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CHROMATOGRAPHIC ANALYSIS OF VOLATILE COMPOUNDS ISOLATED FROM THE *NIGELLA DAMASCENA* L. AND *NIGELLA ARVENSIS L.* SEEDS

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Abstract

The Nigella (*Nigella* L.) genus includes 18 species of annual herbaceous plants which seeds are used in folk medicine and cooking around the world. Only *Nigella sativa* L. has been characterized sufficiently in terms of chemical constituents or pharmacological properties. The aim of the study was to conduct the gas chromatography mass spectrometric (GC-MS) analysis of the compositions of volatiles in the *Nigella damascena* L. and *Nigella arvensis* L. seeds. The raw materials were harvested from the plants cultivated in Ternopil region (Ukraine). The conducted GC-MS analysis gives the possibilities to identify more than 25 volatile compounds in the seeds of both investigated species. Carvone (5.448 mg/kg) and squalene (0.966 mg/kg) dominated among the volatile ingredients of *Nigella arvensis* seeds. Dihydrofarnesol (0.957 mg/kg) and squalene (0.525 mg/kg) prevailed in the *Nigella damascena* raw material. Besides the terpenoids, there were found also the long-chained carboxylic acids and unsaturated hydrocarbons in the investigated seeds. Generally, the studied raw materials could be regarded as prospective sources of new medicines and food additives.

Keywords: *Nigella*, seeds, essential oil, gas chromatography mass spectrometry

Introduction

The Nigella (*Nigella* L.) genus belonging to the Buttercup (*Ranunculaceae* L.) family includes 18 species of annual herbaceous plants [1]. Their seeds are often used in cooking as well as in folk medicine to prevent and treat asthma, diarrhea, hepatotoxicity, and other diseases around the world [2]. Only a few of these species have been characterized sufficiently in terms of chemical constituents or pharmacological properties [3–7].

A different number of reviews and original articles concerning chemical composition and bioactivities of the different *Nigella* representatives were published up to the 1st of October 2021 in the scientific Journals indexed in the PubMed database: genus '*Nigella*' – 1784 results, including '*Nigella sativa*' – 1656, '*Nigella damascena*' – 43, '*Nigella arvensis*' – 14, etc. As can be seen from the represented data, the most studied species is *Nigella sativa* L., and the least attention of scientists was paid to *Nigella arvensis* L.

The *Nigella sativa* seeds which are commonly known as 'black cumin' are the most widely used as a spice or medicine [5–7]. The seeds of *Nigella damascena* L. are sporadically applied in cooking, mostly in the Asian countries [3, 8, 9]. The experimental studies revealed the anti-inflammatory, antioxidant, antihypertensive, immunomodulatory, hepatoprotective, neuro-protective, antimicrobial, anti-cholesterol, and wound-healing properties of essential oils and extracts from the *Nigella sativa* raw material [5–7, 10].

It was found that the researches were focused on the of chemical constituents of the fixed oil of the *Nigella* species. Therefore, more attention should be paid to the profile of the other compounds in their seeds, for instance, to terpenoids. For instance, the bioactive compounds of the *Nigella sativa* seeds such as thymiquinone, α-hederin, and nigellidine are regarding as promising herbal medicines to combat COVID-19 [10, 11].

As it is known, essential oils are the multicomponent mixtures of volatile organic compounds which are synthezided in diffferent organs of plants and cause their odor as well as biological properties [13, 14]. Among the components of essential oils dominate terpenoids (hydrocarbons, ketones, aldehydes, alcohols, and esters) [14]. The main attention of researchers was paid to the composition and bioactivity of *Nigella sativa* essential oil while the volatiles of the other species attracted much less scientific interests.

The aim of the study was to conduct the gas chromatography mass spectrometric (GC-MS) analysis of the component compositions of volatiles in the *Nigella damascena* and *Nigella arvensis* seeds.

