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RESEARCH ARTICLE

Chemical Composition and Antimicrobial Activity of *Hesperis isatidea* (Boiss.) D.A.German & Al-Shehbaz Essential Oil

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ABSTRACT

The essential oils of aerial parts of *Hesperis isatidea* were analyzed using gas chromatography-mass spectrometry for the first time in the present study. The major components were determined as benzyl salicylate (13.40%), phytol (9.66%), benzyl benzoate (8.57%), undecanal (8.45%), and linalool (5.71%). The essential oil exhibited notable antibacterial activity against *Clostridium perfringens*, with a minimum inhibitory concentration (MIC) of 125 µg/mL, and *Escherichia coli*, with an MIC of 62.5 µg/mL. However, it showed limited effectiveness against *Staphylococcus aureus* and *Listeria monocytogenes*, both with an MIC of 250 µg/mL, and no antibacterial activity against *Enterococcus faecalis* and *Bacillus cereus*. The results demonstrate that the essential oil possesses selective antibacterial properties, especially against specific Gram-negative bacteria, indicating its potential as a valuable complementary antimicrobial agent.

1 | Introduction

The family Brassicaceae comprises 352 genera and approximately 3700 species worldwide. *Hesperis* L. (Brassicaceae) species, comprising 56 species globally, are widely distributed across the northern hemisphere, with a significant concentration in Asia and Europe [1]. Thirty-two species are naturally distributed in Turkey, exhibiting an 82% endemism ratio, suggesting that Turkey is the probable center of diversity for this species [1, 2]. *Hesperis isatidea* (Boiss.) D.A. German & Al-Shehbaz, also known as *Tchihatchewia isatidea* Boiss. and *Neotchihatchewia isatidea* (Boiss.). [3] *H. isatidea* is a species endemic to Turkey's eastern regions [4]. *Hesperis* sp. are utilized globally for sedative and hypnotic [5], antiscorbutic, diuretic, diaphoretic [6], and expectorant [5–7]. *H. isatidea* flowers are used in folk medicine as a wound healer [8], antitussive [9], sinusitis [10], bronchitis, [10], and skin diseases [8]. Previous studies have reported the possesses of antibacterial [11], antioxidant [11], acetylcholinesterase inhibition [12], and antidiabetic activities [13] of ethanolic and methanolic extracts of *Hesperis* species. A variety of biologically active compounds, such as phenolic and organic acids [14], as well as flavonoids [14], have been identified from *H. isatidea*. These compounds are responsible for antioxidant [15], anti-diabetic [14], antibacterial [15], anti-tyrosinase [14], and cytotoxic [16] activities.

The rising prevalence of multidrug-resistant pathogens poses a critical threat to global health, driving the need for alternative antimicrobial agents [17]. Essential oils, complex mixtures of volatile compounds derived from plants, have gained attention for their broad-spectrum antimicrobial properties, including

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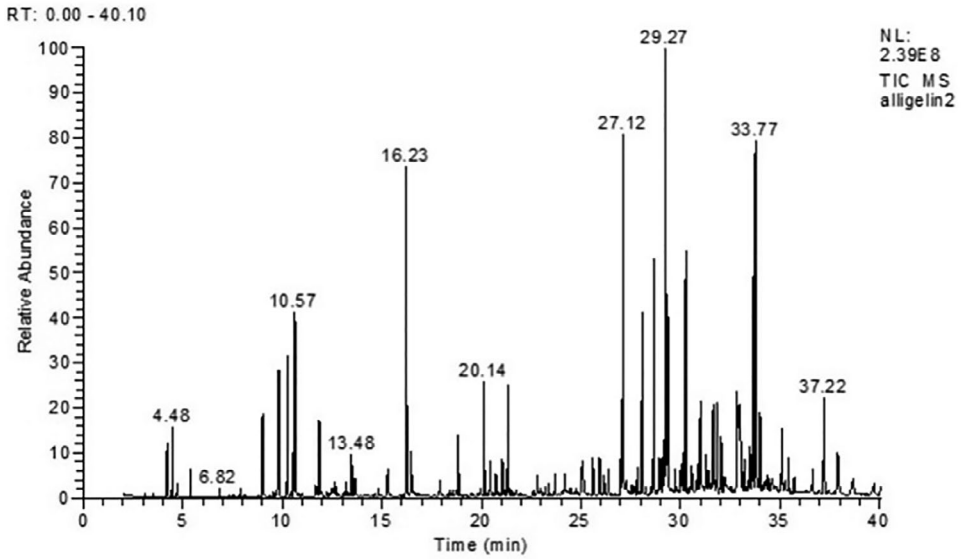


