CHEMICAL COMPOSITION OF THE TUNISIAN *NIGELLA SATIVA*. NOTE II. PROFILE ON FATTY OIL

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Abstract

Nigella sativa L., *Ranunculaceae*, commonly named black cumin, is a spontaneous plant. *Nigellae sativae semen* has been used for a long time in the Arabian and Romanian ethnopharmacological field for its tonic effect. The scientific basis for using this plant was confirmed by phytochemical studies. These studies represent the scientifical confirmation of the empirical use of this vegetal product. The lipophylic fraction from *Nigellae sativae semen* contains, besides essential oils, fatty oils. The qualitative and quantitative analysis of fatty acids from mentioned oil were performed using GLC (Gas-Liquid Chromatography). The obtained fatty oil was found highly unsaturated, 84.35% from the total fatty acids representing the mono- and polyunsaturated fatty acids. The main fatty acid of the Tunisian *Nigella sativa* seeds was found the linoleic acid (C18:2) representing about 63.71% of the total fatty acids.

Rezumat

Nigella sativa L. (*Ranunculaceae)* denumită popular chimen negru sau negrilică este o plantă medicinală spontană, utilizată în medicina tradiţională arabă şi română datorită efectului tonic. Fundamentarea ştiinţifică a utilizarii plantei a fost dovedită prin studii fitochimice, care constituie confirmarea ştiinţifică a uzanţei sale empirice. Fracţia lipofilă a produsului vegetal *Nigellae sativae semen* conţine, alături de ulei volatil şi grăsimi. Analizele calitativă şi cantitativă ale acizilor graşi din grăsimi au fost realizate prin CGL. Uleiul gras obţinut este constituit majoritar (84,35%) din acizi graşi mono- şi polinesaturaţi. Acidul gras principal din seminţele de *Nigella sativa* din Tunisia este acidul linoleic (C18:2) reprezentând 63,71% din totalul acizilor graşi.

Keywords: *Nigellae sativa*e *semen*, *Ranunculaceae*, seeds, fatty acids, GLC.

Introduction

The lipophilic fraction from *Nigella sativa* seeds is composed of essential (volatile) oil and fatty oil[1]. The essential oil was studied and results were reported in Note I [6].

The aim of the present work was to study the *Nigella sativa* species from the chemical point of view. For this purpose, we focused on the *Nigella sativa*'s fatty oil extraction, respectively on the analysis of these extracts by GLC (Gas Liquid Chromatography).

Materials and Methods

Chemicals

Hexane, used in the experiments, was HPLC grade and was purchased from Merck. The other chemicals used were of analytical grade.

Plant material

Mature seeds of *Nigella sativa* were collected in April and May 2005 from cultivated plants from the Nabeul and Menzel Temime regions (Northeast of Tunisia). They were soaked in water, washed, air-dried and stored in hermetic bags and deep freezer until use.

Preparation of seed extracts

Air-dried and finely ground *Nigella sativa* seeds samples (27.13 g) were extracted in a Soxhlet extractor with 250 mL hexane (Merck) at 50^{o}C. The extraction procedure was continued for six hours and was repeated three times. The solvent from collected extract was removed in vacuum, using a rotary evaporator, yielding 2.4 g of a blackish-brown concentrate (% oil /dry matter = 1.2 x100/27.13=4.42 %).

Fatty acids methylation and GLC analysis

0.1 g of concentrated oil extract was dissolved in 2 mL heptane and 0.2 mL 2M methanolic KOH was added. The mixture was shaken for two minutes and allowed to stand for 10 minutes. The upper layer was removed and washed with water. The oil (as the methyl esters of the fatty acids) was analyzed by Gas-Liquid chromatography (GLC) using a Hewlett-Packard 5890, series II system equipped with a hydrogen flame ionization detector, a capillary column: Quartz HP Innowax column (30 m x 0.25 mm x 0.25 μm), and nitrogen as the carrier gas. The column temperature was programmed from 180 to 240^{o}C at a rate of 12^{o}C/min and kept at this temperature for 3 minutes. The injector and detector temperatures were set at 260^{o}C. The injection volume was 0.1 μL. The identification and quantification of the fatty methyl esters was accomplished by comparing the retention times of

peaks with those of pure standards purchased from Sigma and analyzed under the same conditions [3,4,7,8]. The results were expressed as a percentage of individual fatty acids (% i) in the considered sample, according to equation (1):

$$\%i = \left(\frac{S_i}{\sum S} \right) \cdot 100 \qquad (1)$$

Results and Discussion

The solvent extraction of *Nigella sativa* seeds gave a blackish-brown oil extract with a strong aromatic odour (% oil /dry matter = 1.2/ 27.13 x 100 = 4.42 %). In the obtained extract, twelve saturated and unsaturated fatty acids were identified by gas-liquid chromatography.

The percentage of the fatty acids from the seeds of *Nigella sativa* extract is presented in Table I.

