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## Original Article

# Chemical Composition, Phytochemical Screening, and Antibacterial Potentials of the Aerial Parts of *Eryngium thyrsoideum* Boiss.

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**ABSTRACT**

The chemical composition, phytochemical contents, and antibacterial potentials of the *Eryngium Thyrsoideum* Boiss. aerial parts were evaluated. The RP-HPLC-DAD method were applied to determine the phenolic and flavonoid contents of the extract. The hydrodistillation method with a Clevenger apparatus was used to isolate the essential oil, and the analyses of the oil was performed using GC-MS. Using the disc diffusion method and the broth microdilution assay, three gram-negative and six gram-positive bacterial strains were tested to assess the antibacterial effect of oil. The total flavonoid content of the extract was 0.81 ± 0.05 mg of quercetin equivalent per gram of dry plant materials, while its total phenolic content was 0.24 ± 0.01 mg of gallic acid equivalents per g of dry plant materials. According to the phytochemical screening results, phenols, flavonoids, steroids, glycosides, and saponins were detected in the ethyl acetate, *n*-hexane, and methanolic extracts. Results showed the existence of rutin, quercetin, and benzoic acid in the methanolic extract and revealed 46 compounds that constitute 93.07% of the obtained oil were recognized. Besides, 2,3,6-trimethyl benzaldehyde (24.15%), *trans*-4,10-epoxy-amorphane (15.55%), longifolene (13.72%), germacrene D (4.76%), 2,3,4-trimethyl benzaldehyde (4.67%), *cis*-chrysanthenyl acetate (3.3%), caryophyllene oxide (3.26%), and sesquicineole (2.78%) were identified in the oil as the major compounds. The oil showed *in vitro* significant inhibitory effect on *Bacillus pumilus*, *Bacillus subtilis*, and *Staphylococcus epidermidis*, resulting from its high proportion of sesquiterpenes. Regarding the different bioactive compounds and the antibacterial properties of *Eryngium thyrsoideum*, this plant can be applied in cosmetic, food, and pharmaceutical products.

## INTRODUCTION

The family Apiaceae (Umbelliferae) comprises some of the economically important vegetables, food, ornamental, spice, and medicinal species around the globe with a long history of use [1]. This family is considered one of the most prominent families of flowering plants with 434 genera and 3780 species [2, 3]. As the richest and arguably the most taxonomically complicated genus of the family Apiaceae, the genus *Eryngium* (eryngos) possesses approximately 300 annual or perennial species with a distribution all over the world, particularly in North and South America, Australia, North Africa, and Eurasia [4, 5]. Some of the members of this genus have been long exploited in traditional medicine in different cultures worldwide for the treatment of skin illnesses, cancers, kidney stones, inflammation, cough, snake and scorpion bites, infections, and hypoglycemia. They have been also utilized to control blood pressure and improve diuresis [6, 7].

Considering the existence of ten *Eryngium* species in all parts of Iran, they have been long used in Iranian traditional medicine (ITM) for halitosis, early stages of lymphatic filariasis, cramps and gripes, insect bites, snakebite, and pulmonary disease. These plants have also been applied as analgesic, anti-inflammatory, digestive, diuretic, galactagogue, aphrodisiac, anti-flatulent, emmenagogue, and antidote agents; according to the ITM literature [8].

Recent studies on the *Eryngium* species in different regions of the world have shown that the essential oils and extracts of these plants contain many bioactive secondary metabolites that are considered the main reason for their various biological properties such as antimicrobial effects [9]. In this regard, the extracts and essential oils of the Iranian *Eryngium* species including *E. creticum* [10], *E. kotschyi* [11], *E. caeruleum* [12], *E. glomeratum* [13], and *E. pyramidale* [14] have been reported to possess significant inhibitory effects against bacterial strains. *E. thyrsoideum* Boiss. is a perennial plant found abundantly in Iran and has been known for its blood sugar lowering action in diabetes among Iranian people [15]. Only a few studies have investigated the biological potential of this plant. Hosseini *et al*. (2022) assessed the cytotoxic activity of *E. thyrsoideum* extract fractions on the MCF-7 and MDA-MB-231 breast cancer cells. This study demonstrated that the 80% fraction of the methanolic extract exhibited the highest cytotoxicity against both kinds of BC cell lines 16. As far as we know, no previous research has investigated the antibacterial activity of the essential oil and extracts of *E. thyrsoideum*.

The objective here is to investigate the phenolic and flavonoid contents in the methanolic extract of the *E. thyrsoideum* aerial parts using the RP-HPLC-DAD method and UV spectrophotometry, as well as the determination of the chemical composition of the aerial parts essential oil using the gas chromatography/mass spectrometry (GC-MS) and gas chromatography-flame ionization detection (GC-FID) methods. The present work also aims to study the antibacterial potentials of the oil on both gram-negative and gram-positive bacterial strains according to the broth microdilution assay and the disc diffusion method.

## MATERIAL AND METHODS

### Plant Material

The *E. thyrsoideum* Boiss. aerial parts were collected from the province of Kurdistan in western Iran over the flowering season in July 2018. After the identification of the plant materials by a botanist (Hiva Ghaderi), a voucher specimen (No. 407) was preserved in the Herbarium of the School of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran.