FIGURE 1 | Gas chromatography (GC) chromatogram showing separation and retention times of essential oil.

activity against Gram-positive and Gram-negative bacteria [17, 18]. Additionally, some essential oils also act as chemosensitizers, enhancing the efficacy of existing antibiotics and restoring susceptibility in resistant strains [19]. The mechanisms underlying the antimicrobial effects of essential oils are diverse, including disrupting bacterial cell membranes, increasing permeability, and inducing cell death [20].

The global economy is increasingly recognizing the value of natural antimicrobial agents [21], particularly in the food industry, where essential oils are being explored as preservatives due to their ability to inhibit microbial growth and extend shelf life [21, 22]. The rising consumer preference for natural products over synthetic preservatives further underscores the relevance of essential oils in contemporary food safety practices. Moreover, the integration of essential oils into food preservation strategies not only addresses microbial resistance but also aligns with the growing demand for clean-label products in the marketplace [21–23].

In recent years, there has been growing interest among scientists in discovering aromatic herbal plants with diverse therapeutic properties. In particular, researchers and clinical practitioners are increasingly focused on the study of plant-derived antibacterial essential oils due to their natural abundance and cost-effective treatment potential. Numerous *Hesperis* L. species have been the subject of in-depth research to determine the chemical composition and biological activity of their essential oils. To our knowledge, no research *on H. isatidea* has been conducted. Therefore, we are reporting here for the first time on the chemical composition and antibacterial activity of the *H. isatidea*.

2 | Results

2.1 | Phytochemical Analysis by Gas Chromatography-Tandem Mass Spectrometry

The compositions of the essential oil extracted from the aerial parts of *H. isatidea* are presented in Table 1 and Figure 1. The essential oil was obtained as a yellowish liquid with a yield of 0.026% (v/w), calculated based on fresh weight. The essential oil yield is compatible with the low yield of other *Hesperis* species. [24] According to gas chromatography-mass spectrometry (GC-MS) analysis, 31 compounds (accounting for 98.43% of the total composition) were identified in the essential oil (Table 1). The identified chemicals were classified into aldehydes/ketones (26.61%), alcohol (6.15%), monoterpenes (11.33%), Diterpenoid alcohol (10.57%), hydrocarbons (6.91%), and esters (33.96%). The main components (> 5%) were identified as benzyl salicylate (13.40%), phytol (9.66%), benzyl benzoate (8.57%), undecanal (8.45%), and linalool (5.71%).

2.2 | Antibacterial Activity of *H. isatidea* Essential Oil

Table 2 illustrates the antibacterial activity assay findings, which reveal that *H. isatidea* essential oil has moderate antimicrobial activity against four pathogenic bacteria: *Staphylococcus aureus* (inhibition zone: 9.2 ± 0.2 mm) and *Listeria monocytogenes* (inhibition zone: 7.0 ± 0.3 mm) in the disc diffusion assay. Additionally, the essential oil showed remarkable antibacterial activity, especially against *Escherichia coli* (inhibition zone: 20.1 ± 0.2 mm) and *Clostridium perfringens* (inhibition zone: 13.1 ± 0.5 mm). However, no antibacterial activity was observed against *Enterococcus faecalis* and *Bacillus cereus* strains.

The essential oil exhibited notable antibacterial activity against *C. perfringens*, with a minimum inhibitory concentration (MIC) of 125 µg/mL, and *E. coli*, with an MIC of 62.5 µg/mL. However, it showed limited effectiveness against *S. aureus* and *L. monocytogenes*, both with an MIC of 250 µg/mL and no antibacterial activity against *E. faecalis* and *B. cereus* (Table 2).

The antibacterial activity of the essential oil varied significantly among different bacterial strains ($p < 0.05$, one-way ANOVA). Further post-hoc analysis revealed that the inhibition zone *for E. coli* was significantly higher compared to *L. monocytogenes*

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TABLE 1 | Chemical compositions of *H. isatidea* essential oil.

