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CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF THREE VOLATILE OILS EXTRACTED FROM *NIGELLA SATIVA* L. SEEDS

Sefa GÖZCÜ[1]*, Zeynep AKŞİT[2]

[1]*Erzincan Binali Yildirim University, Faculty of Pharmacy, Department of Pharmacognosy, Erzincan, 24100, Türkiye*
[2]*Erzincan Binali Yildirim University, Tourism and Hospitality Vocational School, Department of Hotel, Restaurant and Service, 24100, Erzincan, Türkiye*

Abstract: *Nigella sativa* L. (Ranunculaceae) and its volatiles have a wide range of benefits. The aim of this study was to investigate the chemical composition and *in vitro* antibacterial activity of three volatile oils from Erzincan (Local market/Provincial Agriculture and Forestry Office in Erzincan) and Konya. These three samples were grown under different edaphic and climatic conditions. The disc diffusion method was used to test the antibacterial activity against ten standard bacterial strains (*Staphylococcus aureus*, *Clostridium perfringens*, *Enterococcus faecium*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, *Salmonella enterica*, *Salmonella enteritidis*, *Bacillus cereus*, *Listeria monocytogenes*, and *Escherichia coli*). Monoterpenes were abundant in the chemical composition of all volatile oils tested. The seeds of Erzincan (from local market), Erzincan (from Erzincan Provincial Authority of Agriculture and Forestry) and Konya were characterized by the presence of *p*-cymene (41.74%-51.98%), *α*-thujene (16.02%-16.49) and nerol (7.91%-8.50%). *Clostridium perfringens* (inhibition zone: 35 to 39.3 mm) and *Pseudomonas aeruginosa* (inhibition zone: 29.7 to 38.7 mm) were found to be particularly sensitive to all volatile oils tested. The results of this study show that the volatile oil of the seeds of *N. sativa* has remarkable antibacterial activity, which may be due to the presence of various secondary metabolites. In view of the uncontrolled development of antibiotic resistance, these compounds can be used for prophylactic or curative purposes.

Keywords: *Nigella sativa* L., Volatile oil, Essential oil, Antibacterial activity

***Corresponding author:** Erzincan Binali Yildirim University, Faculty of Pharmacy, Department of Pharmacognosy, Erzincan, 24100, Türkiye
E mail: sgozcu@erzincan.edu.tr (S. GÖZCÜ)
Sefa GÖZCÜ https://orcid.org/0000-0002-0735-4229
Zeynep AKŞİT https://orcid.org/0000-0002-0349-0223

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1. Introduction

Nigella sativa L. (Ranunculaceae) is a remarkable medicinal herb that has been extensively researched to support its traditional claims. In Islamic medicine, ayurvedic medicine, Unani folk medicine, and indigenous medicine, the seeds or extracted oil of *Nigella sativa* L. (NS) are historically used alone or in conjunction with other products like honey, lint, melted butter, and astringents (Hossain et al., 2021). They are used to treat a variety of diseases or health conditions, such as abscesses, anorexia, worm infections, bronchitis, colic, rhinitis, cough, dermatosis, diabetes, diarrhea, dejection, eye infections, fatigue, fever, liver disorders, respiratory infections, rheumatism, sinusitis (Yarnell and Abascal, 2011; Omidi et al., 2017; Alkis et al., 2021; Khazdair et al., 2021).

Antibiotic resistance has become a severe issue that affects millions of people worldwide. Because of this resistance, there has been a rise in study for novel options, such as medicinal plants (Bakal et al, 2017). Because of this, the World Health Organization (WHO) has produced a list of global priority diseases of multidrug-resistant bacteria for the development of new effective antibiotics (Shrivastava et al., 2018). Currently, several biological investigations on NS are being conducted; the richness of their seeds in volatile chemicals, saponins, and alkaloids provides a wide range of impacts on various disorders (Ahmad et al., 2013). It was recently discovered that NS oil and extracts had antibacterial, immunomodulatory, and anticancer effects (Almatroudi et al., 2020; Daoudi et al., 2022). Volatile oils are regarded as a significant source in a variety of industries, including perfumeries, cosmetics, and pharmaceuticals (Tariq et al., 2019).