### Preparation of Extracts

After grinding the *E. thyrsoideum* aerial parts into fine powder by a mechanical grinder, 50 g of the powder was added to each of the *n*-hexane (500 ml), methanol (500 ml), and ethyl acetate (500 ml) solutions at room temperature for 72 h. In order to remove the solvents, the extracts were filtered with a Whatman filter paper and then concentrated at 40 °C under diminished pressure with a rotary evaporator (Heidolph, Germany). Then, the solvent-free extracts were placed in the dark in a refrigerator (4 °C) until further tests.

### Phytochemical Analysis

To detect different groups of secondary metabolites in the plant extracts, the preliminary phytochemical screening of the methanolic, *n*-hexane, and ethyl acetate extracts was performed using the standard methods [17]. These procedures were employed to identify glycosides, tannins, flavonoids, phlobatannins, steroids, proteins, terpenoids, amino acids, saponins, alkaloids, anthraquinones, and phenols in the extracts.

### Assessment of Total Phenolic Content (TPC)

The total phenolic content (TPC) of the methanolic extract of the *E. thyrsoideum* aerial parts was determined using the Folin-Ciocalteu assay. The reference compound was gallic acid (3,4,5-trihydroxybenzoic acid). In this method, 2 ml of distilled water and 500 µl of the Folin-Ciocalteu reagent were mixed with 20 µl of each extract (10 mg/ml). After the incubation of the resulting mixture for 3 min at room temperature, 300 µl of sodium carbonate solution (7%) was added to the mixture, and the final mixture was kept in the dark for 30 min with intermittent shaking. Using a Synergy HDX ELISA reader, the absorbance of the final mixture was determined at the wavelength of 765 nm. Different dilutions of the standard gallic acid solution were prepared in ethanol. Then, a linear calibration curve (absorbance versus concentration) was obtained for each concentration. The standard calibration curve equation ($R^2$ = 0.99) was used to quantify the total phenolic content of the extract as milligrams of gallic acid equivalent per g of dry plant materials [18].

## Assessment of Total Flavonoid Content (TFC)

Quercetin (standard reference) and aluminum chloride ($AlCl_3$) (reagent) were used to assess the total flavonoid content (TFC) of the methanolic extract of the *E. thyrsoideum* aerial parts based on the procedure reported by Kerdar *et al*. (18). In this procedure, 0.2 ml of the sample extract (10 mg/ml), 0.2 ml of $NaNO_2$ (1 M), and 0.6 ml of ethanol (30%, v/v) were mixed with 0.2 ml of $AlCl_3$ reagent (10%, w/w). Using distilled water, the resulting mixture volume was then diluted to 2 ml. After 60 min of incubation at room temperature, the absorbance was read at the wavelength of 415 nm with a Synergy HDX ELISA reader. Similarly, the absorbance of various dilutions of the standard quercetin solution prepared in ethanol was recorded at 415 nm. A standard calibration curve was then achieved for each concentration by plotting absorbance versus concentration. The total flavonoid content of the extract was calculated based on the standard calibration curve equation ($R^2 = 0.99$) and expressed as milligrams of quercetin equivalent (QE) per gram of dry plant materials.

## Preparation of Essential Oil

The essential oil of the *E. thyrsoideum* aerial parts was isolated by the hydrodistillation method using a Clevenger apparatus. Briefly, 170 g of the finely powdered aerial parts was subjected to hydrodistillation using 1000 ml of distilled water in a Clevenger-type apparatus under the heating mantle for at least 3 h. Next, the obtained oil was dehydrated using anhydrous sodium sulfate ($Na_2SO_4$). Finally, the obtained, yellowish-colored oil was stored in a refrigerator at 4 °C until further tests. The isolation yield of the oil was 3% (w/w).

## Determination of the Chemical Composition of Essential Oil

The quantitative analysis of the constituents in the essential oil was carried out by the gas chromatography-flame ionization detection (GC-FID) on a gas chromatograph (Thermoquest Trace, UK) coupled with a flame ionization detector (FID) (300 °C); a split/split less injector (250 °C); and a DB-5 fused-silica capillary column (J & W Scientific, USA: 60 m x 0.25 mm, film thickness 0.25 µm). The carrier gas was nitrogen at a flow rate of 1 ml/min. The column initial temperature was set at 60 °C during the initial 2 min, gradually raised to 250 °C at a rate of 5 °C/min, and held at the final temperature for 2 min. After the dilution of the essential oil in *n*-hexane (1:4 v/v), a sample of the diluted solution (1 µl) was injected into the GC-FID system with a split ratio of 1:50.

The gas chromatography-mass spectrometry (GC-MS) method was employed for the qualitative analysis of the chemical compositions of the essential oil. This analysis was performed on a Thermoquest-Finnigan GC coupled with a TRACE mass spectrometer (Manchester, UK) with a J & W Scientific column (USA: 60 m x 0.25 mm, film thickness 0.25 µm). The procedures used for the sample injection conditions, sample injection volume, sample preparation, and column temperature program were the same as those utilized for the GC-FID analysis. The mass spectra of the constituents were measured at the ionization energy of 70 eV within a mass scan range of 43 to 456 m/x. Helium was used as the mobile phase with a flow rate of 1.1 ml/min.

To characterize the components of the essential oil, the data of the mass spectra were compared with those of the published mass spectra and the Adams and Wiley libraries. Furthermore, the GC retention indices of the compounds were compared to those previously reported in the literature [19]. The retention indices of the constituents in the essential oil were determined using *n*-alkanes ($C_6$-$C_{30}$) as standards according to the Van den Dool method [20]. On the other hand, the quantification of the oil constituents was carried out using relative area percentages obtained from the GC-FID analysis without the application of correction factors. This analysis was performed three independent times.

