IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
November 2020 Vol.:19, Issue:4

Study of Lipids and Some Biological Compounds of *Vitis vinifera* and *Triticum Sp.* Growing in Georgia

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

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

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

Submission: 23 October 2020
Accepted: 29 October 2020
Published: 30 November 2020

HUMAN JOURNALS
www.ijppr.humanjournals.com

Keywords: lipids, fatty acids, phospholipids, CC-MS

ABSTRACT

The aim of the research was to study the lipids from the seeds of the grape (*Vitis vinifera*) and seeds of wheat (*Triticum sp.*). From the plants, some of neutral (N/L) and polar (P/L) lipids were obtained. Saturated, mono- and poly-unsaturated fatty acids were identified in some of the neutral lipids by Gas Chromatography combined with Mass Spectrometry. Oils used for analysis have been obtained by extraction using the n-hexane at room temperature. Major components of *V. vinifera* seed oil was dodecanoic acid - 4,58%; hexadecanoic acid – 50,06%; octadecanoic acid – 4,98%; 9-octadecenoic acid – 18.1%; 9,12-octadecadienoic acid – 0,67%; 9,12,15-octadecatrienoic acid - 4,73%, eicosanoic acid - 2,27%, eicosenoic acid -2,0% and the major components of *Triticum sp.* oil was tetradecanoic acid - 0,1%; hexadecanoic acid - 9,01%; octadecanoic acid -1,0%; octadecenoic acid -15%; 9,12-octadecadienoic acid -25%; 10,12-eicosadienoic acid - 0,11%. It can be concluded that *V. vinifera* seeds and *Triticum speciosum* grains are an excellent source of essential fatty acids; omega-6 and omega-9. In some of the polar lipids, existences of following phospholipids were determined: phosphatidylcholine, phosphatidylethanolamine, N-acyl lysophosphatidyl-ethanolamine, N-acyl phosphatidylethanolamine, lyso-phosphatidylinositol, phosphatidylinositol. The content of carotenoids was determined quantitatively in the sums of neutral lipids making 2, 23 mg% and 14,4 mg%, respectively. In the raw materials of the plants, existence of amino acids was determined.

INTRODUCTION

Lipids of plant origin are one of the most important biologically active natural compounds. They form a part of all living cells and play an active role in the regulation of vital processes of an organism, evidencing their important physiological role and versatile mechanism of their pharmacological action. They have immunotropic, hepatoprotective, bile-expelling, anti-allergic, anti-inflammatory, cytostatic, anti-oxidant and other pharmacological actions [1, 2, 3].

The goal of this research was to study the content of lipids in grape (*Vitis vinifera*) seeds and wheat (*Triticum sp.)* grains [4, 5].

Vitis vinifera – grapes of family *Vitaceae* is widely spread in East and West Europe and grows in almost all regions of Georgia. As per the literary data, a grapefruit contains vitamins, anthocyanins, hydrocarbons and organic acids. Grape seeds contain vegetable oil pectin, and oil produced with them has hypocholesteremic and cardioprotective actions. It is used to cure avitaminosis, chronic bronchitis, rachitis, anemia, liver and kidney diseases and it is a good restorative [6, 7].

Triticum sp. – wheat of family *Gramineae* is an annual crop. Its culture is known from ancient times. 1/3 of world population uses wheat as food, which is a leading culture among the world cereals. There are 20 annual, autumn and spring wheat, varieties. The homeland of wheat is the Mediterranean coastal countries. Wheat grain contains vitamins of groups A, E, PP, B, calcium, manganese, silicium, chlorine, sodium, sulfur, iron, iodine, copper and selenium, as well as proteins, fats and hydrocarbons. Owing to its rich chemical content, wheat is used not only in food but also in medicinal and cosmetic products. During long processing, wheat loses many of its useful properties. In order to gain maximum benefit from wheat grains, they must be used as whole, with skin when they contain great amount of harsh cellulose, which decreases cholesterol in blood what is important to treat cardiovascular diseases, controls glucose level and suppresses appetite; it is a source of energy for a living organism, improves intestine flora and has a positive impact on skin. Wheat extract is used as an auxiliary means to cure atherosclerosis, gastrointestinal diseases and anemia [8-10].

MATERIAL AND METHODS

Plant material

The seeds of seeds *Vitis vinifera* and grains of wheat *Triticum sp.* Seeds and grains were collected by hand in autumn of 2018 in Kakheti 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).

