

## Chemical compositions of essential oils, antimicrobial effect and antioxidant activity studies of *Hyoscyamus niger* L. from Türkiye

Şule İnci<sup>1</sup>, Pelin Yılmaz Sancar<sup>2\*</sup>, Azize Demirpolat<sup>3</sup>, Sevda Kırbağ<sup>2</sup>,  
Semsettin Civelek<sup>2</sup>

<sup>1</sup>Rumeli University, Vocational School of Health Services, İstanbul, Türkiye

<sup>2</sup>Fırat University, Faculty of Sciences, Department of Biology, Elazığ, Türkiye

<sup>3</sup>Bingöl University, Vocational School of Food, Agriculture and Livestock, Bingöl, Türkiye

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**Abstract:** This study investigated the essential oil components of *Hyoscyamus niger* L. and their antimicrobial and antioxidant properties. Essential oils were extracted separately from the aerial parts and seeds of the plant using the hydrodistillation method. Antimicrobial activity was evaluated using the disk diffusion method, while antioxidant activity was assessed by measuring the total antioxidant status (TAS), total oxidant status (TOS), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity. The primary essential oil components of the aerial parts were identified as phytol (52.09%) and hexahydrofarnesyl acetone (19.66%). Hydrodistillation of the seeds yielded 0.7% (v/w) yellow oils, comprising 41 components that accounted for 99.0% of the oil. The major components in the seed oils were hexahydrofarnesyl acetone (46.36%) and hexanal (9.05%). Methanol extracts of the aerial parts demonstrated inhibitory effects on pathogenic microorganisms, with inhibition zones ranging from 13±0.46 to 32±0.11 mm. The TAS and TOS values of the methanol extracts were calculated as 3.77±0.0 mmol and 6.94±0.0 µmol, respectively. The DPPH radical scavenging activity increased with rising extract concentrations. These findings highlight the potential antimicrobial and antioxidant applications of *H. niger* essential oils and methanol extracts.

## 1. INTRODUCTION

Today, medicinal plants continue to play a vital role in modern medicine and pharmacy, serving as valuable natural sources of pharmaceutical raw materials due to their essential oils and secondary metabolites (Akbaş *et al.*, 2020). The Solanaceae family, which includes many important medicinal plants, is a diverse plant family consisting of 98 genera and approximately 2,700 species. Plants from the Solanaceae family have been utilized for centuries by humans as sources of food, ornamental plants, and medicinal remedies (Guha & Maheshwari, 1966). This family is particularly notable for its richness in alkaloids with potent pharmacological effects, such as scopolamine, atropine, and hyoscyamine, making it highly significant both medically and economically (Maiti *et al.*, 2002).

\*CONTACT: Pelin YILMAZ SANCAR ✉ [peyilmaz@firat.edu.tr](mailto:peyilmaz@firat.edu.tr) 📍 Fırat University, Faculty of Sciences, Department of Biology, Elazığ, Türkiye

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*Hyoscyamus niger* L. (black henbane) is an annual or biennial herbaceous plant belonging to the Solanaceae family. It is commonly found in rocky areas, uncultivated lands, and especially along roadsides (Orbak *et al.*, 1998; Li *et al.*, 2011; Yücel & Yılmaz, 2014). This plant is a valuable resource for the pharmaceutical industry due to its alkaloid content, which includes some of the oldest drugs used in medicine (Orbak *et al.*, 1998; Pudersell *et al.*, 2012; Dehghan *et al.*, 2012). Traditionally, *H. niger* has been used to treat various ailments, including stomach pains, ulcers, and kidney and liver disorders, and is known for its analgesic and antispasmodic effects (Tanker *et al.*, 1998; John *et al.*, 2010). However, the consumption of its roots and leaves can lead to poisoning, as some of its secondary metabolites paralyze the nerve endings of the parasympathetic system in humans (Orbak *et al.*, 1998).

In addition to its medicinal applications, *H. niger* has a history of being used as a hallucinogen. This usage dates back to ancient times, as evidenced by ancient Greek writings, and the plant's hallucinogenic properties were introduced to Europe in the Middle Ages. The seeds and leaves of *H. niger* contain hyoscyamine, scopolamine, and other tropane alkaloids, which are responsible for its hallucinogenic effects as well as symptoms like ataxia and hypertension (Ugur, 2013). Due to its toxicity, *H. niger* is considered extremely poisonous and undesirable for consumption, and it has been historically used as a poison (Haas, 1995).