## HPLC Analysis of Phenolic Compounds

The HPLC analyses of the standard compounds and the methanolic extract were carried out to determine the phenolics and flavonoids in the methanolic extract. The analyses were performed utilizing the reversed-phase high-performance liquid chromatography (RP-HPLC-DAD) method on a liquid chromatographic system (Shimadzu Scientific Instruments; Kyoto, Japan) coupled with a dual solvent pump (LC-20AD), an auto-sampler (SIL-20AC), an auto-injector (SIL-10ADvp), a C18 reversed-phase column (25 cm × 4.6 mm, 3µm), and a photodiode array detector (SPD-M20A) at 25 °C. The mobile phases were as follows: solvent A (the water-acetic acid mixture, 97:3, v/v) and solvent B

(HPLC grade methanol, Merck, Germany) with a flow rate of 1 ml/min. The amount of 20 µl of each sample was loaded into the system. The following gradient elution conditions were employed: 0.0 min, 0% B; 0-10 min, 20% B; 10-30 min, 20-50% B; 30-55 min, 50-100% B; 55-65 min, 100% B. The amount of run time for each sample analysis was 65 min. All spectra were acquired within the wavelength range of 200-400 nm. For the HPLC analysis; naringenin, apigenin, quercetin, ferulic acid, benzoic acid, rutin, *trans*-resveratrol, caffeic acid, (+)-catechin, and gallic acid as standard phenolic compounds were obtained from Sigma-Aldrich Corporation (St. Louis, MO, USA). After the determination of various concentrations of the standard phenolics, each concentration was separately introduced into the HPLC system. The values of the phenolic compounds in the methanolic extract were calculated as mg/100 g of the plant using the calibration curves of the standards. The values of the limit of quantification (LOQ) and the limit of detection (LOD) were calculated as ng/ml. The peak areas were plotted against different concentrations of the phenolics and flavonoids so as to obtain a standard calibration curve for each standard. Finally, the chromatograms of the methanolic extract were compared to those of the standards to identify and quantify the phenolic and flavonoid compounds.

### Antibacterial Activity

The antibacterial effect of the essential oil isolated from the *E. thyrsoideum* aerial parts was assessed against three gram-negative and six gram-positive bacteria based on the procedures published by Baron and Finegold, in which inhibitory zone diameters and minimal inhibitory concentration (MIC) values were determined using the disc diffusion assay on Mueller-Hinton agar medium and the broth microdilution method (BMM), respectively [21]. After incubation for 24 h at 37 °C, the inhibition zone diameter was measured with a caliper (in millimeter), including a 6 mm diameter of the paper disc. The three standard strains of gram-negative bacteria used in this study were *Pseudomonas aeruginosa* (ATCC 85327), *Escherichia coli* (ATCC 25922), and *Klebsiella pneumoniae* (ATCC 10031); however, *Enterococcus faecalis* (ATCC 29737), *Bacillus pumilus* (PTCC 1274), *Staphylococcus aureus* (ATCC 25923) (American Type Culture Collection), *Bacillus cereus* (PTCC 1015) (Persian Type Culture Collection), *Bacillus subtilis* (ATCC 465), and *Staphylococcus epidermidis* (ATCC 12228) were the six standard strains of gram-positive bacteria. The gentamicin (10 mg/disc), tetracycline (30 mg/disc), and ampicillin (10 mg/disc) standard antibiotics were applied as positive controls against the bacteria. The MIC values (mg/ml) in this work were calculated as the lowest concentration that inhibited the growth of the bacteria after 24 h of incubation at 37 °C.

## RESULTS AND DISCUSSION

### Qualitative Phytochemical Analysis

As shown in Table 1, various bioactive compounds and secondary metabolites including phenols, flavonoids, steroids, glycosides, and saponins were identified by the preliminary phytochemical analysis in the *n*-hexane, ethyl acetate, and methanolic extracts of the *E. thyrsoideum* aerial parts.

**Table 1** Qualitative phytochemical analysis of the extracts obtained from the *E. thyrsoideum* Boiss. aerial parts

| Phytochemical Constituents | Methanolic extract | Ethyl acetate extract | *n*-Hexane extract |
|---|---|---|---|
| Proteins | - | - | - |
| Flavonoids | +++ | +++ | + |
| Tannins | - | - | - |
| Amino acids | - | - | - |
| Phlobatannins | - | - | - |
| Steroids | +++ | ++ | + |
| Terpenoids | - | - | - |
| Glycosides | +++ | + | - |
| Anthraquinones | - | - | - |
| Phenols | +++ | +++ | + |
| Alkaloids | - | - | - |
| Saponins | ++ | ++ | - |

[+++]: Abundantly present, [++]: Moderately present, [+]: Slightly present, [-]: Absent.

The results in Table 1 indicated that all of the three plant extracts contained phenols, flavonoids, and steroids. The results also showed that the methanolic extract possessed abundant amounts of flavonoids, steroids, phenols, and glycosides as well as moderate amounts of saponins. The ethyl acetate extract, on the other hand, has been shown to have phenols and flavonoids at abundant levels, but it had steroids and saponins at moderate levels. However, glycosides were slightly detected in this extract. Furthermore, the phytochemical analysis of the *n*-hexane extract revealed the existence of flavonoids, phenols, and steroids at slight levels and also the absence of glycosides and saponins. It can be concluded from these results that the methanolic and ethyl acetate extracts were richer in bioactive compounds than the *n*-hexane extract.

