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Original Research Article

Phytochemical analysis of *Echinops macrophyllus* Boiss & Hausskn

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ABSTRACT

Echinops macrophyllus Boiss & Hausskn, also known as "Shekartighal kohgiluyeh" in Persian, is a prickly perennial herbaceous plant. Various species within the genus *Echinops* have traditionally been used as remedies for severe coughs, nervous attacks, and infectious diseases. This study aimed to isolate and elucidate the structures of the compounds found in the aerial parts of *E. macrophyllus*. The ethyl acetate extract from the aerial parts of the plant was fractionated using column chromatography. The structures of the isolated compounds were characterized using ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY, HSQC-DEPT, HMBC and NOESY techniques. Phytochemical analysis of the plant resulted in the isolation of six compounds (**1-6**), including one triterpenoid (α-amyrin) (**4**), three sterols involving 3-*O*-[β-D-(6′-tetradecanoate)-glucopyranosyl]-β-sitosterol (**6**), β-sitosterol (**3**), and daucosterol (**5**) as well as two phenolic compounds, namely *p*-hydroxybenzoic acid (**1**) and ethyl-2-hydroxy-*trans*-cinamate (**2**), from this species for the first time. It is noteworthy that these compounds have previously been reported to exhibit various biological activities.

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1. Introduction

Asteraceae is one of the most widely distributed plant families, but it does not naturally occur on the Antarctic mainland. Medicinal herbs from the Asteraceae family are widely used in the pharmaceutical and food industries due to their diverse and effective compounds (Amirahmadi et al., 2022; Kazeminia et al., 2022). The genus *Echinops* L. (Asteraceae) comprises approximately 120-130 species found worldwide (Bitew et al., 2017). This genus, known in Persian as Shekartighal, includes 54 species of perennial thorny herbaceous plants in Iran, most of which are endemic (Mozaffarian, 2006). *Echinops* species are primarily distributed in North and tropical Africa, the Mediterranean Basin, and Central Asia. In traditional medicine, the genus *Echinops* is used for pain relief, inflammation, respiratory issues, diseases caused by various microorganisms, as an aphrodisiac, and to eliminate kidney stones (Bitew and Hymete, 2019). Previous phytochemical investigations of the genus have revealed the presence of various phytochemical constituents such as thiophenes, terpenes, flavonoids, alkaloids, and lignans (Liu et al., 2002; Hymete et al., 2005; Sandjo et al., 2016). Researchers have discovered that extracts, isolated compounds, and essential oils from *Echinops* species exhibit numerous biological properties, including anti-proliferative, antioxidant, antimicrobial, and anti-inflammatory effects (Abdallah et al., 2013; Kiyekbayeva et al., 2018). *Echinops macrophyllus* Boiss & Hausskn is an endemic species that grows in various parts of Iran, particularly in Tehran (Alijanpour et al., 2019).

According to the literature review, no previous phytochemical studies have been conducted on *E. macrophyllus* Boiss & Hausskn to date. In our pursuit of discovering new and potentially bioactive secondary metabolites from the Iranian herbal species (Tabefam et al., 2018; Alizadeh et al., 2021), we studied the ethyl

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acetate extract prepared from the aerial parts of *E. macrophyllus* Boiss & Hausskn.

2. Experimental

2.1. Plant material

The aerial parts of the plant (*E. macrophyllus* Boiss & Hausskn) were collected in June 2019 at the flowering stage from Shiraz, located at N 29° 40′, E 52° 33′, and an altitude of 1721 m in Fars Province, Iran. The plant was identified by Dr. Mojtaba Asadollahi (botanist). A voucher specimen (MPH-3202) has been deposited at the herbarium of Medicinal Plants and Drug Research Institute of Shahid Beheshti University, Tehran, Iran.

2.2. Extraction

The dried aerial parts of the plant (2.0 kg) were milled and macerated with ethyl acetate (3 × 15 L) each for 48 hours at room temperature. This process yielded dark gummy extracts, totaling 60 g residue, obtained from the concentrated extract under reduced pressure.

