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Characterization of fatty acids in some plants growing in Georgia

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Abstract
The objective of the study was to evaluate of the fatty acids' composition in the oils from the seeds of *Vitex rotundifolia* and the roots of *Cichorium intybus* by GC-MS. Oils used for analysis have been obtained by extraction using the n-hexane in the room temperature. The oils were esterified to bring them into a vaporous phase, transforming the fatty acid from oil into fatty acids methyl esters. Major components of *V. rotundifolia* seed oil was linoleic acid (36,9%), followed by palmitic acid (15.2%), oleic acid (13.3%) and the major components of C. intybus root oil was palmitic acid (55,9%), linoleic acid (11,8%), followed by, oleic acid (10,2%). It can be concluded that V. rotundifolia seeds and *C. intybus* root oils are an excellent source of essential fatty acids omega-6 and omega-9. In the sum of polar lipids were determined existences of following phospholipids: phosphatidylcholine, phosphatidylethanolamine, N-acyllysophosphatidylethanolamine, N-acylphosphatidylethanolamine, lysophosphatidylinositol, phosphatidylinositol.

Keywords: Lipids, GC-MS, fatty acids, phytochemistry

Introduction
Vitex rotundifolia L., belonging to the *Verbenaceae* family, is a branched, sprawling shrub grown on beaches, rocky shorelines, and sand dunes. The plant is widely spread in Korea, India, China, and Japan [1]. The seeds and fruits of the plant contain fatty acids, vitamins, essential oils, iridoids, phenylpropanoids, flavonoids, lignans and diterpenes [2-4]. Lipids and other accompanied compounds derived from the plant have antioxidant, anti-inflammatory, antiosteoporosis, anticancer, antiviral, immunotropic, hepatoprotective, choleretic, antiallergic and cytotoxic activities [5-8]. *Cichorium intybus* L. (Family: *Asteraceae*) is a perennial plant grown in Georgia [9]. The plant contains vitamins carotinoids, C, K; essential oils, coumarins, iridoids, flavonoids, phytosterols and microelements: Ca, Mg, K. Seeds of the plant and oil gained from them have sedative, analgesic, digestive system regulation and hepatoprotective actions. It is used for prevention of gastritis, diabetes mellitus and cancer diseases [10].

Material and Methods
Plant material
The seeds of *V. rotundifolia* and the roots of *C. intybus* were collected after the flowering season in Adjara and Kartli regions of Georgia in 2018. They were identified by stuff scientists of Department of Pharmacobotany at TSMU Iovel Kutateladze Institute of Pharmacochemistry. Specimen vouchers #18673 and #10232 are stored in the herbarium of Iovel Kutateladze institute of Pharmacochemistry (TBPH). The plant materials were powdered and used for analysis.

Extraction of lipids
10 g. powdered seeds of *V. rotundifolia* and 10 g. powdered, air-dried roots of *C. intybus* were separately extracted with 50 ml n-Hexane at the 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^{0}C, and detector temperature at 280^{0}C. The column temperature was kept at 60 ^{0}C for 2 min followed by linear programming from 60 to 100 ^{0}C (at 2,5 ^{0}C/min) and kept isothermal for 2 min; 100 to 280 ^{0}C (at 7 ^{0}C/min) and kept isothermal for 2 min. The transfer line was heated at 280 ^{0}C. 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].

Quantitative analysis of phospholipid components

Quantity of total phospholipids in the polar lipids was determined by using a spectrophotometric method according inorganic phosphor (Wavelength 620nm) [14, 15].

Results and Discussion

The neutral lipid content from the seeds of *Vitex rotundifolia* and from the air-dried roots of *Cichorium intybus* are respectively 8% and 6%.

The major bioactive compounds from oils of the seeds of *V. rotundifolia* and the roots of *C. intybus* are presented in the Table 1and Figure 1-2.

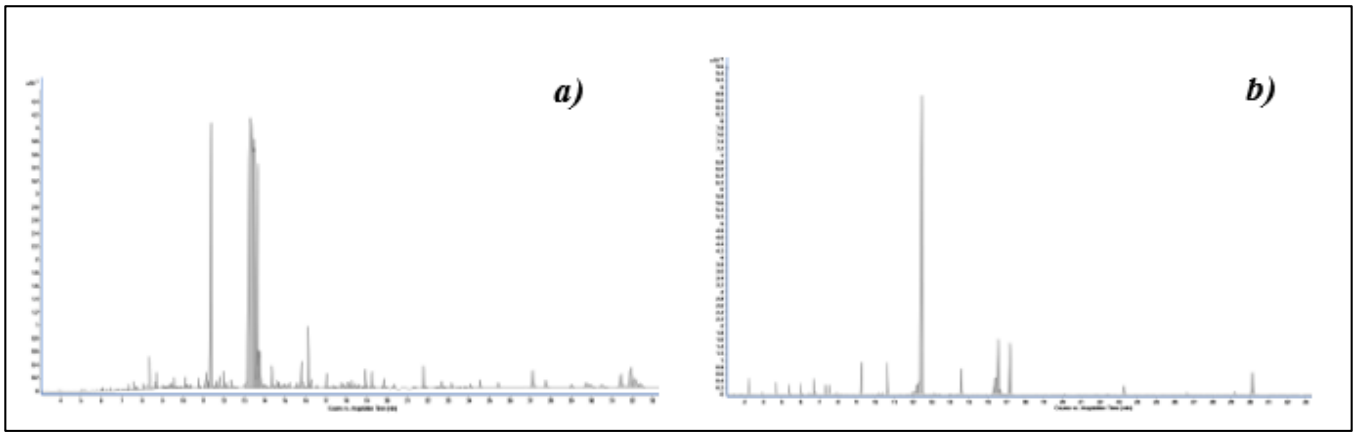

Fig 1: GC-MS Profile of the seeds of a) *V. rotundifolia* and the roots of b) *C. intybus*