## Assessment of the Total Phenolic and Flavonoid Contents

The standard gallic acid solution, the Folin-Ciocalteu reagent, and the standard curve equation were used to assess the total phenolic content (TPC) of the methanolic extract of the *E. thyrsoideum* aerial parts, which was found to be 0.24 ± 0.01 mg of gallic acid equivalent per g of dry plant materials. On the other hand, the total flavonoid content (TFC) value of the methanolic extract determined by the aluminum chloride ($AlCl_3$) reagent, the standard quercetin, and the standard curve equation was 0.81 ± 0.05 mg of quercetin equivalent (QE) per gram of dry plant materials.

As the most abundant and the most important group of secondary metabolites in plants, phenolics are natural plant-derived compounds that possess an aromatic ring with one or more hydroxyl groups [22]. These secondary metabolites are divided into two categories of polyphenols and phenolic acids. Flavonoids are the most common class of polyphenols found in human diets and responsible for most of the colors and flavors in vegetables and fruits [23]. Different studies on phenolic and flavonoid compounds have revealed that phenolics lighten oxidative disease burdens and also exert several beneficial effects such as metal inactivation, peroxide decomposition, oxygen scavenging, and free radical inhibition in biological structures. On the other hand, various pharmacological properties such as anticancer, anti-inflammatory, anti-coagulant, antioxidants, antimicrobial, anti-cholesterol, and anti-allergic effects have been reported for flavonoids [24, 25]. Due to the important role and benefits of phenolic and flavonoid compounds in human health, various *Eryngium* species have been recently investigated for their total phenolic and flavonoid contents. In this regard, Bouzidi *et al*. (2017) assessed the antipyretic, anti-inflammatory, and antioxidant properties of the *n*-butanol extract of the aerial parts and roots of the *E. campestre* L. collected from Algeria. Both aerial parts and roots extracts contained phenolics and flavonoids. However, the maximum value of the total phenolic content was observed in the ethyl acetate extract of the aerial parts (27.77 µg gallic acid equivalent/mg extract), while the roots aqueous extract was found to have the highest amount of the total flavonoid content (9.461 µg quercetin equivalent/mg extract) [26]. Moreover, another study was performed by Daneshzadeh *et al*. (2020) to evaluate the antioxidant and antimicrobial effects in addition to the total phenolic and flavonoid contents of the ethanolic extract of the *E. billardieri* aerial parts. The results indicated that the total phenolic contents of the different concentrations of the extract were from 10.71 to 33.38 mg gallic acid equivalent/g dry extract; however, the total flavonoid contents of these concentrations ranged from 15.04 to 27.13 mg quercetin equivalent/g dry extract [27].

## Chemical Compositions of the Essential Oil

As can be seen in Figure 1 and Table 2, the isolated essential oil of the *E. thyrsoideum* aerial parts was identified and quantified using the GC-MS and GC-FID analyses, respectively. The results obtained from these analyses including the relative percentages of the constituents, the chemical compositions of the oil, the retention time, the relative retention indices, and the relative retention indices from the published literature were given in Table 2. The results in Table 2 revealed that a total of 46 compounds were characterized in the essential oil, which made up 93.07% of all isolated oil. Besides, the oil was composed of monoterpenes (6.64%), oxygenated sesquiterpenes (30.01%), sesquiterpene hydrocarbons (25.96%), and non-terpenoids (30.46%). This demonstrated that the sesquiterpene compounds made up much of the essential oil as compared to monoterpenes and non-terpenoids.