2.3. Isolation

The extract underwent silica gel column chromatography (diameter 4.5 cm, 70-230 mesh, 800 g) with a gradient of *n*-hexane-ethyl acetate (100:0 → 0:100) as the eluent, followed by an increasing concentration of methanol (up to 20%) in ethyl acetate. A total of 234 fractions of 250 mL each were collected, screened by TLC (detection at 254 nm and after spraying with 5% phosphomolybdic acid in ethanol), and then pooled together. Finally, twenty combined fractions (F_1-F_{20}) were obtained. Fraction 7 [5.2 g, eluted with *n*-hexane-ethyl acetate (85:15)] was further separated via silica gel column chromatography using *n*-hexane-$CHCl_3$ (25: 75) as the eluent to produce ten sub fractions (7a-7j). Subfraction 7c (327 mg) was chromatographed on silica gel, using *n*-hexane-$CHCl_3$ (40:60) as the mobile phase, yielding α-amyrin (**4**, 10 mg). A crude solid was obtained from fraction 9 [1 g, eluted with *n*-hexane-ethyl acetate (80:20)], which was recrystallized from acetone to afford β-sitosterol (**3**, 10 mg). Fraction 12 [387 mg, eluted with *n*-hexane-ethyl acetate (65:35)] was separated by silica gel column chromatography using CH_2Cl_2-methanol (99:1) to CH_2Cl_2-methanol (95:5) as the mobile phase, yielding *p*-hydroxybenzoic acid (**1**, 1.5mg). Fraction 15 [900 mg, eluted with *n*-hexane-ethyl acetate (40:60) to (30:70)] was submitted to silica gel column chromatography, eluted with *n*-hexane-CH_2Cl_2-acetone (50:25:25), yielding 3-*O*-[β-D-(6′-tetradecanoate) glucopyranosyl]-β-sitosterol (**6**, 2.4 mg). Fraction 20 [300 mg, eluted with ethyl acetate-methanol (90:10)] was submitted to silica gel column chromatography, eluted with CH_2Cl_2-methanol (95:5) to CH_2Cl_2-methanol (80:20), affording thirteen sub-fractions (20a-20m). The precipitate of subfraction 20d was recrystallized from methanol to afford daucosterol (**5**, 6mg). Subfraction 20i (70 mg) was separated by silica gel column chromatography using $CHCl_3$-acetone (40:60) as the mobile phase to obtain ethyl-2-hydroxy *trans*-cinamate (**2**, 2 mg).

2.4. Structure elucidation

An Avance II 600 spectrometer (Bruker) was utilized to record one-dimensional and two-dimensional NMR experiments, using deuterated chloroform (chloroform-d) and deuterated methanol (methanol-d_4) as the solvents.

3. Results and Discussion

The aerial parts of *E. macrophyllus* Boiss & Hausskn were extracted with ethyl acetate. Fractionation of the resulting extract using open column chromatography on silica gel afforded six compounds (**1-6**) (Fig. 1). Their structures were determined through extensive spectroscopic analysis, including 1D and 2D NMR, and confirmed by comparing their NMR data with those reported in the literature. These compounds were identified as *p*-hydroxybenzoic acid (**1**) (Cho et al., 1998), ethyl-2-hydroxy *trans*-cinamate (**2**) (Ferreira et al., 2016), β-sitosterol (**3**) (Sen et al., 2013), α-amyrin (**4**) (Nnamonu et al., 2016), daucosterol (**5**) (Moghaddam et al., 2007) and 3-*O*-[β-D-(6′-tetradecanoate)-glucopyranosyl]-β-sitosterol (**6**) (Mohamed et al., 2009). Notably, considering the ethyl acetate extraction process, it is crucial to acknowledge that compound **2** might be an artefact formed during extraction (Venditti, 2020).