Despite its toxic nature, recent studies have highlighted potential therapeutic applications of *H. niger*. For instance, Kosari *et al.* (2021) conducted a clinical study in Iran in which both a placebo and a methanolic extract of *H. niger* were administered to 50 participants. The extract was given three times a day for six days, and the results showed beneficial effects in improving COVID-19 symptoms. Symptoms such as dry cough, shortness of breath, sore throat, chest pain, fever, dizziness, headache, abdominal pain, and diarrhea were reported to have improved more significantly in the group receiving *H. niger* extract combined with propolis than in the placebo group.

This study aims to determine the biological activity characteristics of *H. niger*, a medicinal plant. With the growing need for natural therapeutic resources against diseases, identifying the biological properties of plants that grow naturally in specific regions can support their use as valuable pharmaceutical raw materials. In this study, the essential oil properties, antimicrobial activity, and antioxidant properties of *H. niger* are evaluated together for the first time.

## 2. MATERIAL and METHODS

### 2.1. Collection of the Plant Material

The flowering period of *H. niger* occurs from May to June. During this period, plant materials were collected from the Baskil district of Elazığ province and preserved through shade drying. Following the collection, the plant materials were identified at the Firat University Herbarium (FUH) using the Flora of Turkey as a reference. The identification was carried out by Şemsettin Civelek. The general appearance of the plant is presented in [Figure 1](#).

### 2.2. Isolation and Analysis of Essential Oils

Essential oils were extracted from shade-dried *H. niger* plants using the hydrodistillation method. Approximately 250 g of dried plant material was boiled in a Clevenger apparatus for 4 hours, and the essential oils were collected through distillation. The extracted essential oil was stored at +4°C until further analysis (Demirpolat, 2023).

The essential oils were analyzed using gas chromatography-flame ionization detection/mass spectrometry (GC-FID/MS) with an HP 7890A GC system, 5975C MS, and a flame ionization detector (FID) at the Bingöl University Research Laboratory. A series of n-alkanes were employed as reference points to calculate retention indices (RI). The mass spectrometer was operated at 70 eV, scanning a mass range of 35–425 m/z. Compound identification was carried out by comparing the retention indices and mass spectra of the samples with those in the Wiley-NIST W9N11 libraries.



**Figure 1.** General view of *H. niger*.

### **2.3. Determination of the Antimicrobial and Antioxidant Effect**

#### **2.3.1. Antimicrobial analysis**

The antimicrobial effect of the methanol extract of the plant sample was evaluated using the disk diffusion method (Collins & Lyne, 2004). Various bacteria, yeast, and dermatophyte fungi were used as test microorganisms in this study. All microbial cultures were obtained from the Microbiology Laboratory culture collection of the Department of Biology, Faculty of Science, Firat University. The microorganisms used in the study, along with their strain numbers, are listed in [Table 2](#).

Bacterial strains were inoculated into Nutrient Broth and incubated at  $35\pm 1^\circ\text{C}$  for 24 hours, yeast strains were inoculated into Malt Extract Broth (MEB) and incubated at  $25\pm 1^\circ\text{C}$  for 48 hours, while dermatophyte fungi were inoculated into Glucose Sabouraud Broth (GSB) and incubated under the same conditions. The prepared bacterial, yeast, and fungal cultures ( $10^6$  bacteria/mL,  $10^4$  yeast/mL,  $10^4$  fungi/mL) were inoculated into Mueller Hinton Agar (MHA), Sabouraud Dextrose Agar (SDA), and Potato Dextrose Agar (PDA), respectively, at a concentration of 1%. After thorough mixing, the inoculated media were poured into sterile petri dishes.

