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RESULTS OF A COMPARATIVE STUDY OF *NIGELLA SATIVA* L. SEEDS OILS COMPOSITION

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This article presents results of the chemical composition study of the seeds oils lipid complex of *Nigella Sativa* L. grown under various geographic conditions. The task of the comprehensive study of the chemical composition of the plant and its individual parts remains relevant due to the wide spectrum of its pharmacological activity.

The aim of this work is a comparative study of the fatty acid composition, a non-saponifiable fraction and the composition of essential oils of *Nigella Sativa* L. seeds grown in different regions of the world.

Materials and methods. The combination of chromatography-mass spectrometry and 1H-NMR spectroscopy methods made it possible to study the qualitative and quantitative composition of *Nigella Sativa* L. lipid complex seeds. All the experiments were carried out in accordance with the requirements of the State Pharmacopoeia, 14th Ed, given in the corresponding general pharmacopeial monographs.

Results. Profiles have been established and the content of fatty acids, sterines, triterpene alcohols, essential oils and thymoquinone found out in the lipid complex, has been estimated. The saponifiable portion of the complex is represented by triglycerides (81.7–95.3%), di- (3.9–15.2%) and monoglycerides (0.7–4.1%). They mainly contain linoleic (55.8–60.6%), oleic (21.8–24.6%), palmitic (10.0–12.8%), stearic (2.4–3.2 %) and cis-11.14-eicosadiene (2.3–2.6%) acids. In the lipid complex, the contents of sterines and triterpene alcohols were 0.4–0.7%; up to 70% of the fraction was represented by β-sitosterol (22.5–29.2%), cycloartenol (20.1–36.6%) and 24 methylenecycloartanol (9.5–19.9%). In the trace amounts (up to 1.0%), cholesterol has been detected in all the samples. In the lipid complexes, the content of thymoquinone ranged from 0.7 to 2.6%.

Conclusion. A comparative study of the seeds lipid complex of *Nigella Sativa* **L.** grown under various geographic conditions, has been carried out. The marker compounds as well as their content standards for determining the authenticity of raw materials (thymoquinone, para-cimen, cis-11.14-eicosadienic acid), have been identified.

Keywords: *Nigella sativa* L., fatty oil, essential oil, chromatography-mass spectrometry, NMR spectroscopy

РЕЗУЛЬТАТЫ СРАВНИТЕЛЬНОГО ИССЛЕДОВАНИЯ СОСТАВА МАСЕЛ СЕМЯН *NIGELLA SATIVA* L.

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RESEARCH ARTICLE

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В данной статье представлены результаты исследования химического состава липидного комплекса из семян черного тмина, выращенного в различных географических условиях. Актуальной остается задача всестороннего изучения химического состава растения и его отдельных частей, обусловленная широким спектром его фармакологической активности.

Цель данной работы – сравнительное исследование жирнокислотного состава, неомыляемой фракции и состава эфирных масел семян чёрного тмина, выращенного в различных регионах мира.

Материалы и методы. Совокупностью методов хромато-масс-спектрометрии и спектроскопии ЯМР ^{1}H изучен качественный и количественный состав липидного комплекса семян чёрного тмина. Все эксперименты проводили в соответствии с требованиями Государственной фармакопеи XIV издания, приведенными в соответствующих общих фармакопейных статьях.

Результаты. Установлены профили и оценено содержание жирных кислот, стеринов, тритерпеновых спиртов, эфирных масел и тимохинона, обнаруженных в липидном комплексе. Омыляемая часть комплекса представлена триглицеридами (81,7–95,3%), присутствуют ди- (3,9–15,2%) и моноглицериды (0,7–4,1%). Они содержат в составе преимущественно линолевую (55,8–60,6%), олеиновую (21,8–24,6%), пальмитиновую (10,0–12,8%), стеариновую (2,4–3,2%) и *цис*-11,14-эйкозадиеновую (2,3–2,6%) кислоты. Содержание стеринов и тритерпеновых спиртов в липидном комплексе составило 0,4–0,7%, до 70% фракции представлено β-ситостерином (22,5–29,2%), циклоартенолом (20,1–36,6%) и 24-метиленциклоартанолом (9,5–19,9%). В следовых количествах (до 1,0%) во всех образцах был обнаружен холестерин. Содержание тимохинона в липидных комплексах варьировалось в пределах 0,7–2,6%.

Заключение. Проведено сравнительное изучение липидного комплекса из семян черного тмина, выращенного в различных географических условиях, выявлены соединения-маркеры, а также нормы их содержания для определения подлинности сырья (тимохинон, пара-цимен, *цис*-11,14-эйкозадиеновая кислота).

Ключевые слова: чёрный тмин, *Nigella sativa* L., жирное масло, эфирное масло, хромато-масс-спектрометрия, спектроскопия ЯМР

INTRODUCTION

Since classical times, *Nigella sativa* L. has been cultivated in the Mediterranean, North Africa, Central Asia, India, and the Middle East. On the territory of Russia, it can grow and ripe in the North Caucasus, in Tatarstan [1–4].

