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

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ABSTRACT

In this study, essential oil components of the *Hyoscyamus niger* L. plant and their antimicrobial and antioxidant properties were determined. The essential oils were obtained separately from both the aerial parts and seeds of the plant using the hydrodistillation method. Antimicrobial activity was determined using the disk diffusion method. Total antioxidant level (TAS), total oxidant level (TOS), and 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity were detected for the antioxidant activity of the plant. The main essential oil components of the above-ground parts *Hyoscyamus niger* were determined as major components the phytol 52.09%, hexahydrofarnesyl acetone 19.66 %. *H. niger* seeds were hydrodistilled separately, obtaining yields of 0.7% (v w^{-1}) of yellow oils. In the essential oils of *H. niger* seeds, forty-one components were identified representing 99.0% of the oils. According to the results of the analysis hexahydrofarnesyl acetone 46.36%, hexanal 9.05% as major components. It was determined that the methanol extracts of the above-ground *H. niger* inhibited the growth of pathogenic microorganisms at different rates (13-32 mm). TAS value of methanol extracts of *H. niger* was calculated as 3.7705 mmol, while the TOS value was calculated as 6.9403 µmol. It was determined that the scavenging effects of the DPPH radical increase, depend on increasing concentrations.

Keywords: *Hyoscyamus niger*, Essential oil, Antimicrobial, Antioxidant, Turkey

INTRODUCTION

Today, medicinal plants, which have an important place in modern medicine and pharmacy, maintain their importance as a natural source of pharmaceutical raw materials due to the essential oils and secondary metabolites they contain (Akbaş et al., 2020).

The Solanaceae family, which includes many important medicinal plants, is a plant family with a wide variety of 98 genera and approximately 2700 species. Plants belonging to the Solanaceae family have been used by humans for many years as food, ornamental plants, and medicine (Guha and Maheshwari, 1966). The Solanaceae family, which contains many valuable plants rich in alkaloids with strong effects such as scopolamine, atropine, and

hyoscyamine, therefore has great importance both medically and economically (Maiti et al., 2002).

Hyoscyamus niger (black henbane) is an annual or biennial herbaceous plant belonging to the Solanaceae family and is commonly found in rocky areas, uncultivated lands, and especially on roadsides (Orbak et al., 1998; Li et al., 2011; Yücel and Yılmaz, 2014). Hyoscyamus *niger* is an important resource for the pharmaceutical industry as it contains some important alkaloids, which are among the oldest drugs used in medicine (Orbak et al., 1998; Pudersell et al., 2012; Dehghan et al., 2012).

This plant is generally used in the treatment of stomach pains, ulcers, kidney and liver disorders, as well as it's analgesic and antispasmodic effects (Tanker et al., 1998; John et al., 2010). However, with the consumption of the roots and leaves of the *Hyoscyamus niger*, some secondary metabolites in the plant can also cause poisoning by paralyzing the nerve endings of the parasympathetic system in humans (Orbak et al., 1998).

H. niger has been used as a hallucinogen since ancient times. Although it came to Europe in the middle ages, the hallucinogenic properties of this plant are even found in Ancient Greek writings. In particular, the seeds and leaves of the plant contain hyoscyamine, scopolamine, and other tropane alkaloids. Apart from the hallucinogenic properties of these chemicals, they are also responsible for the effects of ataxia and hypertension (Ugur, 2013). *H. niger* is an extremely poisonous plant and its consumption is undesirable. Because of this feature, it has also been used as a poison in history (Haas, 1995). Both placebo and *H. niger* methanolic extract to a group of 50 people in a clinic in Iran. It has been stated that the mixture applied to each patient three times a day for 6 days has beneficial effects in improving the symptoms of COVID-19. They defined that the clinical symptoms of COVID-19 such as dry cough, shortness of breath, sore throat, chest pain, fever, dizziness, headache, abdominal pain, and diarrhea were reduced with propolis plus *Hyoscyamus niger* L. extract than the placebo group (Kosari et al., 2021).

This study aims to determine the antimicrobial and antioxidant activity and essential oil composition of *Hyoscyamus niger*, a medicinal plant. In this study, while determining the essential oils in the plant, it will also be tried to determine the presence and limits of the antimicrobial effect on some bacterial and fungal species. Thus, it is aimed to contribute to the studies that have been done and will be done about providing raw materials for new therapeutics from plants that naturally grow in our country and have medicinal properties.

MATERIALS AND METHODS

Collection of the plant material

The plant material used in the study was collected from the Baskil district of Elazig province. Detailed locality information of the region where the plant was collected is given in Table 1. It

was collected from its natural habitat in its intense flowering period in May-June, which is the flowering period of the plant, and dried in the shade. The specimens of the plant are preserved in the Herbarium (FUH) of the Faculty of Science, Firat University.

Table 1. Detailed location information

Taxa	Collection date	Location	Lat.-Lon.	Collectors
***Hyoscyamus niger* L.**	24.05.2021	B7 Elazig: Baskil-Sancaklı village inner road, 1410 m.	Lat: 38°35'13.061" Lon: 38°55'23.055"	P. Yilmaz Sancar A. Demirpolat

Isolation of the essential oils

In this study, the plants were air-dried. In recent years, effective methods have emerged in drying methods by drying in isothermal and non-isothermal systems (Demirpolat et al., 2021). The Hydrodistillation method was used to obtain the oil of the plant. Essential oil using a modified Clevenger-type apparatus for 4 h to yield. A total of 250 g of fresh plant parts material has been used. The essential oil obtained was dried over anhydrous sodium sulfate and kept at 4°C until analysis.