No.	RT	RI*	RI**	Component	% Comp***
1	4.48	855	856	3-hexen-1-ol	0.76 ± 0.1
2	9.00	1047	1049	Benzene acetaldehyde	1.33 ± 0.01
3	9.82	1069	1066	*cis*-linalool oxide	2.30 ± 0.05
4	10.25	1096	1094	*trans*-linalool oxide	2.04 ± 0.2
5	10.57	1103	1104	Linalool	**5.71 ± 0.001**
6	10.66	1107	1108	Nonanal	2.18 ± 0.3
7	11.85	1134	1133	*cis*-Verbenol	1.28 ± 0.07
8	13.48	1204	1200	Decanal	1.30 ± 0.06
9	16.22	1310	1308	Undecanal	**8.45 ± 0.02**
10	16.47	1316	1315	2-Methoxy-4-vinylphenol	1.24 ± 0.2
11	18.84	1375	1379	Dodecanal	1.09 ± 0.03
12	20.14	1380	1382	Farnesan	4.72 ± 0.08
13	20.44	1519	1518	Tridecanal	0.73 ± 0.04
14	21.33	1617	1614	Tetradecanal	1.79 ± 0.01
15	27.01	1755	1761	Tetradecanoic acid	0.78 ± 0.5
16	27.12	1763	1760	Benzyl benzoate	**8.57 ± 0.05**
17	28.09	1819	1817	Hexadecanal	3.03 ± 0.09
18	28.67	1830	1827	Hexahydrofarnesyl acetone	3.90 ± 0.6
19	29.26	1870	1869	Benzyl salicylate	**13.4 ± 0.06**
20	29.37	1882	1883	1-Hexadecanol	3.24 ± 0.3
21	30.27	1929	1927	Hexadecanoic acid, methyl ester	3.91 ± 0.06
22	31.01	1963	1964	Hexadecanoic acid	2.12 ± 0.1
23	31.67	1998	1999	9-Octadecenal	2.07 ± 0.9
24	31.85	2011	2010	1-Hexadecanol, acetate	1.56 ± 0.1
25	32.06	2017	2018	Octadecanal	0.74 ± 0.2
26	32.22	2071	2073	Thunbergol	0.91 ± 0.5
27	33.64	2089	2086	9-Octadecenoic acid, methyl ester	3.72 ± 0.4
28	33.77	2122	2119	Phytol	**9.66 ± 0.07**
29	34.00	2129	2128	Octadecanoic acid, methyl ester	1.31 ± 0.7
30	35.45	2210	2208	Acetic acid *n*-octadecyl ester	1.49 ± 0.4
31	37.23	2500	2500	Pentacosane	2.19 ± 0.3
Aldehydes & ketones (2, 6, 8, 9, 11, 13, 14, 17, 18, 23, 25)					26.61
Alcohols (1, 10, 20, 26)					6.15
Monoterpenes (3, 4, 5, 7)					11.33
Diterpenoid alcohols (26, 28)					10.57
Hydrocarbons (12, 31)					6.91
Esters (16, 19, 21, 24, 27, 29, 30)					33,96
Fatty acids (15, 22)					2.90
Total identified					98.43

RI*: retention index as determined on an HP-5MS column using the homologous series of n-hydrocarbons, RI **: Retention indexes according to literature, % Composition***: Percent compositions were expressed as mean ± standard deviation of three independent measures.

($p < 0.05$) and *S. aureus* ($p < 0.05$). No significant differences were observed among other bacterial strains.

Imipenem was chosen as a positive control due to its broad-spectrum activity against both Gram-positive and Gram-negative bacteria. It is a clinically relevant antibiotic commonly used in the treatment of bacterial infections, making it a suitable reference for antimicrobial activity comparisons. Furthermore, imipenem is frequently employed in antimicrobial susceptibility tests, allowing for standardized comparisons with previous studies [25]. Imipenem, in contrast to other β-lactam antibiotics, has also been reported to kill non-growing bacteria [26].

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TABLE 2 | Disc diffusion (mm) and Minimum inhibitory concentrations (μg/mL) results of *H. isatidea* essential oil for tested pathogenic bacteria.

