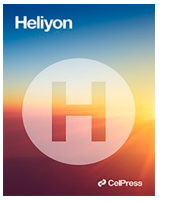
Heliyon 10 (2024) e38389

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Analysis of *Eremostachys hyoscyamoides* essential oil composition and assessing the antibacterial and antioxidant properties of the ethanol extract

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ARTICLE INFO

Keywords:
Antibacterial effect
Antioxidant activity
Flavonoid
Eremostachys hyoscyamoides
Essential oil
GC-MS

ABSTRACT

The increasing issue of antibiotic resistance in bacterial infections has led to challenging and costly treatments. Free radicals significantly contribute to the progression of several diseases, such as cardiometabolic disorders, neurodegenerative conditions, and cancers. Antioxidants can help alleviate or prevent these health problems. This research aimed to assess the antibacterial and antioxidant effects of *Eremostachys hyoscyamoides* ethanol extract. Additionally, the chemical profile of the essential oil obtained from the aerial parts of *E. hyoscyamoides* was characterized through gas chromatography-mass spectrometry (GC-MS). The extract's antibacterial effect was tested against three Gram-positive bacteria (*Micrococcus luteus*, *Staphylococcus epidermidis*, and *S. aureus*), in addition to three Gram-negative bacteria (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli*). The cup-plate method was employed to assess the antibacterial activity, which was subsequently followed by the determination of the minimum inhibitory concentration through the agar dilution method. To evaluate the antioxidant effects, the DPPH radical scavenging assay was used. The total phenolic content (TPC) and flavonoid content (TFC) of the extract were quantified using the Folin Ciocalteu and Aluminum Chloride methods as spectrophotometric-based techniques, respectively. The essential oil of *E. hyoscyamoides* was extracted via hydrodistillation and subjected to GC-MS analysis. The findings demonstrated that the ethanol extract of *E. hyoscyamoides* effectively inhibited the growth of the bacterial strains examined. The IC_{50} value measured in the DPPH test was 48.194 ± 0.61 μg/mL. The TPC was found to be 84.15 ± 2.5 mg GAE/g, while the TFC was determined to be 19.35 ± 1.3 mg RE/g. A total of 50 components, accounting for 93.6 % of the essential oil composition, were identified. High concentrations of elemol (6.8 %), 2,4-di-tert-butylphenol (10.7 %), and linalyl acetate (4.2 %) were putatively identified in the essential oil. In conclusion, the ethanol extract of *E. hyoscyamoides* exhibited hopeful potential as a natural source of antioxidants and antibacterial agents.

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https://doi.org/10.1016/j.heliyon.2024.e38389
Received 3 June 2024; Received in revised form 21 September 2024; Accepted 23 September 2024
Available online 24 September 2024

1. Introduction

For millennia, natural compounds have been of noteworthy significance in the field of pharmaceutical research, particularly in the context of finding potential treatments for diseases such as cancer and infectious diseases [1,2].

The pathogenesis of numerous health conditions, such as heart and lung diseases, metabolic disorders, neurodegenerative conditions like Alzheimer's, and diverse types of cancer, have been linked to the detrimental effects caused by the presence of excessive bodily free radicals [3,4]. Oxidative stress has been implicated in mitochondrial dysfunction, DNA damage, aging, dysregulation of proteins, and lipids metabolism [5–7].

Due to the changes in human lifestyle particularly alterations in the dietary regimens, the prevalence of oxidative stress is increasing. Free radicals can be produced through various external sources, which include environmental pollution, exposure to toxic heavy metals and harmful substances, as well as exposure to various forms of radiation such as ultraviolet, gamma, and X-rays. Furthermore, they can also be generated through the action of intrinsic oxidizing agents [7]. So, the need for antioxidants that can effectively scavenge free radicals is of high interest. Medicinal plants and their specialized metabolites including phenolic compounds and flavonoids can be rich sources of antioxidants [8].