Gas chromatography-mass spectrometry (GCFID/ GC-MS) analysis

The essential oil was analyzed by using GC- FID / MS analysis of the essential oils were done in Research Laboratory, Bingol University, by using HP (GC-MS) 7890A GC, 5975C MS and with a flame ionization detector FID detector were used. The study was performed by simultaneous injection in the same instrument. RXI-5 MS column (30m x 0,25 mm x 0,25 µm) Colon Flow Rate was used in both analyses with helium as the carrier gas in GC-MS. The injection volume was 1.0 µL of diluted solution (1 100^{-1}) of oil in n-hexane. The temperature of the injector was 250°C, and the flow rate was 1.3 mL min^{-1}. (splitless mode). The GC oven temperature was preserved at 70°C for 2 min and automated to 150°C at a rate of 10 °C/min and then kept constant at 150°C for 15 min to 240°C at a rate of 5°C min^{-1}. A series of n-alkanes were used as reference points in the calculation of retention indices (RI). MS were occupied at 70 eV and a mass range of 35-425. The determination of the compounds was based on a comparison of their retention indices (RI), and mass spectra with those acquired from Wiley-Nist W9N11 libraries.

Determination of the antimicrobial activity

In this study; *Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC25322, *Klebsiella pneumoniae* ATCC 700603, *Bacillus megaterium* DSM32, *Candida glabrata* ATCC 66032, *Candida albicans* FMC17, *Epidermophyton* sp., and *Trichophyton* sp. microorganisms were used. Microorganism cultures were obtained from Firat University, Faculty of Science, Department of Biology, Microbiology Laboratory culture collection. The antimicrobial activity of extracts plant obtained from methanol solvents was determined according to the disk diffusion method (Collins et al., 2004). *Bacteria strains* (*Escherichia coli* ATCC25922, *Klebsiella pneumoniae* ATCC700603, *Bacillus megaterium* DSM32, *Staphylococcus aureus* COWAN1, *Candida albicans* FMC17, *Candida glabrata* ATCC 66032, *Epidermophyton sp.* and *Trichophyton* sp) were incubated into Nutrient Broth (Difco) for 24 hours at 35 ± 1°C, yeast strains (*Candida albicans* FMC17 and *Candida glabrata* ATCC 66032) were incubated into Malt Extract Broth (Difco) and dermatophyte fungi (*Trichophyton* sp. and *Epidermophyton sp.*) was inoculated into Glucose Sabouroud Buyyon (Difco) and incubated at 25±1°C for 48 hours. The culture of prepared bacteria, yeast, and fungi in broth are respectively; was inoculated into Müeller Hinton Agar, Sabouraud Dextrose Agar, and Potato Dextrose Agar at a rate of 1% (10^6 bacteria ml^{-1}, 10^4 yeast ml^{-1}, 10^4 fungi ml^{-1}). Then, after shaking well, 25 ml was placed in sterile petri dishes of 9 cm diameter. Homogeneous distribution of the medium was achieved. 6 mm diameter antimicrobial discs (Oxoid), each impregnated with extracts of 100 µl (1000 µg) were lightly placed on the solidified agar medium. After the petri dishes prepared in this way were kept at 4°C for 1.5-2 hours, the plates inoculated with bacteria were incubated at 37 ± 0.1°C for 24 hours, and the plates were inoculated with yeast at 25 ± 0.1°C for 72 hours. As controls, different standard discs were used for bacteria (Streptomycin sulphate 10 µg $disc^{-1}$) and yeasts (Nystatin 30 µg $disc^{-1}$). Zones of inhibition were measured in mm.

Determination of antioxidant activity

Total antioxidant status (TAS) and total oxidant status (TOS) of plant extracts were determined with Rel Assay kits (Rel Assay Kit Diagnostics, Turkey). TAS value was expressed as mmol Trolox equiv. L^{-1} and Trolox was used as the calibrator (Erel, 2004). The TOS value was expressed as µmol H_2O_2 equiv. L^{-1} and hydrogen peroxide was used as the calibrator (Erel, 2005). The antioxidant activity of the methanol extract of the plant was determined according to the 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity method (Cuendet et al., 1997; Kirby and Scmidt,1997). The solution was prepared in methanol at a concentration of 1 mg/ml of the extract obtained. The prepared solution was diluted four times and the calibration curve of DPPH was obtained. By taking 40 µl of the prepared solution, 160 µl of DPPH solution was added. After thorough mixing, the mouth was closed and kept in the dark for 30 minutes. The

same procedures were repeated for all concentrations and methanol was used as a control. At the end of this period, the absorbances of each mixture were read at 570 nm in the spectrophotometer. % inhibition values were calculated;

I (%) = (Acontol - Asample / Acontrol) x 100

Statistical analysis

Statistical analysis of the data was performed in the SPSS 22.0 package program and $p<0.05$ was regarded as statistically significant.

RESULTS

Essential oil studies

The composition of the aerial parts essential oils of *H. niger* and *H. niger* seeds are listed in Table 2, in which the percentage composition and retention index of components are given. *H. niger* seeds were hydrodistilled separately, obtaining yields of 0.5 % (v/w) of yellow oils. In the essential oils of *H. niger* seeds, 41 components were identified representing 99.0% of the oils. According to the results of the analysis hexahydrofarnesyl acetone 46.36%, hexanal 9.05 %as major components. The aerial part of *H. niger* was hydrodistilled, obtaining yields of 0.4% (v/w) of light yellowish oils. In the essential oils of the aerial part of *H. niger*, 22 components were identified representing 97.51% of the oils. More than half of the oil is phytol. According to the results of the analysis, phytol 52.09%, hexahydrofarnesyl acetone 19.66%, as major components. The analysis of the essential oil showed that sesquiterpenes in both oils were found. As a result of GS-MS, the essential oil components of the aerial parts and seeds of *H. niger* were determined in this study. These components are given in Table 2. The essential oils isolated were a complex mixture of sesquiterpenes and hydrocarbons. GC-MS was used to analyze the compositions of the volatile oil in the seeds.