Table I

Fatty acids composition of the *Nigella sativa* seeds oil extract

No	Fatty acid		Retention time [min]	Percentage [%]
1	Myristic acid	C14:0	5.905	0.14
2	Palmitic acid	C16:0	7.647	8.92
3	Palmitoleic acid	C16:1	7.918	0.18
4	Stearic acid	C18:0	10.142	2.44
5	Oleic acid	C18:1	10.451	19.42
6	Linoleic acid	C18:2	11.170	63.71
7	Linolenic acid	C18:3	12.108	0.44
8	Arachidic acid	C20:0	12.727	0.13
9	Eicosenoic acid	C20:1	13.485	0.27
10	Eicosedienoic acid	C20:2	13.863	0.33
11	Behenic acid	C22:0	14.772	2.89
12	Lignoceric acid	C24:0	16.613	1.04

The obtained extract consisted of 15.56 % saturated fatty acids (SFA), 19.87 % monounsaturated fatty acids (MFA), and 64.48 % polyunsaturated fatty acids (PFA) respectively.

As SFA, we identified the myristic acid (C14:0), the palmitic acid (C16:0), the stearic acid (C18:0), arachidonic acid (C20:0), the behenic acid (C22:0) and lignoceric acid (C24:0) (Σ SFA (1+2+4+8+11+12) = 15.56%). The palmitic acid (C16:1), the oleic acid (C18:1) and the eicosanoic acid (C20:1) were identified as MFA (Σ MFA (3+5+9) = 19.87%). The linoleic acid (C18:2), linolenic acid (C18:3) and the eicosadienoic acid (C20:2) were

identified as PFA (Σ PFA (6+7+10) = 64.48 %). It was found that the linoleic acid (C18:2) is the major component (63.71 %) of the total fatty acids (TFA).

The composition in fatty acids of mature seeds of *Nigella sativa* is characterised by the predominant presence (63.71 %) of the linoleic acid (C18:2). The obtained results are similar to those of Atta [2] who showed that the fatty acids composition of the *Nigella sativa* oil is influenced by the extraction system. This author also found out that the oil extracted from mature seeds using the petroleum ether as solvent, is richer in linoleic acid (C18:2) than the one obtained by cold-pressing. The same author found that in the first situation, the percentage of C18:2 to TFA is equal to 49%, and in the second situation, the percentage is about 47.5%. Moreover, in the case of Tunisian seeds of *Nigella sativa*, the literature shows a very similar percentage (61.9 %) [5] as compared to our obtained results (63.71 %).

Conclusions

The studied *Nigella sativa* seeds are a rich vegetal fatty matter source.

The fatty acids are actively biosynthesized and quickly integrated in the triacylglycerol molecules, which are the main constituents of the oil (more than 95 %). This oil was found highly unsaturated (IFA = 84.35 %).

The main fatty acid of the Tunisian *Nigella sativa* seeds was the linoleic acid (C18:2), an essential fatty acid, which can not be biosynthesized by the mammalian's cell. This fatty acid is biosynthesized in the first stage of seeds formation. In mature stage, C18:2 represents about 63.71 % of the TFA of the *Nigella sativa* seeds. This fatty acid has a great importance in human life due to its essential contribution in the biosynthesis of polyunsaturated fatty acids with long chain (C20:5; C22:5; C22:6) and in the synthesis of prostaglandines (PG1, PG2 and PG3).

During their biosynthesis, the fatty acids are stored in the *Nigella sativa* tissues as triglycerides.

References

1. Aitzetmuller K., Werner G., Ivanov S.A., Seed oils of *Nigella* Species and of closely related Genea, Oléagineux, Corps Gras, *Lipides*, 1997, 4 (5), 385-388.
2. Atta M. B., Some Characteristics of *Nigella* (*Nigella sativa* L.) seed cultivated in Egypt and its lipid profile, *Food Chemistry*, 2003, 83, 63-68
3. Codreanu M.V., Istudor V., Dinu M., Dociu N., Pharmacognostical studies on achenes of *Taraxacum officinale* Web., *Farmacia*, 2006, 54 (4),

4. Manea S., Mazilu E., Ristea C., Setnic S., Cretu I., Neagu M., Ivopol G., Popescu M., Comparative determination of certain antioxidants in Sea buckthorn (*Hippophae rhamnoides*) leaves, fruits , juice, oil and oil extraction residues, *Farmacia*, 2006, 54 (4).
5. Ramadan, M.F., Morsel J.T., Neutral lipid classes of black cumin (*Nigella sativa* L.) seed oils, *European Food Research and Technology*, 2002, 214 (3), 202-206.
6. Toma C.C., Simu G.M., Hanganu D., Olah N., Vata F.M.G., Hammami C., Hammami M., Chemical composition of the Tunisian *Nigella sativa*. Note I. Profile on essential oil, *Farmacia*, 2010, 58 (4), 458-464.
7. Toma CC., Pintea A., Bele C., Dulf F., Characterization of neutral lipids composition of *Nigella sativa* L. seeds oil. *Chemicke listy Journal*, 2008, 102, 803-804.
8. Toma C.C., Cercetare farmacognostică asupra speciilor de *Nigella* L. (*Ranunculaceae*), 2004, Ed. Mirton, Timişoara.

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