Infectious agents result in bodily dysfunctions and may cause morbidity and mortality worldwide. Owing to the growing microbial resistance to synthetic antibiotics and their potential adverse effects, the trend for investigating the antibacterial activities of natural products is a significant rationale [9–14]. Medicinal plants have been traditionally used as therapeutic strategies to manage infectious diseases. They produce secondary metabolites, which function as components of their protective mechanisms against microorganisms [1].

Eremostachys is a genus in the Lamiaceae family, comprising 60 species worldwide. Of these, 15 are found in Iran, with 5 being endemic to the region including *E. adenantha*, *E. azerbayjanica*, *E. codonocalyx*, *E. hyoscyamoides*, and *E. pulvinaris*. Previous research on the *Eremostachys* genus has reported the presence of alkaloids, coumarins, flavonoids, terpenes, iridoids, and phenylethanoids in the aerial parts. Additionally, phytochemical investigations have detected a variety of monoterpenes and sesquiterpenes in the essential oils of this genus [15–18]. Numerous pharmacological indications have been reported in *Eremostachys* species including antioxidant, anti-parasitic [19,20], antibacterial, analgesic [21], antidepressant [22], and anti-inflammatory effects [15,23].

Eremostachys hyoscyamoides is a medicinal plant with an ethnobotanical background in the northeast province of Iran. *E. hyoscyamoides* has been traditionally used to treat infected wounds [15].

In this study we assessed the antibacterial and antioxidant properties of *E. hyoscyamoides* ethanol extract. Preliminary phytochemical investigations were performed to determine the TPC and TFC in the extract. Furthermore, the essential oil of *E. hyoscyamoides* was subjected to GC-MS analysis, which enabled the identification of the profile of volatile compounds. This is the first report of investigating the phytochemical and biological aspects of *E. hyoscyamoides*.

2. Materials and methods

2.1. Plant material and extraction

E. hyoscyamoides Boiss. & Buhse was collected in June 2023 from Mashhad, Khorasan Province, Iran. The plant was identified by Dr. F. Mojab, a qualified pharmacognosy professor. A voucher specimen (HPSRC-101) was deposited at the herbarium of the Pharmaceutical Sciences Research Center at Shahid Beheshti University of Medical Sciences. Aerial parts of *E. hyoscyamoides* were shade-dried and ground into fine powder. An aliquot of 150 g of powdered *E. hyoscyamoides* was macerated with ethanol (96 %) (3 × 450 mL) on a GFL 3017 orbital shaker (Burgwedel, Germany) for three days. The extracted solution was subjected to filtration utilizing Whatman filter paper and subsequently concentrated using a Heidolph rotary evaporator (Schwabach, Germany) at 45 °C and 70 rpm. The yield of extraction was 9 %. The dried extract was immediately stored at 4 °C in the dark to maintain its stability.

2.2. Bacterial strains

The antibacterial properties of the ethanol extract from *E. hyoscyamoides* were evaluated against six bacterial strains, including *Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* ATCC 10031, *Kocuria rhizophila* ATCC 9341, *Staphylococcus aureus* ATCC 6538, *S. epidermidis* ATCC 12228, and *Pseudomonas aeruginosa* ATCC 9027. These microorganisms were acquired from the Iranian Research Organization for Science and Technology. The bacterial culture preparation involved the cultivation of the microorganisms on Merck Mueller Hinton Agar-MHA medium(Darmstadt, Germany) and maintaining them incubated at 37 °C for 24 h.

2.3. Antibacterial susceptibility test

The antibacterial properties of *E. hyoscyamoides* ethanol extract were assessed using the Cup-Plate method. In this procedure, 7 mm diameter wells were created in the MHA medium, that had been inoculated with a microbial suspension adjusted to a 0.5 McFarland standard (1×10^8 CFU/mL) in distilled water. The wells were then filled with 100 μL of the *E. hyoscyamoides* ethanol extract at varying concentrations (1000, 500, 250, 125, and 62.5 mg/mL), all dissolved in water. A well containing only the solvent was included as a negative control. The plates were subsequently incubated at 37 °C for 24 h. The experiment was conducted in triplicate, and the mean values of the results were reported [9,24].