Nigella sativa L. seeds are used as a spice (nigella) and oily raw materials. They can contain up to 70% oil [5].

Nigella sativa L. oil has a wide range of pharmacological activities and therefore is widely used in folk medicine of the East. Today, there is a great number of scientific papers devoted to the study of the pharmacological activity of this plant material [6–11].

Nigella sativa L. fatty seed oil is richer in palmitic acid and relatively rare fatty acids of C20 group than sunflower oil. Compared to palm oil, it is much richer in polyunsaturated fatty acids and also contains a lot of palmitic acid [5, 12, 13].

The unsaponifiable components of *Nigella sativa* L. seed oils are represented by a complex of sterines, monoterpenes, diterpenes and triterpenes. In its turn, *Nigella sativa* L. is a valuable resource of the essential oil, which consists of terpenes and products of their oxidation, condensation and cyclization – phenols, thymohydroquinone and thymoquinone. The total content of the essential oil in *Nigella sativa* L. seeds is from 0.5 to 3% of air dried raw materials [3, 14, 15].

Modern instrumental methods including gas chromatography and high-performance liquid chromatography with various types of detection, and a nuclear magnetic resonance method, were used to study the phytochemical composition of *Nigella sativa* L. seeds [14–21].

The composition of *Nigella sativa* L. essential oil contains a rather high content of thymoquinone. It is due to the fact that thymoquinone is the final oxidation product in this chain of terpenes, therefore, it is most accumulated in the oil.

Thymoquinone can undergo further transformations, for example, in the light it dimerizes, forming dithymoquinone, which indicates its photosensitivity. As a product of thymoquinone dimerization, dithoquinone is less studied and presumably, like thymoquinone, may have an antitumor effect [22–25].

Currently, information on the comparative chemical composition of *Nigella sativa* L. seeds according to the growth region, is not registered in the available literature. In this regard, it seems relevant to carry out this kind of research.

THE AIM of this work was a comparative study of the fatty acid composition, the unsaponifiable fraction and the composition of essential oils of *Nigella sativa* L. grown in different regions of the world.

MATERIALS AND METHODS

The samples of *Nigella sativa* L. seeds were obtained from 7 different eco-economic regions of the globe: Yemen, the Russian Federation (Republic of Tatarstan), India, Tajikistan, Ethiopia, Egypt, Israel in the period within 2017–2018. The authenticity of the raw materials was checked by a microscopic method in accordance with the requirements of the State Pharmacopoeia (14th Ed.), general pharmacopeial monograph 1.5.3.0003.15 “Technique of microscopic and microchemical studies of medicinal plant materials and herbal medicines” and general pharmacopeial monograph 1.2.1.0009.15 “Optical microscopy”. The studied oils had been obtained from *Nigella sativa* L. seeds in Soxhlet’s apparatus by method of circulating extraction. The seeds had been pre-crushed to a particle size passing through a 0.5 mm sieve. The test samples in the amount of 50.0 g were placed into a cartridge and loaded into Soxhlet’s apparatus. The extraction was carried out with n-hexane. After the extraction, the extractant was distilled off on a rotary evaporator IR-1MZ at the temperature of 40°C.

By this method of obtaining oils, both the lipid com-

ОРИГИНАЛЬНАЯ СТАТЬЯ
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plex of *Nigella sativa* L. seeds and the essential component of the oil had been extracted [26]. Then the lipid complex was saponified and converted into a mixture of methyl esters.

Study of fatty acid oils composition by gas chromatography

The operating mode of Agilent6890N Chromatograph (Agilent Technologies, USA) was the following: capillary column VF-23 ms (Agilent Technologies, USA, 30 m length, 0.32 mm internal diameter, 0.25 µm phase thickness), the carrier gas was helium, the velocity of the carrier gas was 1.5 ml/min, the injector temperature was 280°C, the initial temperature of the chromatograph furnace was 50°C, then there was isotherm for 2 min; after that it was heated at the speed of 10°C/min up to 180°C, held up for 5 minutes, then heated up to 240°C at the rate of 5°C/min.

The total analysis time was 32 minutes. The sample was injected in a flow split mode (1:10). The fatty acids were identified by comparing the retention times of the peaks in the chromatograms of the test samples with the retention times of the peaks in the chromatogram of a standard sample – a mixture of 37 fatty acid methyl esters (Supelco® 37 component FAME mix, 10 mg/ml, methylene chloride, Cat. No CRM47885, Sigma-Aldrich, USA). Each sample was analyzed three times.

Sample preparation: fatty acid methyl esters were obtained by transesterification of glycerides. A sample weighed quantity of about 10.0 mg was placed in a 7.0 ml glass vial with a screw cap, then 1.0 ml of methanol and 100.0 µl of acetyl chloride were added.

The vial was closed and placed in a laboratory heater for 60 min at 80°C. After cooling the reaction mixture, 3.0 ml of double-distilled water was added to the vial, followed by 1.0 ml of n-hexane, and shaken. 1 µl of the upper layer of n-hexane was injected into a gas chromatograph.

