Phytochemical Study of *Euphorbia turcomanica* Boiss.

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Abstract: The attraction to the *Euphorbia* genus, with its remarkable diversity in species, is due to its variety of chemical compositions. *Euphorbia turcomanica* is one of the species of the spurge family growing wildly in Iran. This research aims to investigate the presence of secondary metabolites, specially terpenoid compounds, in terms of structural determination. Samples of *E. turcomanica* were finely powdered and macerated with acetone/dichloromethane 2:1. Repeated column chromatography using silica gel, MPLC, and HPLC methods followed by the analysis of data obtained from spectroscopic means was carried out to purify and identify the terpenoid constituents. The chemical structures of nine known terpenoids were determined for the first time from *E. turcomanica* during this study. Loliolide (1), a monoterpenoid, and eight steroids and triterpenoids, including simiarenol (2), isomultiflorenol (3), cycloart-25-ene-3β,24-diol (4), β-sitosterol (5), cycloart-23-ene-3β,25-diol (6), 3α, 11α-dihydroxyurs-12-ene (7), 3β, 24β, 25-trihydroxyxycloartane (8), and 7α-hydroxystigmasteryl (9) were isolated and identified. *E. turcomanica*, with a rich terpenoid profile, can be one of the valuable and economic sources providing compounds for drug development.

Keywords: *Euphorbia turcomanica*; phytochemistry; terpenoid; triterpene; steroid

1. Introduction

The family Euphorbiaceae, with nearly around 7500 species and 300 genera, is one of the largest families of flowering plants on earth. More than 2000 species belong to the genus *Euphorbia* or spurge, which is the largest genus of this family. These plants occur naturally in both tropical and temperate regions [1,2]. Iran, with 92 species of *Euphorbia*, is one of the most diverse countries in southwest Asia, with the highest occurrence of endemics which are 21 species until 2020 [3]. *Euphorbia turcomanica* Boiss., one of the species of the spurge family, is an annual herb growing wild in the lower fields and plains of Iran [4].

Secondary metabolites with a wide range of biological activities and broad structural diversity have been isolated from different parts of *Euphorbia* species, including bark, cortex, seeds, latex, lactiferous duct, stem, wood, stem bark, leaves, and flowers. The complex and diverse phytochemical properties of this genus include compounds from different chemical classes, especially the ones that consist of isoprene units known as terpenes and terpenoids, which are considered the largest and most diverse group of natural products. Between the major classes of terpenoids found in *Euphorbia* species, diterpenes and triterpenes are the most important constituents of the secondary metabolites, mainly in their ester forms [5,6]. Diterpenes are prominent constituents isolated from this genus incorporating more than 350 new diterpenes over the last decade with over 20 different skeletal structures. Macro cyclic diterpenes are the most frequent diterpenoids from *Euphorbia* plants. Some of these diterpenoids are scarcely distributed in nature, like tigliane, which can be found in *Euphorbia* species [7,8]. The second most extensive group of chemical constituents in this genus are triterpenes, with more than 130 different triterpenoids varying in 10 different structural forms with a significant diversity amongst the cyclic triterpenes. The tetracyclic and pentacyclic triterpenoids and steroids are isolated triterpenes from many species in this genus. Euphane and tirucallane triterpenoids are not only exclusively found in this genus but occur most frequently in them, which is a distinguished point about members of...
the Euphorbiaceae family and consequently can be considered a valuable source for these compounds [2,9].

In traditional medicine, species of Euphorbia have a wide range of applications, but in particular, the latex of E. turcomanica is used as a laxative and diuretic. Also, it is externally applied to snake bites and scorpion stings and internally used for treating intestinal worms and purifying blood [10]. The attraction to the Euphorbia genus in pharmacological research is due to the variety of chemical compositions in them. Part of these effects is associated with the presence of terpenoids, including diterpenes and triterpenes, especially cycloartanes. Plants-derived terpenoids have been identified to have anticancer and anti-inflammatory activities. The Euphorbia genus, with a rich terpenoid profile, can be one of the valuable and economically important sources providing compounds in drug development [9,11].

Most research has proven the biological and therapeutic properties of E. turcomanica, but only a few phytochemical studies have been carried out to isolate and elucidate the chemical constituents present in this plant, including steroids, flavonoids, phenolics, aromatics, and especially terpenoid compounds, by preliminary screening [12].

