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Research Article

Antimicrobial Activity and Chemical Composition of Essential Oil Extracted from *Hyoscyamus niger* L. Inflorescence

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Abstract
This study aimed to analyze the chemical composition and evaluate the antibacterial activity of essential oil extracted from the *Hyoscyamus niger* L.inflorescence collected from Erzincan, Türkiye. The essential oil was extracted using hydrodistillation and analyzed by GC-MS, identifying 23 components, with major constituents being 10-heneicosene (35.72%), phytol (20.50%), and acetic acid, butyl ester (10.10%). The antibacterial activity was tested against six bacterial strains, including *Staphylococcus aureus*, *Clostridium perfringens*, *Enterococcus faecalis*, *Bacillus cereus*, *Listeria monocytogenes*, and *Escherichia coli*, using the disc diffusion method. The *H. niger* inflorescence essential oil exhibited moderate antibacterial activity, specifically against *C. perfringens*, with a zone of inhibition of 11.7 ± 1.2 mm compared to the positive control imipenem (17.0±1.0 mm). The results suggest that *H. niger* inflorescence essential oil has potential as an antibacterial agent, highlighting the importance of exploring plant-derived compounds as alternatives to traditional antibiotics in combating multidrug-resistant bacteria.
Keywords: *Hyoscyamus niger* L., Essential oil, Antibacterial, GC-MS

Hyoscyamus niger L. Çiçeklerinden Elde Edilen Uçucu Yağın Antimikrobiyal Aktivitesi ve Kimyasal Bileşimi

Öz
Bu çalışmada, Erzincan, Türkiye'den toplanan *Hyoscyamus niger* L. çiçek durumlarından elde edilen uçucu yağların kimyasal bileşimi ve antibakteriyel aktivitesi incelenmiştir. Uçucu yağlar hidrodistilasyon yöntemiyle elde edilmiş ve kimyasal bileşenleri GC-MS ile analiz edilmiştir. Analiz sonucunda, uçucu yağda toplam 23 bileşen tespit edilmiştir; majör bileşenler 10-heneikosan (%35,72), fitol (%20,50) ve asetik asit, bütil ester (%10,10) olarak belirlenmiştir Antibakteriyel aktivite, disk difüzyon yöntemi kullanılarak *Staphylococcus aureus*, *Clostridium perfringens*, *Enterococcus faecalis*, *Bacillus cereus*, *Listeria monocytogenes* ve *Escherichia coli* dahil olmak üzere altı bakteri suşuna karşı test edilmiştir. *H. niger* çiçek durumlarından elde edile*n* uçucu yağ, pozitif kontrol imipenem (17.0±1.0 mm) ile karşılaştırıldığında 11.7 ± 1.2 mm'lik inhibisyon alanıyla özellikle *C. perfringens*'e karşı kabul edilebilir seviyede antibakteriyel aktivite sergilediği gözlenmiştir. Sonuçlar, *H. niger* çiçek durumlarından elde edilen uçucu yağın antibakteriyel potansiyele sahip olduğunu ve çoklu-antibiyotik dirençli bakterilerle mücadelede doğal kaynaklı bileşiklerin antibiyotiklere alternatif olarak araştırılmasının önemi vurgulanmıştır.
Anahtar Kelimeler: *Hyoscyamus niger* L., Uçucu yağ, Antibakteriyel, GC-MS

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1. Introduction

The genus *Hyoscyamus* (Solanaceae) comprises six species in the flora of Turkey: *Hyoscyamus aureus* L., *Hyoscyamus albus* L., *Hyoscyamus muticus* L., *Hyoscyamus niger* L., *Hyoscyamus pusillus* L., and *Hyoscyamus reticulatus* L. [1]. *H. niger* (black henbane) is the most renowned species of the *Hyoscyamus* genus. It has been utilized as a medicinal plant since ancient times in both Greece and Türkiye[2-4]. The mature corolla of *H. niger* is a lurid yellow, typically veined with purple; the fruiting calyx is constricted in the middle; and the upper cauline leaves are amplexicaul [1].

