Фітохімічні дослідження

ФАРМАЦЕВТИЧНИЙ ЧАСОПИС

http://ojs.tdmu.edu.ua/index.php/pharm-chas

DOI https://doi.org/10.11603/2312-0967.2024.3.14867
УДК 615.322:582.675.1

PHYTOCHEMICAL AND MICROMORPHOLOGICAL ANALYSIS OF THE SEEDS COLLECTED FROM THE NEWLY DEVELOPED *NIGELLA DAMASCENA* L. CULTIVAR

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INFORMATION

Надійшла до редакції / Received: 07.08.2024
Після доопрацювання / Revised: 26.08.2024
Прийнято до друку / Accepted: 27.08.2024

Key words:
love-in-a-mist;
new cultivar;
seeds;
volatile compounds;
total phenolic content;
micromorphometry.

ABSTRACT

The aim of the work. In this study, the total phenolic content (TPC) and composition of volatile compounds in the seeds of the newly developed cultivar 'Rizdvyana zirochka' of *Nigella damascena* L. (love-in-a-mist) were analyzed. The morphometric characteristics of these seeds were also evaluated.
Materials and Methods. The gas chromatography-mass spectrometric method (GC/MS) was applied to analyze volatile compounds' qualitative composition and quantitative content. The Agilent Technologies 6890 chromatograph with a mass spectrometric detector was applied. The quantitative content of volatile components was determined using tridecane as the internal standard. The TPC was estimated using the Folin-Ciocaltey reagent following the Singleton and Rossi method. The morphometric analysis of seeds was conducted using AxioCam MRc5 light microscope and AxioVision Rel. 4.8.2 software.
Results and Discussion. Among the volatile compounds detected in the newly developed cultivar 'Rizdvyana zirochka' of *Nigella damascena*, three terpenoids were found to be dominant: *beta*-elemene (34.01 %), germacrene A (28.16 %), and damascenine (21.97 %). The TPC of the 80 % methanol extract from the studied cultivar seeds was measured to be (29.37±0.59) mg GAE/g. Additionally, the conducted morphometric analysis revealed the key morphological parameters of these seeds.
Conclusions. In this study, the TPC and volatile compounds composition in the seeds of the newly developed 'Rizdvyana zirochka' cultivar of *Nigella damascena* were analyzed, and the morphometric characteristics of this raw material were evaluated.

Introduction. The therapeutic properties of medicinal plants are closely linked to their phytochemical composition. Aromatic medicinal plants contain volatile bioactive compounds that exhibit various pharmacological effects [1]. Additionally, polyphenols are an important group of phytoconstituents found in essential oil-bearing plants [2, 3].

Nigella L. is a genus of plants in the Ranunculaceae family, comprising approximately 25 species of annual herbaceous plants. The mature seeds of certain *Nigella*

species are used in cooking and folk medicine in some regions [4-6]. *Nigella sativa* L. (black cumin) has been extensively studied for its chemical composition and medicinal properties in recent decades [7, 8]. Clinical trials have been conducted to assess the effectiveness of using *Nigella sativa* seeds [9–12]. However, clinical studies for *Nigella damascena* L. (love-in-a-mist) have not been carried out yet. *Nigella damascena* is primarily known as an ornamental plant and is less commonly cultivated for its medicinal properties [6].

The seeds of *Nigella damascena* contain valuable bioactive compounds such as essential oils, triterpenoids, polyphenols, and unsaturated fatty acids [3, 6, 13]. These compounds' qualitative composition and quantitative content can vary significantly in different taxa, cultivars, and chemotypes of this species [6, 14].

There is no doubt that morphological examination of the seeds can help identify the diagnostic differences between numerous cultivars and varieties that have appeared in recent years [15].

This study aimed to analyze the total phenolic content (TPC) and composition of volatile compounds in the seeds of the newly developed cultivar 'Rizdvyana zirochka' of *Nigella damascena.* It also aimed to evaluate the morphometric characteristics of its seeds.