This study describes the isolation and structural determination of secondary metabolites from E. turcomanica. The structures of these compounds were mainly established by spectroscopic methods.

2. Materials and Methods

2.1. General Experimental Procedures

Medium pressure liquid chromatography (MPLC) was carried out on a BUCHI® Gradient SystemC605 apparatus equipped with a fraction collector, using a glass column filled with silica gel (particle size 15–40 µm; Merck®, Darmstadt, Germany), HPLC (High-Performance Liquid Chromatography) on a Waters® apparatus equipped with a 515 pump, a Waters®2487 Dual Wavelength Absorbance Detector and a Waters®2414 Refractive Index Detector, using Spherisorb® Sil (250 mm × 20 mm) column, 5 µm silica gel and hexane, with ethyl acetate as the mobile phase. The purity was checked using TLC (Thin-Layer Chromatography) with Merck® silica gel GF254 plates detected by spraying cerium sulfate. Determination of each structure was performed by NMR spectra recorded on a Bruker Avance® AV400 spectrometer.

2.2. Plant Materials

The whole plant of E. turcomanica was gathered in two places, Isfahan-Qom Road, Center of Iran, and Bandar Tourkaman, North of Iran, during the flowering time in July 2012. The plant was identified by Mr. Bahram Zehzad, Department of biology, University of Shahid Beheshti, Tehran, and a voucher specimen (No. 2410) is kept at the Herbarium of Pharmacognosy, Department of Pharmacognosy, Faculty of Pharmacy, Isfahan University of Medical Sciences.

2.3. Extraction Procedure

Air-dried samples of E. turcomanica were finely powdered and extracted with acetone/dichloromethane 2:1. The maceration was carried out at room temperature in 3 repeated procedures that each took 4 days and 10–15 liters of solvent. The extract was concentrated under a vacuum. The greasy extract was subjected to Celite and chromatographed using the VLC method with silica gel coated with paraffin as the stationary phase and MeOH–H₂O (60:40) and MeOH–H₂O (90:10) as a solvent system to eliminate chlorophyll and fats from the extract. Normal-phase (NP)-TLC analysis indicated that the fraction which was separated by MeOH–H₂O (60:40) contained a series of terpenoids and fraction eluted with MeOH–H₂O (90:10) affording steroid and triterpene-containing extract.

2.4. Isolation of Terpenoids

The terpene-containing extract was dissolved in CHCl₃, adsorbed onto Celite, and chromatographed using an open column and a linear gradient solvent system from n-
hexane/ EtOAc 3–30%, which yielded 15 fractions. After comparing the TLC analysis of the obtained fractions, the ones with similar UV characterizations and RI values were combined and concentrated by vacuum. Further purification of these fractions was carried out by MPLC using a linear gradient solvent system from MeOH/H2O (70:30) to MeOH/H2O (100:0), resulting in subfractions [13]. Compound 1 was obtained from open column chromatography using a linear gradient solvent system from n-hexane/acetone 3% to n-hexane/acetone 30% of the first subfraction of fraction 10 in an n-hexane/acetone 20% mobile phase. After further vacuum concentration and analysis, it was identified as pure crystals of loliolide.

The steroid and triterpene-containing extract chromatographed by MPLC using a linear gradient solvent system from n-hexane 100% to EtOAc 100% [13]. Fractions were analyzed by TLC, and similar fractions were mixed and concentrated under a vacuum. Seventeen fractions were obtained and subjected to TLC and preliminary NMR analysis.

Fraction 3 was eluted with Hex/EtOAc (90:10) in MPLC and further purified by HPLC using Hex/EtOAc (90:10) as the mobile phase and resulting in the isolation of compound 2 as simiarenol and [3] as isomultiflorenol. Fractions 5 and 6 were both eluted by Hex/EtOAc (70:30) and applied on HPLC using Hex/EtOAc (80:20) as a solvent. Compound 4 was isolated from fraction 5 as cycloartane. Compound 5 was purified from obtained crystals by recrystallization and identified as pure β-sitosterol. Fractions 7 and 8 were eluted with Hex/EtOAc (70:30) in MPLC and purified by HPLC using Hex/EtOAc (80:20) as a solvent and resulting in the isolation of compound 6 as a cycloartane. Fraction 8 contained pure crystals that were filtered and concentrated by a rotary evaporator, which were further purified by recrystallization and yielded compound 7. Fraction 12, eluted with Hex/EtOAc (50:50), was purified further on HPLC using Hex/EtOAc (37:63) as a solvent to yield compound 8 as a cycloartane-type triterpene. Fraction 14 was eluted with Hex/EtOAc (40:60) in MPLC and further purified by HPLC using Hex/EtOAc (50:50) as solvent. As a result, a significant amount of needle-shaped crystal was obtained and further purified by recrystallization and yielded compound 9 as a steroidal triterpene.

