Chemical Methodologies 8(2024) 47-57

Chemical Methodologies

Journal homepage: http://chemmethod.com

Original Article

Phytochemical Evaluation of Aerial Parts of *Eryngium Billardieri* growing in Iran

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ARTICLEINFO

Article history
Submitted: 2023-10-30
Revised: 2023-11-11
Accepted: 2023-12-24
Manuscript ID: CHEMM-2311-1740
Checked for Plagiarism: Yes
Language Checked: Yes

DOI:10.48309/chemm.2024.427947.1740

KEYWORDS
HPLC
Essential oil
GC-MS
NMR

ABSTRACT

Introduction*: Eryngium* plants represent the most diverse species within the Apiaceae family, with approximately 250 species. Numerous species of *Eryngium*, including *Eryngium campestre* and *E. foetidum*, have been used for centuries in traditional medicine. *E. billardieri* was selected for this study due to its purported applications in traditional Iranian medicine, as well as the absence of prior research on the chemical constituents within this plant.
Materials and methods: The aerial parts of the plant were extracted by *n*-hexane, dichloromethane, and methanol (MeOH) by a Soxhlet apparatus. The MeOH extract was exposed to C18 Sep-Pak fractionation cartridge by a step gradient of MeOH-H2O. Further purification was performed by preparative HPLC and the purified compounds were separated to be elucidated with H NMR and C NMR results. The essential oil was obtained by the Clevenger apparatus. The *n*-hexane extract was transformed into methyl ester through a process involving saponification and esterification and the obtained essential oil and fatty acids were analyzed using a flame ionization detector (FID) and a DB-1 capillary column.
Results: The 40% methanol extract led to the identification of two compounds such as decursine and Chlorogenic acid. The essential oil composition analysis yielded the following results: approximately 92% of the essential oil compounds were identified. The predominant compounds were isofenchol (36.85%), germacrene D (7.69%) and gurjunene (6.67%). The analysis of methyl esters revealed the presence of four fatty acid compounds: oleic acid with 27.2%, elaidic acid with 6.91%, palmitic acid with 4.62%, and stearic acid with 2.55% of the total area.
Conclusion: Two substances (decursine and chlorogenic acid) were isolated from the methanol extract, and oleic acid was isolated and identified from the *n*-hexane extract. Concerning the phytochemical potential inherent in this genus and specifically in this plant, further extensive research in this domain is warranted.