Table 2. Constituents of the essential oils from *H. niger*

No	R.Time	Area	Name	*H. niger* seeds	*H. niger* 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	
5.	6.033	534856	**Hexanal**	**9.05**	0.11
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-β-Ionone	0.42	-
31.	43.130	43977	Tetradecanal	0.74	-
32.	45.410	27984	Hexadecamethylcyclo octasiloxane	0.47	-
33.	45.546	52687	1-4--β-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
42.	51.860	2683617	**Hexahydrofarnesyl acetone**	**46.36**	**19.19**
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
46.	53.747	95393	Heneicosane	-	7.02
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	γ-Stearolactone	0.82	-
55.	60.884	7121450	**Phytol**	**0.96**	**52.09**
56.	61.683	109259	β –ıonone	-	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

Antimicrobial activity

The antimicrobial effect of *H. niger* against the microorganisms used is shown in Table 3. Methanol extract of *H. niger* created 32±0.11, 21±0.23, 20±0.51, 19±0.28, 23±0.17, 20±0.46, 13±0.46 and 16±0.23 mm inhibition zones against *E. coli, S. aureus, K. pneumoniae, B. megaterium, C. albicans, C. glabrata, Trichophyton sp.* and *Epidermophyton* sp., respectively. When the results obtained were compared with the controls, it was determined that it showed the best antimicrobial effect against *E. coli* (Table 3).

Table 3. Antimicrobial effect of *H. niger*

Microorganism	***H. niger***	**Standart Control**
Escherichia coli	32±0.11	30±0.23

Staphylococcus aureus	21±0.23	20±0.17
Klebsiella pneumoniae	20±0.51	19±0.05
Bacillus megaterium	19±0.28	25±0.00
Candida albicans	23±0.17	25±0.23
Candida glabrata	20±0.46	20±0.11
Trichophyton	13±0.46	22±0.06
Epidermophyton	16±0.23	25±0.40

Antioxidant activity

While the TAS value of the methanol extract of *H. niger* at 1 mg ml^{-1} concentration was determined as 3.77±0.00 mmol, the TOS value was determined as 6.94±0.00 µmol at the same concentrations. In the results obtained, the TAS value of *H. niger* was very good, while the TOS value was found to be between normal values (Table 4). The scavenging effect of *H. niger* on DPPH radical is shown in Table 5. The DPPH radical scavenging effect of *H. niger*'s methanol extracts at 1000 µg mL^{-1} was determined as 73.07±0.02 %. 125 µg mL^{-1} showed a very low antioxidant effect. In the results obtained, it is seen that the scavenging effect of the DPPH radical increases depending on the increasing concentrations of *H. niger*.

Table 4. TAS and TOS values of *H. niger*

	TAS (mmol Trolox equiv./L)	**TOS (µmol H_2O_2 equiv./L)**
***H. niger*-MetOH**	3.77±0.00	6.94±0.00

Table 5. Percent inhibition of the DPPH radical of root and leaf parts of *H. niger*

Concentrations	*H. niger* -MetOH
1000 µg/mL	73.07±0.02
500 µg/mL	52.73±0.07
250 µg/mL	27.88±0.44
125 µg/mL	15.78±0.29

DISCUSSION

Essential oil studies

H. niger leaves were studied in HPLC by extraction method, and 29 components were found and acid palmitic acid 28.30%, linoleic acid 26.85%, oleic acid 14.39 %, hexanal 10.24%, and stearic acid 1.81% were determined as major components (Wang et al., 2013). Compared with this study, our results show parallelism in terms of compounds that hexanal 9.05%, oleic acid 1.26%. However, the reason for the difference seen in other components (palmitic, linoleic acid) may be due to geographical and climatic differences. *H. muticus* L. subsp. *falezlez* aerial parts of essential oil analysis results, borneol (76.47%) was the main compound identified, followed by bornyl acetate 4.6% and 1,3-bis(2-cyclopropyl-2- methylcyclopropyl) but-2-en-1-one (4.19%) is the major substance. And they reported the oil yield was 0.50% (v w^{-1}) in ten compounds (Ayari-Guentri et al., 2017). These substances were not found in our study. It can be said that the reason for this variability in the composition of essential oils is the differences in the geographical regions from which some species are taken and the variability of ecological conditions. These factors also play a role in the oil composition. In another study, the constituents of the most active methanol extracts of *H. niger* and *H. reticulatus* were determined by HPLC-Diode Array Detector-Electrospray Ionization Mass Spectroscopy. Three major phenolic constituents were identified and quantified: chlorogenic acid, rutin, and Quercetin-3o-Glucoside-Rhamnoside-Rhamnoside (Jassbi et al., 2014). The oil of *H. niger* and its methanol extract was showed antinematode activity (Kareem, 2020). The ethanol extract of *H. niger* seeds showed high insecticidal activity against the *Aphis laburni* and *Lucilia sericata* (Wang et al., 2006; Küpeli et al., 2020). Our results are compatible with the literature. So, secondary metabolites are generally synthesized in plants as they represent the most important defense mechanisms against pathogens; the amount produced along with the quality may vary as a function of habitat, the organ in which they are produced and climate conditions (Balogun et al., 2017; Moradi et al., 2020).

Hexahydrofarnesyl acetone is the major constituent of the *H. niger* seeds essential oil in this study. Hexahydrofarnesyl acetone has antibacterial, anti-nociceptive, and anti-inflammation activities (Wei, 2016). And the methanol extract of *H. niger* showed the highest antimicrobial effect. So, hexahydrofarnesyl acetone, which was a major compound present in all the oils extracted in this study, showed strong antimicrobial activity against gram-positive and gram-negative bacteria (Filipowicz et al., 2003); essential oil from *Equisetum arvense*, which was rich in hexahydrofarnesyl acetone, showed broad-spectrum inhibition against various bacterial and fungal species (Radulovic et al., 2006). Phytol, diterpene alcohol from chlorophyll, which is widely used as a food additive and in medical fields, has been found as a major substance in the aerial parts of *H. niger* essential oil in our study of phytol, which has promising antischistosomal properties. Phytol has antinociceptive and antioxidant activities as well as anti-inflammatory and antiallergic effects. And it showed that has an antimicrobial effect against *Mycobacterium tuberculosis* and *Staphylococcus aureus* (de Moras et al., 2014). As a result of our study, phytol, which is found at the rate of 52.09 percent in *H. niger* essential oil, was found to be high enough to form a source for other studies.