3.1. Structural identification

Compound **6** was obtained as white powder. Its molecular formula was determined to be $C_{49}H_{86}O_7$ by HRESIMS at *m/z* 785.6292 $[M-H]^-$. The 1D and 2D NMR spectra (Fig. 2, Fig. 3 and Fig. 4) of this compound were in accordance with a known compound, daucosterol, except for the presence of a long alkyl chain. The ^1H NMR and HSQC spectra (Table 1) showed an olefinic proton at δ 5.36 (m, H-6), an oxymethine proton at δ 3.55 (m, H-3) and six methyl groups at δ 0.67 (3H, s, H-18), 1.00 (3H, s, H-19), 0.92 (3H, d, *J* = 6.4 Hz; H-21), 0.83 (3H, d, *J* = 6.8 Hz; H-26), 0.81 (3H, d, *J* = 6.8 Hz; H-27), and 0.84 (3H, d, *J* = 7.1 Hz; H-29). The spectra indicated the presence of an alkyl chain and a sugar hexose unit. An anomeric proton with β-configuration was observed at δ 4.38 (1H, d, *J* = 7.7 Hz; H-1′). In the ^{13}C NMR spectrum, 6 carbon signals were assigned to hexose moiety, 29 carbon signals to aglycone moiety, and the remaining 14 carbon signals to fatty acyl residue. The hexose unit consisted of five oxy-methines and one oxy-methylene, as confirmed by the HMQC spectrum. Comparison of the ^1H and ^{13}C NMR data with those for daucosterol, together with the HMBC correlations from H_2-6′ to C-5′ and C-4′ confirmed the structure of the sugar moiety as a glucose unit. The ^{13}C NMR spectrum exhibited an ester carbonyl at δ_c 174.9, two olefinic carbons at δ_c 140.4 and 122.3 corresponding to the double bond between C-5 and C-6, oxymethine signal at δ_c 79.7 for C-3, and an anomeric carbon at 101.3. Key HMBC cross