3. Results

3.1. Spectral Data of Compounds 1–9

Nine compounds were extracted and purified from *Euphorbia turcomanica*, as shown in Figure 1. They were identified using spectroscopic analysis. The 1H NMR and 13C NMR data of each compound are reported here.

1. Loliolide (Compound 1)

White crystal, MW (g/mol): 196.1; 1H NMR data (CDCl3, 400 MHz): δH 1.55 (dd, J = 14.8–3.6 Hz, H-2a), 1.98 (t, J = 2.4 Hz, H-2b), 4.35 (m, H-3), 1.80 (dd, J = 13.2–4.0 Hz, H-4a), 2.02 (t, J = 2.8 Hz, H-4b), 5.72 (m, H-7), 1.49 (s, H-9), 1.29 (s, H-10), 1.80 (d, J = 0.8 Hz, H-11), 13C NMR data (CDCl3, 100 MHz): 30.7 (C-1), 45.6 (C-2), 66.8 (C-3), 47.3 (C-4), 86.7 (C-5), 171.9 (C-6), 112.9 (C-7), 182.4 (C-8), 26.5 (C-9), 27.0 (C-10), 30.7 (C-11).

2. Simiarenol: 3β-hydroxy-E:B-friedo-hop-5-ene (Compound 2)

Amorphous white powder, MW (g/mol): 426.70; 1H NMR data (CDCl3, 400 MHz): δH 3.47 (dd, J = 10.9–5.7 Hz, H-3a), 5.62 (dd, J = 6.0–6.0 Hz, H-6), 1.35 (m, H-22), 1.25 (s, H-23), 1.14 (s, H-24), 1.04 (s, H-25), 1.00 (s, H-26), 0.92 (s, H-27), 0.77 (s, H-28), 0.84 (d, J = 6.5 Hz, H-29), 0.88 (d, J = 6.5 Hz, H-30), 13C NMR data (CDCl3, 100 MHz): 18.20 (C-1), 27.90 (C-2), 76.52 (C-3), 40.97 (C-4), 42.08 (C-5), 122.17 (C-6), 24.18 (C-7), 44.37 (C-8), 34.97 (C-9), 50.35 (C-10), 34.27 (C-11), 34.27 (C-12), 38.74 (C-13), 39.09 (C-14), 29.08 (C-15), 35.53 (C-16), 42.92 (C-17), 51.86 (C-18), 20.05 (C-19), 28.46 (C-20), 60.15 (C-21), 30.94 (C-22), 29.11 (C-23), 25.62 (C-24), 18.01 (C-25), 15.89 (C-26), 15.14 (C-27), 16.21 (C-28), 22.10 (C-29), 23.07 (C-30).
3. Isomultiflorenol (Compound 3)

Amorphous white powder, MW (g/mol): 426.7; $^1$H NMR data (CDCl$_3$, 400 MHz): $\delta_{H}$ 3.23 (dd, $J = 11.6$-4.6 Hz, H-3a), 0.80 (s, H-23), 0.95 (s, H-25), 1.05 (s, H-26), 0.96 (s, H-27), 1.06 (s, H-28), 0.98 (s, H-29), 1.00 (s, H-30); $^{13}$C NMR data (CDCl$_3$, 100 MHz): 34.33 (C-1), 26.34 (C-2), 79.17 (C-3), 38.94 (C-4), 50.81 (C-5), 19.36 (C-6), 28.04 (C-7), 135.19 (C-8), 133.63 (C-9), 37.72 (C-10), 20.95 (C-11), 30.80 (C-12), 37.39 (C-13), 41.13 (C-14), 27.63 (C-15), 36.89 (C-16), 30.93 (C-17), 44.23 (C-18), 34.48 (C-19), 28.47 (C-20), 35.15 (C-21), 36.84 (C-22), 18.19 (C-23), 15.76 (C-24), 19.96 (C-25), 19.14 (C-26), 24.78 (C-27), 31.65 (C-28), 34.74 (C-29), 33.09 (C-30).