Disks impregnated with 100  $\mu\text{l}$  of the plant extract were gently placed onto the solidified media in the petri dishes. The plates were kept at  $4^\circ\text{C}$  for 1–2 hours to allow the diffusion of the extract and subsequently incubated. Plates containing bacteria were incubated at  $37\pm 0.1^\circ\text{C}$  for 24 hours, while those containing yeasts were incubated at  $25\pm 0.1^\circ\text{C}$  for 72 hours. Different standard disks were used as controls: Streptomycin sulphate (10  $\mu\text{g}/\text{disc}$ ) for bacteria and Nystatin (30  $\mu\text{g}/\text{disc}$ ) for yeasts. The resulting inhibition zones were measured in millimeters (mm).

#### **2.3.2. Antioxidant assays**

In this phase of the study, the total oxidant status (TOS) and total antioxidant status (TAS) of the plant samples were measured using Rel Assay kits. Trolox was used as the calibrator for TAS, while hydrogen peroxide served as the calibrator for TOS. The TAS value was expressed as mmol Trolox equivalent/L, and the TOS value was expressed as  $\mu\text{mol H}_2\text{O}_2$  equivalent/L (Erel, 2004; 2005).

The antioxidant activity of the plant's methanol extract was also assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging capacity method (Cuendet *et al.*, 1997; Kirby & Schmidt, 1997). A stock solution of the plant extract was prepared in methanol at a

concentration of 1 mg/mL, and this solution was serially diluted to generate the DPPH calibration curve. From the prepared solutions, 40  $\mu$ L was mixed with 160  $\mu$ L of DPPH solution. The mixture was kept in the dark for 30 minutes to prevent photodegradation, and this process was repeated for all concentrations. Methanol was used as the control.

At the end of the incubation period, absorbance values were measured at a wavelength of 570 nm using an ELISA reader. The percentage of inhibition was calculated using the following formula:

$$I (\%) = (A_{\text{control}} - A_{\text{sample}} / A_{\text{control}}) \times 100$$

This methodology enabled a quantitative evaluation of the antioxidant activity of the plant extract.

## 2.4. Statistical Analysis

The results obtained from antimicrobial and antioxidant analyses were statistically evaluated using the SPSS 22.0 package program and  $p < 0.05$  was considered significant.

## 3. FINDINGS

### 3.1. Essential Oil Studies

The composition of the essential oils extracted from the aerial parts and seeds of *H. niger* is presented in Table 1, which details the percentage composition and retention indices of the identified components. The seeds of *H. niger* were hydrodistilled separately, yielding 0.5% (v/w) of yellow oils. The analysis of the seed essential oils identified 41 components, accounting for 99.0% of the total oil composition. The major components were hexahydrofarnesyl acetone (46.36%) and hexanal (9.05%). Similarly, the aerial parts of the plant were hydrodistilled, yielding 0.4% (v/w) of light yellowish oils. The analysis identified 22 components in the essential oils from the aerial parts, representing 97.51% of the total oil composition. The predominant component was phytol (52.09%), followed by hexahydrofarnesyl acetone (19.66%). Further analysis of the essential oils revealed the presence of sesquiterpenes in both the seed and aerial part oils, highlighting the diverse chemical profile of *H. niger*.

**Table 1.** Constituents of the essential oils from *H. Niger*.

No	R.Time	Area	Name	<i>H. niger</i> seeds	<i>H. niger</i> aerial parts
1.	5.159	48718	1-Pentanol	0.82	-
2.	5.618	14757	3-Hexanone	0.56	-
3.	5.761	58489	2-Hexanone	0.99	-
4.	5.945	25972	3-Hexanol	0.44	-
<b>5.</b>	<b>6.033</b>	<b>534856</b>	<b>Hexanal</b>	<b>9.05</b>	<b>0.11</b>
6.	8.647	112848	1-Hexanol	1.91	-
7.	9.497	55310	2-Heptanone	0.94	-
8.	9.942	73828	Heptanal	1.25	-
9.	12.240	45719	Hydroperoxide, 1-ethylbutyl	0.77	0.45
10.	12.459	96863	Undecanone	1.64	-
11.	12.605	29708	Benzaldehyde	-	0.22
12.	13.261	44576	1-Heptanol	0.75	-
13.	13.693	43030	Amyl vinyl carbinol	0.73	-
14.	13.904	30506	2,3-Octanedione	0.52	-
15.	14.224	175344	Furan, 2-pentyl-	2.97	-
16.	14.786	105944	Octanal	1.79	-
17.	15.129	30388	Cloroben	0.51	-
18.	16.610	50606	3-Octen-2-one	0.86	-
19.	17.541	90244	Octenal	1.53	-
20.	18.310	55427	1-Octanol	0.94	-
21.	19.918	144251	Nonanal	2.44	-