Microorganisms	Disc Diffusion (mm)-MIC (μg/mL)	Imipenem* (10 mcg)
Gram-positive		
Clostridium perfringens	[c] 13.1 ± 0.5 mm (125 μg/mL)	17.0 ± 1.0 mm (3.90 μg/mL)
Listeria monocytogenes	[b] 7.0 ± 0.3 mm (250 μg/mL)	36.3 ± 2.1 mm (<3.90 μg/mL)
Bacillus cereus	ni (nt)	30.0 ± 1.0 mm (<3.90 μg/mL)
Staphylococcus aureus	[b] 9.2 ± 0.2 mm** (250 μg/mL ***)	45.3 ± 0.6 mm (<3.90 μg/mL)
Enterococcus faecalis	ni (nt)	45.0 ± 1.0 mm (<3.90 μg/mL)
Gram-negative		
Escherichia coli	[a]20.1 ± 0.2 mm (62.5 μg/mL)	35.3 ± 2.1 mm (<3.90 μg/mL)

ni: no inhibition, nt: not tested, *: Positive control (Imipenem), **: inhibition zone diameters in mm (mean ± sd, $n = 3$), ***: MIC in μg/mL.

3 | Discussion

The essential oil composition of *H. isatidea* shows notable differences compared to other *Hesperis* species reported in the literature. According to our knowledge of the literature [27], benzyl salicylate, undecanal, benzyl benzoate, and phytol were detected for the first time in studies conducted on plants of *Hesperis* sp [24, 27]. The essential oil from *Hesperis* sp. was primarily characterized by the presence of linalool. Linalool has previously been reported in essential oils obtained from *H. bicuspidate* (1.3%) [27], *H. bottae* (1.0%) [27], *H. podocarpa* (1.1%) [28], and *H. matronalis* (1.4%) [29]. In the presented study, it was determined that the linalool content (5.71%) was higher in the *H. isatidea* aerial parts essential oil compared to other *Hesperis* sp. The chemical composition differences among *Hesperis* sp. can be attributed to genetic factors and environmental conditions of their growing regions (such as soil type and climate). This study also provides novel insights into the metabolic capacity of *H. isatidea*, shedding light on the ecological adaptation processes of this species. Similar findings have been reported in other plant taxa [30], where environmental and genetic factors significantly influence secondary metabolite profiles [30, 31].

According to our knowledge of the literature, the antibacterial effects of the essential oil from *H. isatidea* are being documented for the first time in this study. Jirovetz and colleagues reported that benzyl salicylate and benzyl benzoate exhibited antibacterial activity on *S. aureus*, *E. faecalis*, and *E. coli* bacterial chains [32]. In another study, it was reported that benzyl benzoate [33] and linalool [34] had antibacterial activity on *E. coli* and *L. monocytogenes*. The effect of Linalool against *L. monocytogenes* has been reported to effectively inhibit cell growth by disrupting cell membrane structure [34].

Again, the antibacterial activity of undecanal against the *Salmonella choleraesuis* spp. *choleraesuis* bacterial chain has been reported [35]. Phytol, the other major component, is known to have high antibacterial activity on *E. coli* [36], *Pseudomonas aeruginosa* [36], *Clostridium sporogenes* [37], *Sarcina lutea* [37], and *E. faecalis* bacterial chains [36, 37]. It has been emphasized that phytol exerts antibacterial effects by dysregulating the function of eukaryotic cells through a series of effects, including disruption of membrane permeability and depolarization of the mitochondrial membrane [36]. In light of this information, the antibacterial activity of *H. isatidea* essential oil can be attributed to its major components, and the mechanism of action of the compounds responsible for the antibacterial activity can be explained in this way.

This study gives important information on the antibacterial activity of essential oils derived from *H. isatidea*. In vitro techniques were used to examine antibacterial activity (disc diffusion method), and MIC values were calculated to estimate the efficiency of the essential oils against specific bacterial strains. These findings contribute to the growing body of research on natural antimicrobial agents. Future research should focus on validating these effects under physiological settings using in vivo/clinical trials, researching molecular mechanisms of action, and investigating possible synergistic effects with conventional antibiotics to improve their therapeutic potential.

4 | Conclusion

The present study marks the first comprehensive analysis of the chemical composition and antibacterial activity of the essential oil derived from *H. isatidea*. The GC-MS analysis identified 31 compounds with benzyl salicylate, phytol, benzyl benzoate, undecanal, and linalool being the major constituents. The essential oil demonstrated moderate antibacterial activity against *S. aureus, C. perfringens, L. monocytogenes, and E. coli*. Notably, the antibacterial properties of *H. isatidea* essential oil can be attributed to its major components, such as benzyl salicylate and phytol, which have known antimicrobial effects. This study provides new insights into the potential use of *H. isatidea* essential oil as a natural antibacterial agent and underscores the importance of further research to explore its therapeutic applications.

5 | Materials and Methods

All experimental details, including extraction procedures and static analysis, are available in the Supporting Information.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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