Antimicrobial activity

The antimicrobial effect (26.2 mm-9.6 mm) of the seeds of *H. niger* against some microorganisms (*Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis,* and *Candida albicans*) was determined. The results obtained, showed the best antimicrobial effect against E. coli (Dulger and Dulger, 2015). In a different study, it was reported that *H. niger* has antifungal effects (Dulger et al., 2010). It was reported that the methanol extract obtained from the seeds of the same species showed strong antimicrobial activity (25.0 mm) against *S. aureus* (Dulger et al., 2010). It was reported that alkaloid extracts obtained from flower stems and roots of *H. niger* have strong antimicrobial effects (Chalabian et al., 2012). Crude protein extracts of the same species were tested against *E. coli, S. aureus, P. aeruginosa*, and *P. vulgaris*. It showed growth inhibition zone diameters of 14, 15, 14, and 20 mm against these pathogens, respectively (Mateen et al., 2015). It has been reported that *H. reticulatus* seed extract has an antimicrobial effect against *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pasteurella multicoda, Pseudomonas aeruginosa, Salmonella enteritidis, Staphylococcus aureus, Yersinia enterocolitica* microorganisms (Akbaş et al., 2020). It has been determined that the leaf parts of give have a significant antimicrobial effect (Hossain et al. 2021). When the antimicrobial results of *H. niger* are compared with the literature studies, it is seen that the results obtained are different from each other. Alkaloids, glycosides, flavonoids, and their derivatives, which are usually found in plant extracts, are responsible for the antimicrobial effect (Dulger et al., 2010a-b; Benhouda et al., 2014; Dulger and Dulger, 2015; Ayari-Guentri et al., 2017; Elsharkawy et al.,

2018; Akbaş et al., 2020). In addition, the plant species, concentrations, and microorganisms used to affect the antimicrobial results.

Antioxidant activity

The DPPH radical scavenging effect of the ethanol extract of *H. niger* was reported as 2% at a concentration of 10 mg/ml however, this value gradually decreases at high concentrations (Hajipoor *et al.*, 2015). The antioxidant activity of the aerial parts of *Hyoscyamus niger* extracts was investigated by DPPH (2, 2-diphenyl-1-picrylhydrazyl) and iron-reducing antioxidant power (FRAP) activities. Antioxidant (EC50) for methanol extract was 377±1.21 μg ml^{-1}, 21±0.68, and was 4.8±0.32 μg ml^{-1} for butylated hydroxytoluene and ascorbic acid (Koçpınar, 2020). Free radical scavenging activity of seven fractions of *Hyoscyamus niger* alkaloidal extract was investigated by 2, 2-diphenyl-1-picrylhydrazyl activity. Only a fraction of the alkaloidal extract showed moderate free radical scavenging activity compared to positive and negative controls (Jassbi et al., 2014). Methanolic extracts of *Hyoscyamus niger* showed antioxidant activity (IC50=1.64 μg) compared to α-tocopherol (IC50=0.60 μg) used as positive control (Souri et al. 2004). The IC50 values of the DPPH radical scavenging effect of the methanol extracts of the seed, leaf, and epicalyx parts of *H. niger* were determined as 1485±34.9, 270.2±8.3, and 451.3±2.2 μg mL^{-1}, respectively (Hajipoor et al., 2015). The methanolic extract of *Hyoscyamus albus* leaves displayed the highest antioxidant activity (76.00%) (Benhouda et al., 2014). *Hyoscyamus reticulatus* aqueous extract exhibited significant antioxidant scavenging properties (533.26 μmol TE g^{-1} dry extract weight) (Akbaş et al., 2020). The antioxidant testing showed that the methanolic extract of *H. muticus* has a noticeable antioxidant activity with an IC50 of 8.1±0.65 mg/ml and an EC50 of 12.74±1.12 mg ml^{-1} (Dehghan et al., 2012) The results of DPPH radical scavenging activity of *H. muticus* L. subsp. *falezlez* methanolic extracts showed the highest IC50 value of stem (0.54 ± 0.19 mg mL^{-1}), followed by leaf (0.65 ± 0.29 mg mL^{-1}) and flower (0.92 ± 0.39 mg mL^{-1}) (Ayari-Guentri et al., 2017). When the results obtained are compared with the literature, it is seen that the antioxidant effects vary according to the type used. Because of the plant species used, the habitat collected and the presence of bioactive components in the plant materials affect the antioxidant results.

CONCLUSIONS

It was determined that the highest antimicrobial effect of *H. niger* was against *E. coli*. When the study results are compared with previous studies, the results vary depending on the parts of the plant material used, the microorganism, the solvent, and the concentrations. It was determined that the antioxidant effect of the species is good at 1 mg ml^{-1} concentration. When the results are compared with the literature, it is seen that there are some differences. Because it varies depending

on the parts of the plant materials used, the concentration, and the solvent. *Hyoscyamus* species are known to have important pharmacological effects thanks to the phytochemicals they contain. It is important to investigate these at a sufficient level and especially to evaluate the phytochemicals they contain in terms of bringing them to the literature. It is a medicinal plant that should be studied for this purpose in *H. niger*. The bioactive substances, especially hexahydrofarnesyl acetone and phytol, in the essential oils from seeds and aerial part *H. niger* may be of therapeutic interest.

Competing Intererest

The authors declare no competing or financial interests.

Author contributions

ŞİI, PYS, AD, SK SC, designed the study and experiments and analyzed the data conceived and and drafted and revised the paper.