Methods

Plant material

The raw materials were harvested from the plants cultivated in Ternopil region (Ukraine). The seeds were previously obtained from the collection of M.M. Gryshko National Botanical Garden (National Academy of Sciences of Ukraine).

Sample preparation

The sample of grinded seeds (2.0 g) was placed in a 20 mL vial, and tridecane (50 μg) as an internal standard was added according to the method described by Chernohorod and Vinogradov [15]. 10 mL of distilled water wass added to the sample and the volatile compounds were distilled for 2 h using an air-cooled reflux condenser. During the hydrodistillation process, the volatiles were adsorbed on the inner surface of the reflux condenser. After cooling the system, the adsorbed substances were washed off by slowly adding 3 mL of pentane to a 10 mL vial.

The washed substances were concentrated by blowing (100 mL/min) of ultrapure nitrogen to a residual volume of the extract of 10 μL, which was completely withdrawn with a chromatographic syringe. Further concentration of the sample wass carried out in the syringe to a volume of 2 μL.

Chromatographic analysis

Gas chromatograph Agilent Technologies 6890 with 5973 mass spectrometric detector was used in the study. The chromatographic column was capillary DB-5 (0.25 mm x 30 m). The injection of the sample into the chromatographic column was carried out in the splitless mode (rate 1.2 mL/min during 0.2 min). The flow rate of helium as a carrier gas was 1.2 mL/min. The temperature of thermostat was programmed from 50 to 320°C with a speed of 4 °C/min.

The NIST05 and WILEY 2007 mass spectra libraries in combination with the AMDIS and NIST programs were used for the identification of the components. The internal standard method was applied for the quantitative calculations of the contents of volatile compounds.

Results and Discussion

The conducted GC-MS analysis gives the possibilities to identify more than 25 volatile compounds in the seeds of each investigated species (Figures 1-3). The components whose content exceeded 0.03 mg/kg are shown in Table 1. A number of minor compounds contained in the studied raw materials were not identified.

As can be seen from the Table 1, carvone (5.448 mg/kg) and squalene (0.966 mg/kg) dominated among the volatile ingredients of *Nigella arvensis* seeds. Dihydrofarnesol (0.957 mg/kg) and squalene (0.525 mg/kg) in the *Nigella damascena* raw material. Havlik et al. [16] found that carvacrol methyl ether (26.4%), *β*-pinene (21.4%) and *n*-undecane (13.2%) dominated among the constituents of *Nigella arvensis* essential oil grown in Czech Republic. Thus, the chemical composition of essential oils depends on growing and weather conditions as well as the genetic peculiarities of plants such as belonging to a certain subspecies, variety or chemotype [13, 14, 17, 18].

It should be mentioned that found among the *Nigella arvensis* volatiles monocyclic monoterpenoid carvone was also the main component of *Carum carvi* essential oil [19]. Carvone possesses the proven antioxidant, anti-inflammatory, carminative, antimicrobial and other valuable properties [20, 21].

Acyclic triterpene squalene is an intermediate in the biosynthesis of steroids [22]. It is used as the immunologic adjuvant with a vaccine as well as anti-cancer, detoxifying, and anti-aging agent [22, 23].

Acyclic sesquiterpene dihydrofarnesol can inhibit the growth of dermatophytes [24]. The studied *Nigella damascena* seeds contained 0.370 mg/kg of monocyclic sesquiterpene *β*-elemene which plays a significant role in cancer therapy [25]. *β*-Elemene was detected as the main compound of Polish *Nigella damascena* essential oil possessed the noticeable antimicrobial and anti-inflammatory potential [26, 27].

The content of aromatic monoterpenoid carvacrol with proven antiseptic properties [28] was significantly higher in the *Nigella arvensis* seeds (0.373 mg/kg) comparatively to *Nigella damascene* ones (0.042 mg/kg). Such monoterpenoid as methylcarvacrol (0.157 mg/kg) as well as *cis*-carveol (0.116 mg/kg) were detected only in the *Nigella arvensis* raw material.