22.	22.712	50773	2-Nonenal, (E)-	0.86	-	
23.	25.031	32352	Decanal	0.55	-	
24.	27.750	73534	2-Decenal, (E)-	1.24	-	
25.	28.249	75682	Silane	1.28	-	
26.	30.345	186050	2,4-Decadienal, (E,E)-	3.15	-	
27.	32.529	72146	2-Undecenal	1.22	-	
28.	33.020	116446	2-Octenal, 2-butyl-	1.97	-	
29.	37.239	28290	6-Undecanol	0.48	-	
30.	38.006	55612	trans- $\beta$ -Ionone	0.42	-	
31.	43.130	43977	Tetradecanal	0.74	-	
32.	45.410	27984	Hexadecamethylcyclo octasiloxane	0.47	-	
33.	45.546	52687	1-4- $\gamma$ -H-Pregna	0.89	-	
34.	45.971	55390	2,2,6,7-Tetramethyl-10-oxatricyclo[4.3.0.1(1,7)]decan-5-one	0.94	-	
35.	46.496	35940	2-Pentadecanone	0.61	-	
36.	46.509	50401	Nonadecane	-	0.38	
37.	47.079	87288	Tetradecanal	1.48	0.31	
38.	50.552	78277	2-Ethylhexyl salicylate	-	0.59	
39.	50.827	63244	Tetradecanal	1.07		
40.	51.040	43682	1-Dodecanol, 2-octyl-	0.74		
41.	51.633	70940	Neophytadiene	-	0.53	
<b>42.</b>	<b>51.860</b>	<b>2683617</b>	<b>Hexahydrofarnesyl acetone</b>	<b>46.36</b>	<b>19.19</b>	
43.	52.295	74391	Versalide	-	0.56	
44.	52.528	63003	2-Pentadecanone, 6,10,14-trimethyl-	-	0.47	
45.	52.720	35371	Phthalic acid	-	0.27	
<b>46.</b>	<b>53.747</b>	<b>95393</b>	<b>Heneicosane</b>	-	<b>7.02</b>	
47.	54.443	228392	Farnesyl acetone	-	1.71	
48.	54.653	91116	Hexadecanoic acid, methyl ester	-	0.68	
49.	55.413	204847	Isophytol	-	1.54	
50.	57.106	34439	2-methyloctacosane	-	0.26	
51.	57.352	116782	Spathulenol	-	0.88	
52.	60.193	88314	8,11-Octadecadienoic acid, methyl ester	1.49	0.60	
53.	60.391	74347	Oleic acid	1.26	-	
54.	60.509	48743	$\gamma$ -Stearolactone	0.82	-	
<b>55.</b>	<b>60.884</b>	<b>7121450</b>	<b>Phytol</b>	<b>0.96</b>	<b>52.09</b>	
56.	61.683	109259	$\alpha$ -ionone	-	0.82	
57.	62.019	46342	Neophytadiene	-	0.35	
58.	63.413	526003	Pentacosane	-	3.90	
59.	63.621	392579	Adenine	-	2.90	
60.	64.032	110852	Phytol, acetate	-	0.83	
61.	64.277	191580	Sulfurous acid	-	1.44	
				Total	99.99	98.52

### 3.2. Antimicrobial Effect

The antimicrobial activity of *H. niger* methanol extract against the tested microorganisms is summarized in Table 2. The extract produced inhibition zones of  $32 \pm 0.1$  mm against *E. coli*,  $21 \pm 0.2$  mm against *S. aureus*,  $20 \pm 0.5$  mm against *K. pneumoniae*,  $19 \pm 0.2$  mm against *B. megaterium*,  $23 \pm 0.1$  mm against *C. albicans*,  $20 \pm 0.4$  mm against *C. glabrata*,  $13 \pm 0.4$  mm against *Trichophyton* sp., and  $16 \pm 0.2$  mm against *Epidermophyton* sp. Among these, the methanol extract showed the strongest antimicrobial activity against *E. coli* (see Table 2).