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

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ABSTRACT

In this study, essential oil components of the *Hyoscyamus niger* L. plant and their antimicrobial and antioxidant properties were determined. The essential oils were obtained separately from both the aerial parts and seeds of the plant using the hydrodistillation method. Antimicrobial activity was determined using the disk diffusion method. Total antioxidant level (TAS), total oxidant level (TOS), and 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity were detected for the antioxidant activity of the plant. The main essential oil components of the above-ground parts *Hyoscyamus niger* were determined as major components the phytol 52.09%, hexahydrofarnesyl acetone 19.66 %. *H. niger* seeds were hydrodistilled separately, obtaining yields of 0.7% (v w^{-1}) of yellow oils. In the essential oils of *H. niger* seeds, forty-one components were identified representing 99.0% of the oils. According to the results of the analysis hexahydrofarnesyl acetone 46.36%, hexanal 9.05% as major components. It was determined that the methanol extracts of the above-ground *H. niger* inhibited the growth of pathogenic microorganisms at different rates (13-32 mm). TAS value of methanol extracts of *H. niger* was calculated as 3.7705 mmol, while the TOS value was calculated as 6.9403 μmol. It was determined that the scavenging effects of the DPPH radical increase, depend on increasing concentrations.

Keywords: *Hyoscyamus niger*, Essential oil, Antimicrobial, Antioxidant, Turkey

INTRODUCTION

Today, medicinal plants, which have an important place in modern medicine and pharmacy, maintain their importance as a natural source of pharmaceutical raw materials due to the essential oils and secondary metabolites they contain (Akbaş et al., 2020).

The Solanaceae family, which includes many important medicinal plants, is a plant family with a wide variety of 98 genera and approximately 2700 species. Plants belonging to the Solanaceae family have been used by humans for many years as food, ornamental plants, and medicine (Guha and Maheshwari, 1966). The Solanaceae family, which contains many

valuable plants rich in alkaloids with strong effects such as scopolamine, atropine, and hyoscyamine, therefore has great importance both medically and economically (Maiti et al., 2002).

Hyoscyamus niger (black henbane) is an annual or biennial herbaceous plant belonging to the Solanaceae family and is commonly found in rocky areas, uncultivated lands, and especially on roadsides (Orbak et al., 1998; Li et al., 2011; Yücel and Yılmaz, 2014). Hyoscyamus *niger* is an important resource for the pharmaceutical industry as it contains some important alkaloids, which are among the oldest drugs used in medicine (Orbak et al., 1998; Pudersell et al., 2012; Dehghan et al., 2012).

This plant is generally used in the treatment of stomach pains, ulcers, kidney and liver disorders, as well as it's analgesic and antispasmodic effects (Tanker et al., 1998; John et al., 2010). However, with the consumption of the roots and leaves of the *Hyoscyamus niger*, some secondary metabolites in the plant can also cause poisoning by paralyzing the nerve endings of the parasympathetic system in humans (Orbak et al., 1998).

H. niger has been used as a hallucinogen since ancient times. Although it came to Europe in the middle ages, the hallucinogenic properties of this plant are even found in Ancient Greek writings. In particular, the seeds and leaves of the plant contain hyoscyamine, scopolamine, and other tropane alkaloids. Apart from the hallucinogenic properties of these chemicals, they are also responsible for the effects of ataxia and hypertension (Ugur, 2013). *H. niger* is an extremely poisonous plant and its consumption is undesirable. Because of this feature, it has also been used as a poison in history (Haas, 1995). Both placebo and *H. niger* methanolic extract to a group of 50 people in a clinic in Iran. It has been stated that the mixture applied to each patient three times a day for 6 days has beneficial effects in improving the symptoms of COVID-19. They defined that the clinical symptoms of COVID-19 such as dry cough, shortness of breath, sore throat, chest pain, fever, dizziness, headache, abdominal pain, and diarrhea were reduced with propolis plus *Hyoscyamus niger* L. extract than the placebo group (Kosari et al., 2021).

This study aims to determine the antimicrobial and antioxidant activity and essential oil composition of *Hyoscyamus niger*, a medicinal plant. In this study, while determining the essential oils in the plant, it will also be tried to determine the presence and limits of the antimicrobial effect on some bacterial and fungal species. Thus, it is aimed to contribute to the studies that have been done and will be done about providing raw materials for new therapeutics from plants that naturally grow in our country and have medicinal properties.

MATERIALS AND METHODS

Collection of the plant material

The plant material used in the study was collected from the Baskil district of Elazig province. Detailed locality information of the region where the plant was collected is given in Table 1. It was collected from its natural habitat in its intense flowering period in May-June, which is the flowering period of the plant, and dried in the shade. The specimens of the plant are preserved in the Herbarium (FUH) of the Faculty of Science, Firat University.

Table 1. Detailed location information

Taxa	Collection date	Location	Lat.-Lon.	Collectors
***Hyoscyamus niger* L.**	24.05.2021	B7 Elazig: Baskil-Sancaklı village inner road, 1410 m.	Lat: 38°35'13.061" Lon: 38°55'23.055"	P. Yilmaz Sancar A. Demirpolat

Isolation of the essential oils

In this study, the plants were air-dried. In recent years, effective methods have emerged in drying methods by drying in isothermal and non-isothermal systems (Demirpolat et al., 2021). The Hydrodistillation method was used to obtain the oil of the plant. Essential oil using a modified Clevenger-type apparatus for 4 h to yield. A total of 250 g of fresh plant parts material has been used. The essential oil obtained was dried over anhydrous sodium sulfate and kept at 4°C until analysis.