The composition of unsaponifiable components of *Nigella sativa* L. seeds oils was studied by chromatography-mass spectrometry.

The operating mode of Agilent6890N Chromatograph (Agilent Technologies, USA) was the following: capillary column VF-23 ms (Agilent Technologies, USA, 30 m length, 0.25 mm internal diameter, 0.25 µm phase thickness), the carrier gas was helium, the velocity of the carrier gas was 1.5 ml/min, the injector temperature was 280°C, the initial temperature of the chromatograph furnace was 60°C, then there was isotherm for 3 min; after that it was heated at the speed of 10°C/min up to 290°C and held up for 20 minutes. The total analysis time was 46 minutes. The mass spectra recording mode was the following: magnetic sector mass detector JMSG C Mate II (JEOL, Japan), ionization energy of 70 eV, the source temperature of 270°C, scanning in the range of 40–400 Da at the speed of 2 scans/sec. The volume of the injected sample was 1 µl.

For the identification, standard samples of individual compounds and the NIST 14 mass spectral database were used; in case of the absence of mass spectra of the detected components in it, the structure was established on the basis of characteristic fragmentation processes and the data on the chromatographic properties of the studied compounds.

To calculate the retention indices, an analysis of the mixture of normal hydrocarbons (C6–C35) was performed under the selected chromatographic conditions. When determining the relative percentage of the components of essential oils in terms of their total content, the ionization coefficients were equalized. When determining the quantitative content of sterols and triterpene alcohols in terms of the internal standard, their ionization coefficients were equalized.

Sample preparation: using an automatic pipette dispenser, 10 µl of the essential oil was taken and placed in a 2.0 ml glass vial for chromatography, 1 ml of chloroform was added, and the vial was vigorously shaken. Then 1 µl of the solution was injected into GC-MS.

To isolate the unsaponifiable fraction, 100.0 mg of the sample was placed in a 5 ml glass vial, then 1 ml of potassium hydroxide solution, 2M, and 20 µl of an internal standard solution (cholestanol, 10.0 mg/ml) were added. Then the samples were kept for an hour at the temperature of 80°C and after cooling the reaction mass, 3 ml of bidistilled water was added. The unsaponifiable fraction was extracted in three portions of 1 ml of diethyl ester, the extracts were combined, passed through a cartridge with sodium sulfate, blown dry under the nitrogen current, and silicated before the analysis. To do this, 300 µl of BSTFA: acetonitrile (1:2) mixture was added to the dry residue and kept for 30 minutes at 80°C, then 1 µl of the solution was injected into the GC-MS device.

Study of oils composition by NMR spectroscopy

Quantitative NMR spectra of *Nigella sativa* L. seeds lipid complexes were recorded and processed using the Delta program (JEOL, Japan), which provides an instrument control, data collection and analysis.

The 1H-NMR spectra were recorded under quantitative conditions (32K points per spectrum, 16 accumulations, 90° pulse, 40 with a delay between pulses). For the quantitative determination, the integral signal intensity of chloroform was taken as 1. The content of thymoquinone in the sample was determined by the following formula:

$m(T) = n(CHCl_3) * I(T) * M(T)$,

where:

m (T) is the mass of thymoquinone in the sample,

n (CHCl3) is the content of the residual proton-containing deuterochloroform isotopomer in moles,

I (T) is the integrated signal intensity of thymoquinone at 6.51 or 6.57 ppm,

M (T) is the molecular weight of thymoquinone, equal to 166 Da.

RESULTS AND DISCUSSION

Physico-chemical properties of the obtained *Nigella sativa* L. seeds lipid complexes are presented in Table. 1.

The content of *Nigella sativa* L. seeds lipid complex-

es in terms of absolute dry raw materials amounted to about 30.4-37.8%. The highest yield was observed for the seeds from Russia (Tatarstan). The smallest yield was observed for the seeds from Ethiopia.

Figure 1 shows typical chromatograms of fatty acid methyl esters obtained from *Nigella sativa* L. seeds lipid complexes and methyl esters of Supelco® 37 component FAME mix by transesterification of triglycerides.

As Figure 1 shows, 5 main components have been revealed in the samples, which constitute a total of 98.7–98.9% of the fatty acids content; the remaining components have been detected in the trace amounts. The content of the predominant unsaturated acids (linoleic C18: 2 and oleic C18: 1) in the samples amounts to a total from 80.4% to 83.9%. The content of ichtrans isomers (elaidic – C18: 1n9t and linoleidic – C18: 2n6t acids) is very insignificant and does not exceed 0.05%. In its fatty acid composition, Nigella sativa L. seeds oil is close to sunflower oil, but contains much more palmitic acid. According to this indicator, it exceeds palm oil twice (Table 2). Cis-11.14-eicosadiene acid has also been detected in lipid complexes; in the samples, its content varied within 2.3–2.6%. This component can be considered one of the markers of the authenticity of *Nigella sativa* L. seeds.

