // Bull. of the State Nikit. Botan. Gard. – 2015. – № 114. – P. 22-27

The article presents study results of biologically active substances being contained in needles and cones of Cupressus torulosa D. Don under conditions of South Cimea. Prevailed substances in an essential oil of needles were sabinene (33%), terpinene-4-ol (15%), α- pinene (8%). Essential oil composition of cones for the most part contained terpinene-4-ol (42%), sabinene (11%) and γ-terpenine (8%).

Key words: ***Cupressus torulosa D. Don****, essential oil, terpenine-4-ol, sabinene.*