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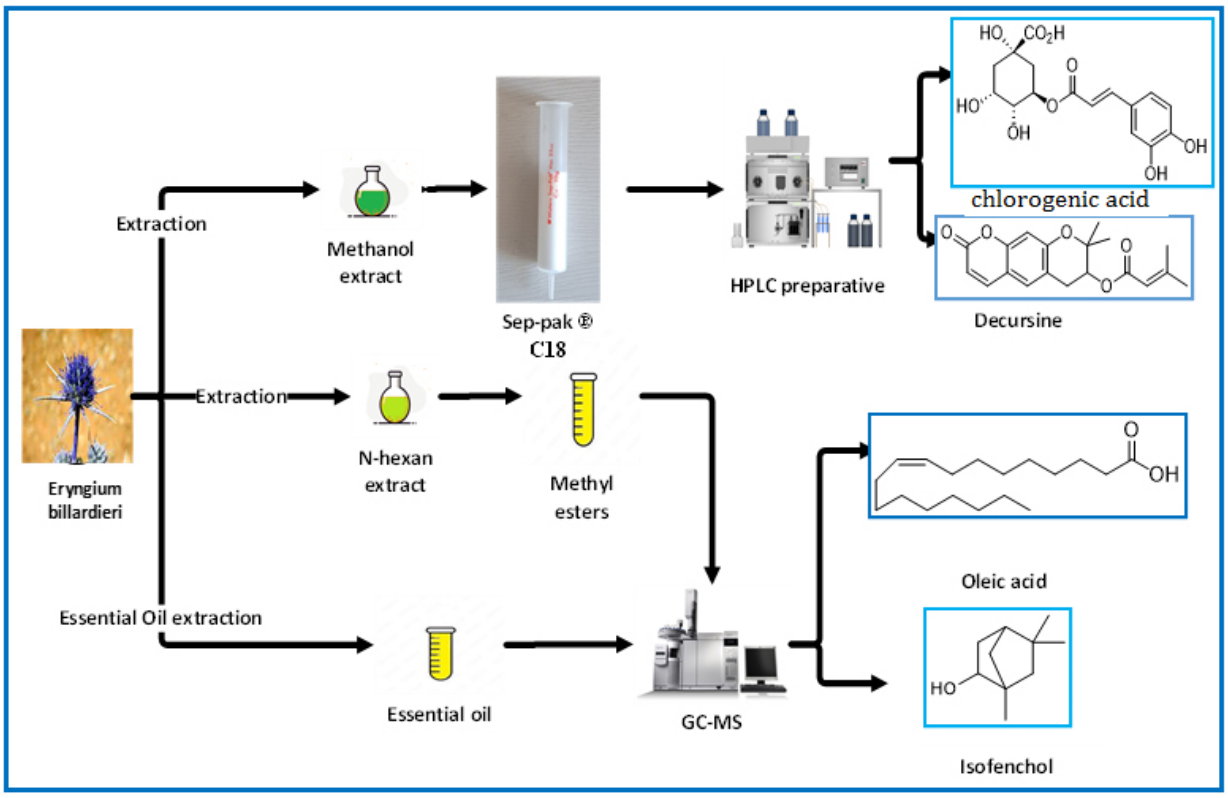
GRAPHICAL ABSTRACT



Introduction

Botanically, *Eryngium* plants represent the most diverse species within the Apiaceae family, comprising approximately 250 acknowledged species. These perennial plants are usually bisexual, featuring prickly leaves and petals, and can reach heights of up to 150 cm. Certain species undergo a notable color transformation, shifting toward a blue–violet hue during the growth season [1, 4]. Numerous species of *Eryngium*, including *Eryngium campestre*, *E. serbicum*, and *E. foetidum*, which are part of the Apiaceae family, have been used for centuries in traditional medicine as medicinal plants [3, 4]. Moreover, recent studies have demonstrated that various species within the genus *Eryngium* are rich sources of diverse phytochemicals. Both *in vitro* and *in vivo* studies have unveiled a spectrum of biological and pharmacological activities associated with *Eryngium* species [2, 5, 7]. *E. caucasicum* and *E. campestre* are extensively utilized as leafy vegetables in regions spanning Asia and Spain [8,9]. In Nigeria, both the seeds and fruits of *E. foetidum* hold a prominent place in nutrition. Originating from the West Indies and Tropical America, these plants are valued for their medicinal properties and culinary uses. Over time, they have been intentionally cultivated in South Asia, Pacific islands, various tropical regions of Africa, and southern Europe [10]. *Eryngium* campestre is a favored option in Turkish traditional medicine. Preparations made from both the aerial parts and roots of the plant have been used as remedies for a range of conditions, including coughs, kidney issues, diminished appetite, fatigue, and sexual dysfunction [11, 12].

In Iranian traditional medicine, numerous medicinal applications of *Eryngium* have been documented. It is known as a diuretic, anti-bloating agent, antidote for poisoning and snakebite, digestive aid, enhancer of sexual potency, anti-inflammatory agent, and pain reliever. These plants are also used to alleviate the effects of insect bites and address issues related to conditions such as elephantiasis and intestinal disorders, including colic and cramps. Furthermore, the leaf of *E. caeruleum* is used as a vegetable in the northern regions of Iran. In

addition, it has been reported that *E. billardieri* is taken orally to address constipation and kidney stones [8, 13, 17].