2.4. Minimum inhibitory concentration (MIC)

The MIC was determined using the agar dilution technique following the recommendations set by the Clinical & Laboratory Standards Institute. Ethanol extract from *E. hyoscyamoides* was mixed with melted MHA to get homogeneous concentrations of 250, 125, 62.5, 31.25, 15.62, 7.81, 6.25, and 3.9 mg/mL. Bacterial suspensions, standardized to a 0.5 McFarland turbidity, were prepared and then diluted 10 times by using sterile normal saline. A volume of 2 μL from each diluted bacterial suspension was carefully applied to the surface of the agar mediums containing different concentrations of the *E. hyoscyamoides* ethanol extract. One plate served as a negative control, without the plant extract. All plates were then incubated at 37 °C for 24 h, and the MIC determinations were performed in triplicate for reliable results [24].

2.5. The DPPH radical scavenging assay

The DPPH radical scavenging assay was performed using a range of concentrations (6.25–800 μg/mL) of the ethanol extract from *E. hyoscyamoides*. These dilutions were transferred to 96-well plates and kept in the dark at 37 °C for 30 min. Following the addition of DPPH, the absorbance was read at 517 nm using a BioTek microplate reader (Winooski, Vermont, USA). Rutin and vitamin C were used as positive controls at concentration ranges of 1.563–6.25 and 0.781–6.25 μg/mL, respectively [25,26].

2.6. Total phenolic content

The analysis of the TPC of *E. hyoscyamoides* ethanol extract was performed using the Folin-Ciocalteu reagent. Absorbance was measured at 765 nm with a Multispec-1501 Shimadzu UV spectrophotometer (Kyoto, Kyoto, Japan). A calibration curve was established using gallic acid as the standard. All measurements of TPC were conducted in triplicate, and the TPC was reported as gallic acid equivalents (mg GAE per gram of sample) [27,28].

2.7. Total flavonoid content

The TFC was evaluated using the $AlCl_3$ colorimetric technique. The measurement of absorbance was conducted at 420 nm. A calibration curve was constructed utilizing rutin as the standard, and the determination of TFC in *E. hyoscyamoides* ethanol extract was performed in triplicate. The quantification of these compounds was calculated based on the equation derived from the aforementioned line diagram. TFC was expressed as the amount of rutin equivalents in the dry extract matter, measured in milligrams per gram (mg RE/g) [29,30].

2.8. Extraction of the essential oil

The EO of *E. hyoscyamoides* was extracted through hydrodistillation employing a Clevenger-type apparatus for 4 h. Following extraction, the EO was desiccated utilizing anhydrous sodium sulfate and kept refrigerated at 4 °C until further analysis.

2.9. GC-MS analysis of the essential oil and identification of volatile compounds

The chemical composition of the nonderivatized EO obtained from *E. hyoscyamoides* was subjected to GC-MS analysis. The analysis was conducted on an Agilent 7000 Series MS instrument (Santa Clara, CA, USA) equipped with an HP-5ms column (30 m × 0.25 mm inner diameter, 0.25 μm film thickness), a triple quadrupole mass detector, and a computing system integrated with Wiley 7n.l and NIST version 2.0 libraries. The GC-MS procedure involved an initial temperature of 60 °C maintained for 5 min, followed by a gradual increase from 60 °C to 250 °C over a period of 30 min, and a final at 250 °C for 10 min, resulting in a total run time of 60 min. The injector temperature was set at 250 °C, with a 1 μl injection of the EO and a split ratio of 1:50. Helium served as the carrier gas, maintaining a flow rate of 1.1 mL/min. The mass spectrometer functioned under an ionization potential of 70 eV, an ionization current of 150 μA, an ion source temperature of 230 °C, a quadrupole temperature of 150 °C, and a mass range spanning from 25 to 1000 amu in full scan mode.