Statistical analysis revealed that the antimicrobial effects of the methanol extract against *E. coli*, *S. aureus*, *K. pneumoniae*, *B. megaterium*, *C. albicans*, and *C. glabrata* were similar (Table 2,  $p > 0.05$ ). However, the results for *Trichophyton* sp. and *Epidermophyton* sp. were significantly different (Table 2,  $p < 0.05$ ). When compared with the control group, the differences in antimicrobial activity across all tested microorganisms were statistically significant (Table 2,  $p < 0.05$ ).

**Table 2.** Antimicrobial effect of *H. niger*.

Microorganism	<i>H. niger</i>	Standart Control
<i>Escherichia coli</i> (ATCC25322)	32±0.1 <sup>a</sup>	30±0.2 <sup>b</sup>
<i>Staphylococcus aureus</i> (ATCC25923)	21±0.2 <sup>a</sup>	20±0.1 <sup>a</sup>
<i>Klebsiella pneumoniae</i> (ATCC700603)	20±0.5 <sup>a</sup>	19±0.0 <sup>a</sup>
<i>Bacillus megaterium</i> (DSM32)	19±0.2 <sup>a</sup>	25±0.0 <sup>b</sup>
<i>Candida albicans</i> (FMC17)	23±0.1 <sup>a</sup>	25±0.2 <sup>b</sup>
<i>Candida glabrata</i> (ATCC66032)	20±0.4 <sup>a</sup>	20±0.1 <sup>a</sup>
<i>Trichophyton</i> sp.	13±0.4 <sup>a</sup>	22±0.0 <sup>b</sup>
<i>Epidermophyton</i> sp.	16±0.2 <sup>a</sup>	25±0.4 <sup>b</sup>

<sup>a, b</sup> Values shown with the same letters in each line are not different from each other, Values are means of three replicates ±SD

### 3.3. Antioxidant Activity

The TAS value of the *H. niger* methanol extract at a concentration of 1 mg/mL was measured as 3.77±0.0 mmol, while the TOS value at the same concentration was recorded as 6.94±0.0 µmol. These results indicate that the TAS value of the plant was notably high, whereas the TOS value was within the normal range (see Table 3).

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the methanol extract at 1000 µg/mL was determined to be 73.07±0.02%. In contrast, the antioxidant effect at 125 µg/mL was very low (see Table 4). The results demonstrate that the DPPH radical scavenging effect of *H. niger* methanol extracts increases proportionally with higher extract concentrations.

**Table 3.** TAS and TOS values of *H. niger*.

	TAS (mmol Trolox equiv./L)	TOS (µmol H2O2 equiv./L)
<i>H. niger</i> -MetOH	3.77±0.0 <sup>a</sup>	6.94±0.0 <sup>b</sup>

<sup>a, b</sup> Values shown with different letters in each line are different from each other ( $p < 0.05$ ), Values are means of three replicates ±SD

**Table 4.** Percent inhibition of the DPPH radical of root and leaf parts of *H. niger*.

	<i>H. niger</i> -MetOH
1000 µg/mL	73.07 ±0.02 <sup>d</sup>
500 µg/mL	56.73 ±0.07 <sup>c</sup>
250 µg/mL	27.88 ±0.44 <sup>b</sup>
125 µg/mL	16.82 ±0.29 <sup>a</sup>

<sup>a, b, c, d</sup> Means in the same column with the different superscripts are significantly different ( $p < 0.05$ ), Values are means of three replicates ±SD

## 4. DISCUSSION

In this study, the essential oil components of the aerial parts and seeds of *Hyoscyamus niger* were analyzed using GC-MS. The essential oils were found to be complex mixtures of sesquiterpenes and hydrocarbons. Compared to previous studies, such as Ting-guo (2013), who identified 29 components in *H. niger* leaves through HPLC, including palmitic acid (28.30%), linoleic acid (26.85%), oleic acid (14.39%), hexanal (10.24%), and stearic acid (1.81%) as major compounds, the current study showed similar results with hexanal (9.05%) and oleic acid (1.26%) identified. Differences in other components like palmitic and linoleic acids could be attributed to geographical and climatic variations, as well as the methods and equipment used.