Extraction of lipids

100 g. air-dried powdered seeds of *V. vinifera* and grains of *Triticum sp.* were separately extracted with 500 ml n-hexane (with the ratio of 1:5), the room temperature by shaking 30 min, followed by reducing of extracts on vacuum-evaporator at 60°C. Polar lipids were obtained from the residual plants by extracting with the mixture of chloroform-methanol (2:1).

Separation of neutral lipids by TLC

The neutral lipids were separated by TLC as follows: the polar lipid extract was applied to the head of a silica gel LSL 5/40 TLC plate (20 cm × 20 cm, 0.5 mm thick, Chemapol, Prague, Czech Republic) along with suitable standards and R_f value. The chromatogram was developed using solvent systems: petroleum-ether-diethyl ether-glacial acetic acid (85:14:1). Bands were visualized with iodine vapor and 30% sulfur acid solution. Plates were heated at 105^0C temperature in dry chamber [11].

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* [12].

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 280ºC, and detector temperature at 280ºC. The column temperature was kept at 60ºC for 2 min followed by linear programming from 60 to 100ºC (at 2, 5ºC/min) and kept isothermal for 2 min; 100 to 280ºC (at 7ºC/min) and kept isothermal for 2 min. The transfer line was heated at 280ºC. 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 LSL 5/40 TLC plate (20 cm × 20 cm, 0.5 mm thick, Chemapol, Prague, Czech Republic) 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* [13-14].

Quantitative analysis of phospholipid components

Quantity of total phospholipids in the polar lipids was determined by using a spectrophotometric method according inorganic phosphor (Wavelength 620 nm) [15].

Separation of Amino acid by TLC

In order to determine Amino acids, 80% ethanol extracts of the grape seeds and wheat grains, were separated by TLC as follows: extracts were applied to the head of a silica gel 60 F254TLC plate (20 cm × 20 cm, E. Merck, Darmstadt, Germany) along with suitable standards. The chromatogram was developed using solvent systems: butanol-acetic acid-

water (6:2:2). Bands were visualized with 1% ninhydrin solution [16].

Quantitative analysis of carotenoids

Quantity of total of carotenoids was determined in the sum of neutral lipids by using a spectrophotometric method according inorganic phosphor (Wavelength 451 nm) [15].

RESULTS AND DISCUSSION

The neutral lipid content from the seeds of Grape seeds and Wheat grains are respectively 12% and 16%. The major classes presented in them were identified as: hydrocarbons, fatty acid ethers, triglycerides, free fatty acids and sterols.

It can be observed that the fatty acids were identified by their time of retention from derivatized oils are in order of their retention time: seeds of *V. vinifera* dodecanoic acid - 4,58%; **hexadecanoic acid – 50,06**%; octadecanoic acid– 4,98%; **9-octadecenoic acid – 18.1%**; 9,12-octadecadienoic acid – 0,67%; 9,12,15-octadecatrienoic acid-4,73%, eicosanoic acid - 2,27%, eicosenoic acid -2,0% and the grains of *Triticum sp.* tetradecane - 0,1%; **hexadecanoic acid - 9,01%;** octadecanoic acid -1,0%; 9- **octadecenoic acid -15%**; **9,12-octadecadienoic acid - 25%**; 10,12-eicosadienoic acid - 0,11%. All the fatty acids were expressed in methyl esters **Table 1-2 and *Figure 1.***

