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Study of Lipid Constituent of the Cones of *Humulus lupulus* Growing in Georgia

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Bela Kikalishvili[1]*, Tsisana Sulakvelidze[1], Malkhaz Getia[1], Mariam Malania[1], Durmishkhan Turabelidze[1]

Iovel Kutateladze Institute of Pharmacochemistry of Tbilisi state medical university, 36 P. Sarajishvili, 0159, Tbilisi, Georgia

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ABSTRACT

This paper describes to study lipid constituent of the cones of *Humulus lupulus* L. From the cones of the plant were obtained sum of neutral (N/L) and polar (P/L) lipids. Was evaluated fatty acids' composition in the oils from the cones of H. lupulus by Gas Chromatography combined with Mass Spectrometry. Oils used for analysis have been obtained by extraction using the n-hexane at room temperature. The samples of *H. lupulus* cones oils were esterified to bring them into a vaporous phase, transforming the fatty acids into the methyl ester derivatives. The results showed that the major components of the oil were: tetradecanoic acid - 3.17%, tetradecenoic acid – 1.02%; hexadecanoic acid – 3.7%; hexadecenoic acid – 0.47%; octadecanoic acid - 1,61%; octadecenoic acid - 0,05%; 9,12-octadecadienoic acid – 17.95 %; 9,12,15-octadecatrienoic acid - 13,23%; eicosanoic acid - 0,58%, all the fatty acids were expressed in methyl esters. It can be concluded that *H. lupulus* cones oil is an excellent source of omega-6 (linoleic acid). Were studied physical-chemical characteristic of the neutral lipids. In the sum of polar lipids were determined existences of following phospholipids: lysophosphatidylinositol, phosphatidylcholine, phosphatidylethanolamine, N-acyllysophosphatidylethanolamine. In the raw material were determined existence of carotenoids and amino acids.

INTRODUCTION

Lipids and other accompanied compounds derived from the plant have antioxidant, anti-inflammatory, antiosteoporosis, anticancer, antiviral, immunotropic, hepatoprotective, choleretic, antiallergic and cytotoxic activities [1-4].

Humulus lupulus L. *(Moraceae)* is widespread plant in Georgia [5]. The plant is widely spread in Europe [6]. The cones of the plant contain fatty acids, vitamins, essential oils, iridoids, phenylpropanoids, flavonoids, lignans and diterpenes. The plant has antiallergic, sedative, analgesic, anti-inflammatory and bacteriocidic activity. Crude extract of the cones of *Humulus lupulus* are used in dermatology and cosmetology [7-10].

MATERIALS AND METHODS

Plant material: The seeds of *Humulus lupulus* were collected after the flowering season in around Akhaltsikhe, Georgia in 2019. They were identified by stuff scientists of Department of Pharmacobotany at TSMU Iovel Kutateladze Institute of Pharmacochemistry. Specimen voucher #2154 is stored in the herbarium of Iovel Kutateladze Institute of Pharmacochemistry (TBPH). The plant materials were powdered and used for analysis.

Extraction of lipids: 100 g. powdered cones were separately extracted with 50 ml n-Hexane at room temperature by shaking 30 min.

Polar lipids were obtained from the residual plants by extracting with the mixture of chloroform-methanol (2:1).

Methylation Procedures: Transesterification reactions were done in 16 × 125 mm glass culture tubes according to a one-step procedure (methanolic HCl for 2 h at 70 °C) as described by Sukhija and Palmquist [11].

GC-MS analysis of fatty acids methyl esters: Gas chromatography-mass spectrometry (GC–MS) analysis of the fatty acids was carried out on a GC system (Agilent technologies 7890B). The instrument was equipped with a split/splitless injector. The auto-sampler was attached to HP-5ms Ultra Inhert capillary column (30m×250µm×25µm film thickness) and fitted to Mass Detector (Agilent technologies 5977A MSD). Helium was used as carrier gas with flow rate of 1 mL/min. Injector temperature at 280^0C, and detector temperature at 280^0C. The column temperature was kept at 60^0C for 2 min followed by linear programming from 60 to 100^0C (at $2,5^0$C/min) and kept isothermal for 2 min; 100 to 280^0C (at 7^0C /min) and kept isothermal for 2 min. The transfer line was heated at 280^0C. Mass spectra were acquired in scan mode (70

eV) in range 50–550 m/z. The components of the oil were separated and the chromatogram obtained was identified by comparing the mass spectra to those from National Institute of Standards and Technology (NIST) libraries.

Separation of polar lipids by TLC: In order to determine the phospholipid composition, polar lipids were separated by TLC as follows: the polar lipid extract was applied to the head of a silica gel LS5/40 chromatoplate (20 cm × 20 cm, 0.5 mm thick, E. Merck, Darmstadt, Germany) along with suitable Phospholipids standards. The chromatogram was developed using solvent systems: 1. chloroform-methanol-25% ammonium hydrate (65:30:5); 2. Chloroform-methanol-acetic acid-water (170:25:25:6). Bands were visualized with iodine vapor and Vaskovsky's reagent [12-13].