The moderate amount of acyclic monoterpenoid linalool possessing antimicrobial and sedative actions [29, 30] was found in the *Nigella arvensis* seeds (0.180 mg/kg), while the *Nigella arvensis* raw material accumulate only 0.033 mg/kg of linalool. Thus, the studied raw materials differed significantly in the presence and content of a lot of detected terpenoids.

Besides the terpenoids, there were found also the long-chained carboxylic acids and unsaturated hydrocarbons in the studied raw materials. These results are consistent with the data of Mahboubi et al. [31]. Researchers reported that fixed oil of *Nigella sativa* containing a lot of fatty acids as well as terpenoids, could be used as alternative treatment of rheumatoid arthritis as a pain inhibitor and anti-inflammatory drug [31]. Polyunsaturated fatty acids (ω-3 and ω-6) are used for the preventing and treating of atherosclerosis, obesity and cancer [32, 33].

Thus, the anti-inflammatory, antioxidant, antiviral, anticholesterinemic, anticancer, anesthetic, and analgesic effects are shown by various classes of biologically active substances of primary and secondary synthesis which are synthesized in plants [34–44].

Generally, the studied raw materials could be regarded as prospective sources of new medicines and food additives.

References

1. The Plant List (2021). *http://www.theplantlist.org*
2. Niu, Y., Zhou, L., Meng, L., Chen, S., Ma, C., et al. (2020). Recent progress on chemical constituents and pharmacological effects of the genus *Nigella*. *Evidence-based complementary and alternative medicine,* 6756835.
3. Farag, M. A., Gad, H. A, Heiss, A. G., & Wessjohann, L. A (2014). Metabolomics driven