While many species within the *Eryngium* genus remain unexplored from a phytochemical perspective, various sesquiterpenes and monoterpenes have been identified in this genus. Furthermore, triterpene saponins have been isolated from *E. foetidum*, *E. yuccifolium*, *E. campestre*, *E. caerulum* and *E. kotschky* [18, 23]. Furthermore, flavonoids have been isolated in investigations of *E. creticum*, *E. campestre*, *E. giganteum*, *E. macrocalyx*, *E. maritimum*, *E. planum*, and *E. dichotomum* [24,27]. Studies involving polar and semipolar extracts of *E. campestre*, *E. creticum*, *E. biebersteinianum*, *E. bourgatti*, and *E. ilicifolium* have revealed the presence of coumarins [28,32], while investigations of *E. yuccifolium*, *E. alpinum*, *E. maritimum*, and *E. creticum* have led to the extraction and isolation of phenolic compounds [19,31,33].

E. billardieri was selected for this study due to its purported applications in traditional Iranian medicine, as well as the absence of prior research on the chemical constituents within this particular plant.

Materials and Methods

Chemicals

Methanol and dichloromethane were procured from Samchun Chemicals in South Korea and Mojallali Chemicals in Iran. *n*-Hexane was supplied by Merck in Germany.

Plant Material

The aerial parts of *E. billardieri* were collected from the Marmishiow region, near Urmia, West Azerbaijan Province, Iran. The plant was authenticated at the Herbarium of the Pharmacy Faculty, Tabriz University of Medical Sciences, and assigned the herbarium code TBZ-FPH-4045.

Extracting and Separating Fractions

The aerial parts of the plant were first dried and powdered. They were then subjected to extraction using a Soxhlet apparatus. Initially, n-hexane solvent was employed for extraction, followed by dichloromethane and methanol to obtain different extracts from the same plant material. The methanol extract (2 g) was fractionated using solid-phase extraction (SPE) with a Sep-Pak cartridge (C18, 35 cc, 10 g) employing a stepwise gradient of MeOH-water. The previous step yielded six fractions (10:90, 20:80, 40:60, 60:40, 80:20, and 100:0), each measuring 200 ml. All SPE fractions were subsequently concentrated and dried using a rotary evaporator apparatus (Heidolph, Germany) with a maximum temperature of 40 °C. According to the data obtained from analytical HPLC, the 40% fraction was selected for further purification. Preparative HPLC (KNAUER D-14163) with a WATERS column (250 mm × 20 mm, 20 µm) was used for further purification. The mobile phase consisted of the following gradient: 0 to 50 min, 25% - 45% MeOH in water; 50-62.5 min, 45% MeOH in water; 62.5-67.5 min, MeOH in water 45%-25%; 67.5-75 min, 25% MeOH in water. The flow rate was set at 8 mL/min. Two compounds were isolated from the 40% MeOH-SPE fraction: one coumarin with a retention time of 48 minutes and one phenolic compound (RT = 17.6 min).

Essential Oil Isolation

The leaves, flowers, seeds, stems, and spiny sepals of the plant, totaling 100 g, were left at room temperature for drying. Subsequently, they were finely ground and subjected to a Clevenger tool for 2 hours. *n*-Hexane (2 mL) was employed as a collecting agent. The resulting volatile oil from *E. billardieri* exhibited a yellow hue. The essential oil was then separated from the solvent using anhydrous sodium sulfate. Following this, the solvent was removed, leaving behind the obtained oil.