The objective of this research is to examine at the chemical composition variations of different NS volatile oils from Erzincan (local market/ Erzincan Provincial Authority of Agriculture and Forestry) and Konya and determine the way they affect ten common pathogenic bacteria including five of gram positive; *Staphylococcus aureus* (ATCC 6538), *Clostridium perfringens* (ATCC 13124), *Enterococcus faecalis* (ATCC 8459), *Bacillus cereus* (ATCC 10876), *Listeria monocytogenes* (ATCC 51774), and five of gram negative; *Pseudomonas*

fluorescens (ATCC 13525), *Pseudomonas aeruginosa* (ATCC 15442), *Salmonella enterica* (ATCC 14028), *Salmonella enteritidis* (ATCC 15442), and *Escherichia coli* (ATCC 25922).

2. Material and Methods

2.1. Materials

Nigella sativa L. (Ranunculaceae) seeds were obtained from three different origins of Türkiye. *N. sativa* seeds obtained from Erzincan and Konya were purchased from the local market (first and second *N. sativa*). Erzincan Provincial Authority of Agriculture and Forestry provided the third *N. sativa* seeds. The shaded chamber was used to naturally dry *N. sativa* seeds until they reached a steady weight after around 7 days. Before hydrodistillation, the completely dried samples were crushed into a fine powder.

2.2. Volatile Oil Extraction

A total of 400 grams of *Nigella sativa* (NS) seeds were mixed and hydrodistilated for 3 hours using a Clevenger apparatus. The extraction procedure was carried out in triplicate. Following that, the volatile oils were carefully gathered and stored in sealed sample tubes, which were then maintained at 4 °C until the analyses were performed.

2.3. GC-MS Analysis Conditions

According to previously published procedures *(Aksit et al., 2022)*, GC-MS studies were performed using a Thermo Scientific Trace 1310 GC-MS (Trace 1310, Thermo Scientific, Milano, Italy) system furnished with an HP-5MS capillary column (30 m x 0.25 mm and 0.25 m ID). Helium was used as a carrier gas at a constant flow rate of 1.2 mL/min in split mode with a 50:1 ratio. Both the injection site and the mass transfer line were set to 280 °C. The column oven temperature was designed to start at 60 °C for 3 minutes, then grow to 200 °C at a rate of 3 °C/min for 0 minutes, and lastly ramp up to 240 °C at a rate of 5 °C/min for 5 minutes. The mass spectrometer parameters were configured as follows: the ion source temperature was held at 280 °C, and electron ionization (EI) mode with ionization energy of 70 eV was utilized. The retention index (RI) for all secondary metabolites was calculated using the Van den Dool and Kratz equation, which is based on a homolog *n*-alkane series (C8-C40). Wiley and NIST2004 MS libraries were used to validate chemical identification. Based on the peak areas obtained from MS chromatograms, the relative peak area percentages of each chemical were determined.

2.4. Antibacterial Activity

Staphylococcus aureus (ATCC 6538), *Clostridium perfringens* (ATCC 13124), *Enterococcus faecalis* (ATCC 8459), *Pseudomonas fluorescens* (ATCC13525), *Pseudomonas aeruginosa* (ATCC 15442), Salmonella *enterica* (ATCC 14028), *Salmonella enteritidis* (ATCC 15442), *Bacillus cereus* (ATCC 10876), *Listeria monocytogenes* (ATCC 51774), and *Escherichia coli* (ATCC 25922) were examined for antibacterial properties. The disc diffusion test was carried out following the standard procedure (Wayne, 1999) 100 μL of bacteria (10^8 cells/ml) was inoculated on Nutrient Agar medium and sterile blank discs in 6 mm were placed on. 5 μL of the sample was injected on blank disc and Imipenem antibiotic disc as positive control was placed on the medium. Plates incubated at 37 °C for 24 hours. With the aid of a digital caliper, inhibition zones were measured, and the mean diameter of three replications in mm was recorded.

2.5. Statistical Analysis

The Duncan test was used to compare means in trials, and ANOVA was performed to examine the significance of differences between treatments. For statistical analysis, the SPSS 15 software was utilized.