Table 2 shows the results of the study of fatty acids compositions of seven *Nigella sativa* L. *seeds* samples from different regions of the world.

The results presented in Table 2, indicate the presence of comparable amounts of fatty acids in all the studied samples. A noticeable difference is observed only in the case of palmitic acid – up to 3.0%.

The results of the study of the unsaponifiable fraction of the *Nigella sativa* L. seeds lipid complexes by GC-MS, are presented in Table 3.

The above data shows that the content of sterols and triterpene alcohols depends on the country of origin of the seeds, however, a characteristic profile is preserved in all oil samples. The total content of sterols and triterpene alcohols was 400.3–719.7 μg/100.0 mg (0.4–0.7%). It has been found out that β-sitosterol is the main component of the fraction, its content was about 22.5–29.2%, campesterol and stigmasterol were detected in the amounts of 4.8–6.0% and 7.7–9.6%, respectively. Cycloartenol (20.1–36.0%) and 24-methylenecycloartanol (9.5–19.9%) are the dominant triterpene alcohols found in the lipid complexes of *Nigella sativa* L. seeds. In the trace amounts (up to 1.0%), cholesterol has also been detected in all the samples.

The low molecular weight fraction of unsaponifiable substances is represented by a set of mono-terpenes and their oxidation products: pinenes, limonene, r-cimol, carvacol, thymoquinone, trace amounts of free fatty acids – palmitic, stearic, linoleic, oleic and cis-11.14-eicosadiene. Free fatty acids indicate the residual activity of the lipase enzyme present in *Nigella sativa* L. seeds; this enzyme causes hydrolysis of acylglycerides.

A typical chromatogram of *Nigella sativa* L. essential oil is shown in Figure 3, and the composition of the essential oils of the seeds grown in various regions of the world, is shown in Table 4.

The composition of the essential oils includes more than 40 compounds, however up to 99.0% are represented by 16 compounds. The most common component of the essential oils of all the samples is para-cymol. Its content is 38.2–52.0%. A significant part (16.2–23.9%) is represented by monoterpenes - hydrocarbons formed by the combination of two isoprene fragments with the general molecular formula $C_{10}H_{16}$ C10H16 (136 Da). Thymol, which is a hydroxy derivative of para-cimol, has been detected in 2.5–4.9%. The proportion of thymoquinone in essential oils ranged from 10.1 to 22.9%.

A typical 1H NMR spectrum of *Nigella sativa* L. oil is shown in Figure 4. Unlike peaks in GC or HPLC chromatograms, where the signals correspond, as a rule, to individual components, the signals in the NMR spectra are associated with the presence of certain functional groups in the compounds. The 1H NMR spectra of vegetable oils contain, as a rule, 9 main signals corresponding to the functional groups of fatty acids that make them up: -CH_3 (0.82–0.94 ppm), -$(CH_2)_n$- (1.20–1.43 ppm), -OCO-CH_2-$\underline{CH_2}$ (1.55–1.69 ppm), -$\underline{CH_2}$-CH=CH- (1.93–2.13 ppm), -OCO-CH_2- (2.25–2.36 ppm), =CH-CH_2-CH= (2.73–2.87 ppm), -CH = CH- (5.29–5.43 ppm), as well as methylene and methine protons of the glycerol fragment -CH_2OCOR (4.10–4.35 ppm) and CHOCOR (5.23–5.29 ppm). If the experiment is performed correctly, a strict linear relationship between the signal areas and the content of the molecule fragments responsible for these signals in the sample under study is observed; it is based on the physical principle of NMR spectroscopy. By reference to the relationships between the signal areas, the content of various oil components can be calculated. The composition of *Nigella sativa* L. seeds lipid complexes determined by the 1H NMR method, is shown in Table. 5.

The results on the fatty acids composition obtained by the 1H NMR method, are quite close to the results obtained by the GC-FID method. There are also slight variations in the fatty acids compositions of oils from different regions of the world. The registration of 1H NMR spectrum of a thymoquinone standard sample, made it possible to identify characteristic signals in the spectrum that do not overlap with the main signals of fatty acids and glycerol functional groups. There are three different signals located at 6.57, 6.51 and 3.01 m. d., respectively, suitable to identify thymoquinone in the oils (Fig. 4). For the purpose of the quantitative assessment of the thymoquinone content, two signals located about 6.5 m. d., were selected. NMR spectroscopy is a direct method of the quantitative analysis that does not require any use of standard samples of the compounds being determined to assess their content in the objects of different Genesis. The results of 1H NMR show that the content of thymoquinone in the samples varies within the range of 0.7–2.6%.