Gas chromatography-mass spectrometry (GCFID/ GC-MS) analysis

The essential oil was analyzed by using GC- FID / MS analysis of the essential oils were done in Research Laboratory, Bingol University, by using HP (GC-MS) 7890A GC, 5975C MS and with a flame ionization detector FID detector were used. The study was performed by simultaneous injection in the same instrument. RXI-5 MS column (30m x 0,25 mm x 0,25 µm) Colon Flow Rate was used in both analyses with helium as the carrier gas in GC-MS. The injection volume was 1.0 µL of diluted solution (1 100^{-1}) of oil in n-hexane. The temperature of the injector was 250°C, and the flow rate was 1.3 mL min^{-1}. (splitless mode). The GC oven temperature was preserved at 70°C for 2 min and automated to 150°C at a rate of 10 °C/min and then kept constant at 150°C for 15 min to 240°C at a rate of 5°C min^{-1}. A series of n-alkanes were used as reference points in the calculation of retention indices (RI). MS were occupied at 70 eV and a mass range of 35-425. The determination of the compounds was based on a

comparison of their retention indices (RI), and mass spectra with those acquired from Wiley-Nist W9N11 libraries.

Determination of the antimicrobial activity

In this study; *Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC25322, *Klebsiella pneumoniae* ATCC 700603, *Bacillus megaterium* DSM32, *Candida glabrata* ATCC 66032, *Candida albicans* FMC17, *Epidermophyton* sp., and *Trichophyton* sp. microorganisms were used. Microorganism cultures were obtained from Firat University, Faculty of Science, Department of Biology, Microbiology Laboratory culture collection. The antimicrobial activity of extracts plant obtained from methanol solvents was determined according to the disk diffusion method (Collins et al., 2004). *Bacteria strains* (*Escherichia coli* ATCC25922, *Klebsiella pneumoniae* ATCC700603, *Bacillus megaterium* DSM32, *Staphylococcus aureus* COWAN1, *Candida albicans* FMC17, *Candida glabrata* ATCC 66032, *Epidermophyton sp.* and *Trichophyton* sp) were incubated into Nutrient Broth (Difco) for 24 hours at 35 ± 1°C, yeast strains (*Candida albicans* FMC17 and *Candida glabrata* ATCC 66032) were incubated into Malt Extract Broth (Difco) and dermatophyte fungi (*Trichophyton* sp. and *Epidermophyton sp.*) was inoculated into Glucose Sabouroud Buyyon (Difco) and incubated at 25±1°C for 48 hours. The culture of prepared bacteria, yeast, and fungi in broth are respectively; was inoculated into Müeller Hinton Agar, Sabouraud Dextrose Agar, and Potato Dextrose Agar at a rate of 1% (10^6 bacteria ml^{-1}, 10^4 yeast ml^{-1}, 10^4 fungi ml^{-1}). Then, after shaking well, 25 ml was placed in sterile petri dishes of 9 cm diameter. Homogeneous distribution of the medium was achieved. 6 mm diameter antimicrobial discs (Oxoid), each impregnated with extracts of 100 µl (1000 µg) were lightly placed on the solidified agar medium. After the petri dishes prepared in this way were kept at 4°C for 1.5-2 hours, the plates inoculated with bacteria were incubated at 37 ± 0.1°C for 24 hours, and the plates were inoculated with yeast at 25 ± 0.1°C for 72 hours. As controls, different standard discs were used for bacteria (Streptomycin sulphate 10 µg $disc^{-1}$) and yeasts (Nystatin 30 µg $disc^{-1}$). Zones of inhibition were measured in mm.

Determination of antioxidant activity

Total antioxidant status (TAS) and total oxidant status (TOS) of plant extracts were determined with Rel Assay kits (Rel Assay Kit Diagnostics, Turkey). TAS value was expressed as mmol Trolox equiv. L^{-1} and Trolox was used as the calibrator (Erel, 2004). The TOS value was expressed as µmol H_2O_2 equiv. L^{-1} and hydrogen peroxide was used as the calibrator (Erel, 2005). The antioxidant activity of the methanol extract of the plant was determined according to the 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity method (Cuendet et al., 1997; Kirby and Scmidt,1997). The solution was prepared in methanol at a concentration of 1 mg/ml of the extract obtained. The prepared solution was diluted four times and the calibration

curve of DPPH was obtained. By taking 40 µl of the prepared solution, 160 µl of DPPH solution was added. After thorough mixing, the mouth was closed and kept in the dark for 30 minutes. The same procedures were repeated for all concentrations and methanol was used as a control. At the end of this period, the absorbances of each mixture were read at 570 nm in the spectrophotometer. % inhibition values were calculated;

I (%) = (Acontol - Asample / Acontrol) x 100

Statistical analysis

Statistical analysis of the data was performed in the SPSS 22.0 package program and $p<0.05$ was regarded as statistically significant.

RESULTS

Essential oil studies

The composition of the aerial parts essential oils of *H. niger* and *H. niger* seeds are listed in Table 2, in which the percentage composition and retention index of components are given. *H. niger* seeds were hydrodistilled separately, obtaining yields of 0.5 % (v/w) of yellow oils. In the essential oils of *H. niger* seeds, 41 components were identified representing 99.0% of the oils. According to the results of the analysis hexahydrofarnesyl acetone 46.36%, hexanal 9.05 %as major components. The aerial part of *H. niger* was hydrodistilled, obtaining yields of 0.4% (v/w) of light yellowish oils. In the essential oils of the aerial part of *H. niger*, 22 components were identified representing 97.51% of the oils. More than half of the oil is phytol. According to the results of the analysis, phytol 52.09%, hexahydrofarnesyl acetone 19.66%, as major components. The analysis of the essential oil showed that sesquiterpenes in both oils were found. As a result of GS-MS, the essential oil components of the aerial parts and seeds of *H. niger* were determined in this study. These components are given in Table 2. The essential oils isolated were a complex mixture of sesquiterpenes and hydrocarbons. GC-MS was used to analyze the compositions of the volatile oil in the seeds.