**Table 2** Percentage composition of the essential oil isolated from the *E. Thyrsoideum* Boiss. aerial.

| No. | Components [a] | RI [b] | Area | RI [c] | RT [d] |
|---|---|---|---|---|---|
| 1 | α-Pinene [f] | 932 | 0.44 | 922 | 3.93 |
| 2 | Sabinene | 969 | 0.05 | 961 | 4.59 |
| 3 | β-Pinene | 974 | 0.25 | 978 | 4.88 |
| 4 | Mesitylene | 994 | 1.04 | 984 | 4.98 |
| 5 | n-Octanal | 998 | 0.11 | 994 | 5.14 |
| 6 | 1,2,4-trimethyl Benzene | 1021 | 0.27 | 1015 | 5.57 |
| 7 | Sylvestrene | 1025 | 0.25 | 1019 | 5.66 |
| 8 | γ -Terpinene | 1054 | 0.15 | 1048 | 6.31 |
| 9 | *cis*-Verbenol | 1137 | 0.58 | 1138 | 8.31 |
| 10 | Eucarvone | 1146 | 0.22 | 1153 | 6.42 |
| 11 | Terpinen-4-ol | 1174 | 0.11 | 1172 | 9.21 |
| 12 | Safranal | 1196 | 0.08 | 1195 | 9.78 |
| 13 | β-Cyclocitral | 1217 | 0.03 | 1216 | 10.31 |
| 14 | *cis*-Chrysanthenyl acetate | 1261 | 3.3 | 1252 | 11.36 |
| 15 | Isobornyl acetate | 1283 | 0.1 | 1276 | 11.88 |
| 16 | *trans*-Sabinyl acetate | 1289 | 0.01 | 1283 | 12.07 |
| 17 | *trans*-Verbenyl acetate | 1291 | 1.07 | 1290 | 12.24 |
| 18 | 2,3,4-trimethyl Benzaldehyde | 1313 | 4.67 | 1311 | 12.76 |
| 19 | 2,3,6-trimethyl Benzaldehyde | 1352 | 24.15 | 1358 | 13.99 |
| 20 | α-Copaene | 1373 | 0.39 | 1367 | 14.02 |
| 21 | β-Bourbonene | 1387 | 0.94 | 1376 | 14.43 |
| 22 | β-Elemene | 1389 | 0.5 | 1382 | 14.59 |
| 23 | Longifolene | 1407 | 13.72 | 1412 | 15.34 |
| 24 | β-Caryophyllene | 1417 | 0.45 | 1419 | 15.51 |
| 25 | 6,9-Guaiadiene | 1442 | 1.72 | 1444 | 16.11 |
| 26 | *trans*-4,10-Epoxy-amorphane | 1478 | 15.55 | 1475 | 16.86 |
| 27 | Germacrene D | 1484 | 4.76 | 1488 | 17.18 |
| 28 | β-Selinene | 1489 | 1.53 | 1494 | 17.34 |
| 29 | Sesquicineole | 1515 | 2.78 | 1503 | 17.56 |
| 30 | Dauca-4(11),8-diene | 1530 | 0.48 | 1513 | 17.78 |
| 31 | Germacrene B | 1547 | 1.47 | 1547 | 18.58 |
| 32 | (3Z)-Hexenyl benzoate | 1565 | 0.22 | 1563 | 18.95 |
| 33 | Spathulenol | 1577 | 1.61 | 1570 | 19.13 |
| 34 | Caryophyllene oxide | 1582 | 3.26 | 1574 | 19.21 |
| 35 | Salvial-4(14)-en-1-one | 1598 | 1.14 | 1585 | 19.46 |
| 36 | β-Atlantol | 1608 | 0.33 | 1604 | 19.92 |
| 37 | Eremoligenol | 1629 | 1.18 | 1642 | 20.76 |

| | | | | | |
|---|---|---|---|---|---|
| 38 | Agarospirol | 1646 | 1.44 | 1647 | 20.86 |
| 39 | Himachalol | 1652 | 0.02 | 1661 | 21.19 |
| 40 | 14-hydroxy-(Z)-Caryophyllene | 1666 | 0.29 | 1668 | 21.34 |
| 41 | 14-hydroxy-9-epi-(E)-Caryophyllene | 1668 | 0.17 | 1674 | 21.48 |
| 42 | Khusinol | 1679 | 1.32 | 1678 | 21.57 |
| 43 | Amorpha-4,9-dien-2-ol | 1700 | 0.24 | 1700 | 22.04 |
| 44 | 14-hydroxy-α-Humulene | 1713 | 0.22 | 1706 | 22.18 |
| 45 | β-(Z)-Curcumen-12-ol | 1754 | 0.34 | 1756 | 23.25 |
| 46 | 14-oxy-α-Muurolene | 1767 | 0.12 | 1762 | 23.38 |
| | Monoterpenes | | 6.64 | | |
| | Sesquiterpene hydrocarbons | | 25.96 | | |
| | Oxygenated sesquiterpenes | | 30.01 | | |
| | Other compounds | | 30.46 | | |
| | Total | | 93.07 | | |

[a]: Compounds are listed in order of elution from DB-5 MS column; RI [b]: Retention indices to C6-C30 n-alkanes on DB-5MS column; RI[c]: Retention indices according to the literature; RT [d]: Retention time (min); [f]: The identification was also confirmed by co-injection with the standard.