ОРИГИНАЛЬНАЯ СТАТЬЯ
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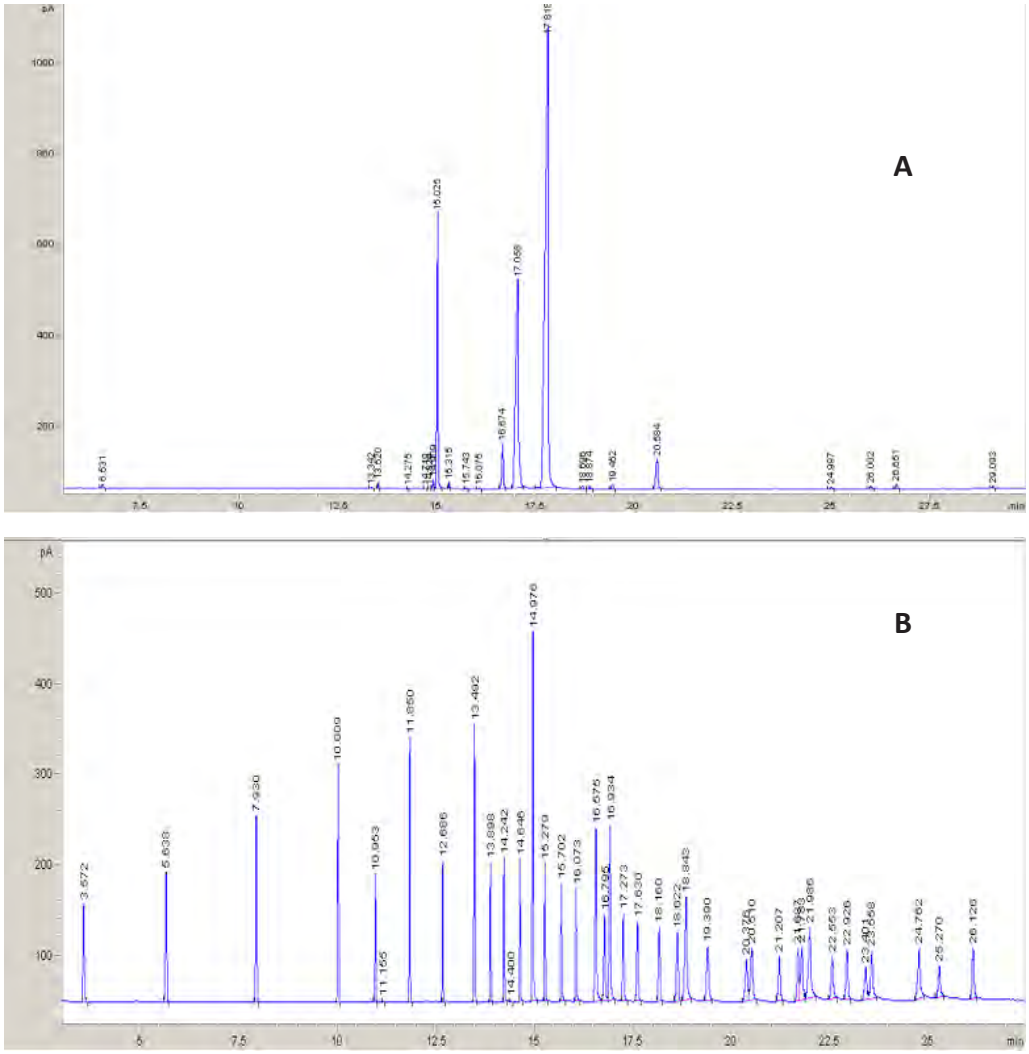

Figure 1 – Chromatogram of FA methyl esters from the lipid complex obtained from *Nigella sativa* L. seeds (A); chromatogram of methyl esters obtained from Supelco® 37 component FAME mix (B)

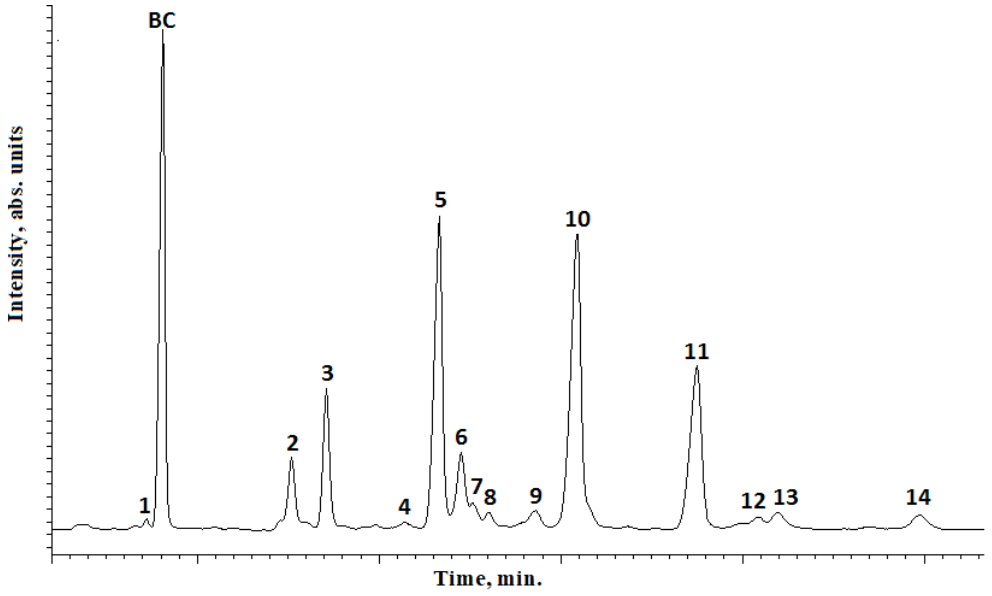

Figure 2 – A typical chromatogram for the total ion current of the *Nigella sativa* L. unsaponifiable fraction (the output region of sterols and triterpene alcohols)