4. Cycloart-25-ene-3β,24-diol (Compound 4)

Amorphous white powder, MW (g/mol): 442.38; $^1$H NMR data (CDCl$_3$, 400 MHz): $\delta_{H}$ 3.28 (dd, $J = 11.0$-4.5 Hz, H-3a), 0.96 (s, H-18), 0.33 (d, $J = 4.0$ Hz, H-19a), 0.55 (d, $J = 4.0$ Hz, H-19b), 0.88 (d, $J = 6.4$ Hz, H-21), 4.02 (t, $J = 6.03$ Hz, H-24), 1.72 (s, H-27), 0.88 (s, H-28), 0.80 (s, H-29), 0.96 (s, H-30); $^{13}$C NMR data (CDCl$_3$, 100 MHz): 32.04 (C-1), 30.49 (C-2), 78.99 (C-3), 40.61 (C-4), 47.21 (C-5), 21.25 (C-6), 28.22 (C-7), 48.14 (C-8), 20.09 (C-9), 26.48 (C-10), 26.15 (C-11), 35.67 (C-12), 45.38 (C-13), 48.93 (C-14), 32.08 (C-15), 26.56 (C-16), 52.27 (C-17), 18.19 (C-18), 28.95 (C-19), 36.05 (C-20), 18.44 (C-21), 30.12 (C-22), 28.28 (C-23), 76.52 (C-24), 147.87 (C-25), 111.10 (C-26), 17.74 (C-27), 19.45 (C-28), 25.56 (C-29), 14.15 (C-30).

5. β-sitosterol: Stigmast-5-en-3β-ol (Compound 5)

White crystal, MW (g/mol): 414; $^1$H NMR data (CDCl$_3$, 400 MHz): $\delta_{H}$ 1.85 (m, H-1), 3.52 (m, H-3a), 5.36 (br s, H-6), 0.68 (s, H-18), 1.01 (s, H-19), 0.92 (d, $J = 6.4$ Hz, H-21), 0.81 (d, $J = 6.9$ Hz, H-26), 0.85 (d, $J = 7.7$ Hz, H-27), 0.86 (t, $J = 7.5$ Hz, H-29), 0.96 (s, H-30); $^{13}$C NMR data (CDCl$_3$, 100 MHz): 37.37 (C-1), 29.23 (C-2), 71.95 (C-3), 42.42 (C-4), 140.88 (C-5), 121.88 (C-6), 32.02 (C-7), 32.04 (C-8), 50.23 (C-9), 36.63 (C-10), 21.21 (C-11), 39.89 (C-12), 42.44 (C-13), 56.88 (C-14), 24.44 (C-15), 28.40 (C-16), 56.15 (C-17), 12.00 (C-18), 19.55 (C-19).
6. Cycloart-23-ene-3β,25-diol (Compound 6)

White crystal, MW (g/mol): 442.38; 1H NMR data (CDCl3, 400 MHz): δH 3.28 (dd, J = 11.0-4.5 Hz, H-3a), 0.97 (s, H-18), 0.33 (d, J = 4.3 Hz, H-19a), 0.85 (d, J = 4.4 Hz, H-19b), 0.86 (d, J = 6.4 Hz, H-21), 5.60 (m, H-23), 5.60 (m, H-24), 1.32 (s, H-26), 1.32 (s, H-27), 0.88 (s, H-28), 0.81 (s, H-29), 0.97 (s, H-30), 13C NMR data (CDCl3, 100 MHz): 32.08 (C-1), 30.49 (C-2), 78.98 (C-3), 40.61 (C-4), 47.20 (C-5), 21.25 (C-6), 28.21 (C-7), 48.12 (C-8), 20.09 (C-9), 26.14 (C-10), 25.57 (C-11), 32.88 (C-12), 45.41 (C-13), 48.94 (C-14), 35.70 (C-15), 26.54 (C-16), 52.12 (C-17), 18.24 (C-18), 30.00 (C-19), 36.51 (C-20), 18.41 (C-21), 39.16 (C-22), 139.42 (C-23), 125.76 (C-24), 70.73 (C-25), 30.10 (C-26), 30.04 (C-27), 19.43 (C-28), 14.15 (C-29), 24.85 (C-30).

