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FATTY ACID PROFILE AND ANTIOXIDANT ACTIVITY OF NIGELLA SATIVA FATTY OIL

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The chemical composition of *Nigella sativa* L. (black cumin) fatty oil was studied by gas chromatography with mass-selective detection in a search for promising antioxidant agents among edible vegetable oils with high contents of unsaturated fatty acids. Commercial products (Egypt, Pakistan, Saudi Arabia) had fatty-acid profiles dominated by essential fatty acids and were confirmed to contain essential oils. Considering these results, the antioxidant activities of the fatty-oil samples were compared using chemiluminescence. Black cumin fatty oil was proven to be capable of suppressing generation of reactive oxygen species and lipid peroxidation in model systems. The antioxidant properties were most pronounced at a concentration of 5 mg/mL for a system simulating generation of reactive oxygen species and 1 mg/mL for a system simulating lipid peroxidation.

Keywords: *Nigella sativa* L., black cumin, fatty oil, fatty acids, antioxidant properties, chemiluminescence.

Environmental pollution, dietary imbalance, and a wide range of pathologies are responsible for increased free-radical formation in the human body [1]. The significant increase in free-radical oxidation (FRO) associated with an elevated content of reactive oxygen species (ROS) leads to oxidative stress [2, 3], which initiates the development of cardiovascular diseases, including atherosclerosis [4], hypertension [5], diabetes [6], oncological diseases, and several others.

Antioxidants capable of inactivating free radicals by forming inactive or less active species play an important role in regulating the occurrence of free-radical reactions in the body and have significant effects on its condition. Many studies both *in vitro* and *in vivo* demonstrated the positive effects of various classes of chemical antioxidants on the course of many diseases including infections and inflammations [7 – 9].

An assessment of the antioxidant properties of various compounds is an extremely urgent problem. However, a standardized method for assessing antioxidant activity does not exist because methodical approaches are highly variable [10].

In our opinion, vegetable oils with high contents of unsaturated fatty oils are promising as natural antioxidants for medical use. A rather broad array of fatty oils based on triglycerides of polyunsaturated fatty acids needs in-depth studies both as individual antioxidants and as components and excipients (solvents) for antioxidants of other chemical classes. The present research investigated fatty oil from the annual herb black cumin (*Nigella sativa* L.) of the family Ranunculaceae. Black-cumin oil is a specialized food product that is produced in many countries and is commonly available as a supplement in pharmacy chains, specialty stores, and the appropriate departments of trade networks. According to the instructions for use, this product is recommended as a source of unsaturated and saturated fatty acids, fat-soluble vitamins, phospholipids, essential oils, and macro and trace elements; for internal and external use as antioxidants; and as a supplement to prescribed treatment for diseases associated with metabolic disorders [11].

A literature analysis showed that black-cumin fatty oil contains various biologically active compounds (unsaturated fatty acids, terpenoids, vitamins, etc.) that have been reported in the global literature to have antioxidant, lipolytic, antibacterial, and expectorant properties [12 – 15].

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Five samples of black-cumin oil from various manufacturers were analyzed by us at the request of the pharmacy chain Pharmland Co. to study the composition, determine the biological properties, and identify the most preferred samples.

The goal of the present research was to establish the fatty-acid profile and study the influence of black-cumin fatty oil on FRO in *in vitro* model systems using a rapid method for determining the antioxidant activity based on recording chemiluminescence (CL).

