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Research Article

Study of The Lipids from The Fruits of Yuglans Regia L. Growing in Georgia

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Abstract

The goal of the study was to explore the lipids gained from Juglans regia L. cultivars grown and collected in the East Georgia. By using high performance liquid chromatography, fatty acids were identified qualitatively and quantitatively.

The study evidenced that linoleic and linolenic acids were predominant and together, they constitute the principal basis of study composition. Palmitic and stearic acids were found in less amounts.

Their relative concentrations are expressed as percentages of total fatty acids components. The study showed different sensitivity of components contained in Juglans regia L.

Keywords: *Lipids; Fatty Acids; Phospholipids.*

Introduction

The natural flora of Georgia is characterized by a great generic diversity allowing conducting complex studies of the vegetation sources. Their qualitative and quantitative analyses, bio-screening and pharmacological assessment help identifying active natural compounds necessary to make practically new medicinal phyto-preparations. The good perspectives of phyto-chemical studies ensue from the high clinical effect, factual absence of side effects and wide spectrum of the pharmacological effects of phytogenic medicinal products.

Based on unique natural wealth of Georgian flora, at present, the I. Kutateladze Institute of Pharmacochemistry is accomplishing wide-spectrum active studies. Perspective phytochemical components with expressed antimicrobial, antiviral, anticancer, analgesic and gastroprotective effects are identified.

Following the deep-rooted many-century tradition, the Georgian population commonly uses walnut oil. Walnut (*Juglans regia L.*) is a tree of the *Juglandaceae* Family, with the height of 4 to 20 m. The walnut tree crown is dense and wide. The walnut fruit has a thick coriaceous-fiber-like shell and hard stone [1]. Walnut trees are commonly grown in the eastern regions of Georgia (the lower reaches of the Alazani River) and western part of Georgia. It is a high-calorie nutrition product as it contains great amounts of fats and a unique complex of physiologically active components.

It is well known that the value and quality of food products much depend on lipids, whose amount and composition vary significantly depending on a number of factors, with the climatic conditions of the plant growth as an important one.

It is a common practice to divide the analytical methods used to study the fats and oils into the following groups:

1) determination of the properties by using the methods approved by relevant standards: acid number, saponification ratio, iodine index, hydroxyl value, content of nonsaponifying substances;

2) determination of individual components, content and structure of glycerides: content of fatty acids and mono-, di- and tri-glycerides;

3) determination of physical constants to identify and characterize the sample: light refraction coefficient, viscosity, density.

4) determination of properties to establish the physical state of a study fat or oil (consistence, solid phase content, polymerization).

Tri-glycerides are a main fraction of natural lipids. When characterizing them by chromatographic analysis, it is a common practice to use so called “carbonic number”, which is a sum of carbon atoms of fatty-acid residues. Generally, over 90% of vegetable oils are tri-glycerides [2-4]. Therefore, for the purpose of their primary characterization, only the content of fatty-acid lipids is determined. It is well known that the ecological peculiarities of growth may change the composition and quantitative content of vegetable oils. The analysis of a lipid composition of oil extracted from walnut seeds grown in eastern regions of Georgia is of a great interest in a medical-pharmacological view and has never been accomplished thoroughly so far.

Plant material: The FRUITS OF YUGLANS REGIA L.grains were collected by hand in autumn of 2018 in Kaspi Region, Georgia. They were identified by stuff scientists of Department of Pharmacobotany at TSMU Iovel Kutateladze Institute of Pharmacochemistry.

Chemicals and reagents: Methanol and Diethyl ether were obtained from Honeywell (USA), Acetic acid was purchased from Ak-Kim-Kimya San. TiC. A.S. (Turkey) Chloroform and n-Hexane were also acquired from CarlRoth (Germany).

Goal of the paper: The object of the study was the walnut fruit cultivar collected in the period of full ripeness in an eastern region of Georgia (Kaspi Region). Neutral lipids were extracted at a room temperature with n-hexane for four times. The extracts were combined and distilled in a vacuum-rotational manner [5-7]. The yield was of up to 70% as compared to air-dry raw material. Isolation was done on plates covered with silica gel LSL5/40 TLC plate (20 cm × 20 cm, 0.5 mm thick, Chemapol, Prague, Czech Republic) along with suitable standards and Rf value.

Systems: 1) petroleum -ether-diethyl ether- glacial acetic acid (85:14:1); 2) petroleum ether-diethyl ether (1:1). Exposure – 30% of sulfuric acid with further heating and impact with iodine vapor. The following substances were identified qualitatively in walnut oil: hydrocarbons, tryacylglycerides, free fatty acids and sterols. The physical-chemical constants of oil were determined: acid number was within the limits of 1,8-2 mg KOH, iodine index was within the limits of 106-110, refraction index: 1,478; specific weight: 0,926. From the residue of chloroform-methanol extraction cake (2:1), polar lipids were extracted; the extracts were combined, thickened (60,0°C), divided in two dimensions and exposed with iodine vapor and Vaskovsky reagent.

The following phospholipids were identified qualitatively and quantitatively in polar lipids: lysophospatidylcholine Rf-0,25 (11%); phosphatidylinositol Rf-0,36 (21%); phosphatidylcholine Rf-0,58 (14%); phosphatidyl ethanolamine Rf-0,69 (45%). Phospholipids were determined quantitatively for the content of inorganic phosphor by using a spectrometric method at 820 nm wavelength.

Fatty acids were identified with the method of high performance liquid chromatography (HPLC) (chromatograph PTC-1, refractometric detector R-401; Waters; USA). Column: metallic (200,0x3,0 mm) filled with inverted phase μ-Bondopac C18. Eluents: 1) methanol – water (2:1) + 0,1% acetic acid solution; 2) tetrahydro Furan, acetonitrile, water (4:7:9) + 0,1% acetic acid solution. Consumption – 2,0 ml/min. The results were processed with OASIS-740,4 software.

Study Results

As the results of the accomplished analysis evidence, walnut oil contains saturated, unsaturated and polyunsaturated fatty acids. The following saturated acids were identified: dodecanoic (0,10-0,15mg%); tetradecanoic (0,12-0,20mg%); hexadecanoic (4,9-6,2mg%); octadecanoic (1,4-2,0mg%); eicosanic (0,21-0,25mg%); docosanoic (0,15-0,20mg%) and tetracosanic acids (0,10-0,18mg%).

Thus, walnut oil contains seven basic saturated fatty acids, which are commonly found in all vegetable oils.

In addition to the saturated fatty acids, unsaturated oils were identified in walnut seeds: one monoene – 9-Octadecene acid with its amount in the investigated oil having reached 68,8-72,0mg % and two polyenoic – 9,12-Octadecadiene and 9,12,15-Octadecatriene acids. Their total amount is quite impressive: 18,6-34,5 mg% and 1,0-1,8 mg%, respectively. In addition to the above-given electronic signals, one more signal was fixed, which was impossible to identify. We suppose (by considering the detention time) that it is 15-tetracosane acid.

Conclusions

Thus, walnut seed oil grown in the eastern regions of Georgia contains unique combination of saturated as well as mono- and polyenoic fatty acids with unsaturated acids found in the greatest amount. The content of hexa decanal acid is highest of the saturated acids and the content of 9-octadecene acid is highest of the unsaturated acids found in the studied oil. Those vegetation oils with a high content of unsaturated fatty acids have the highest biological value and the oil of fruit of walnut grown in Georgia is undoubtedly such oil having an expressed gastroprotective action.

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