Materials and Methods. The mature seeds of the newly developed cultivar 'Rizdvyana zirochka' [16] of *Nigella damascena* were gathered in 2023 from the plants (Fig. 1) cultivated in the Ternopil region of Ukraine (49°38′03″ N; 25°28′32″ E). After the plant material was dried at 25–30 °C, it was either used whole (for morphometric analysis) or crushed (for phytochemical research).

The gas chromatography-mass spectrometric (GC/MS) method was applied to analyze the qualitative composition and quantitative content of volatile compounds according to [17]. The Agilent Technologies 6890 chromatograph with a mass spectrometric detector and an HP-5ms capillary column (internal diameter - 0.25 mm, length – 30 m) was applied for the analysis. The carrier gas (helium) was set at a velocity of 1.0 mL/min. The temperature of the sample introduction heater was maintained at 250 °C, and the thermostat temperature was programmed to increase from 50 to 320 °C at a rate of 4 °C/min during the analysis. The components were identified based on the general patterns of fragmentation of organic compounds and by comparing the results with the NIST 08 mass spectrum libraries in combination with AMDIS and NIST 08 identification programs. The quantitative content of volatile components was determined using tridecane as the internal standard.

The TPC was estimated using the Folin-Ciocaltey reagent following the Singleton and Rossi method [18]. The extraction was carried out using 80 % methanol with a ratio of 1:20 of raw materials to extractant. Maceration (24 hours at room temperature) and ultrasonic bath (30 min at 45 °C) were used for the extraction of polyphenols. The resulting extract was centrifuged at 5000 rpm, and the supernatant was used for spectrophotometric analysis of the TPC with a Genesys 20 spectrophotometer. The mixture with reagents was left to stand in a dark place for 60 min before measuring its absorbance. The TPC was measured at 760 nm. The TPC was then calculated using gallic acid equivalent (GAE).

The morphometric analysis of seeds was conducted on a total of 20 randomly selected seeds using AxioCam MRc5 light microscope and AxioVision Rel. 4.8.2 Software (Carl Zeiss, Switzerland). The following measurements were taken: seeds' length (μm) and diameter (μm). The appearance of the seeds from the surface and on the section was also evaluated.

A B C

Fig. 1. The view of the newly developed cultivar 'Rizdvyana zirochka' of *Nigella damascena* on the plots in Ternopil region (Ukraine): A – flower; B – inflated capsules; C – ripe seeds.

Results and Discussion. Dozens of volatile components were found in the seeds of the newly developed cultivar 'Rizdvyana zirochka' of *Nigella damascena*; however, the content of only eight of them exceeded 1 % (Table 1, Fig. 2).

Among the detected volatile compounds, the following three terpenoids dominate (Fig. 3): *beta*-elemene (34.01 %), germacrene A (28.16 %), and damascenine (21.97 %). In the scientific sources, there is information that the predominant components identified by us have pronounced pharmacological effects. For example, sesquiterpene *beta*-elemene has been found to exert an anti-tumor effect by suppressing glycolysis in cancer cells [19, 20] and shows anti-mycobacterial properties [21]. Germacrene A is a precursor of *beta*-elemene [22]. Damascenine could be regarded as an important chemo-taxonomical marker among the volatile compounds of the *Nigella damascena* seeds. Therefore, the seeds of this cultivar can be considered a prospective source for developing new medicines and dietary supplements based on their essential oil.