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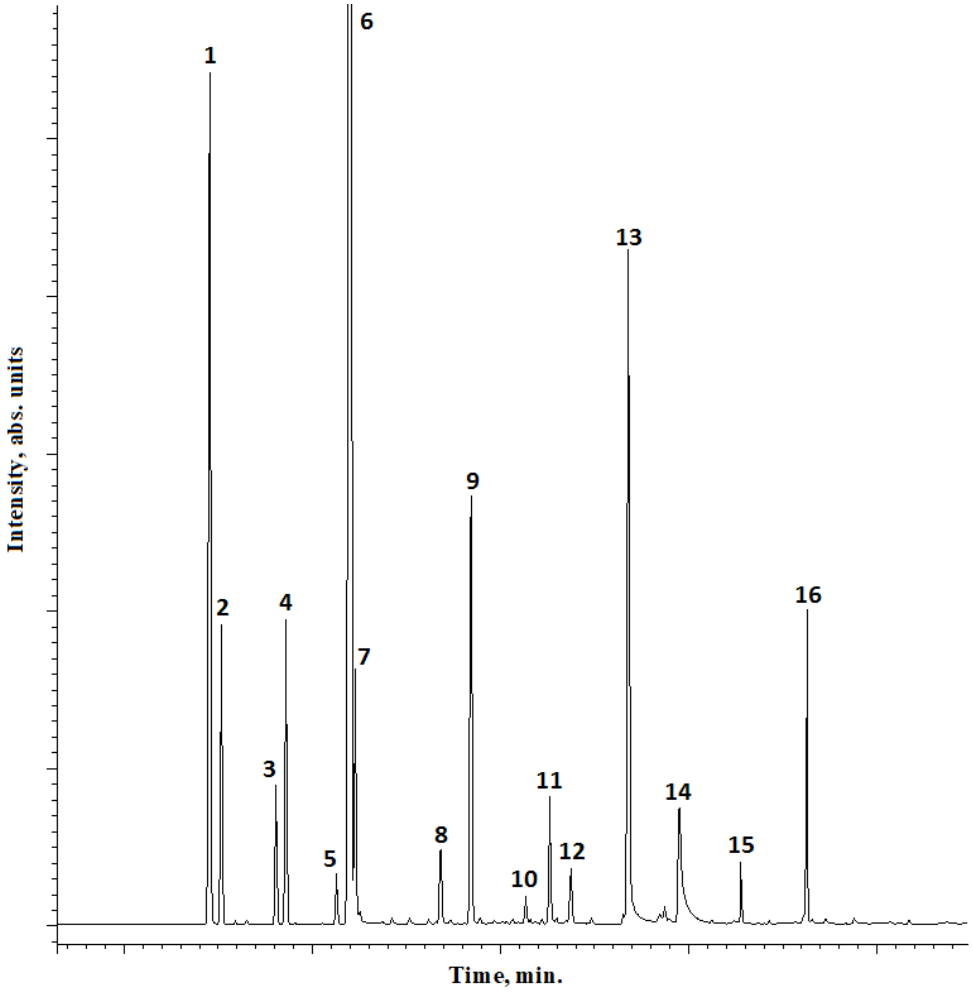

Figure 3 – Typical chromatogram of the total ion current of *Nigella sativa* L. essential oil