analysis of six *Nigella* species seeds via UPLC-qTOF-MS and GC-MS coupled to chemometrics. *Food Chem*, 15; 151: 333–342.
4. Salehi, B., Quispe, C., Imran, M., Ul-Haq, I., Živković, J., et al. (2021). *Nigella* plants traditional uses, bioactive phytoconstituents, preclinical and clinical studies. *Frontiers in pharmacology*, *12*, 625386.
5. Ahmad, M. F., Ahmad, F. A., Ashraf, S. A., Saad, H. H., et al. (2021). An updated knowledge of Black seed (*Nigella sativa* Linn.): Review of phytochemical constituents and pharmacological properties. *Journal of herbal medicine*, *25*, 100404.
6. Kooti, W., Hasanzadeh-Noohi, Z., Sharafi-Ahvazi, N., Asadi-Samani, M., & Ashtary-Larky, D. (2016). Phytochemistry, pharmacology, and therapeutic uses of black seed (*Nigella sativa*). *Chinese journal of natural medicines*, 14(10), 732–745.
7. Usama, H. Ramadhan, Munther A. Mohammedali, & Huda S. Abood (2011). Study the analgesic activity of *Nigella sativa* volatile oil against pain in mice. *Journal of Current Pharmaceutical Research*, 5 (1), 36–38.
8. Shanaida, M. (2019). Carboxylic acids of *Nigella sativa* and *N. damascena* seeds. *Ukr biopharm J*, (60), 71–76.
9. Rudenka, D., & Shanaida, M (2015). [Аналіз ефірної олії з насіння *Nigella arvensis* L.]. *Medical chemistry*, 16, 3 (60), 134. [In Ukrainian].
10. Islam, M. N., Hossain, K. S., Sarker, P. P., Ferdous, J., Hannan, M. A., et al. (2021). Revisiting pharmacological potentials of *Nigella sativa* seed: A promising option for COVID-19 prevention and cure. *Phytotherapy research*, *35*(3), 1329–1344.
11. Khazdair, M. R., Ghafari, S., & Sadeghi, M. (2021). Possible therapeutic effects of *Nigella sativa* and its thymoquinone on COVID-19. *Pharmaceutical biology*, 59(1), 696–703.
12. Gontova, T. M., Sokolova, O. O., Kotov, A. G., Kutsenko, S. A., & Mashtaler, V. V. (2018). Determination of essential oil component composition of common sunflower marginal flowers. *Research Journal of Pharmacy and Technology*, 11(5), 1971–1973.
13. Shanaida M., Hudz N., Bialon M., Kryvtsova M., Svydenko L., et al. (2021). Chromatographic profiles and antimicrobial activity of essential oils obtained from some species and cultivars of the *Mentheae* tribe (*Lamiaceae*). *Saudi Journal of Biological Sciences*. https://doi.org/10.1016/j.sjbs.2021.06.068
14. Sharifi-Rad, J., Sureda, A., Tenore, G.C., Daglia, M., Sharifi-Rad, M., et al. (2017). Biological activities of essential oils: from plant chemoecology to traditional healing systems. *Molecules*, 22, 70.
15. Chernohorod, L. B., & Vinogradov, B. A (2006). [Эфирные масла некоторых видов рода *Achillea* L., содержащие фрагранол]. *Rastit. res.* 42 (2), 61–68. [In Russian].
16. Havlik, J., Kokoska, L., Vasickova, S., & Valterova, I. (2006). Chemical composition of essential oil from the seeds of *Nigella arvensis* L. and assessment of its actimicrobial activity. *Flavour Fragr. J*, 21, 713–717.
17. Hudz, N., Makowicz, E., Shanaida, M., Białoń, M., Jasicka-Misiak, I., et al. (2020). Phytochemical evaluation of tinctures and essential oil obtained from *Satureja montana* herb. *Molecules*, 25, 4763.
18. Vaičiulytė, V., Butkienė, R., & Ložienė, K., 2016. Effects of meteorological conditions and plant growth stage on the accumulation of carvacrol and its precursors in *Thymus pulegioides*. *Phytochemistry, 128*, 20–26.
19. Hajlaoui, H., Arraouadi, S., Noumi, E., Aouadi, K., Adnan, M., et al. (2021). Antimicrobial, antioxidant, anti-acetylcholinesterase, antidiabetic, and pharmacokinetic properties of *Carum carvi* L. and *Coriandrum sativum* L. essential oils alone and in combination. *Molecules*, 26(12), 3625.
20. Asle-Rousta, M., Amini, R., & Aghazadeh, S. (2020). Carvone suppresses oxidative stress and inflammation in the liver of immobilised rats. *Arch of physiology and biochemistry*, 1–6. doi.org/10.1080/13813455.2020.185172
21. Frolova, N., Yushchenko, N., Korablova, O., Voitsekhivskyi, V., Ocheretna, A., & Synenko, T. (2021). Comparative study of carvones from various essential oils and their ability to increase the stability of fat-containing products. *J Ecol Eng*, 22(3), 239–248.
22. Kim, S. K., & Karadeniz, F. (2012). Biological importance and applications of squalene and