EXPERIMENTAL CHEMICAL PART

The fatty-acid compositions of black-cumin oils (from Egypt, Pakistan, and Saudi Arabia) were studied using gas chromatography with mass spectrometric detection (GC-MS) after conversion of the fatty acids to the methyl esters according to GOST 31665–2012, i.e., transesterification with methanolic KOH [16]. The fatty-acid composition was determined using a MAESTRO 7820 gas chromatograph (INTERLAB LLC, Russia) with an Agilent 5975 mass-spectrometer (Agilent Technologies, Inc., USA) and an autosampler. The analyses used an HP-5ms quartz capillary column (30 m × 0.25 mm × 0.25 μm; stationary phase 5% diphenyl 95% dimethylsiloxane; Agilent Technologies, Inc., USA). The chromatography conditions were column temperature thermostat programmed for isothermal at 50°C for 1 min, heating to 180°C at 15°C/min, heating to 280°C at 4°C/min, and isothermal at 280°C for 5 min; He carrier gas; carrier-gas flow rate 1 mL/min; vaporizer temperature 280°C; ion-source temperature 150°C; quadrupole temperature 230°C; transfer chamber temperature 280°C; and injected liquid sample volume 1 μl with flow division.

Linear retention indices were determined and results were compared with total mass spectra in libraries (NIST 2.0 mass spectra libraries) and the literature to identify the constituents. Only constituents determined from the library with >90% probability were considered.

EXPERIMENTAL BIOLOGICAL PART

Measurement of antioxidant activity of black-cumin fatty oils in model systems (MSs)

Six commercial samples of black-cumin fatty oil of various geographical origins and manufacturers (Russian Federation, Egypt, Pakistan, Saudi Arabia) that were obtained by cold pressing (within the declared shelf life) were compared. Antioxidant activity was determined by recording chemiluminescence on a KhLM-003 chemiluminescence spectrometer from systems simulating (1) generation of ROS and (2) lipid peroxidation (LPO).

The MS generating ROS used phosphate buffer (20 mL, 20 mM KH_2PO_4, 105 mM KCl) with added luminol solution (10^{-5} M) and sodium citrate (50 mM). All studied samples were dissolved beforehand in DMSO calculated for 0.2 mL of the studied oil in 1 mL of the resulting sample. The pH of the obtained MS solution was adjusted to 7.5 by titration with saturated KOH solution. The reactions accompanied by ROS formation were initiated by adding a solution (1 mL, 50 mM) of an Fe^{2+} salt. Emission was recorded for 5 min with constant stirring. CL of the MS was characterized by spontaneous emission, a fast flash, and a slow flash that then developed. The most informative CL characteristics were the emission light sum, which was determined from the emission intensity, and the amplitude of the maximum emission [18, 19].

The effects of the studied samples on LPO were studied using egg-yolk lipids, which have a composition similar to that of human blood lipids. Lipids were obtained as a liposome by homogenization of egg yolk in phosphate buffer in a 1:5 ratio followed by a 20-fold dilution. Aliquots of 20 mL were taken. The system was treated with Fe^{2+} solution (1 mL, 50 mM), which initiated oxidation of unsaturated

TABLE 1. Fatty-acid Composition of Black Cumin Oil

Sample No.	Identified constituent (fatty acids)	Egyptian Black Seed Oil (Egypt), %	Royal Oil (Egypt), %	Black seeds oil (Pakistan), %	Huile de Nigelle (Saudi Arabia), %	Ethiopia Gold (Egypt), %
1	Palmitic acid (C16:0)	9.26	3.97	6.41	12.36	7.60
2	Linoleic acid (C18:2)	**64.08**	**36.64**	**63.33**	**48.92**	**64.82**
3	Oleic acid (C18:1)	23.32	55.84	26.56	31.53	24.09
4	11-Octadecenoic acid (C18:1)	0.45	–	0.63	1.79	0.47
5	Linolenic acid (C18:3)	–	**0.24**	**0.02**	–	–
6	Stearic acid (C18:0)	1.30	1.24	1.41	2.62	1.42
7	Eicosadienoic acid (C20:2)	**0.87**	**0.19**	**0.61**	**1.48**	**0.73**
8	11-Eicosenoic acid (C20:1)	0.10	0.24	0.12	–	0.10

Note: averages (n = 3) are given.