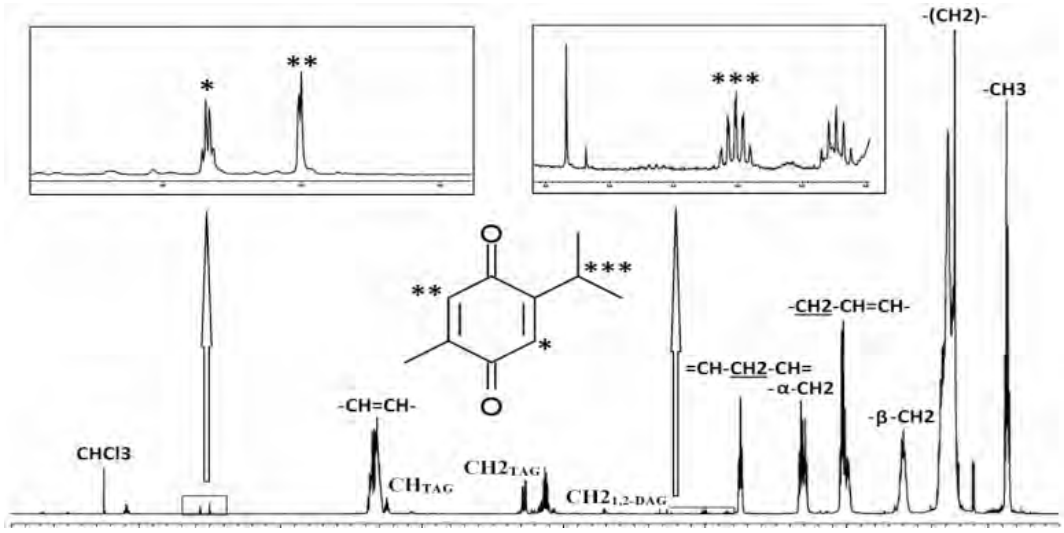

Figure 4 – Typical quantitative 1H NMR spectrum of *Nigella sativa* L. oil

ОРИГИНАЛЬНАЯ СТАТЬЯ
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Table 1 – Physico-chemical properties of the obtained *Nigella sativa* L. seeds lipid complexes

No	Country of origin	Oil content, %	Oil characteristics			
			Viscosity, cPs	Refraction index	Density, мg/cm^3	Appearance
1	Yemen	35.8	39.3	1.4688	906.592	Yellow orange viscous opalescent liquid with an aromatic odor and a bitter spicy acrid taste
2	Tatarstan	37.8	40.8	1.4654	908.924	
3	India	34.8	41.1	1.4675	903.945	
4	Tajikistan	37.2	39.2	1.4644	892.137	
5	Ethiopia	30.4	37.0	1.4638	897.590	
6	Egypt	35.5	38.2	1.4677	908.304	
7	Israel	32.4	37.5	1.4687	907.865	

Table 2 – Fatty acids composition of *Nigella sativa* L. seeds lipid complexes according to the results of GC-FID

No	Acid name	Formula	Sanple, growth region, relative content of FA in the lipid complex, %						
			Yemen	Tatarstan	India	Tajikistan	Ethiopia	Egypt	Israel
1	Palmitic	C16:0	12.1	10.0	12.2	12.8	2.3	12.4	12.3
2	Stearin	C18:0	2.6	2.4	2.7	3.2	3.1	2.8	2.9
3	Oleic	C18:1	3.3	23,3	24.4	24.6	4.7	21.8	22.2
4	Linoleic	C18:2	8.3	60.6	57.1	55.8	6.3	59.4	59.0
5	Cis-11,14-eicosadiene	C20:2	2.6	2.5	2.3	2.3	2.3	2.4	2.5
6	Other fatty acids		1.1	1.2	1.3	1.3	1.3	1.2	1.1
7	Total		100.0	100.0	100.0	100.0	100.0	100.0	100.0

* The table shows average values of three parallel detections

Table 3 – Sterols and triterpene alcohols found in *Nigella sativa* L. seeds lipid complexes according to the results of GC-MS

No on chromatogram (Fig. 2)	Compound	Sample, growth region, content, mcg / 100 mg						
		Yemen	Russia (Tatarstan)	India	Tajikistan	Ethiopia	Egypt	Israel
BC	Cholestanol	200.0	200.0	200.0	200.0	200.0	200.0	200.0
1	Cholesterol	2.6	1.4	2.7	3.7	9.0	4.3	2.5
2	Campesterol	34.7	30.9	25.4	26.6	31.3	25.7	21.0
3	Stigmasterol	57.6	49.4	37.1	37.0	45.2	39.1	38.3
4	Cleosterol	3.6	5.8	4.1	2.4	1.3	3.3	2.6
5	Sitosterol + Lanosterol (traces)	165.6	143.7	108.4	117.3	119.9	129.2	116.9
6	Δ5-Avenasterol + sitostanol (traces)	44.1	41.9	28.6	37.5	35.1	39.5	20.3
7	β-amyrin	13.6	12.6	9.3	0.0	5.6	6.9	7.4
8	Otusifoliol	7.6	8.0	6.6	4.4	5.5	3.9	5.9
9	Gramisterin	13.3	16.4	11.2	4.5	5.1	6.6	7.6
10	Cycloartenol + Δ7-Avenasterin	210.7	190.8	120.9	164.6	188.8	89.9	95.1
11	24-methylenecycloartanol	126.1	106.3	69.5	54.6	49.9	89.1	73.0
12	Erythrodiol	10.6	8.8	6.4	2.5	2.0	3.7	3.9
13	Cytrostadienol	14.8	12.1	7.2	5.3	4.0	3.4	3.9
14	Uvaol	14.8	11.0	3.8	17.7	21.2	2.9	1.9
	Total	719.7	639.1	441.2	478.1	523.9	447.5	400.3

* The table shows the average values of three parallel detections

Table 4 – Compounds found in *Nigella sativa* L. essential oils seeds, according to the results of GC-MS