squalane. *Advances in food and nutrition research*, 65, 223–233.
23. Ronco, A. L., & De Stéfani, E. (2013). Squalene: a multi-task link in the crossroads of cancer and aging. *Functional Foods in Health and Disease*, 3 (12), 462–476.
24. Brasch, J., Horter, F., Fritsch, D., Beck-Jendroschek, V., Tröger, A., & Francke, W. (2014). Acyclic sesquiterpenes released by *Candida albicans* inhibit growth of dermatophytes. *Medical mycology*, 52(1), 46–55.
25. Zhai, B., Zhang, N., Han, X., Li, Q., Zhang, M., et al. (2019). Molecular targets of *β*-elemene, a herbal extract used in traditional Chinese medicine, and its potential role in cancer therapy: A review. *Biomedicine & Pharmacotherapy*, *114*, 108812.
26. Sieniawska, E., Sawicki, R., Golus, J., Swatko-Ossor, M., Ginalska, G., & Skalicka-Wozniak, K. (2018). *Nigella damascena* L. essential oil-a valuable source of *β*-elemene for antimicrobial testing. *Molecules*, *23*(2), 256.
27. Sieniawska, E., Michel, P., Mroczek, T., Granica, S., & Skalicka-Woźniak, K. (2019). *Nigella damascena* L. essential oil and its main constituents, damascenine and *β*-elemene modulate inflammatory response of human neutrophils *ex vivo*. *Food and chemical toxicology*, 125, 161–169.
28. Memar, M. Y., Raei, P., Alizadeh, N., Aghdam, M.A., & Kafil, H.S. (2017). Carvacrol and thymol: strong antimicrobial agents against resistant isolates. *Reviews in Medical Microbiology*, 28(2), 63–68.
29. Barchuk, O., Pryshlyak, A., Shanaida, M. (2021). Chemical compositions and sedative activities of the *Dracocephalum moldavica* L. and *Ocimum americanum* L. essential oils. *Pharmacologyonline*, 2, 179–187.
30. Herman, A., Tambor, K., & Herman, A. (2016). Linalool affects the antimicrobial efficacy of essential oils. *Curr. Microbiol.* 72(2), 165–172.
31. Mahboubi, M., Mohammad Taghizadeh Kashani, L., & Mahboubi, M. (2018). *Nigella sativa* fixed oil as alternative treatment in management of pain in arthritis rheumatoid. *Phytomedicine*, 46, 69–77.
32. D'Angelo, S., Motti, M. L., & Meccariello, R. (2020). ω-3 and ω-6 polyunsaturated fatty acids, obesity and cancer. *Nutrients*, *12*(9), 2751.
33. Marangoni, F., Agostoni, C., Borghi, C., Catapano, A. L., Cena, H., et al. (2020). Dietary linoleic acid and human health: Focus on cardiovascular and cardiometabolic effects. *Atherosclerosis*, 292, 90–98.
34. Boozari, M., & Hosseinzadeh, H. (2021). Natural products for COVID-19 prevention and treatment regarding to previous coronavirus infections and novel studies. *Phytotherapy research : PTR*, *35*(2), 864–876.
35. Kumar, A., Agarwal, K., Singh, M., Saxena, A., Yadav, P., et al. (2018). Essential oil from waste leaves of *Curcuma longa* L. alleviates skin inflammation. *Inflammopharmacology*, *26*(5), 1245–1255.
36. Lv, L., Yin, B., You, Y., Sun, Z., He, J., & Cao, Y. J. (2020). Protective effects of total alkaloids from *Menispermum dauricum* against airway inflammation in asthmatic mice. *Planta medica*, 86(10), 665–673.
37. Mykhailenko, O., Korinek, M., Ivanauskas, L., Bezruk, I., Myhal, A., et al. (2020). Qualitative and quantitative analysis of Ukrainian *Iris* species: A fresh look on their content and biological activities. *Molecules*, 25(19), 4588–4612.
38. Pop, R. M., Sabin, O., Suciu, Ș., Vesa, S. C., Socaci, S. A., et al. (2020). *Nigella sativa*'s anti-inflammatory and antioxidative effects in experimental inflammation. *Antioxidants*, 9(10), 921.
39. Quispe, C., Cruz-Martins, N., Manca, M. L., Manconi, M., Sytar, O. et al. (2021). Nano-derived therapeutic formulations with curcumin in inflammation-related diseases. *Oxidative Medicine and Cellular Longevity*, 3149223.
40. Rytsyk, O., Soroka, Y., Shepet, I., Vivchar, Z., Andriichuk, I., et al. (2020). Experimental evaluation of the effectiveness of resveratrol as an antioxidant in colon cancer prevention. *Nat. Product. Commun*, 15 (6), 1–10.
41. Shanaida, M., Pryshlyak, A., & Golembiovska O. (2018). Determination of triterpenoids in some *Lamiaceae* species. *Research Journal of Pharmacy and Technology*, 7: 3113–3118.
42. Skakun, M. P., Stepanova, N. Iu. (1988). Comparative evaluation of the hepatoprotective,

antioxidant and cholagogic activities of flavonoid preparations. *Vrach Delo*, 12: 52–54. [Article in Russian]