Volatile compound identification was achieved by matching the obtained EI-MS fragmentation patterns at 70 eV with those in the NIST MS Search algorithm (version 2.0) library. Retention indices (RI) were also used as supplementary identifiers, with the experimental values compared to literature reference indices within a tolerance of ±15 units. To calculate the RI for each respective peak, a 1 μL injection of an n-alkane mixture (ranging from C_7 to C_{40}; Sigma-Aldrich, St. Louis, Mo, USA) was performed under similar GC-MS conditions. The RI was obtained utilizing the formula.

$$(1)\quad RI = 100n + 100(tx - tn)/(tn + 1 - tn)$$

In this formula, t_x represents the R_t of the compound, while n and n + 1 denote the alkanes' number of carbon atoms that elute just before and after the compound, respectively [31].

2.10. Statistical analysis

In all tables, data were shown as mean ± SD. The statistical analyses were implemented utilizing GraphPad Prism 9.1 (San Diego, CA, USA) and Microsoft Office Excel 2023. Each experiment was performed at least three times. IC_{50} values were derived from logarithmic regression curves and are conveyed with 95 % confidence intervals.

3. Results

3.1. Results of antibacterial activities of E. hyoscyamoides ethanol extract by well diffusion method

Table 1 illustrates the inhibition zone diameters produced by the ethanol extract of *E. hyoscyamoides*. The negative control, distilled water, demonstrated no inhibitory impact on the examined bacterial strains. The obtained results from Table 1 indicated that the ethanol extract had antimicrobial activity against the selected microorganisms with a diameter zone of inhibition that ranged from 11 to 24 mm. Among the bacteria tested, *S. epidermidis* was the most sensitive to the *E. hyoscyamoides* ethanol extract, represented by the larger inhibition zone.

3.2. Results of minimum inhibitory concentration (MIC) of E. hyoscyamoides ethanol extract in agar dilution method

Table 2 represents the results of an agar dilution test conducted on several types of bacteria. The purpose of this test was to determine the MIC of an extract against these bacteria. The extract effectively inhibited Gram-positive species (*S. epidermidis* and *S. aureus*) at higher concentrations (15.62 mg/mL and 31.25 mg/mL, respectively). However, it inhibited Gram-negative species (*P. aeruginosa*, *K. pneumoniae*, and *E. coli*) at lower concentrations (≤125 mg/mL). Additionally, *K. rhizophila* exhibited intermediate susceptibility, inhibited at 62.5 mg/mL.

As shown in Table 3, the MIC values of *E. hyoscyamoides* ethanol extract on tested microorganisms are expressed. The MIC values obtained in this investigation ranged from 15.62 to 125 mg/mL. *E. hyoscyamoides* ethanol extract exhibited the lowest MIC (15.62 mg/mL) against *S. epidermidis*. This suggested that *S. epidermidis* displayed the highest level of sensitivity among the six microorganisms examined in this study. *K. pneumoniae* and *P. aeruginosa* demonstrated the greatest resistance (MIC of 125) to *E. hyoscyamoides* ethanol extract.

Table 1
The mean diameter of the growth inhibition diameter (mm) of *E. hyoscyamoides* ethanol extract by well diffusion assay. *ATCC (American Type Culture Collection), diameter well (7 mm), mean ± SD value of three independent experiments (n = 3).