3. Results and Discussion

3.1. Chemical Composition of Volatile Oil

The hydrodistillation technique was used to produce volatile oil from three *Nigella sativa* (NS) samples from three origins with yields ranging from 0.047% to 1.7%. The yield of volatile oil obtained through steam distillation of NS seeds can vary but is generally around 0.4-0.7% (Mozaffari et al., 2000). This yield varies according to the climate and environmental conditions in which the plant grows (Mehalaine and Chenchouni, 2021). The GC-MS technique was used to analyze the composition of the volatile oil, and the identification of their constituents was performed by comparing their MS data with those held in the National Institute of Standards and Technology (NIST147) and Wiley Library. The chemical composition of volatile oil was given in Table 1 showing the percentage of each component, retention time (RT), and retention indices (RI). Table 1 show that the major components were found to be *p*-cymene (41.74%-51.98%), α-thujene (16.02%-16.49), and nerol (7.91%-8.50%). Previous studies have demonstrated that the *p*-cymene content ranged from 7% to 60% (Burits and Bucar, 2000; Erdoğan et al., 2023; Mehalaine and Chenchouni, 2021; Wajs et al., 2008). In the presented study, *p*-cymene was found to be in the range of 41.74%-51.98%. In contrast, α-Thujene had efficacy (1%-10%) in previous studies but ranged from 16.02% to 16.49% in the current study (Burits and Bucar, 2000; Erdoğan et al., 2023; Mehalaine and Chenchouni, 2021; Wajs et al., 2008). Also in the present study, nerol was reported for the first time in NS seeds.

3.2. Antibacterial Activity

The results of the antibacterial activity test volatile oil samples according to the disc diffusion method are given in Table 2. The antibacterial activity of the volatile oils was compared to imipenem which is effective for both gram-negative and positive bacteria, and was utilized as a positive control at a concentration of 10 mcg. Volatile oils (5 µL) showed antibacterial activities against *Staphylococcus aureus*, *Clostridium perfringens*, *Enterococcus faecalis*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, Salmonella *enterica*, *Salmonella enteritidis*, *Bacillus cereus*, *Listeria monocytogenes*,

Escherichia coli. Clostridium perfringens (zone inhibition: 35 to 39.3 mm) and *Pseudomonas aeruginosa* (zone inhibition: 29.7 to 38.7 mm) were found to be most sensitive to all volatile oils tested. While imipenem had a 17 mm inhibition zone on *Clostridium perfringens* bacterial chain, it showed 24,3 mm inhibition zone on *Pseudomonas aeruginosa* bacterial strain. Additionally, Erzincan Provincial Authority of Agriculture and Forestry (EAF) volatile oil (40,3 mm) exhibited higher sensitivity to *Bacillus cereus* compared to imipenem (30 mm). Furthermore, all volatile oils exhibited moderate antibacterial activity compared to imipenem. The antibacterial effects of *Nigella sativa* (NS) volatile compounds in the examined volatile oils might be attributed to the various molecules found with the GC-MS, such as *α*-thujene, *p*-cymene and nerol. When examined by previous studies, it has been reported that *α*-thujene, *p*-cymene, and nerol secondary metabolites exhibit high antibacterial effects against various bacterial strains (Joshi et al., 2019; Marchese et al., 2017; Yang et al., 2023). Similar to our results, a study was reported that NS volatile oil had antibacterial effects on *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Escherichia coli* (Kazemi, 2014). Again, in many studies conducted on NS seeds, volatile oils containing similar major monoterpenes showed high antibacterial effects (Daoudi et al., 2022; Salman et al., 2016; Ugur et al.,2016). It is also claimed that *p*-cymene inhibits *Escherichia coli* and *Staphylococcus aureus* by disturbing of bacterial membran's lipids (Cristani et al., 2007).

According to experts, the search for antibiotic alternatives is important since the antibiotic age is coming to an end. Volatile oils, being natural extracts with limited side effects, may become reliable antibacterial agents. Furthermore, the fight against multidrug resistant bacteria must be won by ways other than antibiotics, and volatile may play a role in this. Overall, previous studies were suggested that volatile oils rich in *α*-thujene, *p*-cymene, and nerol exhibit the highest antibacterial activity against various pathogenic bacteria. Consequently, the potent antibacterial activity of *N. sativa* volatile oils is attributed to its key components.