It could be mentioned that *Nigella damascena* seeds grown in Morocco contain a dominant amount of sesquiterpene *beta*-elemene (54.7 %) [23]. The essential oil extracted from the *Nigella damascena* seeds collected in Italy was characterized by nearly 100 % sesquiter-

Table 1
The major volatile compounds detected in the seeds of cultivar 'Rizdvyana zirochka' of *Nigella damascena*

Compound Name	Retention time	Content, %
Linalool	12.09	3.68
Damascenine	13.97	21.97
Caryophyllene	14.51	1.34
Naphthalene	15.87	4.43
Germacrene A	16.07	28.16
alpha-Panasinsen	16.28	1.29
beta-Elemene	17.09	34.01
Dihydrofarnesol	20.73	3.89

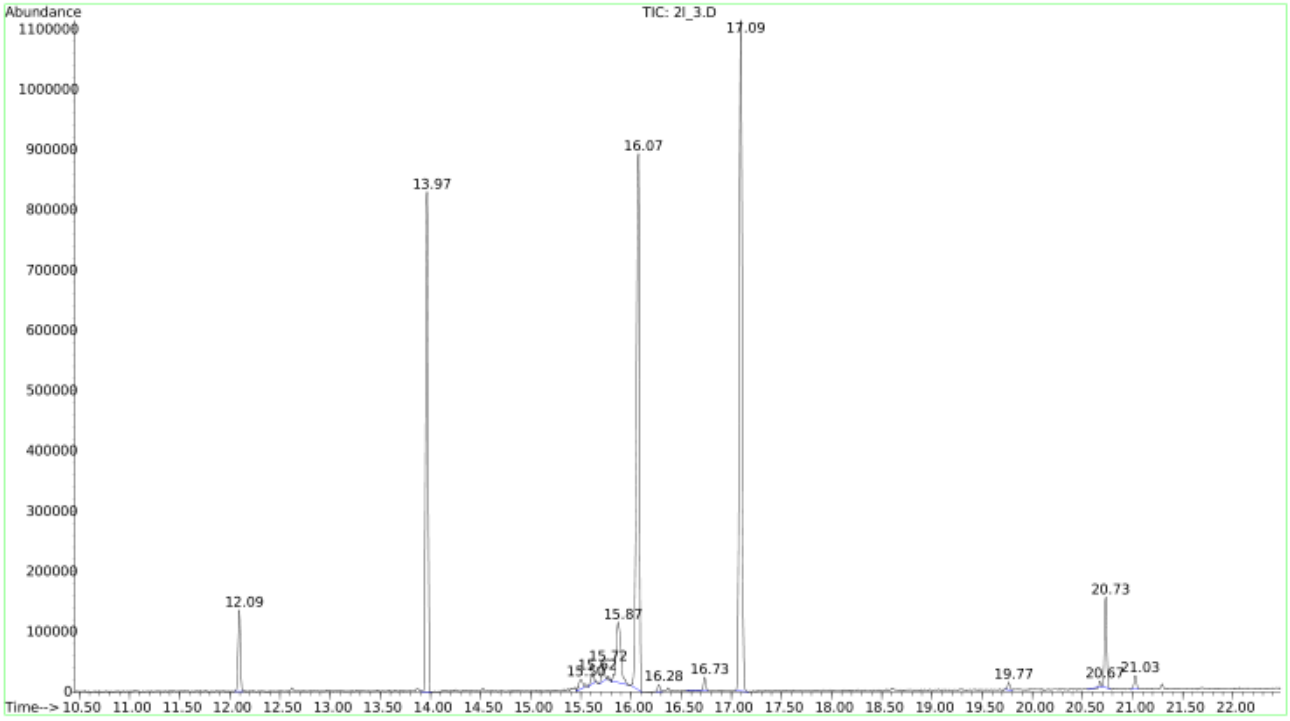
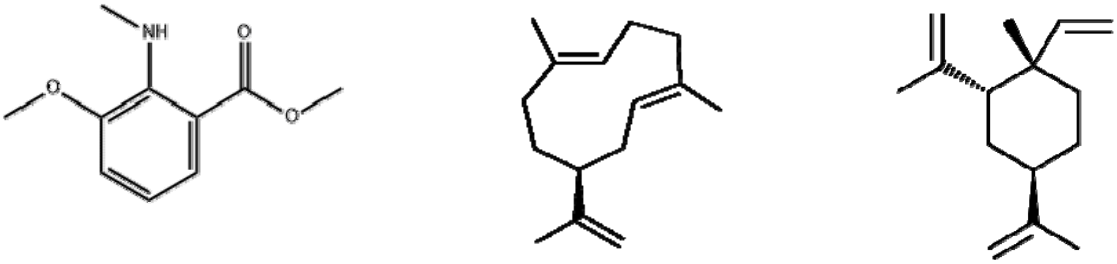