No.	Bacteria	Gram	ATCC*	Extract concentration (mg/ml)	Mean (mm) ± SD
1	*Staphylococcus epidermidis*	+	12228	1000	24 ± 0.5
				500	18 ± 0.4
				250	17 ± 1
				125	13 ± 0.5
				62.5	11
2	*Staphylococcus aureus*	+	6538	1000	16 ± 1
				500	15 ± 0.2
				250	13.5 ± 0.5
				125	11
				62.5	–
3	*Pseudomonas aeruginosa*	_	9027	1000	16 ± 0.1
				500	14 ± 0.8
				250	13 ± 0.5
				125	–
				62.5	–
4	*Escherichia coli*	_	8739	1000	20 ± 0.4
				500	16 ± 1
				250	14
				125	12.5 ± 1
				62.5	–
5	*Klebsiella pneumonia*	_	10031	1000	14 ± 0.6
				500	13 ± 0.4
				250	12 ± 0.5
				125	–
				62.5	–
6	*Kocuria rhizophila*	+	9341	1000	14 ± 1
				500	12.5 ± 0.5
				250	11 ± 0.5
				125	–
				62.5	–

Table 2
Results of agar dilution test on studied bacteria, (n = 3). ✓: growth of bacterial colonies.

Extract concentration (mg/mL)	250	125	62.5	31.25	15.62	7.81	3.9
Bacteria							
S. epidermidis	×	×	×	×	✓	✓	✓
S. aureus	×	×	×	✓	✓	✓	✓
P. aeruginosa	×	✓	✓	✓	✓	✓	✓
K. pneumoniae	×	✓	✓	✓	✓	✓	✓
K.rhizophila	×	×	✓	✓	✓	✓	✓
E. coli	×	×	✓	✓	✓	✓	✓

Table 3
MIC (mg/ml) of *E. hyoscyamoides* ethanol extract (n = 3).

Microorganism	MIC (mg/mL)
Staphylococcus epidermidis	15.62
Staphylococcus aureus	31.25
Pseudomonas aeruginosa	125
Klebsiella pneumonia	125
Kocuria rhizophila	62.5
Escherichia coli	62.5

3.3. Results of DPPH radical scavenging assay

As shown in Fig. 1, *E. hyoscyamoides* ethanol extract exhibited noticeable concentration-dependent anti-radical activity. The ethanol extract of *E. hyoscyamoides* exhibited notable antioxidant activity, with an IC_{50} value of 48.19 ± 0.61 μg/mL. The standards including rutin and vitamin C showed antioxidant activity, with IC_{50} values of 9.26 ± 0.25 and 2.29 ± 0.22 μg/mL, respectively.

3.4. Results of TPC and TFC in E. hyoscyamoides ethanol extract

TPC was calculated as 84.15 ± 2.5 mg GAE/g which was equal to 21.03 % TPC/extract according to the standard calibration curve ($y = 0.005x + 0.044$, $r^2 = 0.99$).

TFC was calculated as 19.35 ± 1.3 mg RE/g which was equal to 4.83 % TFC/extract based on the standard calibration curve ($y = 0.0142x + 0.0472$, $r^2 = 0.99$).

3.5. Results of GC-MS analysis of E. hyoscyamoides essential oil

Table 4 presents the detailed results of a GC-MS analysis of the EO extracted from *E. hyoscyamoides*. This analysis was conducted to elucidate the chemical composition of the EO, which is crucial for understanding its biological activities and potential applications. According to the comprehensive GC-MS analysis, a total of 93.6 % of the EO's chemical composition was successfully identified, encompassing a diverse array of 50 compounds. Several terpenes were present, including limonene, terpinolene, α-terpineol, linalyl acetate, neryl acetate, geranyl acetate, (E)-caryophyllene, humulene, germacrene D, bicyclogermacrene, β-bisabolene, nerolidol. Compounds like methyl palmitate, linolenic acid methyl ester, oleic acid, and n-octadecyl ethanoate suggested the presence of fatty acids and their esters. A series of n-alkanes were identified, ranging from n-decane to n-nonacosane.