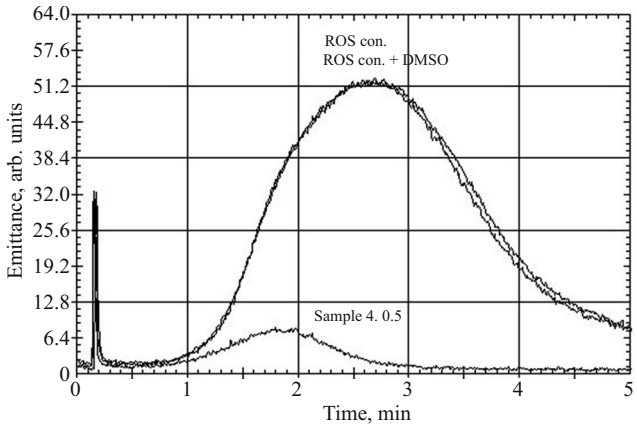

Fig. 1. Influence of black cumin oil (Huile de Nigelle, Saudi Arabia) on FRO in ROS model system.

fatty acids included in the lipids that was accompanied by CL. LPO processes were evaluated from the emission intensity. The spontaneous emission intensity characterized the LPO level before addition of the catalyst. The amplitude of the fast flash reflected the oxidation rate of Fe^{2+} and the formation of ROS and lipid peroxides. The length of the latent period correlated with the antioxidant activity of the studied sample. The light sum determined the ability of the lipids to undergo oxidation.

The controls were MSs without added preparation (normal saline of the same volume was added) and with added DMSO (control + DMSO). The studied preparations, i.e., black-cumin oil samples, were added to the MS as DMSO solutions. The reference preparation was an oil solution of á-tocopherol acetate. A solution (0.5 mL) of a prepared sample was optimally selected and added to the MS in series of experiments for determining ROS. A solution (0.1 mL) of a prepared sample, i.e., black-cumin oil of various origins, was added to the MS for determining LPO.

RESULTS AND DISCUSSION

GC-MS was used to establish the fatty-acid composition of black-cumin oil (from Egypt, Pakistan, and Saudi Arabia). A total of 29 constituents were identified. The fatty-acid profile was characteristically dominated by unsaturated fatty acids (90% and greater) such as linoleic and oleic, an insignificant amount of eicosadienoic, and a minor amount of linolenic acid. Also, saturated fatty acids (<10% in most samples) such as palmitic and stearic were present. A total of 15 fatty acids were detected, the main ones of which are listed in Table 1. Furthermore, examination of the oils for essential-oil constituents soluble in them showed trace amounts relative to other black-cumin oil constituents (fatty acids and their triglycerides). The black-cumin essential oil fraction contained mainly *p*-cymene, the content of which dominated in all oil samples (0.09 – 0.85%). Lower contents of β-thujene (0.03 – 0.07%), longifolene (0.03 – 0.08%), α-pinene, and *trans*-4-methoxythujone were observed. This were detected in small amounts in all fatty-oil samples. Trace amounts of β-pinene, thymoquinone, sabinene, limonene, γ-terpinene, *cis*-4-methoxythujone, terpinen-4-ol, camphor, bornyl acetate, longipinene, and apiol were present [17].

The antioxidant activity of the black-cumin fatty oils in the MS was measured considering the large amount of observed essential fatty acids.

CL was recorded to establish the substantial inhibitory effect of the studied black-cumin oil samples on the FRO kinetics in systems simulating ROS generation and LPO.

Addition to MS generating ROS of black-cumin oil samples decreased the amplitude of the fast flash and lengthened the latent period. The slow flash began later and decayed earlier. The maximum emission decreased. The samples with the best (Fig. 1) and worst parameters (Fig. 2) were determined.