Gas Chromatography-Mass Spectrometry (GC-MS)

The essential oil was analyzed using a flame ionization detector (FID) and a DB-1 capillary column with dimensions of 60 m × 0.25 mm and a thickness of 0.25 µM. The analysis was

conducted under the following conditions: helium was used as the carrier gas at a flow rate of 1.3 ml/min. Initially, the column temperature was maintained at 50 °C for 2 minutes, and then increased at a rate of 3.0 °C/min, reaching 275°C. It was held at 275 °C for an additional 3 minutes. The injector and detector temperatures were set at 250 °C and 280 °C, respectively. The injection volume was 1 µL, and the fractionation ratio was 1:20. Mass spectra were recorded at 70 eV, covering a mass range of 30-600 amu, with a source temperature of 260 °C and a solvent hysteresis of 2.0 minutes. The constituents of the essential oil were identified by comparing mass spectrometry results and Kovat's index to entries in the Wiley and NIST mass spectrometry databases or with authentic compounds. The samples were stored in sealed bottles at -4 °C for subsequent analysis [34, 43].

Preparation of the Fatty Acid Component of n-Hexane Extraction Methyl Ester

The *n*-hexane extract was transformed into methyl ester through a process involving saponification and esterification. Specifically, 5 mL of *n*-hexane and 2 mL of 2 M potassium hydroxide in methanol were combined and vortexed for 2 minutes at room temperature. The resulting mixture was then subjected to a 60 °C water bath for 15 minutes, followed by a cooling phase with tap water. After that, 5 mL of *n*-hexane was added, and the mixture was vortexed and subsequently centrifuged for 10 minutes. Finally, after a 5-minute interval, 1 µL of the uppermost layer from the tube was introduced into the GC for analysis [44].

Analysis of the Fatty Acid Component of n-hexane Extraction by Gas Chromatography

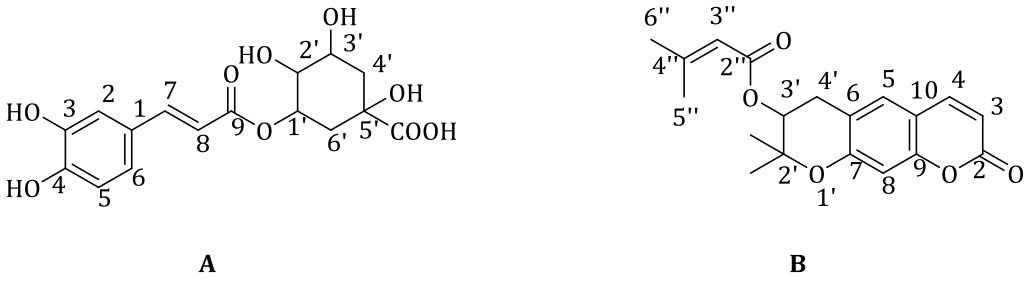
The chromatography was outfitted with a Shimadzu GC–MS-QP5050A, featuring a flame ionization detector (FID), a DB-1 capillary column (inner diameter of 60 m and outer diameter of 0.25 mm, with a film thickness of 0.25 mm), and an additional flame ionization detector. The temperature profile for the initial column commences at 80 °C, experiences a 20 °C/min ascent to 120 °C, followed by a 3 °C/min elevation to 260 °C. Subsequently, the temperature was maintained at 260 °C for 10 minutes. The injector was operated at 260 °C, while the detector was set to 280 °C. The split ratio was 1:50, and the carrier gas (helium) flowed at a rate of 2 mL/min.

