Postprint: Triterpenoid Constituents of Small-fruited Vaccinium bracteatum

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Abstract

Lanostane triterpenoids are one of the main active components in Vaccinium genus plants. To further investigate the material basis for the efficacy of plants in this genus, combined with the traditional folk application of Vaccinium smallii, this study employed phytochemical techniques including silica gel, MCI, Sephadex LH-20, and semi-preparative high-performance liquid chromatography to separate and purify the 95% ethanol extract of this plant, and identified the compounds based on comprehensive physicochemical properties and spectroscopic data. The results showed that 14 triterpenoids and their saponins were isolated from the stem and leaf extracts of Vaccinium smallii, which were identified as mollic acid 3-O-α-L-arabinopyranoside (1), mollic acid3-O-β-D-glucopyranoside (2), cycloart-3,7-dihydroxy-24-en-28-oic acid (3), betulinic acid (4), 1β,3α, 11α-trihydroxy-urs-12-ene (5), oleanderolide (6), (Z)-maslinic acid 3-O-p-coumarate (7), friedelin (8), pomolic acid (9), 2α, 3α-dihydroxy-urs-12-en-28-oic acid (10), corosolic acid (11), oleanolic acid (12), ursolic acid (13), and tormentic acid (14). Among them, compounds 1-7 were isolated from Vaccinium genus plants for the first time, while compounds 8-11 and 14 were isolated from Vaccinium smallii plants for the first time.

Full Text

Preamble

Triterpenoids from *Lyonia ovalifolia* var. *elliptica*

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Abstract

Lanostane triterpenes represent one of the main active components in *Lyonia* plants. To further investigate the functional material basis of this genus and incorporate ethnobotanical applications of *Lyonia ovalifolia* var. *elliptica*, we conducted phytochemical analysis of the plant's 95% ethanol extract using silica gel, MCI, Sephadex LH-20, and semi-preparative HPLC. Fourteen triterpenes and their saponins were isolated and purified from the stem and leaf extracts, and their structures were elucidated based on physicochemical properties and spectroscopic data. The compounds were identified as: mollic acid 3-O-α-L-arabinopyranoside (1), mollic acid 3-O-β-D-glucopyranoside (2), cycloart-3,7-dihydroxy-24-en-28-oic acid (3), betulinic acid (4), 1β,3α,11α-trihydroxy-urs-12-ene (5), oleanderolide (6), (Z)-maslinic acid 3-O-p-coumarate (7), friedelin (8), pomolic acid (9), 2α,3α-dihydroxy-urs-12-en-28-oic acid (10), corosolic acid (11), oleanolic acid (12), ursolic acid (13), and tormentic acid (14). Compounds 1–7 are reported from the *Lyonia* genus for the first time, while compounds 8–11 and 14 are isolated from this plant species for the first time.

Keywords: *Lyonia ovalifolia* var. *elliptica*; chemical constituents; triterpenes; extraction and isolation; structure identification

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Introduction

Lyonia ovalifolia var. *elliptica* (family Ericaceae), locally known as "Xiao Guo Nan Zhu," is a shrub that grows in sunny hillside thickets and is distributed primarily in Hunan, Guangxi, Sichuan, Guizhou, and Yunnan provinces south of the Yangtze River. The plant has been used in traditional medicine for dispelling wind and toxins, promoting blood circulation, and strengthening tendons, with ethnobotanical applications in treating closed fractures (Editorial Committee of Flora of Guizhou, 1990).

Previous phytochemical investigations of *Lyonia* species have primarily reported grayanane and isopimarane diterpenes, lanostane triterpenes, and other triterpenoid saponins. The toxic grayanane diterpenes have been a particular focus of attention. Additional constituents include iridoids, lignans, and flavonoids (Sakakibara et al., 1974; Zhao et al., 2018; Kashima et al., 2010). Pharmacological activities demonstrated include antimicrobial, antiviral, sodium channel modulation, antifeedant, analgesic, and antioxidant effects (Lv et al., 2017; Lv et al., 2016; Wu et al., 2011; Li et al., 2013a; Li et al., 2013b), with the active compounds primarily being grayanane and isopimarane diterpenes, as well as lanostane triterpenes and their saponins.