The emission light sum was the indicative characteristic of the CL and was less than the control, the control + DMSO,

TABLE 2. Influence of Black Cumin Fatty Oils on CL in MS Generating ROS

No.	Expt.	Volume, mL	Light sum abs./rel. control	Max. emission
1	Control		123 ± 0.2/102.5%	52 ± 0.2
2	**Control + DMSO**		**120 ± 0.1/100%**	**51 ± 0.1**
3	Egyptian Black Seed Oil (Organic Co. for Natural oil, Egypt)	0.1	110 ± 0.2/91.7%	52 ± 0.2
		0.5	18 ± 0.1/15%	11 ± 0.1
4	Royal Oil (Habet el Baraka, Egypt)	0.5	31 ± 0.2/25.8%	20 ± 0.2
5	Black seeds oil (Hemani, Pakistan)	0.5	26 ± 0.2/21.7%	17 ± 0.2
6	Huile de Nigelle (Modern Beauty, Saudi Arabia)	0.5	13 ± 0.1/10.8%	8 ± 0.1
7	Ethiopia Gold (Arabian secrets, Egypt, Giza Province)	0.5	17 ± 0.1/14.2%	10 ± 0.1
8	Unrefined cumin black oil (Oleos LLC, Russia, Podol'sk)	0.5	69 ± 0.2/57.5%	40 ± 0.2
9	Reference preparation (oil solution of α-tocopherol acetate)	0.5	115 ± 0.3/95.8%	50 ± 0.3

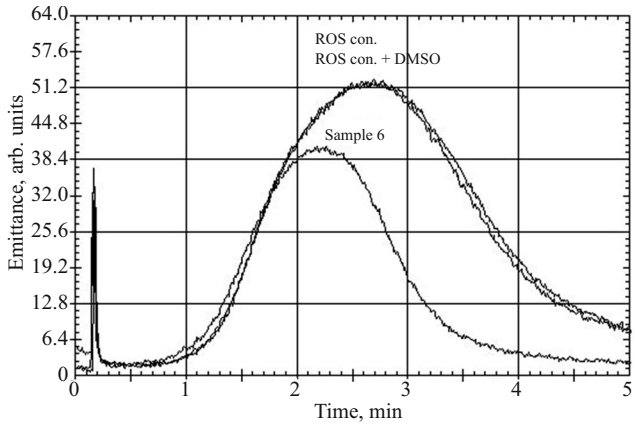

Fig. 2. Influence of black cumin oil (Oleos, Russia) on FRO in ROS model system.

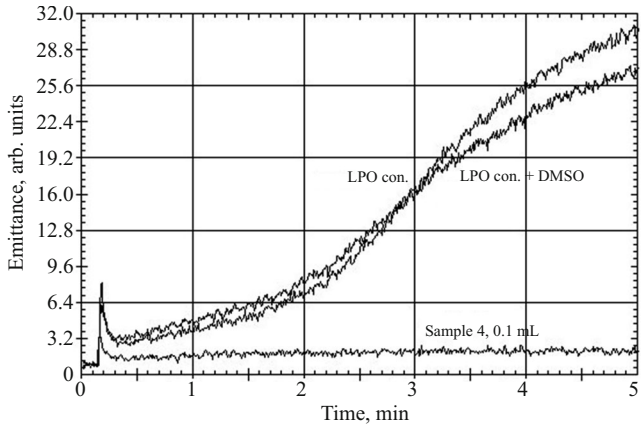

Fig. 3. Influence of black cumin oil (Huile de Nigelle, Saudi Arabia) on FRO in LPO model system.

and the reference preparation (α-tocopherol acetate) for all six studied samples of black-cumin oil (Table 2). Table 2 presents results for the effects of the six samples of black-cumin fatty oil on CL of the ROS model systems. Suppression of CL depended on the fatty oil concentration in the MS. A sample volume of 0.5 mL (5 mg/mL in the MS) was selected for this analysis by using one oil sample as an example. A sample of 0.1 mL (1 mg/mL in the MS) had a comparable value because the positive effect on the decrease of FRO was insufficient. It was found that the greater the oil concentration in the MS was, the stronger the suppression of the emission was. This indicated that the effects of the studied fatty-oil samples were dose-dependent.