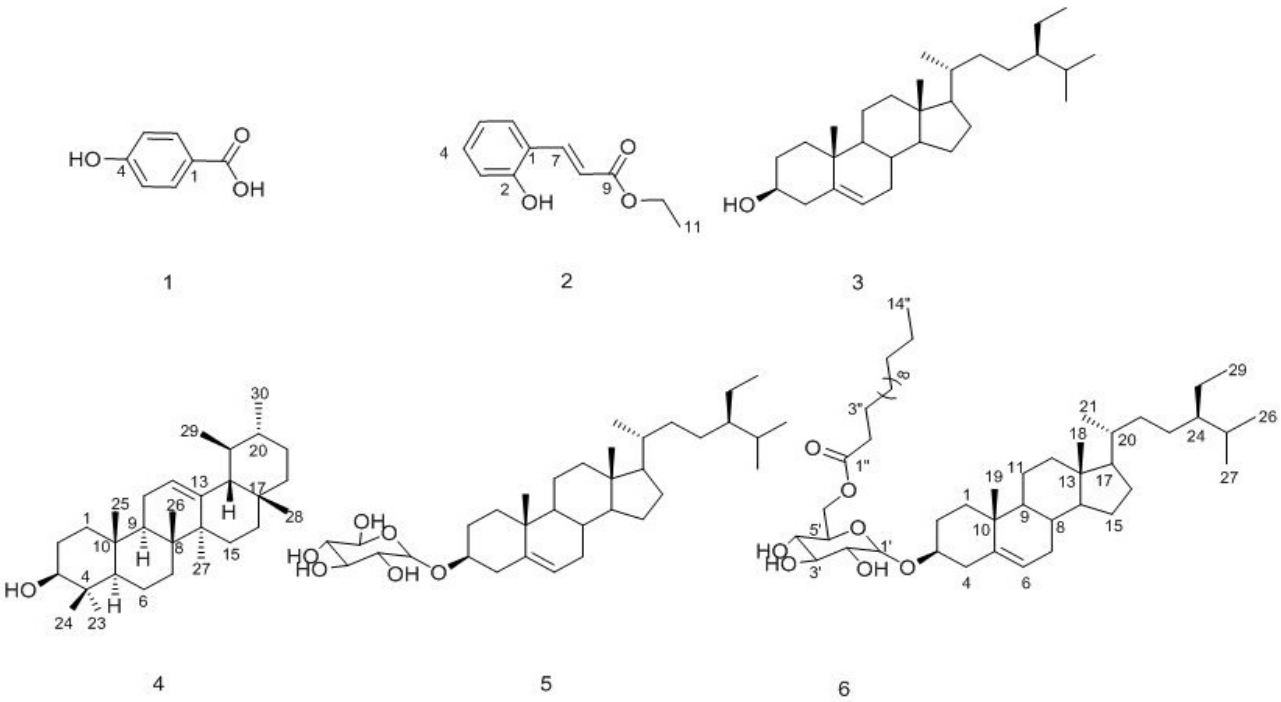

Fig. 1. Structure of compounds (**1-6**) isolated from the aerial parts of *E. macrophyllus* Boiss & Hausskn.

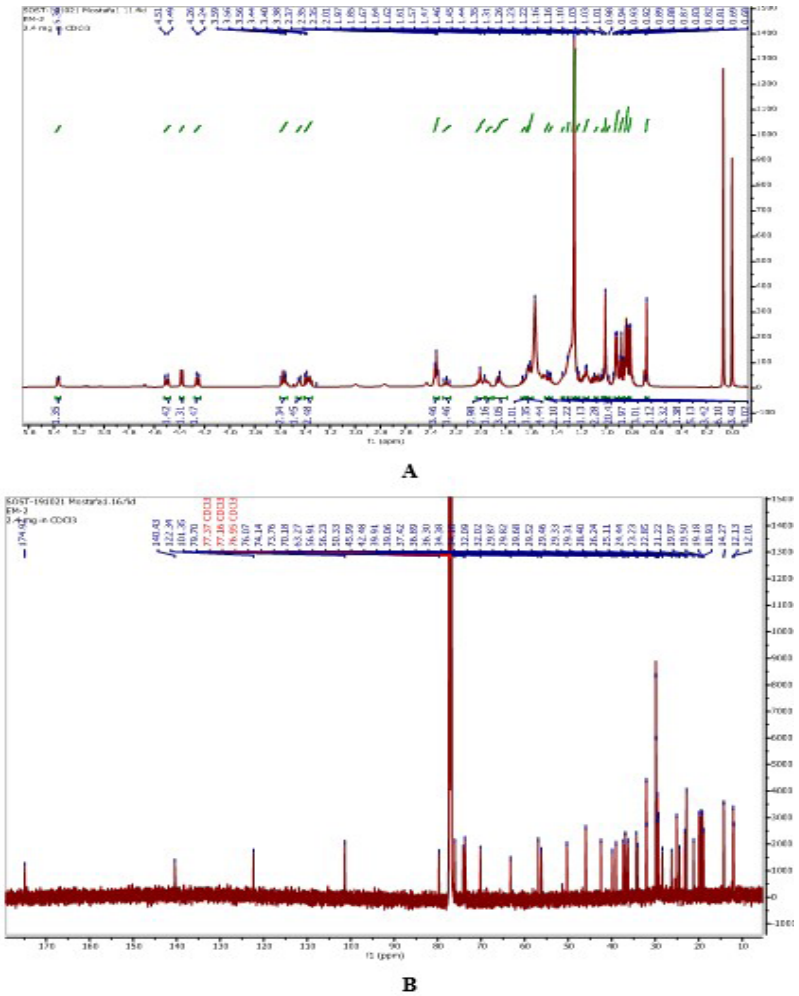

Fig. 2. (A) ^{1}H NMR and (B) ^{13}C NMR spectra of compound **6**.