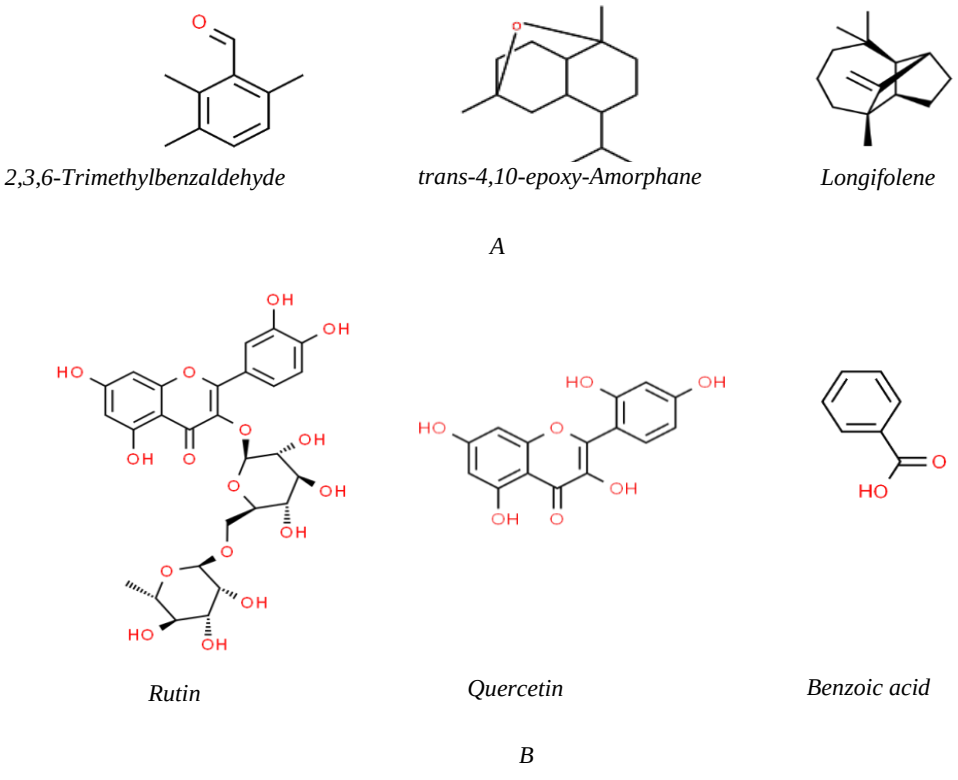


**Fig. 1** Chemical structures of the main constituents in the essential oil (A) and the methanolic extract (B) of the *E. Thyrsoideum* Boiss. aerial parts.

Additionally, terpenoids were significantly more abundant than non-terpenoids in the essential oil. The data in Figure 1 and Table 2 also showed that 2,3,6-trimethyl benzaldehyde (24.15%), *trans*-4,10-epoxy-amorphane (15.55%), and longifolene (13.72%) were found to be the three main components with the highest percentage in the essential oil isolated from the *E. thyrsoideum* aerial parts. However, the other substantial constituents identified in the isolated oil were as follows: germacrene D (4.76%), 2,3,4-trimethyl benzaldehyde (4.67%), *cis*-chrysanthenyl acetate

(3.3%), caryophyllene oxide (3.26%), and sesquicineole (2.78%).

Both 2,3,6-trimethyl benzaldehyde and 2,3,4-trimethyl benzaldehyde isomers have been reported to be the main constituents in the essential oils of some *Eryngium* species. For instance, in a study conducted by Kremer *et al*. (2021) on the essential oil of *E. amethystinum* from Croatia, 2,3,6-trimethyl benzaldehyde (9.3%) was one of the three major compounds in the isolated oil [28].

In another investigation, Kikowska *et al*. (2020) found that 2,3,4-trimethyl benzaldehyde (11.3%) was the main compound in the shoot culture essential oil of *E. maritimum*, while 2,3,4-trimethyl benzaldehyde (11.3%) and germacrene D (10.5%) dominated in the oil isolated from the leaves (29). On the other hand, *trans*-4,10-epoxy-amorphane and longifolene have not been identified in the essential oils of *Eryngium* species as the main constituents in previous studies. Therefore, the presence of these two compounds in the essential oil makes *E. thyrsoideum* special among other investigated *Eryngium* species. Considering the high concentrations of these constituents in the essential oil, the aerial parts of this plant can be used as a natural source of these two secondary metabolites. However, earlier studies on different *Eryngium* species such as *E. campestre* (30), *E. amethystinum* (31), *E. planum* (32), *E. alpinum* (7), *E. thorifolium* (33), *E. aquifolium* (34), and *E. bornmuelleri* (35) have shown that the essential oils obtained from these plants were rich in germacrene D, *cis*-chrysanthenyl acetate, caryophyllene oxide, and sesquicineole, which are directly in line with the findings in this study.

## HPLC Analysis

Regarding the considerable amounts of the total phenolic and flavonoid contents and also the existence of phenolics in the methanolic extract, the RP-HPLC-DAD method was applied to the identification and quantification of 10 standard phenolic and flavonoid compounds in the methanolic extract of the *E. thyrsoideum* aerial parts. These analyses indicated the presence of rutin, quercetin, and benzoic acid in the methanolic extract (Figure 2 and Table 3). The chemical structures of these phenolic and flavonoid compounds are shown in Figure 1. Figure 2 illustrates the HPLC chromatograms of the methanolic extract, in which the HPLC peaks of quercetin, benzoic acid, and rutin can be observed. Table 3 gives the quantitative HPLC results of benzoic acid, rutin, and quercetin with the concentrations of 0.46 ± 1.7, 1.00 ± 1.4, and 0.05 ± 1.6 mg/100 g of the plant, respectively. Table 3 also presents the summary of retention time, absorbance maximum wavelength, the limit of detection (LOD), and the limit of quantification (LOQ) of the three quantified compounds in the extract.