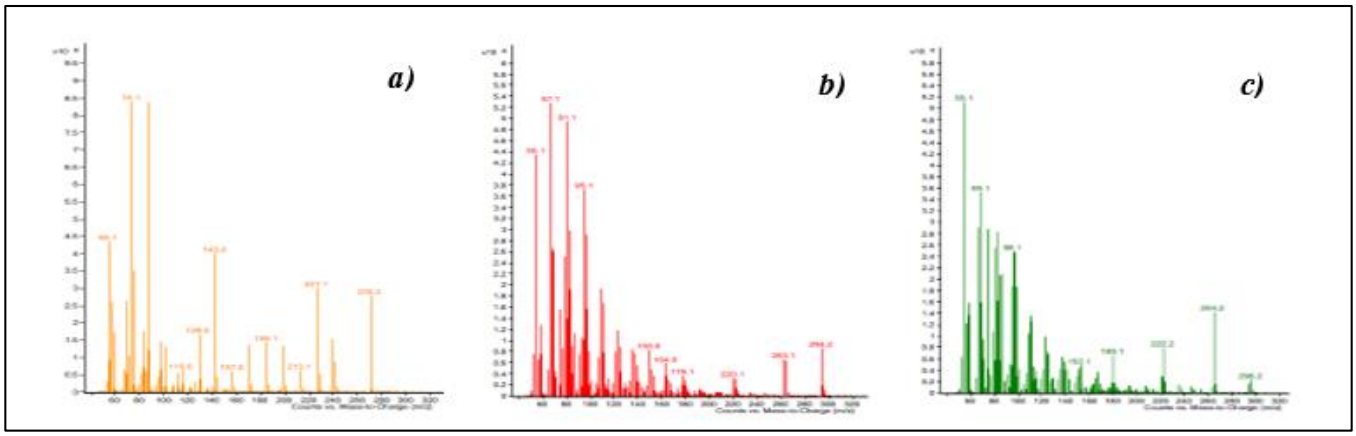

Fig 2: GC-MS spectra of a) *Palmitic acid* methyl ester, b) *Linoleic acid* methyl ester, c) *Oleic acid* methyl ester

We can observe that the fatty acids that were identified by their time of retention from derivatised oils of the seeds of *V. rotundifolia* and the roots of *C. intybus* are in order of their retention time: myristic acid, palmitic acid, oleic acid, linoleic and linolenic acid.

The results showed that the major components of *V. rotundifolia* seed oil was linoleic acid (36,9%), followed by palmitic acid (15.2%), oleic acid (13.0%) and the major components of *C. intybus* root oil was palmitic acid (55,9%), linoleic acid (11,8%), followed by, oleic acid (10,2%), all the fatty acids were expressed in methyl esters.

Regarding the physical-chemical parameters of the oils from the seeds of *V. rotundifolia* and from the air-dried roots of *C. intybus*, the determination results are presented in the Table 2. 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, because the oils can be used as fuel after transesterification, that has the purpose to decrease viscosity of it to not damage the engine. 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.

Table 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. rotundifolia* %	*C. intybus* %
Palmitic acid, methyl ester	$C_{17}H_{34}O_2$	270.5	15,02	55,95
Linoleic acid, methyl ester	$C_{19}H_{34}O_2$	294.5	36,99	11,8
Oleic acid, methyl ester	$C_{19}H_{36}O_2$	296.5	13,3	10,29

Table 2: Physical-chemical parameters of the oils from the seeds of *V. rotundifolia* and from the air-dried roots of *C. intybus*.

No.	Physical-chemical indicators	Value	
		V. rotundifolia	*C. intybus*
1	Refraction index n^{20}	1,487	1,466
2	Density d^{20}	0,912	0,936
3	Acid value (KOH)	-	2,7
4	Iodine value	114	-

Polar lipids obtained from the seeds of *V. rotundifolia* contains: phosphatidylcholine, phosphatidylethanolamine, N-acyllysophosphatidylethanolamine, N-acylphosphatidylethanolamine. The amount of total phospholipids - 0,2%.

Polar lipids obtained from the air-dried roots of *C. intybus* contains: lysophosphatidylinositol, phosphatidylinositol, phosphatidylcholine, phosphatidylethanolamine. The amount of total phospholipids - 0.17%.

Conclusion

Oils of the seeds of *Vitex rotundifolia* and air-dried roots of *Cichorium intybus* contain mixture of saturated and unsaturated fatty acids. The results showed that the major components of *V. rotundifolia* seed oil were linoleic acid (36,9%), followed by palmitic acid (15.2%), oleic acid (13.0%) and the major components of *C. intybus* root oil was palmitic acid (55,9%), linoleic acid (11,8%), followed by, oleic acid (10,2%), all the fatty acids were expressed in methyl esters. It can be concluded that the seeds of *V. rotundifolia* and *C. intybus* root oils is an excellent sources of essential fatty acids omega-6 (linoleic acid) and omega-9 (oleic acid). In the sum of polar lipids were determined existences of following phospholipids: phosphatidylcholine, phosphatidylethanolamine, N-acyllysophosphatidylethanolamine, N-acylphosphatidylethanolamine, lysophosphatidylinositol, phosphatidylinositol. Physical-chemical properties of triglyceride and its applications depends upon fatty acid constituents in molecule and are very important in the determination of the composition of the oils. 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 seeds of *V. rotundifolia* and roots of *C. intybus* are excellent sources of essential fatty acids.

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