Additionally, Ayari-Guentri *et al.* (2017) analyzed the aerial parts of *H. muticus* L. subsp. *falezlez* and found borneol (76.47%) as the main compound, followed by bornyl acetate (4.6%) and 1,3-bis(2-cyclopropyl-2-methylcyclopropyl) but-2-en-1-one (4.19%), with an oil yield of 0.50% (v/w). These compounds were absent in this study, likely due to variations in

geographical regions and ecological conditions, which significantly influence the composition of essential oils.

In a separate study, the methanolic extracts of *H. niger* and *H. reticulatus* were analyzed using HPLC, revealing and quantifying three major phenolic compounds: chlorogenic acid, rutin, and quercetin-3-O-glucoside-rhamnoside-rhamnoside-rhamnoside (Jassbi et al., 2014). Additionally, the oil and methanol extract of *H. niger* demonstrated antinematode activity (Kareem, 2020). The ethanol extract of *H. niger* seeds exhibited significant insecticidal activity against *Aphis laburni* and *Lucilia sericata* (Küpeli et al., 2020; Wang et al., 2006). The findings of this study align with existing literature. Secondary metabolites produced in plants during infections are critical defense mechanisms against pathogens and display substantial biological activity. The quality, quantity, and diversity of these secondary metabolites can vary based on habitat, soil, climatic conditions, and the specific plant organ where they are synthesized (Moradi et al., 2020; Balogun et al., 2017).

Hexahydrofarnesyl acetone was identified as the major constituent of the *H. niger* seed essential oil in this study. This compound is known for its antibacterial, anti-nociceptive, and anti-inflammatory properties (Wei, 2016). The methanol extract of *H. niger* demonstrated the highest antimicrobial effect, which can be attributed to the presence of hexahydrofarnesyl acetone. As a key compound in all the oils extracted in this study, hexahydrofarnesyl acetone exhibited strong antimicrobial activity against both gram-positive and gram-negative bacteria (Filipowicz et al., 2003). Additionally, essential oil from *Equisetum arvense* rich in hexahydrofarnesyl acetone has been shown to inhibit a broad spectrum of bacterial and fungal species (Radulovic et al., 2006).

Phytol, a diterpene alcohol derived from chlorophyll, was identified as a major component in the essential oil of the aerial parts of *H. niger* in this study. Phytol, widely used as a food additive and in medical applications, has promising antischistosomal properties. It also exhibits antinociceptive, antioxidant, anti-inflammatory, and antiallergic activities. Furthermore, phytol has demonstrated antimicrobial effects against *Mycobacterium tuberculosis* and *Staphylococcus aureus* (de Moras et al., 2014). These findings underscore the potential pharmacological applications of the major constituents of *H. niger* essential oil. As a result of the current study, phytol, which constitutes 52.09% of the essential oil of *H. niger*, was found in sufficient quantities to serve as a significant resource for future research.

Previous studies have demonstrated the antimicrobial properties of *H. niger* seeds against various microorganisms, with inhibition zones ranging from 9.6 mm to 26.2 mm. Notably, the strongest antimicrobial effect was observed against *Escherichia coli* (Dulger and Dulger, 2015). Additional research has reported antifungal activity for *H. niger* (Dulger et al., 2010a). Methanol extracts obtained from the seeds of this species exhibited strong antimicrobial activity (25.0 mm) against *Staphylococcus aureus* (Dulger et al., 2010b). Similarly, alkaloid extracts derived from the flower stems and roots of *H. niger* showed potent antimicrobial effects (Chalabian et al., 2002). Crude protein extracts of the same species were tested against *E. coli*, *S. aureus*, *Pseudomonas aeruginosa*, and *Proteus vulgaris*, yielding inhibition zone diameters of 14 mm, 15 mm, 14 mm, and 20 mm, respectively (Mateen et al., 2015).