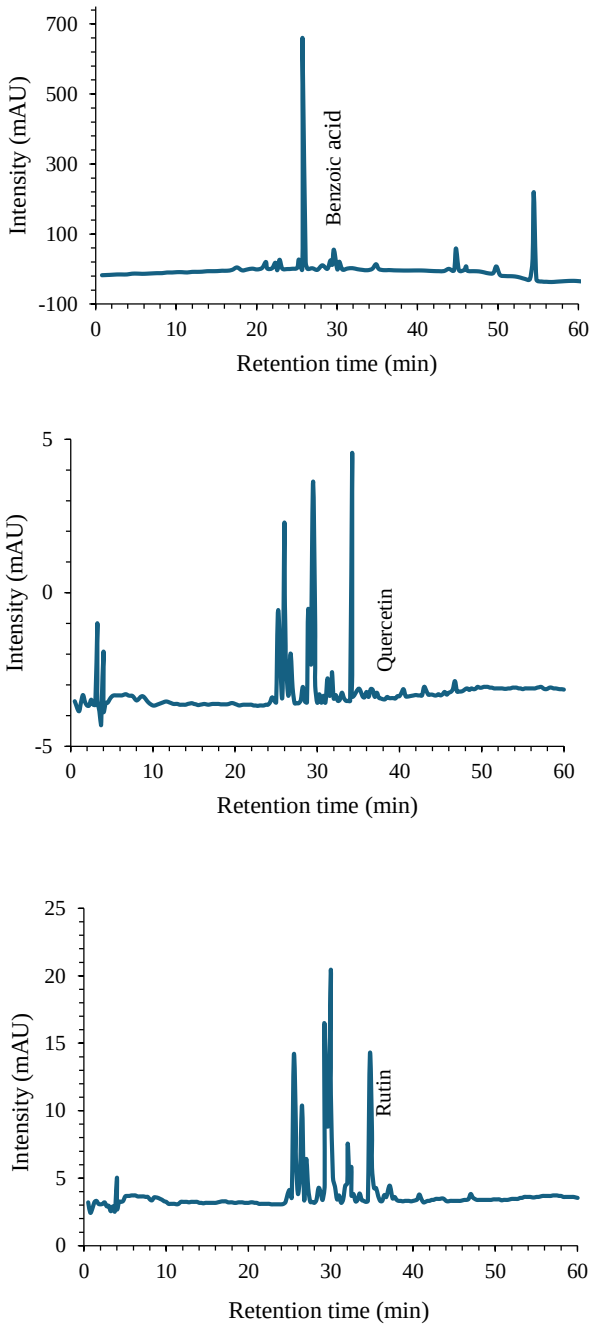


**Fig. 2** HPLC peaks of benzoic acid, quercetin, and rutin in the HPLC chromatograms of the methanolic extract of the *E. thyrsoideum* Boiss. aerial parts

**Table 3** Quantitative HPLC analyses of phenolic compounds in the methanolic extract of the *E. thyrsoideum* Boiss. aerial parts.

| Compounds | Retention time (min) | Absorbance maximum wavelength (nm) | Amount of compound in extract (mg/100 g of plant) | LOD (ng/ml) | LOQ (ng/ml) |
|---|---|---|---|---|---|
| Benzoic acid | 27.8 | 254 | 0.46±1.7 [a] | 5 | 25 |
| Rutin | 34.1 | 360 | 1.00±1.4 | 20 | 50 |
| Quercetin | 40.3 | 370 | 0.05±1.6 | 20 | 50 |

[a]: The results are presented as the mean ± standard deviation (SD) of three replications

According to these results, rutin had the highest concentration in the methanolic extract, followed by benzoic acid. On the other hand, quercetin was found to have the lowest concentration among these phenolic compounds. Rutin or 3,3′,4′,5,7-pentahydroxyflavone-3-rhamnoglucoside is a citrus flavonoid glycoside that is known as vitamin P (Figure 1). Many plants such as vegetables, passion flowers, tea, apple, buckwheat, and citrus fruits are rich sources of this polyphenolic bioflavonoid (36, 37). Several biological and pharmacological properties such as antidiabetic, antiprotozoal, anti-inflammatory, antioxidant, anticarcinogenic, antibacterial, antimalarial, antifungal, antiviral, antimicrobial, antitumor, antiallergic, and hypolipidemic have been reported for rutin. It also indicates cardioprotective, vasoactive, retinoprotective, antihypertensive, neuroprotective, anti-fatigue, and sunscreen effects (38, 39). Rutin is one of the main components in the extracts of a few *Eryngium* species such as *E. bornmuelleri* (40), *E. amethystinum*, and *E. alpinum (28)*. This flavonoid was found to be the dominant compound in the methanolic extract in this investigation. The amount of rutin in this work was considerable as compared to those of other investigated *Eryngium* species. However, it was not comparable with the rutin amount in our previous study on *Eryngium pyramidale* (14). Benzoic acid is a phenolic phytochemical that has been applied for a long time as an antimicrobial preservative in foods and beverages, particularly in pickled foods and carbonated beverages (Figure 1). The highest level of benzoic acid used in foods and beverages ranges from 0.05 to 0.1%. Benzoic acid shows antimicrobial effects against bacteria, yeasts, and molds, which are the main reasons for food spoilage (41, 42). Quercetin is a widespread antioxidative flavonoid that occurs commonly in fruits, vegetables, and tea. This polyphenol presents a variety of biological activities such as antiviral, anti-carcinogenic, anti-aging, and anti-inflammatory properties. It also shows protective effects on cardiovascular and pulmonary diseases and diminishes platelet aggregation, capillary permeability, and lipid peroxidation (43, 44).

**Table 4** *In vitro* antibacterial activities of the essential oil isolated from the *E. thyrsoideum* Boiss. aerial parts.

| Sample | Microorganism | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *B. pumilus* | *B. subtilis* | *S. aureus* | *B. cereus* | *K. pneumoniae* | *E. faecalis* | *E. coli* | *S. epidermidis* | *P. aeruginosa* |
| Essential Oil | 17[a] (7.5) b | 17 (7.5) | 12 (15) | 12 (15) | 11 (15) | 14 (15) | 14 (15) | 15 (15) | - |
| Tetracycline[c] | nt | 21 (3.2) | 20 (3.2) | nt | nt | nt | - (nt) | 34 (1.6) | nt |
| Gentamicin[d] | nt | - (nt) | - (nt) | nt | nt | nt | 23 (3.2) | - (nt) | nt |
| Ampicillin[e] | 15 (15) | 14 (15) | 13 (15) | nt | nt | nt | 12 (15) | 19 (15) | nt |

a: Zone of inhibition (in mm) includes diameter of the disc (6 mm); b: Minimum inhibitory concentration values as mg.ml-1; [-]: Inactive, (7-13): Moderately active, (> 14): Highly active, [nt]: Not tested; c: Tested at 30 µg/disc; d: Tested at 10 µg/disc; e: Tested at 10 µg/disc.