43. Stechyshyn, I., Pavliuk, B., Chornij, N., Kritsak, M., & Prokopovych, O. (2021). Substantiation of attention around antioxidants of plant origin for use in complex pharmacotherapy of diseases of the oral cavity. *Polski Merkuriusz Lekarski,* 49(291), 238–241.
44. Xagorari, A., Papapetropoulos, A., Mauromatis, A., Economou, M., Fotsis, T., & Roussos, C. (2001). Luteolin inhibits an endotoxin-stimulated phosphorylation cascade and proinflammatory cytokine production in macrophages. *J Pharmacol Ex. Ther*, 296(1), 181–187.

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Table 1. Chemical compositions of the main volatile compounds from the seeds of two *Nigella* species determined by GC-MS, mg/kg

Retention time, min	Component	*Nigella arvensis*	*Nigella damascena*
4.94	Decane	0.042	0.031
9.14	Benzaldehyde	–	0.049
10.32	Dodecane	0.033	0.020
10.50	Linalool	0.180	0.033
13.83	Terpinene-4-ol	0.040	–
14.63	α-Terpineol	0.041	–
15.57	*trans*-Dihydrocarveol	0.056	–
15.75	*p*-Cymene-α-ol	0.044	–
16.05	*cis*-Carveol	0.116	–
16.11	*cis*-Dihydrocarveol	0.050	–
16.20	Dihydrocarvone	0.069	–
16.35	Methylcarvacrol	0.157	–
16.49	*trans*-Carveol	0.093	–
16.58	Tetradecane	0.080	0.040
17.97	Carvone	5.448	–
18.86	*β*-Elemene	–	0.370
19.12	Carvacrol	0.373	0.042
19.36	Pentadecane	0.084	0.035
20.31	2-oxo-3-methoxy-4-methylacetophenone	0.279	–
21.47	Hexadecane	0.059	0.036
21.70	Geranilacetone	0.052	–
22.87	2,6,10,14-Tetramethylpentadecane	0.052	–
23.21	Heptadecane	0.091	0.034
23.55	*γ*-Octalactone	0.033	–
24.22	Viridiflorol	–	0.033
24.73	Octadecane	0.073	0.037
25.70	Methyl-2-amino-3-methoxy benzoate	–	0.100
25.37	Dihydrofarnesol	0.152	0.957
26.02	Miristic acid	0.096	–
27.40	Eicosane	0.086	0.039
28.63	Palmitic acid	0.530	0.365
29.76	Phthalate	0.075	0.046
30.94	Oleic acid	0.141	0.090
31.08	Ethyl linoleate	0.095	–
31.89	Tetracosane	0.046	0.030
32.90	Pentacosane	0.101	0.164
33.86	Hexacosane	0.073	0.054
34.79	Heptacosane	0.111	0.328
35.70	Octacosane	–	0.043
36.56	Nonacosane	0.097	0.155
37.30	Squalene	0.966	0.525

