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Chromatography-mass spectrometry determination of volatile compounds in intravaginal combined gel on the basis of extract of hop cones

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Abstract

With chromatography-mass spectrometry volatile compounds from raw cones of hops, liquid extracts of hop cones (1:2)-(LEHC), ethanol extraction 70% and intravaginal gel based on it have been investigated. For their identification an Agilent Technologies chromatograph, equipped with a chromatographic column (internal diameter 0.25 mm and a length of 30 mm), 6890 series with mass-spectrometer series 5973 was used. As to the results of the research the composition and total content of volatile compounds in raw cones of hops, LEHC and combined vaginal gels were identified.

Keywords: vaginal gel, ordinary hop cones, liquid extract, hromato-mass spectrometry, terpenoids.

Introduction

The application of vegetable based medicines is a promising direction in the treatment of diseases, in therapy which has ordinary antibacterial schemes were used. One of such pathology groups are the of infectious and inflammatory diseases in gynecology, in which treatment antibacterial and hormone-replacement therapy, depending on the etiology and pathogenesis is applied. Recently, the range of medicines of this group increased significantly [2]. However, this nosological group is not represented with the vegetable medicines. For further research and development of the drug ordinary hop (*Humulus lupulus*) was selected. As the drug raw material female inflorescences, which are official in Ukraine, Russia, Germany, France, Spain, Portugal, Greece, Romania, Mexico, United States are used. Cones contain a large number of biological active substances, the common are bitterness, polyphenol compounds, essential oils, organic and fatty acids [1, 4]. Valuable biologically active substances in hop cones are prenyl flavonoids, in particular, xanthogumol and its derivatives- izoxanthogumol, 6-prenilargenin, 8-prenilnaringenin, the latter of which has a powerful effect in estrogen-like effect [6, 7].

Objective

By using the method of chromat-mass spectrometry to identify the volatile compounds in raw cones of hops, LEHC and in the combined vaginal gel based on it, in order to determine their influence on pharmacological activity of the drug (antibacterial, anti-inflammatory, regenerative effects) for use in gynecology. Also the development of methods of quality control for the combined vaginal gel on the basis of LEHC.

Materials and methods

The object of study are raw hop cones, LEHC and vaginal gel with 6% of received extract. For the detection of volatile compounds in raw hop cones crushed and dried up raw materials (0.5-5.0 g) were placed in Vialen 20 ml, internal standard was added. As the internal standard tridekan at the rate of 50 mg at weight was used. 10 ml of water was added to the sample and volatile compounds of the sample with steam were sublimated for 2 hours with the use of air cooled reverse fridge. When the detection of volatile compounds *in liquid extract* method was similar, instead of weight 1.0-5.0 ml of liquid extract was measured. For the detection of volatile compounds in the content of *intravaginal gel* also gel weight in mass 0,5-5,0 g was used.

In the process of distillation volatile substance were adsorbed on the inner surface of reverse fridge. Adsorbed substance after cooling of the system were washed with slow addition of 3 ml of especially pure pentane in dry Vialen to 10 ml. Washing was concentrated with blowing (100 ml/min) of particularly pure nitrogen to the residual volume of 10 ml, which are completely sampled with chromatographic distribution syringe. Further concentration of the

sample was carried out in the syringe to the volume of 2 ml. Sample introduction in chromatographic column was carried out in the splitless mode, i.e. without separating of the flow, which allowed to introduce the sample without losing for the separation and to increase significantly (10-20 times) the sensitivity of the method with chromatography. Speed of sample introduction is 1.2 ml/min within 0.2 min. An Agilent Technologies chromatograph + 6890 with mass spectrometric detector 5973. Chromatographic column - capillary DB-5 with int. diam. 0.25 mm and a length of 30 mm. The speed of the carrier gas (helium) is 1.2 ml/min. The temperature of the heater of the sample input is 250 °C. Temperature of the thermostat is programmable from 50 to 320 °C with the speed of 4 °C/min. To identify the components a library of mass

spectra NIST05 and WILEY + 2007 with total number of spectra over 470,000 was used in conjunction with programs to identify AMDIS and NIST. For quantitative calculations the method of internal standard was used. Calculation of components content was carried by the formula:

$C = K_1 * K_2, \text{ mg / kg}$, where $K_1 = P_1 / P_2$ (P_1 is the area of the peak of the investigated substance, P_2 is the area of the peak of the standard).
 $K_2 = 50/M$ (50 is the weight of internal standard (mcg) injected into the sample, M is the sample of weight (g)) [3]. The research results are given in Figure 1 and Table 1.