Table 2. Constituents of the essential oils from *H. niger*

No	R.Time	Area	Name	*H. niger* seeds	*H. niger* 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	
5.	6.033	534856	**Hexanal**	**9.05**	0.11
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-β-Ionone	0.42	-
31.	43.130	43977	Tetradecanal	0.74	-
32.	45.410	27984	Hexadecamethylcyclo octasiloxane	0.47	-
33.	45.546	52687	1-4--β-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
42.	51.860	2683617	**Hexahydrofarnesyl acetone**	**46.36**	**19.19**
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
46.	53.747	95393	Heneicosane	-	7.02

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	γ-Stearolactone	0.82	-
55.	60.884	7121450	**Phytol**	**0.96**	**52.09**
56.	61.683	109259	β –ıonone	-	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

Antimicrobial activity

The antimicrobial effect of *H. niger* against the microorganisms used is shown in Table 3. Methanol extract of *H. niger* created 32±0.11, 21±0.23, 20±0.51, 19±0.28, 23±0.17, 20±0.46, 13±0.46 and 16±0.23 mm inhibition zones against *E. coli, S. aureus, K. pneumoniae, B. megaterium, C. albicans, C. glabrata, Trichophyton sp.* and *Epidermophyton* sp., respectively. When the results obtained were compared with the controls, it was determined that it showed the best antimicrobial effect against *E. coli* (Table 3).

Table 3. Antimicrobial effect of *H. niger*

Microorganism	*H. niger*	Standart Control
Escherichia coli	32±0.11	30±0.23
Staphylococcus aureus	21±0.23	20±0.17
Klebsiella pneumoniae	20±0.51	19±0.05
Bacillus megaterium	19±0.28	25±0.00
Candida albicans	23±0.17	25±0.23
Candida glabrata	20±0.46	20±0.11
Trichophyton	13±0.46	22±0.06
Epidermophyton	16±0.23	25±0.40

Antioxidant activity

While the TAS value of the methanol extract of *H. niger* at 1 mg ml^{-1} concentration was determined as 3.77±0.00 mmol, the TOS value was determined as 6.94±0.00 µmol at the same concentrations. In the results obtained, the TAS value of *H. niger* was very good, while the TOS value was found to be between normal values (Table 4). The scavenging effect of *H. niger* on DPPH radical is shown in Table 5. The DPPH radical scavenging effect of *H. niger*'s methanol extracts at 1000 µg mL^{-1} was determined as 73.07±0.02 %. 125 µg mL^{-1} showed a very low antioxidant effect. In the results obtained, it is seen that the scavenging effect of the DPPH radical increases depending on the increasing concentrations of *H. niger*.

Table 4. TAS and TOS values of *H. niger*

	TAS (mmol Trolox equiv./L)	TOS (µmol H_2O_2 equiv./L)
***H. niger*-MetOH**	3.77±0.00	6.94±0.00

Table 5. Percent inhibition of the DPPH radical of root and leaf parts of *H. niger*

Concentrations	***H. niger* -MetOH**
1000 µg/mL	73.07±0.02
500 µg/mL	52.73±0.07
250 µg/mL	27.88±0.44
125 µg/mL	15.78±0.29

DISCUSSION

Essential oil studies

H. niger leaves were studied in HPLC by extraction method, and 29 components were found and acid palmitic acid 28.30%, linoleic acid 26.85%, oleic acid 14.39 %, hexanal 10.24%, and stearic acid 1.81% were determined as major components (Wang et al., 2013). Compared with this study, our results show parallelism in terms of compounds that hexanal 9.05%, oleic acid 1.26%. However, the reason for the difference seen in other components (palmitic, linoleic acid) may be due to geographical and climatic differences. *H. muticus* L. subsp. *falezlez* aerial parts of essential oil analysis results, borneol (76.47%) was the main compound identified, followed by bornyl acetate 4.6% and 1,3-bis(2-cyclopropyl-2- methylcyclopropyl) but-2-en-1-one (4.19%) is the major substance. And they reported the oil yield was 0.50% (v w^{-1}) in ten compounds (Ayari-Guentri et al., 2017). These substances were not found in our study. It can be said that the reason for this variability in the composition of essential oils is the differences in the geographical regions from which some species are taken and the variability of ecological conditions. These factors also play a role in the oil composition. In another study, the constituents of the most active methanol extracts of *H. niger* and *H. reticulatus* were determined by HPLC-Diode Array Detector-Electrospray Ionization Mass Spectroscopy. Three major phenolic constituents were identified and quantified: chlorogenic acid, rutin, and Quercetin-3o-Glucoside-Rhamnoside-Rhamnoside (Jassbi et al., 2014). The oil of *H. niger* and its methanol extract was showed antinematode activity (Kareem, 2020). The ethanol extract of *H. niger* seeds showed high insecticidal activity against the *Aphis laburni* and *Lucilia sericata* (Wang et al., 2006; Küpeli et al., 2020). Our results are compatible with the literature. So, secondary metabolites are generally synthesized in plants as they represent the most important defense mechanisms against pathogens; the amount produced along with the quality may vary as a

function of habitat, the organ in which they are produced and climate conditions (Balogun et al., 2017; Moradi et al., 2020).

Hexahydrofarnesyl acetone is the major constituent of the *H. niger* seeds essential oil in this study. Hexahydrofarnesyl acetone has antibacterial, anti-nociceptive, and anti-inflammation activities (Wei, 2016). And the methanol extract of *H. niger* showed the highest antimicrobial effect. So, hexahydrofarnesyl acetone, which was a major compound present in all the oils extracted in this study, showed strong antimicrobial activity against gram-positive and gram-negative bacteria (Filipowicz et al., 2003); essential oil from *Equisetum arvense*, which was rich in hexahydrofarnesyl acetone, showed broad-spectrum inhibition against various bacterial and fungal species (Radulovic et al., 2006). Phytol, diterpene alcohol from chlorophyll, which is widely used as a food additive and in medical fields, has been found as a major substance in the aerial parts of *H. niger* essential oil in our study of phytol, which has promising antischistosomal properties. Phytol has antinociceptive and antioxidant activities as well as anti-inflammatory and antiallergic effects. And it showed that has an antimicrobial effect against *Mycobacterium tuberculosis* and *Staphylococcus aureus* (de Moras et al., 2014). As a result of our study, phytol, which is found at the rate of 52.09 percent in *H. niger* essential oil, was found to be high enough to form a source for other studies.