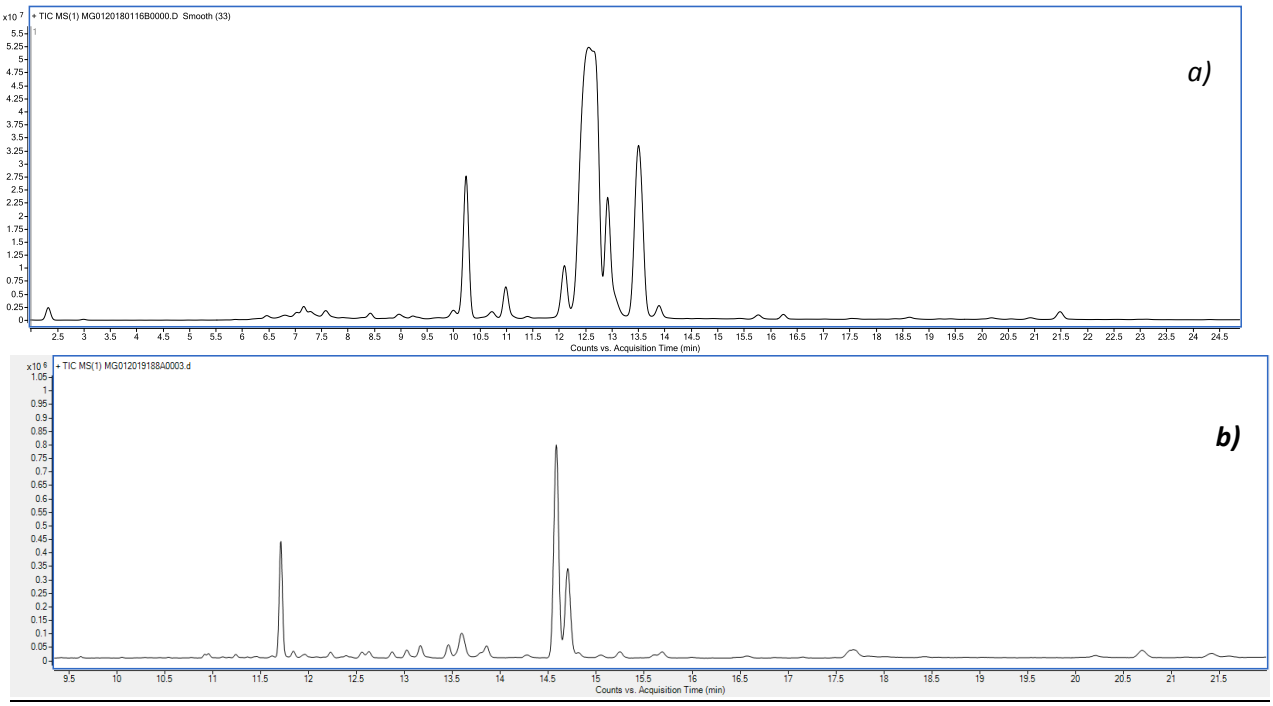

Figure No. 1: GC-MS Profile of the seeds and grains of a) *V. vinifera* and b) *Triticum. Sp.*

Table No. 1: Phytochemical components from seeds of *V. rotundifolia* and roots of *C. intybus* using GC-MS

Fatty acids	**Molecular formula**	**Molecular weight (g/mol)**	***V. vinifera* %**	***Triticum. Sp.* %**
hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5	50.06	9.01
octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294.5	0.7	15
9-octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.5	18.01	25

Some physical-chemical properties of neutral lipids were determined ***Table 2.*** [17]

Table No. 2: Physical-chemical parameters of the oils from the seeds of *V. vinifera* and from the grains of *Triticum spp*.

No.	**Physical-chemical indicators**	**Value**	
		V. vinifera	***Triticum spp.***
1	**Refraction index n^{20}**	1.478	1.465
2	**Density d^{20}**	0.919	0.920
3	**Acid value (KOH)**	3.1	3.2
4	**Iodine value**	135	86

The physical and chemical parameters are very important because they are giving information about the composition of the oils, for example the refractive value is in correlation with molecular weight and degree of unsaturation of fatty acids from the oils, the density and viscosity are very important parameters. Acid value is used to quantify the amount of acid present in the oils and shows the level of freshness for the oil, if the concentration is lower than the quality of the will be higher, iodine value is important because it gives us information about the composition in unsaturated fatty acids of the oils, the value of it is high and that means that the oils has a high content of unsaturated fatty acids and this was also shown in the present study by determination of fatty acid composition of the oils using GC-MS method.

The content of carotenoids was determined quantitatively in the sums of neutral lipids of the seeds of *V. vinifera* and grains of *Triticum spp*. - 2.23 mg% and 14.4 mg %, respectively.

The following amino acids were identified in the alcoholic extracts of the raw materials: *V. vinifera* - lysine, asparagine, alanine, serine, phenylalanine, valine *Triticum spp*. - histidine, asparagine, glycine, serine, alanine, phenylalanine.

The following phospholipids were determined in the sums of polar lipids obtained after secondary extraction of row materials: *V. vinifera* - Lyso-phosphatidylcholine, phosphatidylinositol, phosphatidylcholine, phosphatidylethanolamine, N-acyl-phosphatidylethanolamine and *Triticum spp*. Lyso-phosphatidylinositol, phosphatidylinositol, phosphatidylcholine, lyso-phosphatidylethanolamine, phosphatidylethanolamine, N-acyl-phosphatidylethanolamine.