7. 3α, 11α-dihydroxyurs-12-il (Compound 7)

White crystal, MW (g/mol): 442.38; 1H NMR data (CDCl3, 400 MHz): 3.21 (m, H-3b), 3.98 (m, H-11), 4.90 (d, J = 3.2Hz, H-12), 0.90 (s, H-23), 0.69 (s, H-24), 0.94 (s, H-25), 0.76 (d, J = 5.4 Hz, H-26), 1.00 (s, H-27), 0.82 (m, H-30), 13C NMR data (CDCl3, 100 MHz): 33.5 (C-1), 25.4 (C-2), 76.0 (C-3), 37.5 (C-4), 48.8 (C-5), 18.2 (C-6), 35.2 (C-7), 43.5 (C-8), 55.8 (C-9), 38.2 (C-10), 68.4 (C-11), 128.7 (C-12), 142.9 (C-13), 42.2 (C-14), 27.9 (C-15), 27.7 (C-16), 33.6 (C-17), 58.1 (C-18), 39.4 (C-19), 39.3 (C-20), 31.1 (C-21), 41.3 (C-22), 28.7 (C-23), 22.4 (C-24), 16.6 (C-25), 18.0 (C-26), 23.3 (C-27), 28.6 (C-28), 17.5 (C-29), 21.3 (C-30).

8. 3β, 24β, 25-trihydroxy-cycloartan (Compound 8)

Amorphous white powder, MW (g/mol): 460.39; 1H NMR data (CDCl3, 400 MHz): δH 3.27 (m, H-3a), 0.96 (s, H-18), 0.33 (d, J = 4.2 Hz, H-19a), 0.85 (d, J = 4.2 Hz, H-19b), 0.88 (d, J = 5.2 Hz, H-21), 3.31 (m, H-24), 1.16 (s, H-26), 1.22 (s, H-27), 0.89 (s, H-28), 0.96 (s, H-29), 0.80 (s, H-30), 13C NMR data (CDCl3, 100 MHz): 32.10 (C-1), 30.49 (C-2), 78.99 (C-3), 40.62 (C-4), 47.23 (C-5), 21.26 (C-6), 28.54 (C-7), 48.14 (C-8), 20.10 (C-9), 26.58 (C-10), 26.15 (C-11), 36.68 (C-12), 45.42 (C-13), 50.59 (C-14), 33.03 (C-15), 26.68 (C-16), 52.44 (C-17), 18.22 (C-18), 30.05 (C-19), 36.52 (C-20), 18.57 (C-21), 33.66 (C-22), 28.68 (C-23), 79.77 (C-24), 73.36 (C-25), 23.36 (C-26), 26.72 (C-27), 19.44 (C-28), 14.15 (C-29), 25.57 (C-30).

9. 7α-hydroxy-stigmasterol (Compound 9)

Needle white crystal, MW (g/mol): 428.70; 1H NMR data (CDCl3, 400 MHz): δH 3.58 (m, H-3a), 2.33 (dd, J = 5.2-1.8 Hz, H-4), 2.28 (t, J = 2.0 Hz, H-5), 5.64 (dd, J = 5.4-1.9 Hz, H-6), 3.85 (br s, H-7), 0.68 (s, H-18), 0.99 (s, H-19), 0.93 (d, J = 6.5 Hz, H-21), 5.16 (dd, J = 15.2-8.6 Hz, H-22), 0.81 (d, J = 6.9 Hz, H-26), 0.85 (d, J = 6.9 Hz, H-27), 0.83 (t, J = 7.3 Hz H-29), 13C NMR data (CDCl3, 100 MHz): 37.13 (C-1), 31.50 (C-2), 71.49 (C-3), 42.14 (C-4), 146.39 (C-5), 124.00 (C-6), 65.51 (C-7), 37.64 (C-8), 42.38 (C-9), 36.24 (C-10), 20.84 (C-11), 39.29 (C-12), 42.27 (C-13), 49.55 (C-14), 24.45 (C-15), 28.43 (C-16), 55.82 (C-17), 11.78 (C-18), 18.39 (C-19), 39.92 (C-20), 138.39 (C-22), 128.82 (C-23), 51.36 (C-24), 30.23 (C-25), 18.94 (C-26), 19.95 (C-27), 25.99 (C-28), 12.13 (C-29).

3.2. Structure Identification of Compounds 1–9

Compound (1) was identified as C11H16O3. Based on 1H NMR, two methylic protons at δH 1.49 (H-9) and 1.29 (H-10) and one olefinic proton at δH 5.72 (H-7) were detected. The 13C NMR spectral data demonstrates the existence of a carbonyl group on the lactone ring (δC 182.4), a secondary hydroxyl group (δC 66.8), a trisubstituted olefinic bond (δC 112.9 and 171.9), and three singlet methyl groups (δC 26.5, 27.0, and 30.7) which has resemblance with the data from the literature. This structure was identified as loliolide [14,15].