H. niger is used in folk medicine as an antiasthmatic, anthelmintic and for toothaches [2-5]. Phytochemical investigations show that all parts of *H. niger* contain hyoscyamine and scopolamine [6]. In addition to these compounds, lignanamide, tyramine derivative, and non-alkaloid compounds were isolated from *H. niger* seeds, and many secondary metabolites were identified in its essential oil [7,8]. Previous studies have reported the antibacterial, antifungal antiviral, and insecticidal activities of *H. niger* seeds [1, 7-10]. Additionally, the essential oil of *H. niger* has been reported to exhibit antibacterial and antioxidant properties. [8].

Recent studies have demonstrated the wide-ranging pharmacological effects of essential oils, driven by their complex mixtures of monoterpenes, sesquiterpenes, phenolics, and other volatile compounds. For instance, essential oils from medicinal plants have been shown to exhibit significant antimicrobial, anti-inflammatory, and antioxidant activities, with potential applications against antibiotic-resistant pathogens [11–13]. Advances in analytical techniques, such as GC-MS and LC-MS, have enabled the precise characterization of these oils, linking their bioactivities to specific chemical constituents [14].

Antibiotic resistance has become a serious problem affecting millions of people globally. Because of this resistance, there has been an increase in research into alternative solutions, such as medicinal plants [15,17]. As a result, the World Health Organization (WHO) has published a list of global priority diseases caused by multidrug-resistant bacteria to discover novel effective drugs [16]. The aim of this study is to investigate the chemical composition of the essential oil from *H. niger* inflorescence collected in Erzincan, Türkiye, and to evaluate their antibacterial activity against six bacterial strains: five gram-positive strains, *Staphylococcus aureus, Clostridium perfringens, Enterococcus faecalis, Bacillus cereus*, *Listeria monocytogenes,* and one gram-negative strain, *Escherichia coli*.

2. Materials And Methods

a. Plant Material

The plant material was collected in June 2024 at inflorescence stage from Kurutilek village in Erzincan, Turkiye (39° 46' 33.47" N, 39° 29' 38.57"E), located at an altitude of 1740 m. Fresh inflorescences of *Hyoscyamus niger* were used for the essential oil extraction. The herbarium specimens were prepared by drying the collected plant material under shaded conditions with adequate ventilation to ensure preservation and prevent degradation of morphological and

chemical characteristics. The authentication of voucher specimens was conducted by Prof. Dr. Ali Kandemir and deposited at the Herbarium of Erzincan Binali Yıldırım University in Erzincan, Turkiye (EBYU 000005).

b. Essential Oil Extraction

A total of 500 grams of fresh *Hyoscyamus niger* inflorescence (HN) were mixed and subjected to hydrodistillation for 3 hours using a Clevenger apparatus according to the method recommended in the European Pharmacopoeia. The extraction procedure was carried out in triplicate. Subsequently, the essential oil were carefully collected and stored in sealed sample tubes, which were then kept at 4 °C until analysis [31].

2.3. GC-MS Analysis Conditions

GC-MS analyzes were carried out utilizing a Thermo Scientific Trace 1310 GC-MS (Trace 1310, Thermo Scientific, Milano, Italy) system equipped with an HP-5MS capillary column (30 m x 0.25 mm and 0.25 m ID), in accordance with previously reported techniques [17,18]. In split mode, helium was employed as the carrier gas, with a 50:1 ratio and a constant rate of flow of 1.2 mL/min. The mass transfer line and injection site were both set to 280 °C. Starting at 60 °C for three minutes, the temperature of the column oven was set to increase to 200 °C at a rate of 3 °C/min for 0 minutes, and then ramp up to 240 °C at a rate of 5 °C/min for five minutes. The mass spectrometer was set up with the following parameters: electron ionization (EI) mode was used with an ionization energy of 70 eV, and the ion source temperature was maintained at 280 °C. Based on a homolog n-alkane series (C8–C40), the Van den Dool and Kratz equation was used to compute the retention index (RI) for each secondary metabolite. Chemical identity was validated using NIST2004 MS libraries and Wiley. The relative peak area percentages of each chemical were calculated using the peak areas from the MS chromatograms.