The major compounds present in the EO were 2,4-di-tert-butylphenol (10.7 %), n-eicosane (10.5 %), elemol (6.8 %), n-docosane (6.7 %), and Linalyl acetate (4.2 %). 2,4-di-tert-butylphenol, an alkylphenol, is accomplished by scavenging free radicals and

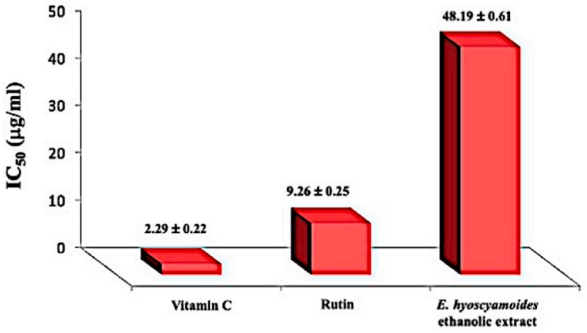

Fig. 1. IC_{50} of extract, rutin, and vitamin C.

Table 4
Chemical compounds identified in *E. hyoscyamoides* essential oil by GC-MS analysis.

No.	Compound	R_t	Area (%)	Exp. RI	L. RI
1	n-Decane	5.24	0.1	997	1000
2	Limonene	5.91	0.1	1027	1027
3	Terpinolene	7.50	1.0	1099	1098
4	α-Terpineol	9.82	1.5	1195	1200
5	**Linalyl acetate**	**11.41**	**4.2**	1255	1257
6	Neryl acetate	14.20	0.8	1362	1362
7	Geranyl acetate	14.68	2.3	1381	1381
8	(E)-Caryophyllene	15.64	2.4	1420	1420
9	Humulene	16.49	0.5	1454	1455
10	Germacrene D	17.16	3.7	1482	1482
11	Bicyclogermacrene	17.53	0.5	1497	1495
12	β-Bisabolene	17.78	0.1	1507	1509
13	**2,4-Di-tert-butylphenol**	**17.90**	**10.7**	1512	1512
14	**Elemol**	**18.78**	**6.8**	1550	1549
15	Nerolidol	19.57	1.2	1584	1583
16	n-Hexadecane	19.83	1.0	1595	1600
17	10-epi-γ-Eudesmol	20.70	0.2	1634	1632
18	(E)-4-Hexadecen-6-yne	21.13	0.1	1653	MS
19	α-Eudesmol	21.20	0.1	1656	1653
20	3-Methylhexadecane	21.52	0.4	1671	1673
21	n-Heptadecane	22.04	0.7	1694	1700
22	n-Octadecane	24.18	9.3	1799	1800
23	3-cyclohexyl- dodecane	24.35	0.9	1807	MS
24	Hexahydrofarnesyl acetone	25.13	2.9	1846	1846
25	Farnesyl acetone	25.65	0.2	1871	1881
26	n-Nonadecane	26.21	0.4	1899	1900
27	Farnesyl acetone	26.64	0.1	1921	1919
28	Methyl palmitate	26.75	0.1	1927	1927
29	2-Methylnonadecane	27.21	0.9	1951	1966
30	Dibutyl phthalate	27.52	2.7	1967	1967
31	2,6,10,14-Tetramethylheptadecane	27.60	0.7	1971	MS
32	n-Heptadecanol	28.03	0.1	1993	1986
33	**n-Eicosane**	**28.15**	**10.5**	1999	2000
34	5E-Eicosene	28.40	3.3	2013	MS
35	n-Heneicosane	30.00	0.4	2101	2100
36	Linolenic acid, methyl ester	30.04	0.5	2103	2101
37	Oleic acid	30.13	0.4	2108	2110
38	Phytol	30.37	0.7	2121	2119
39	5-Methylheneicosane	30.92	0.5	2151	2151
40	8-Heptylpentadecane	31.29	0.4	2172	MS
41	**n-Docosane**	31.78	**6.7**	2198	2200
42	n-Octadecyl ethanoate	32.00	0.3	2211	2211
43	n-Heptadecylcyclohexane	32.10	1.7	2217	MS
44	n-Tricosane	33.49	3.4	2298	MS
45	n-Tetracosane	35.13	2.3	2398	2400
46	n-Pantacosane	36.71	0.5	–	2500
47	n-Hexacosane	37.96	0.8	–	2600
48	n-Heptacosane	39.99	1.4	–	2700
49	n-Octacosane	42.11	0.7	–	2800
50	n-Nonacosane	44.84	2.4	–	2900
Total Identified	**93.6 %**				