The reference preparation (oil solution of α-tocopherol) lengthened insignificantly the latent period and decreased the CL light sum. The studied black-cumin fatty oils significantly lengthened the latent period and decreased the CL light sum by an average of 5.7 times (not considering the sample of Russian origin).

The optimal sample for analysis in the MS of liposomes was 0.1 mL of solution (1 mg/mL in the MS). A comparison was made using 0.5 mL of solution (5 mg/mL in the MS, for following the dose-dependent effect). The MS emission level was observed to be suppressed. The amplitude of the fast and slow flashes decreased more. The duration of the latent period increased. The maximum emission decreased. Also, the sample with the best parameters was sample No. 6 (Fig. 3), like for the MS for ROS; with the worst parameters, sample No. 8 (Fig. 4), which requires more detailed examination of a larger number of samples (from other product batches).

Therefore, black-cumin fatty oil could be considered an antioxidant of LPO. Foreign samples (Saudi Arabia, Egypt, Pakistan) of fatty oil had the greatest antioxidant activity. Table 3 presents the effects of the black-cumin fatty oils on the CL of LPO MSs.

Thus, the results indicated that the ability of black-cumin fatty oil to suppress ROS generation and LPO in the used MSs, which characterized their antioxidant properties, was

TABLE 3. Influence of Black Cumin Fatty Oils on CL in MS Generating ROS

No.	Expt.	Volume, mL	Light sum abs./rel. control	Max. emission
1	Control		69 ± 0.1/104.5%	30 ± 0.1
2	**Control + DMSO**		**66 ± 0.2/100%**	**27 ± 0.2**
3	Egyptian Black Seed Oil (Organic Co. for Natural oil, Egypt)	0.1	19 ± 0.2/28.8%	12 ± 0.2
		0.5	7.8 ± 0.3/11.8%	2.7 ± 0.3
4	Royal Oil (Habet el Baraka, Egypt)	0.1	18 ± 0.2/27.3%	11 ± 0.2
5	Black seeds oil (Hemani, Pakistan)	0.1	20 ± 0.2/30.3%	11 ± 0.2
6	Huile de Nigelle (Modern Beauty, Saudi Arabia)	0.1	9 ± 0.3/13.6%	3 ± 0.3
7	Ethiopia Gold (Arabian secrets, Egypt, Giza Province)	0.1	10 ± 0.7/15.2%	4 ± 0.7
8	Unrefined cumin black oil (Oleos LLC, Russia, Podol'sk)	0.1	29 ± 0.5/43.9%	19 ± 0.5
9	Reference preparation (oil solution of α-tocopherol acetate)	0.1	33 ± 0.2/50.0%	20 ± 0.2

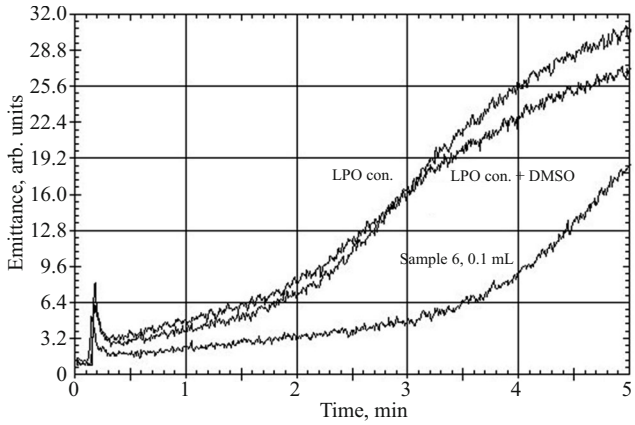

Fig. 4. Influence of black cumin oil (Oleos, Russia) on FRO in LPO model system.

promising for utilization. The properties of fatty oils from domestic and foreign manufacturers differed considerably. This may have been related to the fatty-oil production technology specifics. The results will provide a basis for further studies of the correlation of the fatty-acid profile and the antioxidant activity and will be examined to justify the use of black-cumin fatty oil for medical purposes.

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