Moreover, *H. reticulatus* seed extract has demonstrated antimicrobial activity against *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pasteurella multocida*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Staphylococcus aureus*, and *Yersinia enterocolitica* (Akbaş et al., 2020). It has also been established that the leaf parts of *H. niger* exhibit significant antimicrobial effects (Hossain et al., 2021).

When comparing the antimicrobial results of *H. niger* with findings from the literature, some variations are evident. These differences may be attributed to factors such as the specific plant species studied, the extraction methods and concentrations used, and the microorganisms tested. Alkaloids, glycosides, flavonoids, and their derivatives—commonly found in plant extracts—are primarily responsible for the antimicrobial effects observed (Akbaş et al., 2020; Ayari-

Guentri *et al.*, 2017; Benhouda *et al.*, 2014; Dulger *et al.*, 2010a-b; Dulger and Dulger, 2015; Elsharkawy *et al.*, 2018). These factors, along with the ecological and environmental conditions of the plants, influence the observed antimicrobial activity.

Hajipoor *et al.* (2015) investigated the antioxidant activity of the aerial parts of *H. niger* extracts using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging and iron-reducing antioxidant power (FRAP) assays. They reported that the DPPH radical scavenging effect of the plant's methanol extract was 2% at a concentration of 10 mg/mL, with this value gradually increasing at higher concentrations. Similarly, Koçpınar (2020) examined the heavy metal reduction properties (FRAP and CUPRAC) and DPPH radical scavenging activity of *H. niger*, confirming that its antioxidant activity was stronger than its radical scavenging and heavy metal reduction effects.

Jassbi *et al.* (2014) studied the free radical scavenging activity of seven fractions of *H. niger* alkaloidal extracts using the DPPH assay. Among these, only one fraction demonstrated moderate free radical scavenging activity compared to positive and negative controls. In another study, Ayari-Guentri *et al.* (2017) evaluated the DPPH radical scavenging activity of methanolic extracts from different parts of *H. muticus L. subsp. falezlez*. The highest IC50 value was observed in the stem ( $0.54 \pm 0.19$  mg/mL), followed by the leaf ( $0.65 \pm 0.29$  mg/mL) and the flower ( $0.92 \pm 0.39$  mg/mL).

When these results are compared with the literature, it is evident that antioxidant effects vary depending on the plant species, the specific plant parts used, the habitat where the plants were collected, and the presence of bioactive components in the plant material. These factors significantly influence the observed antioxidant activity.

## 5. CONCLUSIONS

The highest antimicrobial effect of *H. niger* was observed against *E. coli*. When the results of this study are compared with previous studies, variations are evident, which can be attributed to differences in the plant parts used, the microorganisms tested, the solvents applied, and the concentrations studied. The antioxidant effect of the species was found to be significant at a concentration of 1 mg/mL. However, comparisons with the literature reveal some discrepancies, likely due to differences in the plant materials, solvent types, and concentrations used.

*Hyoscyamus* species are recognized for their significant pharmacological effects, largely due to their phytochemical content. A thorough investigation of these plants, particularly their phytochemical composition, is essential to provide valuable contributions to the scientific literature. *H. niger* is a medicinal plant deserving of further study for this purpose. The bioactive compounds in *H. niger*, especially hexahydrofarnesyl acetone, and phytol, identified in the essential oils from its seeds and aerial parts, may offer promising therapeutic potential.

### Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

### Authorship Contribution Statement

**Şule İnci:** Antimicrobial and Antioxidant studies, Writing-original draft. **Pelin Yılmaz Sancar:** Investigation, Resources, Visualization, Formal Analysis and Writing-original draft. **Azize Demirpolat:** Essential oil studies, Writing-original draft. **Sevda Kırbağ:** Antimicrobial-Antioxidant studies, Statistical analysis, Supervision, Validation and Writing-original draft. **Şemsettin Civelek:** Supervision, Validation and Writing-original draft.



## Orcid

Şule Inci  <https://orcid.org/0000-0002-4022-5269>

Pelin Yılmaz Sancar  <https://orcid.org/0000-0002-6134-622X>

Azize Demirpolat  <https://orcid.org/0000-0001-7192-185X>

Sevda Kırbağ  <https://orcid.org/0000-0002-4337-8236>

Şemsettin Civelek  <https://orcid.org/0000-0003-1398-585X>

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