combating oxidative damage as well as the possession of antifungal effects [32,33]. Elemol is a sesquiterpene alcohol acknowledged as a fragrance ingredient and used for insecticidal and anti-termite properties [34,35]. Linalyl acetate, the acetate ester of the monoterpene alcohol linalool has admired sedative and anxiolytic properties [36]. Additionally, n-docosane is a long-chain saturated hydrocarbon.

4. Discussion

E. hyoscyamoides is an endemic plant that has been ethnobotanically used in the wound healing process. This study was the first evaluation of the analysis of *E. hyoscyamoides* chemical composition. We also assessed the antibacterial and antioxidant effects of *E. hyoscyamoides* ethanol extract for the first time.

The application of synthetic preservatives for prevention and antibiotics to combat infectious diseases can yield a variety of undesirable consequences such as allergic reactions, hypersensitivity, and a weakened immune system. Hence, there exists a growing necessity for developing new, natural alternatives that can serve as preservatives or antimicrobial agents [37,38].

By considering the antibacterial activities of the ethanol extract, it is inferred that among the Gram-positive bacteria strains,

E. hyoscyamoides ethanol extract showed the most effectiveness on *S. epidermidis* with mean growth inhibition diameter of 24 and 18 mm at 1000 and 500 mg/mL concentrations, respectively and MIC value equaled to 15.62 mg/mL. In other Gram-positive bacteria strains, acceptable antibacterial effects were observed as well. Among the Gram-negative bacteria strains, *E. hyoscyamoides* ethanol extract displayed the most effectiveness on *E. coli* with mean growth inhibition diameters of 20 and 16 mm at 1000 and 500 mg/mL concentrations, respectively and MIC values equaled 62.5 mg/mL. In two Gram-negative strains including *P. aeruginosa* and *K. pneumoniae,* no growth inhibition zone was observed at 125 mg/mL consternation and the lower ones. According to the results of the study, the largest inhibition zone in the six tested bacteria strains was observed at 1000 mg/mL concentration, which could confirm that the antibacterial activities of *E. hyoscyamoides* ethanol extract was dose-dependent.

Modarressi et al. discovered that iridoid glycosides including phloyoside I, phlomiol, and pulchelloside I isolated from the rhizomes of *E. laciniata* exhibited low to moderate levels of antibacterial impacts with MIC values ranging from 0.05 to 0.50 mg/mL. Among these phytochemicals, pulchelloside I was the most effective, showing activity against *Bacillus cereus*, *Proteus mirabilis*, penicillin-resistant *E. coli*, and *S. aureus* with a MIC of 0.05 mg/mL [39]. Vahedi et al. reported that the methanol extract of *E. labiosiformis* had antibacterial effects against phytopathogens such as *Xanthomonas campestris*, *Pseudomonas viridiflava*, and *Rathayibacter rathayi* [40]. Hariri et al. found that *E. binalodensis* methanolic extract prevented *S. mutans* biofilm formation at 4.096 mg/mL concentration [41]. The *E. hyoscyamoides* ethanol extract showed promising, species-specific antibacterial activity, warranting further investigation into active compound isolation, in vivo efficacy evaluation, and structure-activity relationships investigation to enhance specificity and potency. These antibacterial potentials were in accordance with findings of previous studies conducted on other species of *Eremostachys*.