Separation of Amino acids by TLC: In order to determine the Amino acids composition, extract was separated by TLC as follows: extract was applied to the head of a silica gel LS5/40 chromatoplate (20 cm × 20 cm, 0.5 mm thick, E. Merck, Darmstadt, Germany) along with suitable amino acids standards. The chromatogram was developed using solvent systems: n-butanol-acetic acid-water (6:1:2). Bands were visualized with 1% Ninhydrin reagent.

Quantitative analysis of Carotinoids: Quantity of carotenoids was determined by using a spectrophotometric method - Wavelength 451 nm [14].

RESULTS AND DISCUSSION

The neutral lipid content from the cones of *Humulus lupulus* is 11%.

The major bioactive compounds from oils of the cones of *Humulus lupulus* are presented in Table 1 and Figure 1.

TABLE NO. 1: PHYTOCHEMICAL COMPONENTS FROM CONES OF *H. LUPULUS* USING GC-MS

Fatty acids	Molecular formula	Molecular weight (g/mol)	*H. lupulus* %
Linoleic acid, methyl ester	$C_{19}H_{34}O_2$	294.5	17.95
Linolenic acid, methyl ester	$C_{19}H_{34}O_2$	292.5	13,3

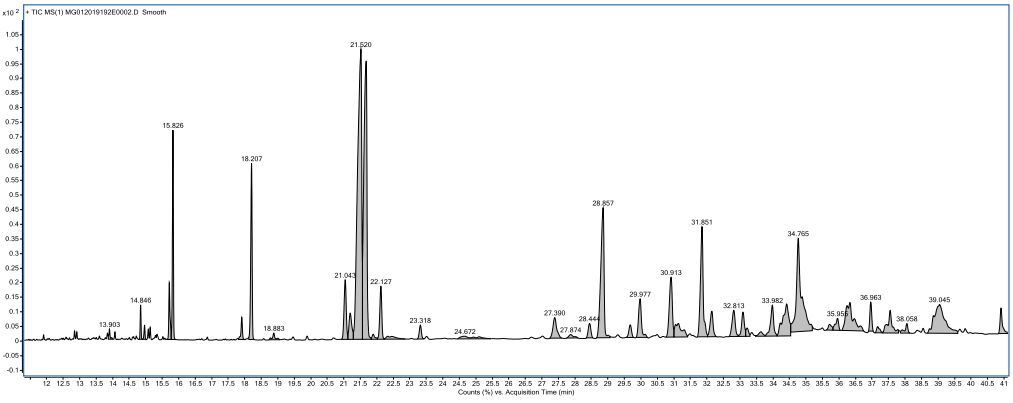

Figure No. 1: Gc-Ms Profile Of The Seeds Of Cones Of *H. Lupulus*

We can observe that the fatty acids that were identified by their time of retention from derivatised oils of the cones of *Humulus lupulus* are in order of their retention time: tetradecanoic acid - 3.17%, tetradecenoic acid – 1.02%; hexadecanoic acid – 3.7%; hexadecenoic acid – 0.47%; octadecanoic acid - 1,61%; octadecenoic acid - 0,05%; 9,12-octadecadienoic acid – 17.95%; 9,12,15-octadecatrienoic acid - 13,23%; eicosanoic acid - 0,58%.

The results showed that the major components of the oil are hexadecanoic acid; 9,12-octadecadienoic acid and 9,12,15-octadecatrienoic acid. All the fatty acids were expressed in methyl esters.

Regarding the physical-chemical parameters of the oils from the cones of *Humulus lupulus*, the determination results are presented in Table 2. The physical and chemical parameters are very important because they are giving information about the composition of the oils.

Polar lipids obtained from the cones of *Humulus lupulus* contains lysophosphatidylinositol, phosphatidylcholine, phosphatidylethanolamine, N-acyllysophosphatidylethanolamine. The amount of total phospholipids - 0,07%.

Amino acids obtained from the cones of *Humulus lupulus* contains Arginine, Asparagine, Glycine, Serine, Alanine, Valine, Phenylalanine.

The content of carotenoids from the cones of *Humulus lupulus* is 31 mg/%.

CONCLUSION

Oils of the seeds of the cones of *Humulus lupulus* contain mixture of saturated and unsaturated fatty acids. The results showed that the major components of the oil are

hexadecanoic acid; 9,12- octadecadienoic acid and 9,12,15-octadecatrienoic acid. All the fatty acids were expressed in methyl esters.

In the sum of polar lipids were determined existences of following phospholipids: lysophosphatidylinositol, phosphatidylcholine, phosphatidylethanolamine, N-acyllysophosphatidylethanolamine. Amino acids obtained from the cones of *Humulus lupulus* contains arginine, asparagine, glycine, serine, alanine, valine, phenylalanine.

The content of carotenoids from the cones of *Humulus lupulus* is 31 mg/%.

The fatty acid profile plays an important role to the chemical properties therefore this is useful knowledge for further researches. The study shows that the cones of *Humulus lupulus* are excellent sources of essential fatty acids (omega-6).

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