Fig. 2. GC/MS chromatogram of volatile compounds in the seeds of cultivar 'Rizdvyana zirochka' of *Nigella damascena.*



Damascenine Germacrene A *beta*-Elemene

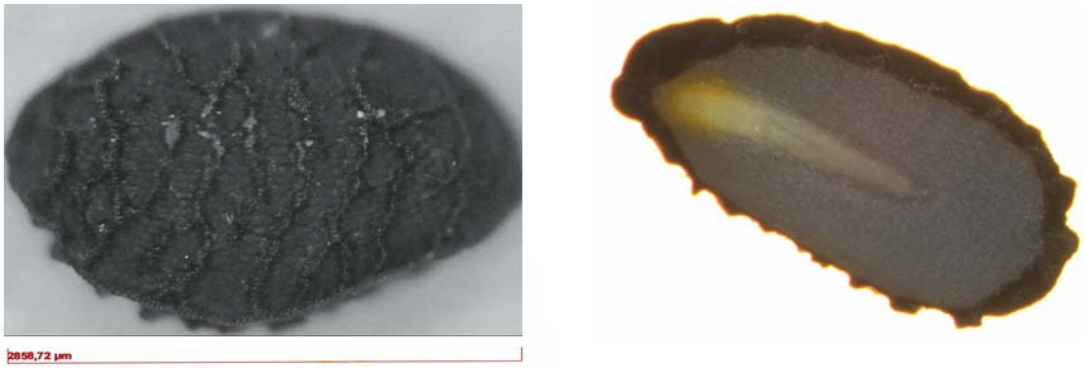
Fig. 3. Structural formulas of the predominant volatile compounds in the studied *Nigella damascena* raw material.

penoids, with *beta*-elemene being the most dominant (73.2 %). In Poland, the essential oil from *Nigella damascena* seeds contained *beta*-elemene (47.37 %), *alpha*-selinene (13.52 %), and *beta*-selinene (10.10 %) as the predominant compounds [21]. This essential oil and isolated *beta*-elemene have shown antimicrobial effects against *Mycobacterium tuberculosis* [21]. Researchers have proposed that *Nigella damascena* essential oil, along with damascenine and *beta*-elemene isolated from it, exhibit multifactorial mechanisms of immunomodulatory activity [24].

The TPC of the 80 % methanol extract from the studied *Nigella damascena* seeds was found to be (29.37±0.59) mg GAE/g. As it was concluded by Khattak et al. [25], the choice of solvents and extraction method played a crucial role in obtaining the highest TPC from *Nigella sativa* seeds. It was determined that methanol is the optimal extractant for polyphenolic compounds from the *Nigella* seeds [25] that was confirmed by our research.

The conducted morphometric analysis of the cultivar 'Rizdvyana zirochka' of *Nigella damascena* seeds shows that they are black and have a triangular-rounded shape with a domed apex (Fig. 4). The length is (92854.8±73) μm and the width is (1905.2±49) μm. The surface has a mesh (striated) structure with three prominent longitudinal stripes on each edge and several transverse stripes. The seeds have hard teguments that are difficult to cut.