Results and Discussion

The reversed-phase HPLC separation of the 40% methanol extract from the plant led to the identification of two compounds: a coumarin and a phenolic compound. The structures of these compounds were elucidated through analysis of data acquired from ^{1}H-NMR and ^{13}C-NMR spectra, corroborated by cross-referencing with established sources. These compounds have been previously documented in the genus *Eryngium.* Compound **A**: molecular formula: $C_{16}H_{18}O_9$: ^{1}H-NMR (DMSO-D6) δ: 1.81 ppm (1H, d, J= 4.0 Hz, H-6'a), 1.83 ppm (1H, d, J= 4.0 Hz, H-6'b), 2.03 ppm (1H, d, J= 4.0 Hz, H-4'a), 2.16 ppm (1H, d, J= 4.0 Hz, H-4'b), 4.05 ppm (1H, dd, J= 4.0 Hz, H-3'), 4.55 ppm (1H, m, H-1'), 6.30 ppm (1H, d, J= 16.0 Hz, H-8), 6.61 ppm (1H, d, J= 4.0 Hz, H-5), 6.78 ppm (1H, d, J= 8.0 Hz, H-6), 7.06 ppm (1H, S, H-2), 7.40 ppm (1H, d, J= 16.0 Hz, H-7). ^{13}C-NMR (DMSO-D6) δ: 37.3 ppm (C-6'), 49.1 ppm (C-4'), 71.3 ppm (C-2'), 72.7 ppm (C-1'), 73.2 ppm (C-3'), 77.9 ppm (C-5'), 115.5 ppm (C-2), 116.3 ppm (C-8), 117.1 ppm (C-5), 123.6 ppm (C-6), 128.2 ppm (C-1), 145.1 ppm (C-7), 145.9 ppm (C-3), 146.5 ppm (C-4), 166.5 ppm (C-9), and 177.2 ppm (C-7'). Based on the reviewed sources and comparative analysis, the identified compound was determined to be chlorogenic acid [33]. Compound **B**: molecular formula: $C_{19}H_{20}O_5$: ^{1}H-NMR (DMSO-D6) δ:1.42 ppm (6H, s, H-6', H-5'), 1.95 ppm (3H, s, H-6''), 2.02 ppm (3H, s, H-5''), 2.83 ppm (1H, dd, J = 6.0 Hz, H-4'a), 5.35 ppm (1H, d, J = 6.0 Hz, H-3'), 5.56 ppm (1H, s, H-3''), 6.41 ppm (1H, d, J = 10.0 Hz, H-3), 6.77 ppm (1H, s, H-8), 7.51 ppm (1H, s, H-5), 7.79 ppm (1H, d, J = 10.0 Hz, H-4). ^{13}C-NMR (DMSO-D6) δ: 20.3 ppm (C-6''), 23.5 ppm (C-5'), 24.8 ppm (C-6'), 26.6 ppm (C-5''), 27.6 ppm (C-4'), 69.4 ppm (C-3'), 84.4 ppm (C-2'), 103.3 ppm (C-8), 113.5 ppm (C-

10), 113.9 ppm (C-3), 115.9 ppm (C-6), 116.6 ppm (C-3''), 125.7 ppm (C-5), 144.4 ppm (C-4), 153.3 ppm (C-9), 156.5 ppm (C-7), 157.6 ppm (C-4''), 160.7 ppm (C-2''), and 166.4 ppm (C-2'').

Based on the reviewed sources and comparative analysis, the identified compound was determined to be decursine (Figure 1(B)) [28, 32, 45]. The analysis of the compounds derived from the essential oil of *Eryngium billardieri* yielded the following results: approximately 92% of the essential oil compounds identified. The predominant compounds were isofenchol, constituting 36.85% of the total area, followed by germacrene D at 7.69% and gurjunene at 6.67%. Upon analysis, it was determined that oxygenated monoterpenes accounted for 43.52% of the compounds, while sesquiterpenes made up 23.58% (Figure 2). In total, 36 compounds were identified using GC–MS, and their calculated and reference Kovat's Indices (K. I) are provided in Table 1.