Note: "–" – the component was not detected

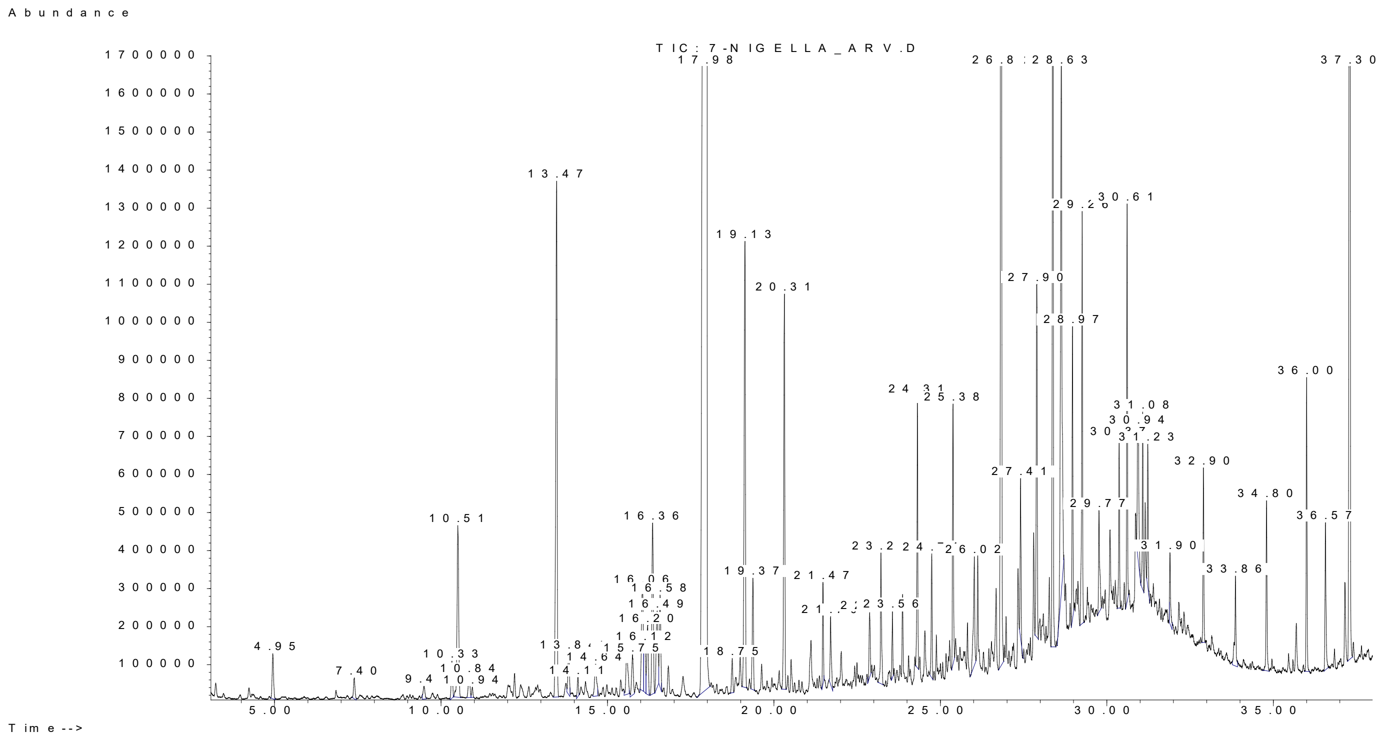

Figure 1. The GC-MS chromatogram of volatiles from the *N. arvensis* seeds

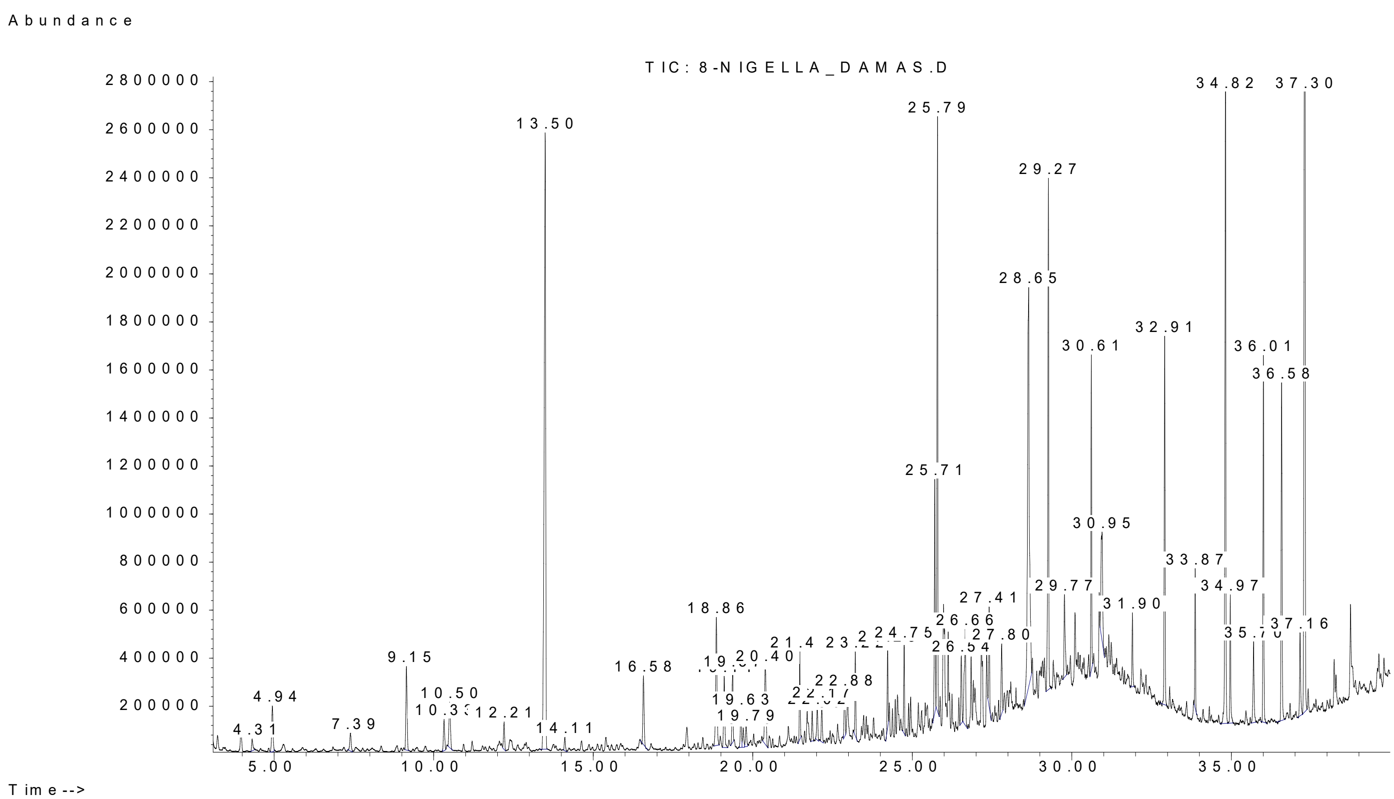

Figure 2. The GC-MS chromatogram of volatiles from the *N. damascena* seeds