Compound 2 was found to be C30H50O. The 1H NMR spectrum shows the existence of six tertiary methyl groups. The presence of the isopropyl group at C21 is confirmed by doublet signals at δH 0.84 and 0.88, which indicate two secondary methyls. Also, the δH 5.62 signal in the 1H NMR spectrum refers to the olefinic proton at H-6. The 1H NMR and 13C NMR spectra signals at δH 3.47 and δC 76.52 suggest that an oxymethine on C3
confirms the hydroxyl group α-orientation. Regarding these data and the findings from the published literature [16,17], compound 2 was identified as simiareno.

Compound 3 with the molecular formula of C₃₀H₅₀O was elucidated as isomultifloranol based on the spectral data analysis and comparing them to the literature [18]. The ¹H NMR signals at δ_H 0.80 (H-23), 0.95 (H-24), 1.05 (H-25), 0.96 (H-27), 1.06 (H-28), 0.98 (H-29), and 1.00 (H-30) revealed eight tertiary methyl groups, and the double doublet signal at δ_H 3.23 (H-3) refers to the axially oriented hydroxyl group. Based on the ¹³C NMR and DEPT data, eight CH₃ carbons, eleven CH₂ carbons, and eight quaternary carbons are revealed. The ¹³C NMR spectrum also indicates the presence of the double bond correlated to the olefinic carbons at δ_C 135.19 (C-8) and 133.63 (C-9).

Compound 4 was isolated with the molecular formula of C₃₀H₅₀O₂ and identified as cycloart-25-en-3β,24-diol according to its ¹H NMR and ¹³C NMR data, compared with published data [19]. The ¹H- NMR signals in the up-field area (δ_H 0.33 and 0.55) revealed a pair of doublets, which is characteristic of a cyclopropane ring. Also, it corroborated one secondary methyl group at 0.88 and five singlet methyls at δ_H 1.72 (H-27), 0.96 (H-18, H-30), 0.88 (H-28), and 0.80 (H-29). A double doublet carbinolic proton axially orientated at δ_H 3.28, assigned to the hydroxyl group as 3β-OH, is also determined from the ¹H NMR data analysis.

Compound 5 was found to be C₂₉H₄₅O, known as β-sitosterol. The ¹H NMR data confirm the resemblance to Δ5-3β-hydroxy sterols by the presence of multiplets at δ_H 3.52, a characteristic peak for H-3α, and δ_H 5.36, determining the presence of the olefinic proton. The ¹H NMR peaks at δ_H 1.01 (H-19) and 0.68 (H-18) reveal two singlet methyls, and the ones at δ_H 0.92 (H-21), 0.81 (H-26), and 0.92 (H-27) shows three secondary methyls and one methyl with a triplet signal at δ_H 0.86 (H-29). The ¹³C NMR spectrum shows two signals correlated with the olefinic carbons at δ_C 140.88 (C-5) and 121.88 (C-6), revealing the presence of the double bond. The signal at δ_C 71.95 suggests an oxymethine on C3, and the ones at δ_C 12.00 and 19.55 confirm the two methyl groups present at C18 and C19. The published spectroscopic data for β-sitosterol are all in agreement with these findings [20–22].

Compound 6 showed the molecular formula of C₃₀H₅₀O₂ and was determined as cycloart-23-ene-3β,25-diol by comparison of the spectral data to those in the published literature [23–25]. Based on ¹H NMR spectra, one oxymethine proton at δ_H 3.28 (H-3), five singlet methyls at 1.72 (H-27), 0.88 (H-28), 0.80 (H-29), 0.96 (H-18, H-30) and one further secondary doublet methyl at 0.88 (H-21) were detected. The pair of doublet signals at δ_H 0.55 and 0.33 in the up-field area are related to the cyclopropane ring in this structure.