A **B**

Figure 1: (A) Chlorogenic acid structure and **(B)** decursine structure

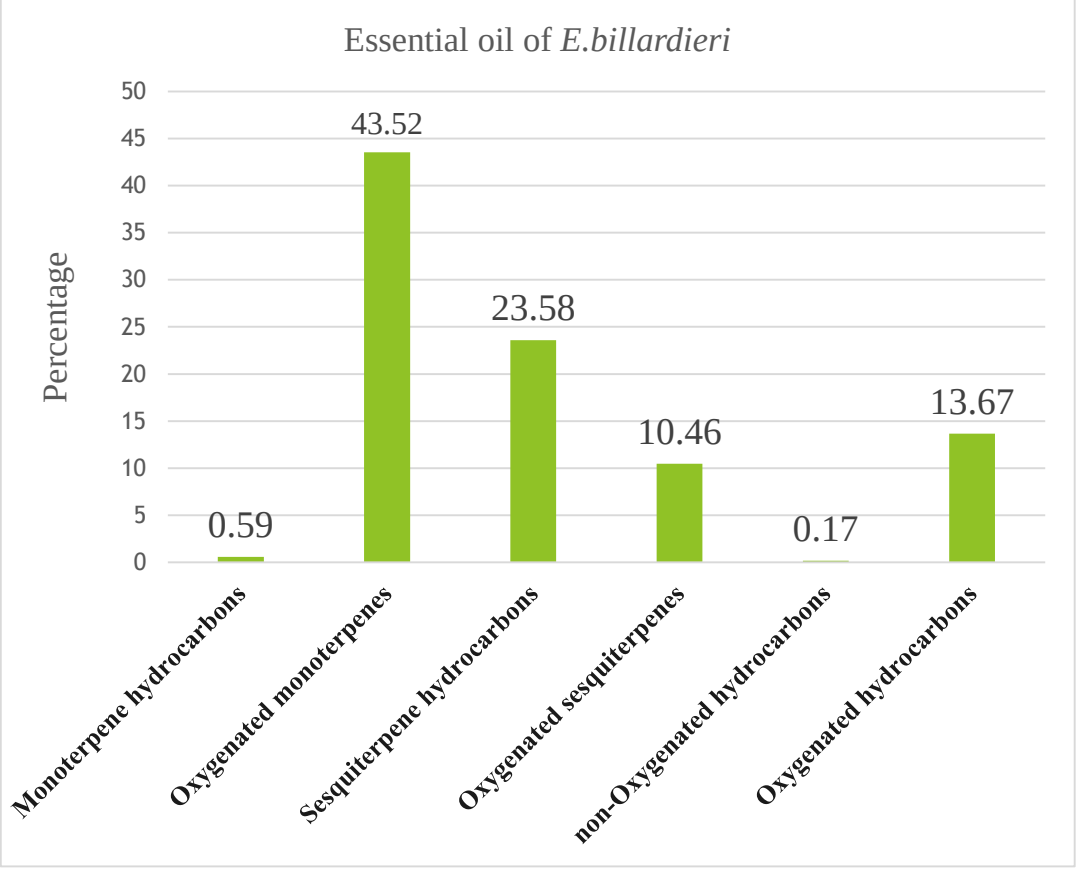


Figure 2: Percentage of essential oil composition of *E. billardieri*

Table 1: Essential oil constituents of the aerial part of *E. billardieri*

Compounds	K.I	Area %	Reported K.I
Hexanal	783	0.75	771
Heptanal	829	0.12	880
Furan, 2-pentyl-	948	0.17	986
Octanal	951	0.69	967
Benzene, 1,2,3-trimethyl-	952	0.17	1000
Nonanal	1041	0.15	1084
Chrysanthenyl actate	1162	1.13	-
Benzaldehyde, 2,4,6-trimethyl-	1256	0.54	1328
Durylaldehyde	1269	1.87	1334
Bicycloelemene	1271	0.38	-
α - Copaene	1339	0.21	1380
Dehydroaromadendrane	1341	0.27	-
β-Elemene	1343	0.99	1407
β -Caryophyllene	1389	2.09	1423
Isoledene	1393	3.7	1377
α -Humulene	1407	1.6	1455
α -Curcumene	1413	0.61	1479
Germacrene - D	1420	7.69	1482
Guaiene	1429	1	1483
Valencene	1440	1.95	1475
Bicyclogermacrene	1443	6.26	1501
Gurjunene	1447	6.67	1460
Isofenchol	1468	36.58	1475
Cadinene	1475	4.06	1518
α-Caryophyllene	1495	0.33	1454
Germacrene B	1521	0.31	1552
Spathulenol	1540	6.57	1571
Caryophyllene oxide	1547	1.3	1579
Cadinol	1470	0.2	1603
Carotol	1569	0.37	1594
Aromadendrene oxide-(2)	1607	0.76	1678
Isospathulenol	1615	0.58	1667
α-Cadinol	1622	0.68	1648
α-Bisabolol	1657	6.09	1658
Tetradecanoic acid	1774	1.1	1750
1,5-Cycloundecadiene, 8,8-dimethyl-9-methylene-	1923	1.06	-
Total		**92.06**	