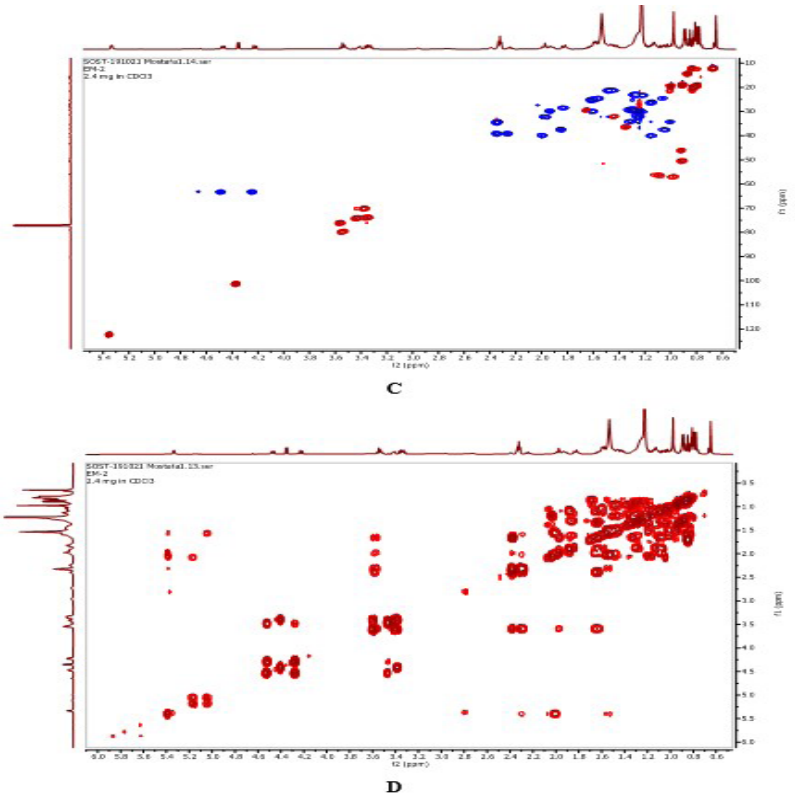

Fig. 3. (C) HSQC-DEPT and (D) ^{1}H-^{1}H COSY spectra of compound **6**.

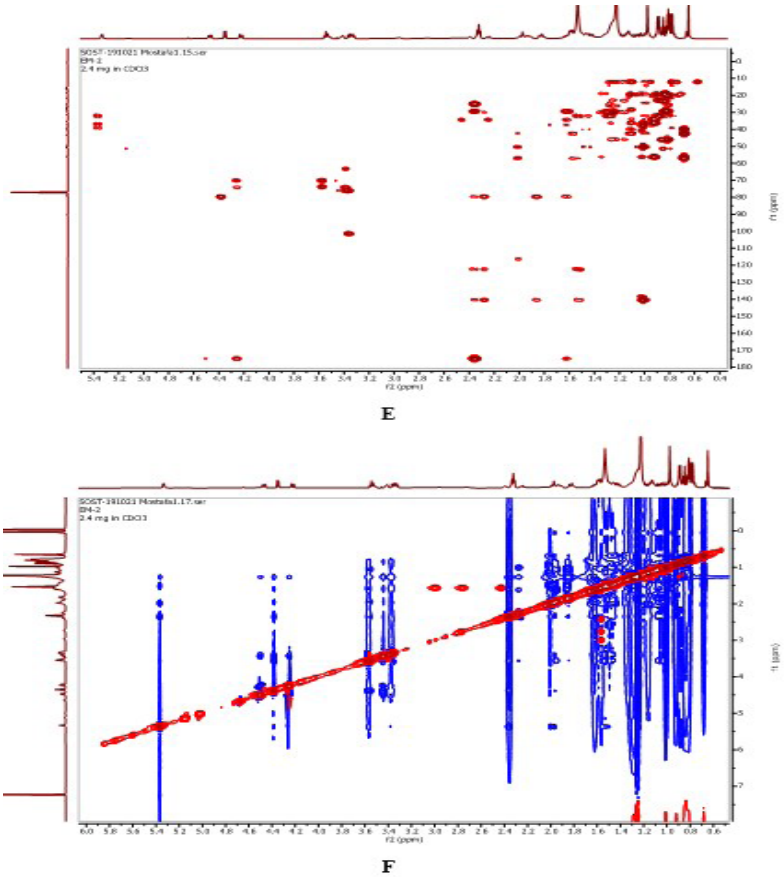

Fig. 4. (E) HMBC and (F) NOESY spectra of compound **6**.