CONCLUSION

The neutral lipids obtained from the grape seeds and wheat grains contain saturated, mono- and poly-non saturated fatty acids grown in Georgia. Hexadecanioc acid dominant saturated fatty acid and 9-octadecenioc acid non-saturated fatty acid are major components of neutral lipids of grape seeds, while hexadecanioc acid saturated fatty acid and 9,12-octadecadienioc acid non-saturated fatty acid are dominants in the sum of neutral lipids of wheat.

Phospholipids were qualitatively identified in the polar sums of the study objects. 5 phospholipids were identified in the fruit of grapes: Lysophosphatidylcholine, phosphatidylinosite, phosphatidylcholine, phosphatidylethanolamine, N-acyl phosphatidylethanolamine and 6 phospholipids were identified in the sum of polar lipids of wheat grains: Lysophosphatidylinosite, phosphatidylinosite, phosphatidylcholine, lysophosphatidylethanolame, phosphatidylethanolamine, N-acyl phosphatidylethanolamine. Content of total phospholipids is 0,14%.

In respect of a practical use, based on the literary data and study results, the lipids obtained from the study objects can be used for curative and prevention purposes, as well as in perfumery and cosmetology.

REFERENCES

1. Nikonov G.K., Manuilov B.M. Basics of modern pharmacotherapy. M. Medicine 2005.107.
2. Shipov A.N., Makarov V.G., Ryzhenkov V.E. Vegetable oils and oil extracts. M. Russian doctor. 2004.119.
3. A. Sh. Ramazanov and K. Sh. Shakhbanov. The study of grape seed oil obtained by extraction by supercritical carbon dioxide. Khim. Rastit. Syr'ya, No. 1, 75 (2018)
4. Gagnidze R. Vascular plants of Georgia, a nomenclatural checklist. Tbilisi, 2005.
5. Shantser, I. A. Rasteniya sredney polosy Evropeyskoy Rossii. Polevoy atlas. 2009.

6. Karomatov I.D., Abdukhukhidov A.T., Grape and grape seed oil for therapeutic purposes. Biology and Integrative Medicine (2018). N1.
7. Kikalishvili B., Sulakvelidze Ts., Malania M., Turabelidze D. Study of the lipid composition of some plants growing in Georgia. Iovel Kutateladze Institute of Pharmacochemistry of Tbilisi Medical University. International Academy Journal. 3 (33), March, 2019.
8. Mosulishvili M., Bedoshvili D., Maisaia I. A consolidated list of Triticum species and varieties of Georgia to promote repatriation of local diversity from foreign gene banks. Annals of Agrarian Science 15 (2017) 61-70.
9. Odintsova I.T., Slezina M. P., Istomina E. A., Korostyleva T. V., Kovtun A. S., Kasianov A. S., Shcherbakova L. A. Kudryavtsev A. M. Non-Specific Lipid Transfer Proteins in Triticum kiharae Dorof. et Migush.: Identification, Characterization and Expression Profiling in Response to Pathogens and Resistance Inducers. Pathogens 2019, 8, 221
10. Ciccoritti Roberto, Carbone Katya, Bellato Silvia, Pogna Norberto, Sgrulletta Daniela. Content and relative composition of some phytochemicals in diploid, tetraploid and hexaploid Triticum species with potential nutraceutical properties. Journal of Cereal Science Volume 57, Issue 2, March 2013, Pages 200-206
11. Sponngord R.Y. Sun. M. Enhancement of an analytical method for the determination of oils in viecine adsorbed formulations. J. Pharm./biomed. Anal. 2008; vol. 52; p. 554-564.
12. Sukhija, P. S. and D. Palmquist. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. J. Agric. Food Chem. 1988, vol. 36, p. 1202-1206.
13. Kates Morris. Techniques of lipidology. Isolation, analysis and identification of lipids.M. 1975.
14. Sultanova Yu.A., Nechaeva A.P. Chromatographic analysis of fats and oils. Moscow Institute of Food Industry. Sat slave M. 2008. 68-76.
15. Russian Pharmacopeia XIII 1.2.3.0020.15.1.2.4.G. method (quantitative determination of phosphorus with an Eiconogenin).
16. Akhrem A. A., Kuznetsova A. I., Thin-layer chromatography Usp. Khim., 1963, Volume 32, Issue 7, Pages 823–859
17. State Pharmacopoeia of USSR, Vol. 10th edition, 1968.