Table 1. Chemical constituents identified in the *Nigella sativa* L. volatile oils

				ELM	EAF	Konya
Compounds	RT	RI	RI (NIST)	Composition (%)	Composition (%)	Composition (%)
Diacetone alcohol	3.70	846	847	0.08	0.08	0.07
α-Thujene	5.58	912	911	16.49	20.56	16.02
α-Pinene	5.76	916	917	3.63	4.73	3.51
Camphene	6.14	934	933	1.06	1.07	1.05
Sabinene	6.79	962	961	2.36	2.08	2.30
α-Myrcene	7.25	982	986	0.41	0.46	0.41
α-Phellandrene	7.63	997	995	0.6	1.10	0.5
α-Terpinene	7.98	1013	1017	0.43	0.31	0.42
p-Cymene	8.25	1018	1021	51.98	41.74	52.39
Limonene	8.34	1020	1030	4.53	3.47	4.54
γ-Terpinene	9.18	1062	1064	2.39	1.81	2.44
trans-Sabinene hydrate	9.46	1098	1101	0.04	0.07	0.04
α-Terpinolene	10.04	1102	1104	0.16	0.07	0.17
Lavandulol	10.27	1160	1170	0.46	1.30	0.51
Linalool	10.38	1085	1080	0.08	0.10	0.09
4-terpineol	12.64	1210	1206	0.62	1.68	0.62
trans-2-Caren-4-ol	13.36	1223	1222	0.69	0.73	0.66
Nerol	14.14	1228	1230	7.91	8.50	8.06
Carvone	14.49	1230	1231	2.08	2.57	2.08
Thymoquinone	14.65	1255	1260	0.48	1.63	0.46
α-Fenchyl acetate	15.63	1262	1265	0.16	0.16	0.16
Carvacrol	16.18	1279	1280	0.34	0.32	0.34
α-Longipinene	17.38	1356	1350	0.34	0.44	0.34
α-Copaene	18.04	1371	1376	0.06	0.05	0.06
Longifolene	18.83	1401	1402	0.51	0.55	0.53
trans-Caryophyllene	19.17	1449	1444	0.20	0.12	0.21
α-Gurjunene	21.75	1469	1490	0.07	0.09	0.07
Elemol	22.34	1537	1547	0.06	0.08	0.07
Total				98.22	95.94	98.12

Compounds were listed in order of elution on HP-5MS column, RT= retention time, RI= Retention Indices, Bolded name = Chemotype
ELM= Erzincan from local market, EAF= Erzincan Provincial Authority of Agriculture and Forestry, K= Konya

Table 2. Disc diffusion results (mm) of Nigella sativa volatile oils for tested pathogenic bacteria

Bacterial strains	Origins			
	ELM (5 µL)	EAF (5 µL)	K (5 µL)	Imipenem (10 mcg)
Staphylococcus aureus	45.3±0.6	27.0±1.0	44.3±0.6	45.3±0.6
Clostridium perfringens	39.3±2.3	35.0±1.0	38.3±2.3	17.0±1.0
Enterococcus faecalis	45.0±1.0	11.7±0.6	45.3±0.6	45.0±1.0
Pseudomonas fluorescens	41.7±2.1	34.3±1.2	21.7±3.5	45.7±1.5
Pseudomonas aeruginosa	37.3±1.5	29.7±1.5	38.7±2.1	24.3±2.5
Salmonella enterica	17.3±1.2	31.7±2.1	16.0±1.7	33.7±1.2
Salmonella enteritidis	18.0±1.0	26.3±1.5	16.0±2.0	43.3±1.5
Bacillus cereus	17.0±1.0	40.3±2.1	11.3±0.6	30.0±1.0
Listeria monocytogenes	16.0±2.0	30.0±2.0	21.3±0.6	36.3±2.1
Escherichia coli	17.3±2.1	35.0±0.6	18.3±0.6	35.3±2.1

ELM= Erzincan from local market, EAF= Erzincan Provincial Authority of Agriculture and Forestry, K= Konya

4. Conclusion

The chemical compounds identified in this study through GC-MS analysis were obtained via hydrodistillation, followed by an investigation into their chemical variation and assessment of their antibacterial efficacy. Despite the wide geographical distribution of *Nigella sativa* (NS) seeds, a remarkable similarity was observed in the composition of the volatile oil, as well as in its demonstrated activity against various bacterial strains. Because NS volatile oils were sensitive to both gram-positive and gram-negative organisms, NS volatile oils provide a rich supply of phytochemical substances that may be employed in the treatment of many infectious disorders caused by microbial pathogens.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	S.G	Z.A
C	50	50
D	50	50
S	100	
DCP	50	50
DAI	50	50
L	50	50
W	80	20
CR	80	20
SR	100	
PM	60	40

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Approval/Informed Consent

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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