Antimicrobial activity

The antimicrobial effect (26.2 mm-9.6 mm) of the seeds of *H. niger* against some microorganisms (*Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis,* and *Candida albicans*) was determined. The results obtained, showed the best antimicrobial effect against E. coli (Dulger and Dulger, 2015). In a different study, it was reported that *H. niger* has antifungal effects (Dulger et al., 2010). It was reported that the methanol extract obtained from the seeds of the same species showed strong antimicrobial activity (25.0 mm) against *S. aureus* (Dulger et al., 2010). It was reported that alkaloid extracts obtained from flower stems and roots of *H. niger* have strong antimicrobial effects (Chalabian et al., 2012). Crude protein extracts of the same species were tested against *E. coli, S. aureus, P. aeruginosa,* and *P. vulgaris*. It showed growth inhibition zone diameters of 14, 15, 14, and 20 mm against these pathogens, respectively (Mateen et al., 2015). It has been reported that *H. reticulatus* seed extract has an antimicrobial effect against *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pasteurella multicoda, Pseudomonas aeruginosa, Salmonella enteritidis, Staphylococcus aureus, Yersinia enterocolitica* microorganisms (Akbaş et al., 2020). It has been determined that the leaf parts of give have a significant antimicrobial effect (Hossain et al. 2021). When the antimicrobial results of *H. niger* are compared with the literature studies, it is seen that the results obtained are different from each other. Alkaloids,

glycosides, flavonoids, and their derivatives, which are usually found in plant extracts, are responsible for the antimicrobial effect (Dulger et al., 2010a-b; Benhouda et al., 2014; Dulger and Dulger, 2015; Ayari-Guentri et al., 2017; Elsharkawy et al., 2018; Akbaş et al., 2020). In addition, the plant species, concentrations, and microorganisms used to affect the antimicrobial results.

Antioxidant activity

The DPPH radical scavenging effect of the ethanol extract of *H. niger* was reported as 2% at a concentration of 10 mg/ml however, this value gradually decreases at high concentrations (Hajipoor *et al.,* 2015). The antioxidant activity of the aerial parts of *Hyoscyamus niger* extracts was investigated by DPPH (2, 2-diphenyl-1-picrylhydrazyl) and iron-reducing antioxidant power (FRAP) activities. Antioxidant (EC50) for methanol extract was 377±1.21 μg ml^{-1}, 21±0.68, and was 4.8±0.32 μg ml^{-1} for butylated hydroxytoluene and ascorbic acid (Koçpınar, 2020). Free radical scavenging activity of seven fractions of *Hyoscyamus niger* alkaloidal extract was investigated by 2, 2-diphenyl-1-picrylhydrazyl activity. Only a fraction of the alkaloidal extract showed moderate free radical scavenging activity compared to positive and negative controls (Jassbi et al., 2014). Methanolic extracts of *Hyoscyamus niger* showed antioxidant activity (IC50=1.64 μg) compared to α-tocopherol (IC50=0.60 μg) used as positive control (Souri et al. 2004). The IC50 values of the DPPH radical scavenging effect of the methanol extracts of the seed, leaf, and epicalyx parts of *H. niger* were determined as 1485±34.9, 270.2±8.3, and 451.3±2.2 μg mL^{-1}, respectively (Hajipoor et al., 2015). The methanolic extract of *Hyoscyamus albus* leaves displayed the highest antioxidant activity (76.00%) (Benhouda et al., 2014). *Hyoscyamus reticulatus* aqueous extract exhibited significant antioxidant scavenging properties (533.26 μmol TE g^{-1} dry extract weight) (Akbaş et al., 2020). The antioxidant testing showed that the methanolic extract of *H. muticus* has a noticeable antioxidant activity with an IC50 of 8.1±0.65 mg/ml and an EC50 of 12.74±1.12 mg ml^{-1} (Dehghan et al., 2012) The results of DPPH radical scavenging activity of *H. muticus* L. subsp. *falezlez* methanolic extracts showed the highest IC50 value of stem (0.54 ± 0.19 mg mL^{-1}), followed by leaf (0.65 ± 0.29 mg mL^{-1}) and flower (0.92 ± 0.39 mg mL^{-1}) (Ayari-Guentri et al., 2017). When the results obtained are compared with the literature, it is seen that the antioxidant effects vary according to the type used. Because of the plant species used, the habitat collected and the presence of bioactive components in the plant materials affect the antioxidant results.

CONCLUSIONS

It was determined that the highest antimicrobial effect of *H. niger* was against *E. coli*. When the study results are compared with previous studies, the results vary depending on the parts of the

plant material used, the microorganism, the solvent, and the concentrations. It was determined that the antioxidant effect of the species is good at 1 mg ml^{-1} concentration. When the results are compared with the literature, it is seen that there are some differences. Because it varies depending on the parts of the plant materials used, the concentration, and the solvent. *Hyoscyamus* species are known to have important pharmacological effects thanks to the phytochemicals they contain. It is important to investigate these at a sufficient level and especially to evaluate the phytochemicals they contain in terms of bringing them to the literature. It is a medicinal plant that should be studied for this purpose in *H. niger*. The bioactive substances, especially hexahydrofarnesyl acetone and phytol, in the essential oils from seeds and aerial part *H. niger* may be of therapeutic interest.

Competing Intererest

The authors declare no competing or financial interests.

Author contributions

Şİ, PYS, AD, SK SC, designed the study and experiments and analyzed the data conceived and and drafted and revised the paper.

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