Compound 7 assigned the molecular formula of C₃₀H₅₀O₂ and, based on the comparison with those reported in the literature, was determined to be 3α, 11α- dihydroxyurs-12-ene [26]. The ¹H NMR spectrum singly supports the existence of two hydroxyl groups at δ_H 3.21 (H-3) and 3.98 (H-11). The peak at δ_H 3.21 (H-3) and the ¹³C NMR peak at δ_C 76.0 (C-3) revealed the presence of a 3α-hydroxyl group that was supported by the coupling constants. It also explains the strengthened α-effect at δ_C 76.0 (C-3) and the diminished β-effect at δ_C 25.4 (C-2) and 37.3 (C-4), comparing to the data from structures with axial orientation. Analyzing the ¹³C NMR spectra suggested the presence of a urs-12-ene by the olefinic carbons at δ_C 128.7 and 142.9. The olefinic signal at δ_H 4.90 from ¹H NMR was assigned to the H-12. The urs-12-ene skeleton is determined from the ¹H NMR signals of eight methyl groups, two of which are on a methine group and six are on quaternary carbons.

Compound 8 showed the molecular formula of C₃₀H₅₂O₃ and, based on the data and comparison of the spectral values with the literature, it was elucidated as 3β, 24β, and 25-trihydroxycycloartane [27]. The ¹H NMR peaks at δ_H 1.16 (H-26), 1.22 (H-27), 0.89 (H-28), 0.96 (H-18, H-29), and 0.80 (H-30) revealed six tertiary methyl groups and the peak at δ_H 0.88 (H-21), a secondary methyl group. In the up-field area, the δ_H 0.33 and 0.55 peaks appeared as a pair of doublets, suggesting the existence of the cycloartane cyclopropane structure. Two signals at δ_H 3.27 (H-3) and 3.31 (H-24) reveal the presence of carbinolic
protons related to the hydroxyl groups attached. The two carbinolic carbon shifts at δC 78.99 (C-3) and 79.77 (C-24) revealed the presence of two secondary hydroxyl groups and a tertiary hydroxyl group from a hydroxylated quaternary carbon at δC 73.36 (C-25).

Compound 9 showed the molecular formula of C29H48O2. From the comparison of the spectroscopic data with other similar compounds published in the literature, it was determined to be 7α-hydroxyxystigmasterol [28,29]. The 1H NMR showed three secondary methyls at δH 0.93 (H-21), 0.81 (H-26), and 0.93 (H-27), two singlet methyls at δH 0.68 (H-18), and 0.99 (H-19), and one triplet signal at δH 0.83 (H-29) indicating a tertiary methyl. The olefinic proton at δH 5.64 (H-6) in down-fields correlated to the double bond between C5 and C6. Another doublet peak at δH 5.16 (H-22) in the same area suggests another double bond between C22 and C23. The shifted signal at δC 146.39 (C-5) from the 13C NMR spectra is a reference to the α-orientation of the hydroxyl group on C27.

4. Discussion

A great deal of ethnopharmacological and ethnomedicinal uses of natural terpenes from different plant resources are related to the presence of isoprenoid units. Recent scientific findings have demonstrated the important role of triterpenes in these therapeutic effects, especially anti-tumor, anti-inflammatory, and immunoregulatory aspects [9].

The rich terpenoid profile of the Iranian Euphorbia species has motivated researchers to carry out further investigations on the biological and pharmacological potentials of these species. As a result, many therapeutic effects are introduced for them together with Euphorbia turcomanica, which is proven to have anti-cancer and immunomodulatory functions [30]. On the other hand, these studies are mainly focused on the medicinal properties of whole or partial plant extracts and cannot specifically show which bioactive component is responsible for the majority of these effects. Hence it is important to carry out phytochemical studies as well to clear the ambiguity and identify each present compound along with its roles individually. This type of study will also be beneficial in order to recognize natural sources of bioactive compounds for drug development.

The present study exhibits that the triterpenoid profile of E. turcomanica is not only remarkable, but also there is a vast variety of them with different subclasses, some of which are even considered to be scarce.

Cycloartanes are triterpenoids identified by their tetracyclic skeletal framework, cyclopropane ring, and side chain. This class of triterpenoids is responsible for a range of biological activities and is considered one of the leading chemical compounds in Euphorbia species, being involved in the biosynthesis of sterols and, therefore, could be considered one of the distinctive chemotaxonomic biomarkers in this genus [9]. Three cycloartane-type triterpenes (4, 6, 8) are elucidated for the first time from E. turcomanica. Compounds 4 and 6 have been isolated from many members of Euphorbiaceae so far studied. They are also found simultaneously in species such as E. schimperi [31] and E. altotibetic [32]. Also, both of these compounds were isolated from E. segetalis and have shown antiviral activities against the African swine fever virus and Herpes simplex virus [33]. Compound 4 has shown notable cancer chemopreventive effects [34], and compound 6, which has also been isolated from E. spinidens [25], is connected to a range of pharmacological effects such as anti-inflammatory, anti-diabetic and antioxidant activities which can lead to further therapeutic properties [35,36].