2.4. Antibacterial Activity

Staphylococcus aureus (ATCC 6538), *Clostridium perfringens* (ATCC 13124), *Enterococcus faecalis* (ATCC 8459), *Bacillus cereus* (ATCC 10876), *Listeria monocytogenes* (ATCC 51774) and *Escherichia coli* (ATCC 25922) were examined for antibacterial activities. The standard protocol was followed for performing the disc diffusion test [19]. Sterile blank discs measuring 6 mm were put on Nutrient Agar medium after 100 µL of bacteria (10^8 cells/ml) was added. A blank disc was injected with 5 µL of the sample, and a positive control disc containing the antibiotic imipenem was put on the medium. Plates were incubated for 24 hours at 37 °C. A digital caliper was utilized to measure the inhibitory zones and report the mean diameter ± standard deviation of three replications in millimeters.

3. Results and Discussion

3.1. The composition of *Hyoscyamus niger* Essential oil

The hydrodistillation technique was used to produce essential oil from *Hyoscyamus niger* inflorescence (HN) with a yield of 0.06% (yellow oil). There were 23 components found in the essential oil of HN, accounting for 97.17% (v/w) of the total oil. Inci at all. detected 22 secondary metabolites in the essential oil obtained from the aerial parts of *H. niger*. It was also reported that 42 secondary metabolites were identified in the essential oil obtained from the *H. niger* seeds [8]. It is known that the difference observed in these yields is due to the climate and environmental conditions in which the plants grow [21]. Table 1 presented the chemical composition of essential oil along with the percentage of each secondary metabolites, retention index (RI), and retention time (RT). Acetic acid, butyl ester (10.10%), 10-heneicosene (35.72%), and phytol (20.50%) were determined to be the major components (Table 1). In the study conducted on *H. niger* aerial parts essential oil, hexahydrofarnesyl acetone (19.19%) and phytol (52.09%) were reported as major components. In the same study again conducted on *H. niger* seeds essential oil, the major component was reported to be hexahydrofarnesyl acetone (46.36%) [8]. In the presented study, hexahydrofarnesyl acetone was found to be 0.11%. In another study, it was reported that the major components of the essential oil obtained from *H. niger* leaves were *n*-Eicosane and phytol [30]. These results support the presented study. Although borneol was detected as the major secondary compound in the essential oils of *Hyoscyamus* sp., borneol was not identified in the presented study [20, 29]. Additionally, In the presented study, the presence of 20 compounds was identified in the essential oil of HN for the first time (Acetic acid, butyl ester, ethylbenzene, *p*-xylene, styrene, methylbutyl propanoate, *α*-pinene, eucalyptol, linalool, camphor**,** caryophyllene, γ-elemene, nerolidol, germacrene B, dodecanoic acid, 8-heptadecene, 2-hexadecanol, 9-nonadecene, 9-eicosene, 10-heneicosene, 9-octadecenoic acid) [8,30].

Table 1. Chemical constituents identified in the *H. niger* L. esential oil

Compounds	RT	RI	RI (NIST)	Composition (%)
Acetic acid, butyl ester	**3.69**	**813**	**814**	**10.10**
Ethylbenzene	4.58	865	867	0.94
p-xylene	4.73	883	882	4.99
Styrene	5.18	894	895	3.68
Methylbutyl propanoate	5.52	969	968	0.22
α-pinene	7.23	945	945	0.20
Eucalyptol	8.68	1043	1045	0.67
Linalool	10.56	1101	1102	0.15

Camphor	11.83	1145	1145	1.84
Caryophyllene	19.21	1443	1442	0.32
γ-elemene	19.52	1445	1440	0.15
Nerolidol	20.05	1531	1533	0.11
Germacrene B	22.55	1562	1559	0.69
Dodecanoic acid	22.70	1571	1572	0.52
8-heptadecene	25.13	1678	1678	1.49
2-hexadecanol	27.22	1705	1706	0.13
Hexahydrofarnesyl acetone	28.67	1848	1847	0.11
9-nonadecene	29.23	1894	1892	3.55
9-eicosene	29.74	1914	1916	9.42
10-heneicosene	**33.49**	**2060**	**2061**	**35.72**
9-octadecenoic acid	34.29	2125	2125	0.11
Phytol	**34.70**	**2149**	**2148**	**20.50**
Phytol, acetate	35.25	2227	2228	1.56
Total				97.17