Results and their discussion

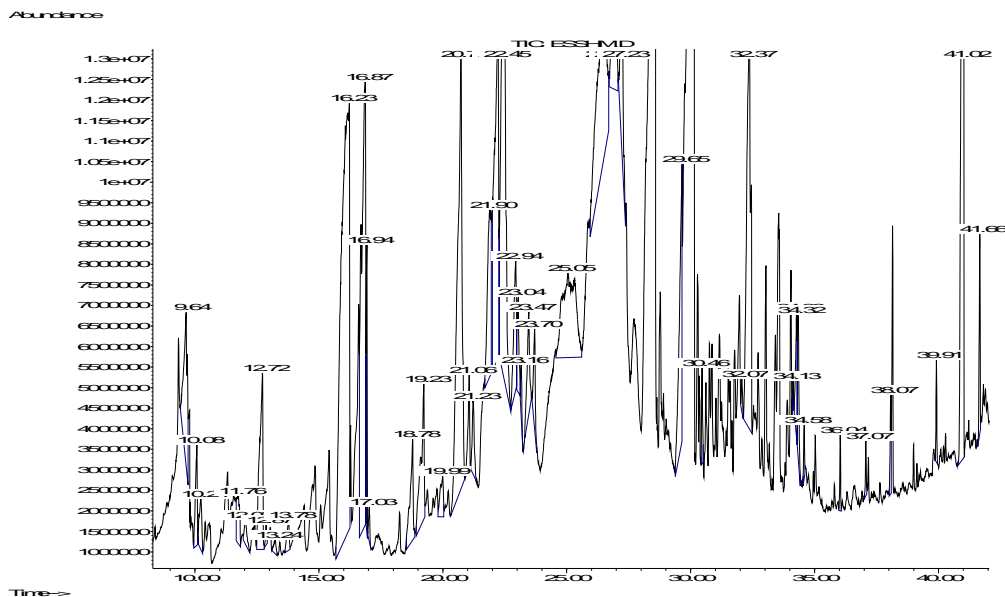


Fig 1: Schedule of chromate-mass spectrometric determination of compounds in samples of ordinary hop cones.

Table 1: Chromate-mass spectrometric determination of compounds in samples of hop cones.

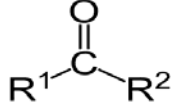
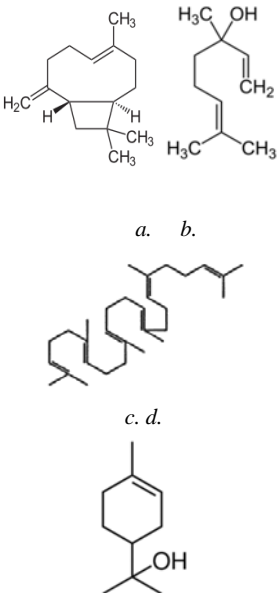
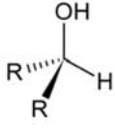
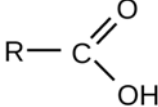
No. of item	The index of the retaining, min.	Compound	Content, mg/kg
1.	9.644	nonan-2-on	28.25
2.	10.076	linalool	9.92
3.	10.238	nonan-2-on	6.96
4.	11.757	capronic acid	7.62
5.	12.027	borneol	3.50
6.	12.72	decane-2-on	31.06
7.	12.875	α -terpenil	1.35
8.	13.777	methyl ether of nonanic acid	3.72
9.	16.229	undecane-2-on	188.95
10.	16.938	metilgeranat	13.37
11.	17.031	capric acid	2.17
12.	18.781	α -copaene	16.64
13.	19.228	dodecane-2-on	24.18
14.	19.991	trans-kariofilen	6.84
15.	20.731	aromadendren	108.05
16.	21.063	gumelen	9.15
17.	21.233	allo-aromadendren	8.24
18.	21.896	valecen	33.52
19.	22.212	β -selinen	74.54
20.	22.451	tridecan-2-on	103.22
21.	22.944	amorfen	19.30
22.	23.044	cis-kalamenen	8.89
23.	23.16	δ -kadinen	3.22
24.	23.469	gvajen	14.51
25.	23.7	izoaromadendreneperoxide	9.03
26.	24.549	kariofilenoxide	61.38