Natural compounds may exhibit antioxidant properties by offering protection against free radicals, which can induce modifications in the composition of DNA, signaling pathways, and cellular membranes [42]. Phenolic compounds represent a group of phytochemicals derived from plants, characterized by the presence of an aromatic ring connected to hydroxyl groups. These compounds are recognized with numerous advantages for human health, including cardiovascular diseases and neurodegenerative disorders [43].

E. hyoscyamoides ethanol extract showed remarkable antioxidant activity with IC_{50} equaled to 48.19 $\pm$ 0.61 μg/mL. TPC was calculated as 84.15 $\pm$ 2.5 mg GAE/g and TFC was measured as 19.35 $\pm$ 1.3 mg RE/g. It is inferred that the promising antioxidant potential of *E. hyoscyamoides* ethanol extract might be attributed to the presence of phenolic and flavonoid compounds. Asnaashari et al. reported that the methanol extract of *E. azerbaijanica* showed significant scavenging activity [44]. Bajalan and coworkers revealed that *E. laciniata* methanol extract showed DPPH radical scavenging activity with IC_{50} equaled to 54.64 μg/mL [45].

This study reported a detailed profile of the EO of *E. hyoscyamoides* for the first time. The different components (n = 50) were identified in *E. hyoscyamoides* EO. Based on the findings derived from the GC-MS analysis, a significant portion of the chemical composition of the EO, amounting to 93.6 %, was successfully identified. This composition consisted of a total of 50 compounds. Among these compounds, the primary ones identified in *E. hyoscyamoides* essential oil were 2,4-Di-tert-butylphenol (10.67 %), n-Eicosane (10.54 %), Elemol (6.82 %), n-Docosane (6.75 %), and Linalyl acetate (4.20 %). According to the literature review, there has been chemical variability reported in the EOs composition of various species from the *Eremostachys* genus and also in different plant parts of each species of varied geographical regions and at different flowering stages [16–18,46–49].

5. Conclusion

This research was done in order to identify the chemical composition of *E. hyoscyamoides* EO and study the antioxidant and antimicrobial effectiveness of its ethanol extract. The GC-MS analysis of the *E. hyoscyamoides* EO yielded the identification of 50 compounds. The high content of 2,4-Di-tert-butylphenol, n-Eicosane, Elemol, n-Docosane, and Linalyl acetate were detected. With the TPC and TFC in high levels, *E. hyoscyamoides* ethanol extract exhibited high antioxidant activities during the DPPH assay. Furthermore, the antibacterial effects of the ethanol extract from *E. hyoscyamoides* were observed against *S. aureus*, *S. epidermidis*, *M. luteus*, *E. coli*, *P. aeruginosa*, and *K. pneumoniae*. Our findings offer a valuable source of information regarding the EO and ethanol extract derived from *E. hyoscyamoides*.

Funding

This work was financially supported by the Research Council of Shahid Beheshti University of Medical Sciences (Grant no. 43008632).

Compliance with ethical standards:

All experiments were conducted according to the ethical guidelines of SBMU Pharmacy School, Tehran, Iran (IR.SBMU.PHARMACY.REC.1402.276).

Data availability statement

All data are within the manuscript.

CRediT authorship contribution statement

Marjan Talebi: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Investigation, Formal analysis, Data curation. **Afsaneh Arefei Oskouie:** Writing – review & editing, Validation, Software, Investigation, Formal analysis. **Arash Mahboubi:** Writing – review & editing, Validation, Software, Investigation. **Mohammad Khani:** Visualization, Validation, Resources, Methodology, Investigation. **Faraz Mojab:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This article is part of Mohammad Khani's Pharm.D. thesis project. The authors gratefully acknowledge the financial support provided by Shahid Beheshti University of Medical Sciences in Tehran, Iran, which made this research possible. Before submission, all authors have thoroughly reviewed the manuscript and approved the final version.

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