No. on chromatogram (Fig. 3)	Compound	Sample, growth region, relative content of component in essential oil, %						
		Yemen	Russia (Tatarstan)	India	Tajikistan	Ethiopia	Egypt	Israel
1	alpha-Thujene	8.8	7.9	12.9	12.1	7.6	9.3	8.6
2	alpha Pinene	0.6	1.9	3.3	3.2	1.7	1.8	1.8
3	Sabinene	1.3	0.8	0.9	1.7	0.7	0.9	1.2
4	beta-Pinene	3.2	2.2	3.3	3.4	2.9	2.5	3.7
5	beta-Myrcene	0.3	0.4	0.2	0.6	0.1	0.2	0.5
6	para-Cymene	44.6	38.2	47.9	46.6	49.2	52.0	49.9
7	Limonene	2.0	3.4	1.8	2.9	2.3	1.9	1.6
8	Cis-methoxythujene	0.8	1.0	1.0	1.2	1.3	0.9	1.3
9	Trans-methoxythujene	4.4	5.3	1.3	6.4	5.3	5.3	4.7
10	Unidentified compound	0.3	0.2	0.3	0.3	0.1	0.1	0.2
11	Terpenine -4-ol	1.2	0.8	0.8	1.5	1.5	0.9	1.4
12	Camphor	0.9	0.6	1.1	0.8	0.8	0.7	1.1
13	Thymoquinone	19.8	22.9	14.5	10.1	17.9	13.5	12.7
14	Thymolum	4.3	4.9	2.9	3.8	2.5	2.7	3.4
15	alpha-Longipinene	0.7	1.7	2.7	0.7	0.9	0.8	0.5
16	alpha-Longifolene	6.0	6.8	4.3	3.6	4.5	5.6	6.3
	Other compounds	0.8	1.1	0.8	0.9	0.7	0.9	1.1
	Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0

* The table shows the average values of three parallel detections

Table 5 – Composition of *Nigella sativa* L. seeds oil lipid complexes from different regions of the world according to the results of quantitative 1H NMR spectroscopy

Compound	Sample, growth region, content, %						
	Yemen	Russia (Tatarstan)	India	Tadjikistan	Ethiopia	Egypt	Israel
Palmitic +Stearic acids	15.2	16.3	16.0	15.9	14.3	15.6	14.7
Oleic	28.4	26.2	28.0	27.3	30.2	26.3	27.1
Linoleic	56.4	57.5	56.0	56.9	55.5	58.1	58.2
Linolenic	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sum of acids	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Triacyglycerides	87.3	86.4	95.3	94.5	85.1	84.1	81.7
1,2-Diacylglycerides	4.7	5.7	1.7	1.9	5.5	5.6	7.2
1,3-Diacylglycerides	6.4	6.0	2.2	2.9	5.3	6.6	8.0
1-Monoacylglycerides	1.4	1.9	0.7	0.7	2.0	2.1	2.5
2-Monoacylglycerides	0.2	0.0	0.1	0.0	2.1	1.6	0.6
Sum of glycerides	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Thymoquinone	1.5	0.7	1.0	2.6	2.2	2.4	2.5

* The table shows the average values of three parallel detections

ОРИГИНАЛЬНАЯ СТАТЬЯ
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The chemical composition data show that linoleic acid is the dominant fatty acid in the lipid complex, which may indicate the potential prospects of this plant material for creating drugs that affect lipid metabolism.

The results of the comparative analysis of *Nigella sativa* L. lipid complex have been obtained for the first time, the data of similar studies in the available literature have not been identified.

CONCLUSION

Thus, the profiles and the contents of fatty acids, sterols, triterpene alcohols, essential oils and thymoquinone found in *Nigella sativa* L. seeds lipid complexes, have been established and estimated. The component composition of the lipid complexes obtained from raw materials grown in different regions of the world, is very similar, their saponified portion is represented by triglycerides (81.7–95.3%), di- (3.9–15.2%) and monoglycerides (0.7–4.1%).

They mainly contain linoleic (55.8–60.6%), oleic (21.8–24.6%), palmitic (10.0–12.8%), stearic (2.4–3.2%) and cis-11.14-eicosadiene (2.3–2.6%) acids. The content of sterols and triterpene alcohols in the lipid complex was 0.4–0.7%; up to 70% of the fraction was represented by β-sitosterol (22.5–29.2%), cycloartenol (20.1–36.6%) and 24-methylenecycloartanol (9.5–19.9%). The content of thymoquinone in lipid complexes ranged from 0.7 to 2.6%.

The chemical composition of *Nigella sativa* L. seeds makes it possible to recommend this plant material as a source of essential fatty acids, thymoquinone and essential oils with a multivalent pharmacological target. This determines the need for further in-depth research of *Nigella sativa* L. from the standpoint of pharmacology and pharmaceutical development.

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AUTHORS` CONTRIBUTION

All authors equally contributed to the research work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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ОРИГИНАЛЬНАЯ СТАТЬЯ

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