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Phytochemical research of *Crataegus submollis* Sarg. leaves lipophilic complex and study of its antibacterial activity

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ABSTRACT

By means of chromatography-mass-spectrometry in lipophilic complex of Crataegus submollis Sarg leaves had been established 16 terpenoids (23.37%), 5 aromatic compounds (2.94%), 10 fatty acids (35.67%), 8 higher alkanes and their derivatives (37.66%). For lipophilic complex was detected antibacterial activity, we are used the test-strains of bacterial cultures: Staphylococcus aureus 25923, Escherichia coli 25922, Pseudomonas aeruginosa 27853, Proteus vulgaris 4636, Bacillus subtilis 6633, Candida albicans 885-663. According the results of experiment was proved that Staphylococcus aureus and Bacillus subtilis are highly sensitive to lipophilic complex, Proteus vulgaris - moderate sensitive.

Keywords: *Crataegus submollis Sarg*., lipophilic complex, chromatography-mass-spectrometry, antibacterial activity.

INTRODUCTION

Quebec hawthorn - *Crataegus submollis* Sarg. it is native to north-eastern North America and has been introduced in Europe [1]. Previous studies of Quebec hawthorn leave biological active compounds (BAC) showed presence of derivatives of flavones and hydroxycinnamic acids. Our recent research of chemical composition of representatives

of *Crataegus* L. genus had shown presence in herbal drugs lipophilic compounds: fatty acids, terpenoids, chlorophylls, which have antimicrobial activity [2-4]. So, it is advisable to investigate the lipophilic compounds of *Crataegus submollis* Sarg. leaves. The object of our research was lipophilic complex of *Crataegus submollis* Sarg. leaves. The aim of our research was to study chemical composition and antibacterial activity of *Crataegus submollis* Sarg. leaves lipophilic complex.

MATERIALS AND METHODS

Plant material. Crataegus submollis Sarg. leaves were collected in August 2015 (Botanical garden of V. N. Karazin Kharkov National University). Lipophilic complex was obtained by the method of circulative extraction (extracting agent – chloroform). The ratio plant material: extragent was 1:10, the time of extraction – 3 hours. After the end of extraction process the extragent was evaporated at a temperature of 35-40 ^{o}C in a vacuum circulation apparatus till complete removal of extragent.

Study of lipophilic complex composition

Qualitative composition and quantitative content of BAC was determined by chromatography-mass-spectrometry in chromatograph 5973N/6890N MSD/DS Agilent Technologies with a mass spectrometer detector 5973N. Conditions of analysis: the capillary chromatographic column Innowax with external diameter 0.25 mm and the length of 30 m; carrier gas - helium; the sample injection rate was 1.2 ml/min for 0.2 min.; the temperature of thermostat 50 ^{0}C with programming 4^{o}/min.; the heater temperature was 250°C. For quantitative calculations used the method of internal standard [5].

Research the antibacterial activity

For determination, antibacterial activity of lipothilic complex we used the test-strains of bacterial cultures*:* *Staphylococcus aureus* 25923, *Escherichia coli* 25922, *Pseudomonas aeruginosa* 27853, *Proteus vulgaris* 4636, *Bacillus subtilis* 6633, *Candida albicans* 885-663. For investigation was used 2% solution in absolute alcohol. Antimicrobial activity was measured as a radius in mm to give a zone of inhibition [6]. The quantification of antibiotic activity and determination of minimal inhibitory concentration had been performed by serial dilutions method. Results have been processed statistically by Glantz [7]. As a control used the alcoholic solution «Chlorophyllipt»