The analysis of methyl esters derived from the *n*-hexane extract of *E. billardieri* revealed the presence of four fatty acid compounds. These include oleic acid, constituting 27.2% of the total area with a retention time (RT) of 30.63 minutes, elaidic acid at 6.91% (RT = 30.12), palmitic acid at 4.62% (RT = 27.70), and stearic acid at 2.55% (RT = 30.44). These fatty acids were identified in the plant extract. Oleic acid, classified as a monounsaturated omega-9 fatty acid, is commonly present in various plant and animal sources, such as olive oil, avocado, nuts, and animal fats. Its chemical formula is $C_{18}H_{34}O_2$. Notably, oleic acid is recognized for its potential health advantages. It is regarded as a beneficial fat and is an important component of the

Mediterranean diet, which is linked to a lowering risk of heart diseases in the Mediterranean population. Furthermore, studies have explored the anti-inflammatory properties of oleic acid and its potential to mitigate inflammation within the body. It may also exhibit antioxidant properties, protecting cells against damage instigated by free radicals [46]. Oleic acid has also been identified in the analysis of the fatty acid composition of other plants within the *Eryngium* genus. Particularly noteworthy are *E. maritimum and E. foetidum* (Table 2) [47, 48].

Table 2: Fatty acid constitution of the aerial part of *E. billardieri*

Major compounds	Chemical formula	Reported KI	Calculated KI	Area (%)
Palmitic acid	$C_{17}H_{34}O_2$	1909	2020	4.62
Elaidic acid	$C_{19}H_{36}O_2$	-	2058	6.91
Stearic acid	$C_{19}H_{38}O_2$	-	2063	2.55
Oleic acid	$C_{18}H_{34}O_2$	-	2066	27.21

Conclusion

This study primarily focused on conducting a comprehensive phytochemical examination of *E. billardieri* plants. Given its traditional medicinal applications and the growing interest in this field, a thorough phytochemical investigation becomes imperative. Apart from the methanol extract, the study also delved into analyzing the essential oil and fatty acids of the plant. Consequently, two substances were isolated from the methanol extract, and oleic acid was successfully isolated and identified from the *n*-hexane extract. Concerning the phytochemical potential inherent in this genus and specifically in this plant, further extensive research in this domain is warranted. Moreover, exploring the bioactivity properties of the plant and establishing the correlation between its compounds and bioactive attributes should be the subject of future investigations.

Acknowledgments

This work was a part of a Ph.D. dissertation and financially supported by Tabriz University of Medical Sciences, Tabriz, Iran [Grant No.: 61088].

Ethical Approval

Ethical approval for this study was obtained from Tabriz University of Medical Sciences. (IR.TBZMED.VCR.REC.1397.304)

Funding

This study was granted by Tabriz University of Medical Sciences.

Conflict of Interest

The authors declare that there is no conflict of interest in this study.

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HOW TO CITE THIS ARTICLE
Keyvan Sardari, Abbas Delazar, Hadi Ghanbari, Solmaz Asnaashari. Parina Asgharian. Phytochemical Evaluation of Aerial Parts of *Eryngium Billardieri* growing in Iran. *Chem. Methodol.*, 2024, 8(1) 47-57
DOI: https://doi.org/10.48309/chemm.2024.427947.1740
URL: https://www.chemmethod.com/article_186127.html