Compound 8 is a less common cycloartane in comparison, but still, it has been reported from E. marschalliana [37] and E. denticulata, which has shown potential cytotoxic activities [38].

Compound 3, identified as isomultiflorenol, is another triterpene isolated from E. turcomanica during the present research, which has already been elucidated from other Euphorbia plants such as E. pubescens [18] and E. supina [39]. During one research experiment on isomultiflorenol, significant anti-cancer activities against human cervical cancer cells were shown [40], which makes this compound, and thus its resources, highly valuable in chemotherapy-related drug development.
Although the biological effects from the triterpene-containing extracts are mostly associated with cycloartanes, there are also other types of triterpenes identified during this research that are noteworthy due to their scarcity in Euphorbia species. Compound 7 is a known pentacyclic ursane-type triterpene that was previously isolated from other plant families [26], but to our knowledge, there are no reports to show its presence in the species of Euphorbiaceae. Not enough studies have been carried out regarding this triterpene, and further investigations can be performed to reveal the potential effects of this natural compound.

Compound 2 is another compound isolated from E. turcomanica during the present research. It is a hopane-type triterpene which is considered one of the rare classes of triterpenes. The uncommon presence of a double bond between C-5 and C-6 causes the rare structural characterization of this compound. This compound has also been found in other Euphorbia species, including E. lathyris [41], and E. aphylla [42]. Moreover, previous studies have shown that this natural compound is associated with the anti-leishmanial activities of E. peplus [17]. Several studies have demonstrated the antimicrobial and antitymocobacterial activities, respectively, from E. nerifolia and Cissampelos muronate containing simiarenol as one of the present compounds in their extracts. Also, one recent research has shown this plant’s probable efficacy in treating a range of infectious diseases such as COVID-19, which might be related to this natural constituent but further investigations should be carried out to determine these claims and specifically relates them to simiarenol [43,44].

Two steroids (5, 9) were also elucidated during the current research. Compound 5 is a commonly occurring phytosterol called β-sitosterol, which is a key component present in plants. Out of the many pharmacological and biological effects that have been reported for this compound over the past decades, anti-inflammatory, angiogenic, anti-adipogenic, anti-diabetic, anti-oxidant, anti-cancer, immunomodulatory activities and a role in cardiovascular diseases prevention can be mentioned, reported from recent findings that can be beneficial in drug developments and other therapeutic applications [45–51]. The hypocholesterolemic effects of E. hirta and its role in curing obesity have been linked to this natural compound [22], as well as the larvicidal activity of E. thymifolia [21]. β-sitosterol is also responsible for the antimicrobial activity of E. segetalis in the presence of other antimicrobial triterpenes [3]. In contrast with the frequency and availability of β-sitosterol in different species, the other isolated steroid (9) has not yet been reported from any of the Euphorbia species. Although this oxidized sterol has been isolated from other plant species [52], not many researchers have focused on its bioactive and pharmacological effects up until now.

One monoterpene (1) is also isolated during this study and identified as loliolide, which is a monoterpenoid lactone with 11 carbons and one of the degradation products of carotenoids that have been isolated from several higher plants and marine algae. Studies have revealed anti-bacterial and anti-fungal properties [53] as well as anti-oxidant [54,55], anti-inflammatory [56,57], potential anti-diabetic [58], and neuromodulatory effects [59,60]. Many studies on plant extracts from other families containing loliolide have been carried out that proved a wide range of anti-cancer activities on different cell lines [61–67]. So far, loliolide has been isolated from other species of the Euphorbia genus, such as E. supina [68], E. micractina [69], E. cooperi [70], and E. alatavica [71] but has never been reported to be identified in E. turcomanica before.

5. Conclusions

In the present study, nine known terpenoids were isolated from E. turcomanica for the first time, including an iridoid monoterpene, as well as steroids and triterpenes. The results suggest that this plant comprises a variety of compounds that can be considered a potential source of natural bioactive constituents with applications in medical and pharmacological sciences. Further investigation should be done for the isolation and identification of other phytochemical compounds in the Euphorbia genus, and it is worth exploring their prospective applications.
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