RESULTS AND DISCUSSION

Composition of lipophilic complex

The lipophilic complex is a viscous substance, dark-green in color, with an unpleasant, specific smell. It was soluble in chloroform and acetone, insoluble in water and water-alcohol mixtures. As a result of chromatography-mass-spectrometry in lipophilic complex was identified 44 lipophilic compounds of different chemical nature: terpenoids (23.37%) - trans-linalooloxide (0.11%), cis-linaloolxide (0.14%), linalool (0.29%), terpen-4-ol (0.09%), *p*-ment-1-en-8-ol (0.42%), nerol (0.11%), geraniol (0.15%), eugenol (4.46%), β-damascenon (0.08%), geranilacetone (0.09%), α-ionone (0.03%), β-ionone-5,6-epoxide (0.18%), β- ionone (0.06%), α-pharnesene (0.24%), neroledol (0.13%), squalene (16.76%); aromatic compounds (2.94%) – benzaldehyde (0.21%), lilac aldehyde (0.01%), 4-vinil-2-methoxyphenol (2.09%), benzophenone (0.03%), phytol (0.58%); fatty acids (35.67%) – capric (0.59%), lauric

(2.48%), myristic (4.01%), pentadecanoic (1.64%), palmitoleic (3.79%), palmitic (13.42%), linolenic (0.08%), linolic (6.28%), oleic (2.80%), stearic (0.56%); higher alkanes and theirs derivatives (37.66%). Composition of *Crataegus submollis* Sarg. leaves lipophilic complex are showed in Table1.

Table: 1. Composition of *Crataegus submollis* Sarg. leaves lipophilic complex

Comp. No	Retention time, min	Compound	Content, mg/kg
1	2	3	4
1	7.26	Benzaldehyde	12.48
2	8.75	Trans-linalooloxide	6.75
3	9.18	Cis-linaloolxide	8.38
4	9.28	Nonanal	1.19
5	9.49	Linalool	17.21
6	10.55	Lilac aldehyde A	0.49
7	10.70	Lilac aldehyde B	0.17
8	11.22	Lilac aldehyde C	0.43
9	12.38	Terpen-4-ol	5.41
10	12.64	Decanal	6.11
11	12.67	*p*-ment-1-en-8-ol	25.22
12	13.74	Nerol	6.74
13	14.63	Geraniol	9.05
14	16.10	4-vinil-2-methoxyphenol	123.32
16	17.50	Eugenol	262.52
17	18.34	β-damascenon	4.73
18	19.22	Capric acid	35.20
19	19.69	Dodecanale	7.57
20	20.50	Geranilacetone	5.73
21	21.27	α-ionone	1.99
22	21.35	β-ionone-5,6-epoxide	10.66
23	21.40	β- ionone	3.70
24	22.69	α-pharnesene	14.24
25	24.57	Neroledol	8.02
26	24.85	*	7.67
27	25.04	Lauric acid	145.84
28	25.17	Benzophenone	1.89
29	27.85	*	12.01
30	29.27	Myristic acid	235.85
31	30.76	Pentadecanoic acid	96.76
32	31.82	Palmitoleic acid	223.34
33	32.32	Palmitic acid	789.54
34	33.97	Phytol	34.67
35	34.17	Linolenic acid	5.12

36	34.28	Linolic acid	369.61
37	34.34	Oleic acid	164.86
38	34.64	Stearic acid	33.01
39	36.13	Tricosane	461.20
40	37.12	Pentacosane	350.12
41	38.20	Hexacosane	718.58
42	39.99	Heptacosane	287.55
43	40.94	Squalene	985.39
44	41.75	Nonacosane	384.30
Total:			5879.22
Note: * - unidentified compound			

Among volatile compounds in quantitative concentration are dominated squalene (16.76%), eugenol (4.46%) and linalool (0.29%). Chromatographic profile of *Crataegus submollis* Sarg. leaves lipophilic complex is shown on Figure 1.