Table 1
^{1}H and ^{13}C NMR spectroscopic data for compounds **6** ($CDCl_3$) (600 MHz for ^{1}H and 150 MHz for ^{13}C NMR; δ in ppm).

	δ_H (*J* in Hz)	δ_C		δ_H (*J* in Hz)	δ_C
1	1.03-1.04, m 1.84-1.87, m	37.4 (CH_2)	22	1.30-1.31, m	34.1 (CH_2)
2	1.62-1.63, m 1.93-1.95, m	29.9 (CH_2)	23	1.14-1.16, m	26.2 (CH_2)
3	3.53-3.55, m	79.7 (CH)	24	0.92-0.94, m	45.9 (CH)
4	2.25-2.29, m 2.35-2.37, m	39.0 (CH_2)	25	1.65-1.67, m	29.3 (CH)
5	-	140.4 (C)	26	0.83, d (6.8)	20.0 (CH_3)
6	5.36-5.37, m	122.3 (CH)	27	0.81, d (6.8)	19.2 (CH_3)
7	1.97-2.00, m	32.0 (CH)	28	1.25m	23.2 (CH_2)
8	1.44-1.45, m	32.1 (CH)	29	0.84, d (7.1)	12.0 (CH_3)
9	0.90-0.92, m	50.3 (CH)	1′	4.38, d (7.7)	101.3 (CH)
10	-	36.9 (C)	2′	3.35-3.37, m	73.7 (CH)
11	1.46-1.48, m	21.2 (CH_2)	3′	3.56-3.59, m	76.0 (CH)
12	1.15-1.17, m 2.01-2.03, m	39.9 (CH_2)	4′	3.37-3.40, m	70.1 (CH)
13	-	42.5 (C)	5′	3.43-3.46, m	74.1 (CH)
14	1.08-1.10, m	56.2(CH)	6′	4.25, dd (12.2, 4.7)	63.2 (CH_2)
15	1.82-1.83, m	28.3 (CH_2)	1″	4.50, dd (12.2, 2.3)	174.9 (C)
16	1.60-1.61, m	24.4 (CH_2)	2″	-	34.4 (CH_2)
17	0.97-0.98, m	56.9 (CH)	3″	2.34-2.36, m	25.1 (CH_2)
18	0.67, s	12.1 (CH_3)	4″-11″	1.57-1.58, m	29.3-29.9 (CH_2)
19	1.00, s	19.5 (CH_3)	12″	1.25, br s	32.0 (CH_2)
20	1.33-1.35, m	36.3 (CH)	13″	1.22-1.23, m	22.8 (CH_2)
21	0.92, d (6.4)	18.9 (CH_3)	14″	1.25m	14.2 (CH_3)

peak from H-1′ to C-3 verified the glycoside group's linkage at position C-3. HMBC correlations of H-6′a and H-6′b to C-1″ confirmed the attachment of the fatty acyl residue to C-6′ of the glycoside moiety. The chemical shift of the H-6′a and H-6′b signals (δ_H 4.25 and 4.50) were in agreement with an acyl group bridge between C-6′ and C-2″ (Ali et al., 2001; Sultana and Afolayan, 2007). Thus, compound **6** was identified as 3-*O*-[β-D-(6′-tetradecanoate) glucopyranosyl]-β-sitosterol.

p-Hydroxybenzoic acid (**1**): White powder, molecular formula: $C_7H_6O_3$, ^{1}H NMR (CD_3OD, 600 MHz): δ 6.75 (2H, d, *J* = 8.6 Hz, H-3, H-5), 7.85 (2H, d, *J* = 8.5 Hz, H-2, H-6), ^{13}C NMR (CD_3OD, 150 MHz): δ 115.5 (CH, C-3, C-5), 125.4 (C, C-1), 132.4 (CH, C-2, C-6), 162.6 (C, C-4), 172.5 (C, COOH).