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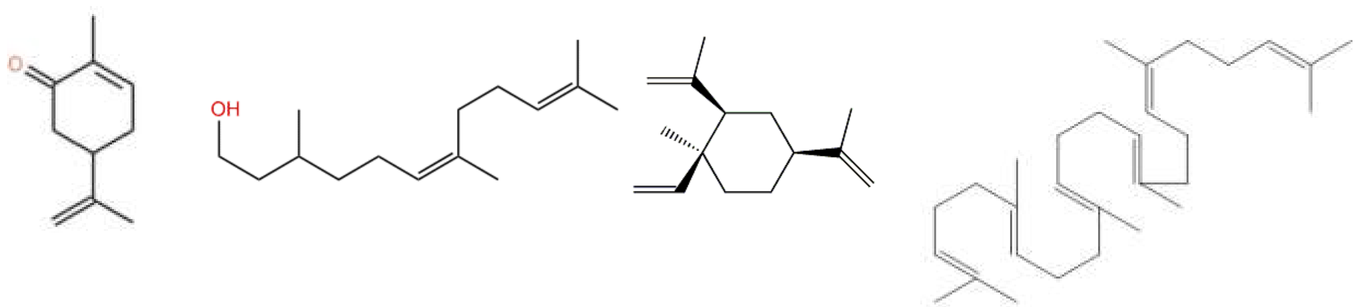

Carvone Dihydrofarnesol *β*-Elemene Squalene

Figure 3. Structural formulas of the predominant terpenoids found in the seeds of investigated *Nigella* species

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