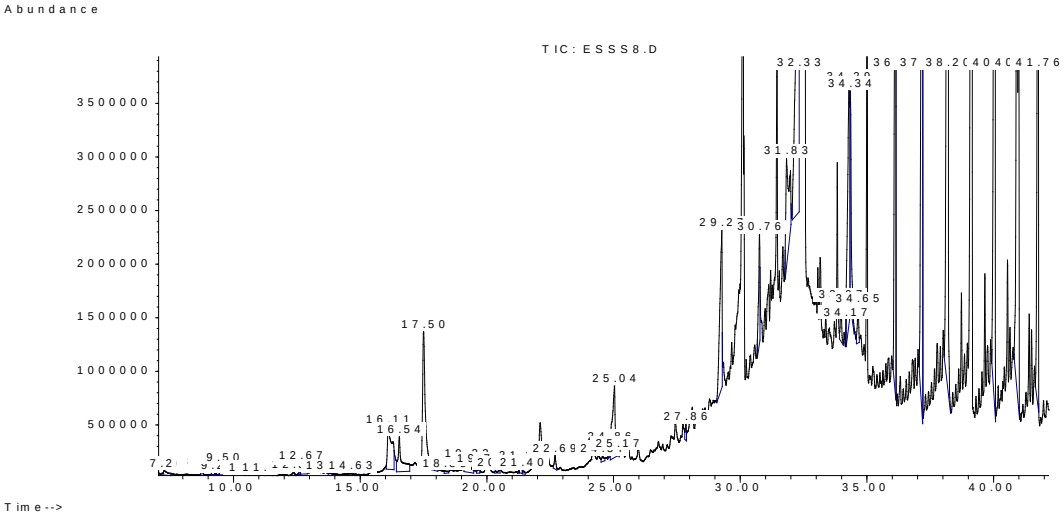

Figure-1: Chromatographic profile of *Crataegus submollis* Sarg. leaves lipophilic complex

Antibacterial activity

The results of research of antimicrobial activity of lipophilic complex have shown, that *Staphylococcus aureus* and *Bacillus subtilis* are highly sensitive to lipophilic complex. Against the *Proteus vulgaris* the lipophilic complex detects a moderate antimicrobial activity. Antibacterial activity of *Crataegus submollis* Sarg. leaves lipophilic complex is shown in Table 2.

When comparing the obtained results with the standard we are established, that the data of minimum bactericidal concentration of *Crataegus submollus* Sarg. leaves lipophilic complex were equal to «Chlorophyllipt» against *Staphylococcus aureus* and were twice as much against *Proteus vulgaris*. According to results of minimum inhibitory concentration, the lipophilic complex was twice as much against *Proteus vulgaris.*

Table: 2. Antibacterial activity of *Crataegus submollis* Sarg. leaves lipophilic complex

Test-strains					
***S. aureus* 25923**	***E. coli* 25922**	***P. aeruginosa* 27853**	***P. vulgaris* 4636**	***B. subtilis* 6633**	***C. albicans* 885-663**
Zone of growth inhibition, mm, (M±m), p≤0,05					
25,5±0,6	x	x	14,0±0,8	29,5±0,8	x
Minimum inhibitory concentration of lipophilic complex, µg/ml					
62,5	>5000	>5000	62,5	250	>5000
Minimum inhibitory concentration of «Chlorophyllipt», µg/ml					
31,25	250	125	125	125	>1000
Minimum bactericidal concentration of lipophilic complex, µg/ml					
125	>5000	>5000	125	500	>5000
Minimum bactericidal concentration of «Chlorophyllipt», µg/ml					
125	500	250	250	250	>1000

Note: x - antimicrobial activity is not detected

CONCLUSION

From *Crataegus submollis* Sarg. leaves were obtained the lipophilic complex and established it chemical composition by chromatography-mass-spectrometry. In lipophilic complex was identified 44 compounds of different chemical structure. The antibacterial activity of *Crataegus submollis* Sarg. leaves lipophilic complex have been study. Was established that *Staphylococcus aureus* and *Bacillus subtilis* are highly sensitive to lipophilic complex, *Proteus vulgaris* – moderate sensitive.

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