27.	26.553	aromadendrenoxide	86.50
28.	26.915	epiglobulol	22.39
29.	27.231	α -bisaboleneperoxide	12.48
30.	29.652	miristin acid	39.46
31.	30.462	pentadecanoic acid	5.09
32.	32.065	palmitoleic acid	1.98
33.	32.374	palmic acid	85.06
34.	34.132	linolenic acid	0.75
35.	34.278	linolic acid	10.74
36.	34.325	oleic acid	11.06
37.	34.579	stearic acid	2.73
38.	36.036	tricosane	3.48
39.	37.069	pentacosane	2.59
40.	38.072	gexacosane	5.48
41.	39.914	geptacosane	4.45
42.	41.017	squalene	100.86
43.	41.665	nonacosane	10.32
Total content			1203.00

As can be seen from Table 1. and Fig. 1, the composition of volatile biologically active ordinary hop cones is presented with 43 volatile compounds of different chemical nature.

Formula, characteristic of the representatives are listed in the table. 2.

Table 2: Classes of organic volatile compounds and their characteristic representatives

Classes of chemical compounds	Structural fragment or formula of a compound
Ketones	 $R_1 - \overset{\text{O}}{\parallel}{\text{C}} - R_2$ <p>$R_1 = (-C_9H_{19}) - (-C_{13}H_{25})$ $R_2 = -CH_3$</p>
Terpenoids a. Borneol b. Trans-kariofilen c. Terpeniol d. Squalene	 <p>a. b. c. d.</p>
Secondary alcohols	 $R - \overset{\text{OH}}{\underset{\text{R}'}{\text{C}}} - \text{H}$ <p>$R = -C_9H_{19}$ $\text{R}' = -CH_3$</p>
Organic acids of aliphatic chain	<p>$R = (-C_{13}H_{27}), (-C_{14}H_{27}), (-C_{15}H_{31}), (-C_{13}H_{35})$ and others</p>  $R - \overset{\text{O}}{\parallel}{\text{C}} - \text{OH}$

As can be seen from Table 1. 2 the most widespread group among identified compounds are terpenoids and aliphatic acids. In the amount of content they constitute a majority among other representatives. Total content of volatile compounds is 1203.00 mg/kg.

The LEHC the following compounds were found in the amount of: compounds: caprine acid-75.84 mg/kg, α -copaen-1.79 mg/kg, dodecane -3.11-2 mg/kg, allo-aromadendren-1.80 mg/kg, β -selinen-2.67 mg/kg, tridecane-2-on-2.08 mg/kg, lauric acid-9.58 mg/kg, myristic acid-42.22 mg/kg, pentadecane acid-17.36 mg/kg, palmitoleic acid-51.30 mg/kg, palmitic acid-203.18 mg/kg, heptadecane acid-9.05 mg/kg, linolenic acid-3.31 mg/kg, linoleic acid-60.79 mg/kg, oleic acid-44.68 mg/kg, stearic acid-36.99 mg/kg, trikosane -2.15 mg/kg, pentacosane - 4.58 mg/kg, heptacosane-1.23 mg/kg, heptacosane - 2.63 mg/kg, squalene-66.83 mg/kg, nonacosane - 7.74 mg/kg. Total content of volatile compounds, including terpenoids and organic acids is 650.91 mg/kg.

In the developed intravaginal gel with LEHC the following compounds were revealed: α -pinen-8.78 mg/kg, camphene - 2.11 mg/kg, octene-3-ol-1.23 mg/kg, myrcene - 1.98 mg/kg, 1,8-cineole-4.79 mg/kg, trans-ocimene-2.77 mg/kg, cis-ocimene-0.45 mg/kg, phellandren-0.90 mg/kg, linalool-120.94 mg/kg, 3-octenilacetat-47.71 mg/kg, terpinen-4-OL-14.74 mg/kg, α -terpineol-21.56 mg/kg, linalilacetat-150.84 mg/kg, TRANS-cariophyllene-19.02 mg/kg, santalen-4.38 mg/kg, β -farnesen-42.0 mg/kg, δ -cadinene-4.9 mg/kg, cariophilenoxide-6.56 mg/kg. Total content of volatile compounds is 409.28 mg/kg.

Thus, we have studied the volatile compounds in raw cones of hops, LEHC, and combined intravaginal gel based on it.

Findings

1. Investigated with the method of chromat-mass-spectrometry volatile substances (ketones, secondary alcohols, organic acid of aliphatic chain) in raw cones of hops, LEHC and in combined intravaginal gel based on them.
2. Due to results of the studies one can make a prediction on pharmacological activity (antibacterial, anti-inflammatory, regenerative) of developed gel for use in gynecological practice.

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