Ethyl-2-hydroxy *trans*-cinamate (**2**): Yellow oil; ESI-MS m/z: 215.0 $[M+Na]^+$, molecular formula: $C_{11}H_{12}O_3$, ^{1}H NMR (CD_3OD, 600 MHz): δ 1.25 (3H, m, H-11), 3.36, 3.49 (2H, m, H-10), 6.41 (1H, m, H-8), 6.74 (1H, m, H-3), 6.78 (1H, m, H-5), 7.39 (1H, m, H-6), 7.46 (1H, m, H-7), 7.54 (1H, m, H-4), ^{13}C NMR (CD_3OD, 150 MHz): δ 29.3 (CH_3, C-11), δ 47.4 (CH_2, C-10), 116.6 (CH, C-3), 116.6 (CH, C-5), 118.3 (CH, C-8), 127.7 (C, C-1), 130.3 (CH, C-6), 141.7 (CH, C-7), 144.3 (CH, C-4), 160.4 (C, C-2), 169.3 (CH, C-9).

β-Sitosterol (**3**): Colorless crystal; ESIMS *m/z* 414.7 and molecular formula $C_{29}H_{50}O$.

α-Amyrin (**4**): White powder; ESIMS *m/z* 449.3 $[M + Na]^+$ and molecular formula $C_{30}H_{50}O$.

Throughout history, plants have been vital sources of medicine, with many pharmaceuticals derived from them (Bitew et al., 2017). The genus *Echinops*, rich in thiophenes and terpenes, has exhibited diverse biological activities, including hepatoprotective, anti-inflammatory, antifungal, and anticancer properties (Bitew et al., 2019). Studies on *Echinops spinosissimus* roots have revealed the presence of phenolic compounds in the ethanol extract inhibiting various bacteria (Khedher et al. 2021). Compounds such as *p*-hydroxybenzoic acid have demonstrated antimicrobial properties against a range of microorganisms, including

both Gram-positive and Gram-negative bacteria (Manuja et al., 2013). Phytosterols are a subcategory of the steroids, as a significant class of bioorganic molecules, general in plants, animals, marines as well as fungi and have similarity to cholesterol in structure. Two phytosterols, including daucosterol and β-sitosterol, have shown antitumor and anticancer effects (Saeidnia et al., 2014; Nguedia et al., 2020). Additionally, α-amyrin has exhibited various beneficial properties, such as analgesic, anti-inflammatory, and hepatoprotective effects (Nogueira et al., 2019).

4. Concluding remarks

The present investigation describes a phytochemical study on *E. macrophyllu*s Boiss & Hausskn, leading to the isolation and identification of six secondary metabolites: *p*-hydroxybenzoic acid (**1**), ethyl-2-hydroxy *trans*-cinamate (**2**), β-sitosterol (**3**), α-amyrin (**4**), daucosterol (**5**) and 3-*O*-[β-D-(6′-tetradecanoate)-glucopyranosyl]-β-sitosterol (**6**) for the first time from this plant. Various biological activities associated with these compounds have been previously reported. Consequently, *E. macrophyllu*s Boiss & Hausskn can be further investigated in terms of these activities.

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Author contribution statement

Conceptualization, literature search, and experimental procedures were performed by Azadeh Khademian. The first draft of the manuscript was prepared by Azadeh Khademian. Mahdi Moridi Farimani designed and coordinated the project. NMR spectra were measured by Mostafa Alilou. Mojtaba Asadollahi identified and collected the plant. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare that there is no conflict of interest.

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