The data obtained by us on the size of the 'Rizdvyana zirochka' seeds show that these seeds are somewhat larger than those of the typical species *Nigella damascena* [26]. It should be noted that the micromorphological characteristics of 12 *Nigella* taxa (species and varieties) collected in Turkey [14] did not include any morphometric analysis. Therefore, we were not able to



A B

Fig. 4. Examples of micrographs of the 'Rizdvyana zirochka' cultivar (*Nigella damascena*) seeds taken from the surface (A) and in long-section (B) using a light microscope.

conduct a comparative analysis with the data that we obtained.

Conclusions. The content of bioactive compounds varies among different varieties and chemotypes within the same species and it has significant influence on medicinal properies of plants. In this study, the TPC and volatile compounds composition in the seeds of the newly developed 'Rizdvyana zirochka' cultivar of *Nigella damascena* were analyzed, and the morphometric characteristics of these seeds were evaluated. Among the volatile

compounds detected, three terpenoids were found to be dominant: *beta*-elemene (34.01 %), germacrene A (28.16 %), and damascenine (21.97 %). These volatiles possess prominent therapeutic potential. The TPC of the 80 % methanol extract from the studied seeds was measured to be (29.37±0.59) mg GAE/g. Additionally, the conducted morphometric analysis revealed the key morphological parameters of the studied raw material.

Acknowledgments and Funding Source. This article was prepared with the active participation of researchers of the international network AGROBIONET as a part of the project MVTS-SR/UA-6/14 "The Use of Lesser-known and Little-used Plant Species to Improve Nutrition, Health, and Quality of Life". The experimental study was conducted in the laboratories of the Institute of Plant and Environmental Sciences at the Faculty of Agrobiology and Food Resources, Slovak Agricultural University in Nitra. The authors would like to acknowledge the financial support received from "The National Scholarship Programme of the Slovak Republic" (SAIA). Mariia Shanaida expresses her gratitude to the SAIA Agency for the financial support of the research stay, during which the experiments were carried out.

Conflicts of interest: authors have no conflict of interest to declare.

Конфлікт інтересів: відсутній.

ФІТОХІМІЧНИЙ ТА МІКРОМОРФОЛОГІЧНИЙ АНАЛІЗ НАСІННЯ НОВОГО СОРТУ *NIGELLA DAMASCENA* L.

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Мета роботи. Проаналізувати загальний вміст фенольних сполук і компонентний склад летких сполук у насінні нового сорту «Різдвяна зірочка» *Nigella damascena* L. (чорнушка дамаська); оцінити морфометричні показники насіння цього сорту.
Матеріали і методи. Для аналізу якісного складу та компонентного вмісту летких сполук застосовано метод газової хромато-мас-спектрометрії (ГХ/МС). Використано хроматограф Agilent Technologies 6890 з мас-спектрометричним детектором. Кількісний вміст летких компонентів визначали з використанням тридекану як внутрішнього стандарту. Загальний вміст фенольних сполук визначали за допомогою реактиву Фоліна – Чекольту (метод Сінглтона й Россі). Морфометричний аналіз насіння проводили під світловим мікроскопом AxioCam MRc5 із застосуванням програми AxioVision Rel. 4.8.2.
Результати й обговорення. Серед виявлених летких сполук домінували терпеноїди: *бета*-елемен (34,01 %), гермакрен А (28,16 %) і дамасценін (21,97 %). Загальний вміст фенольних сполук у 80 % метанольному витязі досліджуваного насіння становила (29,37±0,59) мг/г (у перерахунку на галової кислоти еквівалент). Крім того, проведений морфометричний аналіз дав змогу виявити основні морфологічні параметри цього насіння.
Висновки. У цьому дослідженні проаналізовано загальний вміст фенольних сполук та якісний склад і компонентний вміст летких сполук у насінні нещодавно виведеного сорту «Різдвяна зірочка» *Nigella damascena,* а також оцінено діагностичні морфологічні характеристики цього рослинного матеріалу.

Ключові слова: чорнушка дамаська; новий сорт; насіння; леткі сполуки; загальний вміст фенольних сполук; мікроморфометрія.

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