The existence of these three compounds with a wide range of biological and pharmaceutical actions in the methanolic extract of the *E. thyrsoideum* aerial parts makes this extract an attractive candidate for application in the cosmetic, pharmaceutical, and food industries.

## Antibacterial Activity

The broth microdilution method and the disk diffusion assay were employed to assess the antibacterial activity of the essential oil isolated from the *E. thyrsoideum* aerial parts against six strains of gram-positive and three strains of gram-negative bacteria (Table 4). Based on the results in Table 4, *Bacillus pumilus* and *Bacillus subtilis* were the most sensitive bacterial strains to the essential oil with growth inhibition zone diameters of 17 mm and minimum inhibitory concentration values of 7.5 mg/ml, followed by *Staphylococcus epidermidis* with the growth inhibition zone diameter of 15 mm and the MIC value of 15 mg/ml. It should be noted that the inhibitory activity of the oil against *Bacillus pumilus* and *Bacillus subtilis* was higher in comparison to the standard antibiotic ampicillin. By contrast, the most resistant bacterial strain to the oil was found to be *Klebsiella pneumoniae* (IZ = 11 mm, MIC = 15 mg/ml). However, the essential oil affected both the gram-negative and gram-positive bacteria in this work.

These results, to the best of our knowledge, are the first data reported on the antibacterial activity of the *E. thyrsoideum* essential oil. The extracts and essential oils of several plants in the genus *Eryngium* have been also investigated for their antimicrobial potential using different methods. For instance, Dehghanzadeh *et al*. (2014) assessed the antibacterial activity of the essential oil isolated from the *E. caeruleum* aerial parts using the disc diffusion method and micro-broth dilution assay. The results of their study revealed that the oil effectively inhibited the growth of *Streptomyces scabies*, *Xanthomonas axonopodis pv. citri*, *Erwinia amylovora*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Klebsiella sp*. bacterial cultures [45]. Using the broth microdilution method, Matejić *et al*. (2018) investigated the antimicrobial properties of the essential oils obtained from the aerial parts and/or roots of *E. campestre*, *E. palmatum*, and *E. amethystinum* in addition to their aqueous, acetone, ethyl acetate, and methanolic extracts. The results demonstrated that all tested essential oils in different concentrations showed significant inhibitory effects on gram-positive *Staphylococcus aureus* along with gram-negative *Klebsiella pneumoniae* and *Proteus mirabilis*. The aerial parts extracts also inhibited the tested bacterial strains with the MIC values ranging from 0.004 to 20 mg/ml. Among the extracts, the ethyl acetate and acetone extracts had the highest inhibitory actions on *Proteus mirabilis* [31]. Many studies have stated that the chemical structures of the most abundant constituents in essential oils are associated with the exhibited antimicrobial properties [46, 47]. For example, sesquiterpenes have been reported to be responsible for the antibacterial effects exhibited by the essential oils of several *Eryngium* species [48]. Moreover, the *in vitro* inhibitory actions of some compounds such as longifolene, germacrene D, *cis*-chrysanthenyl acetate, caryophyllene oxide, and sesquicineole in essential oils isolated from many plants have been previously confirmed [14, 49, 50]. Since most of these secondary metabolites are sesquiterpene compounds that constitute 55.97% of the isolated essential oil, the antibacterial activity presented by the oil may be attributed to the sesquiterpenes in the oil. It can be suggested that this oil is a new source of natural antibacterial agents for food, cosmetic, and pharmaceutical products. Future research on the antibacterial potentials of *trans*-4,10-epoxy-amorphane and the 2,3,6-trimethyl benzaldehyde and 2,3,4-trimethyl benzaldehyde isomers might extend the explanations of the inhibitory effects presented by the essential oil of *E. thyrsoideum.*

## CONCLUSION

This study aimed to determine the chemical compositions, phytochemical contents, and antibacterial potentials of the aerial parts of *E. thyrsoideum* Boiss. According to the results, phenolics and flavonoids including rutin, quercetin, and benzoic acid were detected in the methanolic extract using the RP-HPLC-DAD method. In addition, the GC-MS and GC-FID analyses revealed that the major constituents in the essential oil were 2,3,6-trimethyl benzaldehyde, *trans*-4,10-epoxy-amorphane, longifolene, germacrene D, 2,3,4-trimethyl benzaldehyde, *cis*-chrysanthenyl acetate, caryophyllene oxide, and sesquicineole; most of which belong to sesquiterpenes. Furthermore, the antibacterial effect of the *E. thyrsoideum* essential oil on both gram-negative and gram-positive

bacteria was evaluated for the first time by the disc diffusion method and the broth microdilution assay. The oil exhibited *in vitro* considerable antibacterial activity against *Bacillus pumilus*, *Bacillus subtilis*, and *Staphylococcus epidermidis*, which may be due to the high percentage of sesquiterpene compounds in the oil. These results propose that this plant is a natural source of compounds with antimicrobial activities and can be used in the cosmetic, pharmaceutical, and food industries. Regardless, future research could continue to explore other biological aspects of the essential oil and extracts of *E. thyrsoideum.*

## Conflict of Interest

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

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