RT: Retention time, RI: Retention index, RI (NIST): RI literature (NIST webbook)

3.2. Antibacterial Activity

Antibacterial activity test results of *Hyoscyamus niger* inflorescence (HN) essential oil are presented in Table 2. In this context, it was determined that HN essential oil (5 μL) exhibited moderate antibacterial activity against all bacterial chains compared to the positive control imipenem (10 mcg). Inci et al. reported in their study that HN essential oil exhibited higher activity against *E. coli* (inhibition zone: 32 mm) and *S. aureus* (inhibition zone: 21 mm) compared to the positive control streptomycin (zone inhibition: 30 mm- 20mm, respectively) [8]. This difference is attributed to the difference in the components of essential oil in the presented study. In a different study, the methanol extract extracted from the seeds of *H. niger* has been found to have significant antibacterial activity (inhibition zone: 25.0 mm) against *S. Aureus* [22]. Chalabian et al. also reported that the alkaloid extract obtained from *H. niger* root and aerial parts had strong antibacterial activity [23]. Vanitha et al. tested the antibacterial activity of 10-heneicosene, the major component of HN essential oil, on *S. pneumoniae* and *A. fumigatus*. Vanitha et al. reported that 10-heneicosene has strong antibacterial activity [24]. When we look at the antibacterial activity studies on the other major component, phytol, it has been reported that it has strong antibacterial activity on *E. coli* [25] and *P. aeruginosa* [26]

bacterial chains (growth inhibition MIC: 62.5 μg/mL-19 μg/mL, respectively). In another study conducted on phytol, it was shown that it exhibited strong antibacterial activity on *Clostridium sporogenes*, *Sarcina lutea*, and *E. faecalis* bacterial chains [27]. It has been emphasized that phytol exerts antibacterial effects by dysregulating the function of eukaryotic cells through a series of effects, including disruption of membrane permeability and depolarization of the mitochondrial membrane [24-28]. According to this information, the antibacterial activity and antibacterial activity mechanism of HN essential oil can be explained.

Table 2. Disc diffusion results (mm) of *H. niger* L. essential oil for tested pathogenic bacteria

Bacterial strains	**HN (5 μL)**	**Imipenem* (10 mcg)**
Staphylococcus aureus	14.3±0.5	45.3±0.6
Clostridium perfringens	11.7±1.2	17.0±1.0
Enterococcus faecalis	13.0±0.9	45.0±1.0
Bacillus cereus	13.3±1.2	30.0±1.0
Listeria monocytogenes	7.0±1.7	36.3±2.1
Escherichia coli	15.7±1.5	35.3±2.1

HN: *Hyoscyamus niger* inflorescence essential oil, *: Positive control (Imipenem)

4. Conclusion

The chemical compounds identified in this study using GC-MS analysis were produced through hydrodistillation, which was followed by an examination of their chemical variation and antibacterial activity. Despite the broad geographical distribution of *Hyoscyamus niger* inflorescence (HN), there were small variations in the essential oil composition and showed efficacy against various bacterial strains. According to scientists, the search for antibiotic alternatives is critical since the antibiotic era is ending. HN essential oil may be effective antibacterial agents. Furthermore, the war against multidrug-resistant bacteria must be fought by means other than antibiotics, and volatiles may play an essential role in this.

Ethics in Publishing

There are no ethical issues regarding the publication of this study**.**

Conflict of Interest

The authors declare no competing interests.

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Author Contribution

S.G. conceived and designed the research. S.G., Z.A, and H.A. conducted experiments. Z.A. and H.A. evaluated the analysis results. S.G. and H.A. drafted the manuscript. All authors have read and agreed to the published version of the manuscript.

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