Lyonia ovalifolia var. *elliptica* is a variety of *Lyonia ovalifolia*, distinguished from the type species by pubescent lower leaf surfaces, thinner papery leaves, triangular-ovate calyx lobes, and smaller fruits. Prior chemical studies have focused on the toxic grayanane diterpenes (Yasue et al., 1970), with few re-

ports on active triterpenoid constituents. As this plant is occasionally used in Guizhou Miao communities and possesses mild toxicity, elucidating its chemical constituents is essential for ensuring safe medicinal use and guiding future development. To expand the known triterpenoid profile of *L. ovalifolia* var. *elliptica* and provide a foundation for understanding its bioactive compounds, we investigated its triterpenoid constituents. Using multiple chromatographic techniques and semi-preparative HPLC, we isolated fourteen triterpenes and saponins from the 95% ethanol extract of the stems and leaves [Figure 1: see original paper]. Compounds 1–7 represent first-time isolates from the *Lyonia* genus, while compounds 8–11 and 14 are reported from this plant for the first time.

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1. Materials and Instruments

Plant material was collected in Huaxi District, Guiyang, Guizhou Province in September 2017 and identified as *Lyonia ovalifolia* var. *elliptica* (Ericaceae) by Professor Qingwen Sun of Guizhou University of Traditional Chinese Medicine.

Instruments: Bruker 600 MHz NMR spectrometer (TMS internal standard, Bruker, USA); 500 MHz liquid NMR spectrometer (Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences); INOVA 400 MHz NMR spectrometer (TMS internal standard, Varian, USA); Hewlett Packard 110 mass spectrometer (Hewlett Packard, USA); Hanbon NS4101 HPLC system (Jiangsu Hanbon Science & Technology Co., Ltd.); N-1100 rotary evaporator (Eyela, Japan); Sephadex LH-20 (Amersham Biosciences, Switzerland); silica gel (Qingdao Marine Chemical Co., Ltd.). HPLC-grade solvents were used for chromatography, while other solvents were redistilled from industrial grade before use.

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2. Extraction and Isolation

Air-dried and powdered stems and leaves of *L. ovalifolia* var. *elliptica* (14 kg) were refluxed with 95% ethanol three times (2 h each). The solvent was recovered under reduced pressure to yield approximately 4.2 kg of crude extract. The extract was suspended in 25 L distilled water and partitioned three times with two volumes each of petroleum ether and ethyl acetate. Concentration under reduced pressure gave petroleum ether fraction (210 g) and ethyl acetate fraction (500 g).

Petroleum ether fraction: Silica gel column chromatography with a petroleum ether–ethyl acetate gradient (1:0 to 0:1) yielded fractions Fr.1–Fr.31. Combined fractions Fr.19 and Fr.20 were further chromatographed, washed, and recrystallized to afford compound **12** (200 mg). Fraction Fr.16 was subjected to MCI column chromatography to remove pigments, and the methanol eluate was separated on silica gel (petroleum ether:ethyl acetate,

1:0 to 0:1) to give subfractions Fr.16.1–Fr.16.10. Crystallization of Fr.16.1 followed by column purification yielded compound **8** (40 mg). Fraction Fr.16.8 was chromatographed on silica gel with chloroform to obtain four subfractions (Fr.16.8.1–Fr.16.8.4). Subfraction Fr.16.8.3 was further purified on silica gel (petroleum ether:acetone, 10:1) to give compound **4** (30 mg). Combined fractions Fr.23 and Fr.24 were separated by silica gel column chromatography to yield Fr.23.1–Fr.23.15. Subfraction Fr.23.14 was purified by Sephadex LH-20 (chloroform:methanol, 1:1) and preparative HPLC (acetonitrile:water, 85:15) to afford compounds **5** (180 mg), **6** (8 mg), and **9** (25 mg).

Ethyl acetate fraction: Silica gel column chromatography with an ethyl acetate–methanol gradient (1:0 to 0:1) gave four fractions (Fr.1–Fr.4). Fraction Fr.3 was separated using dichloromethane–methanol (1:0 to 0:1) to yield Fr.3.1–Fr.3.18. Crystallization of Fr.3.14, Fr.3.15, and Fr.3.17 afforded compounds **1** (1.25 g), **2** (36 mg), and **3** (10 mg), respectively. Fraction Fr.2 was chromatographed with dichloromethane–methanol (1:0 to 0:1) to give Fr.2.1–Fr.2.15. Crystallization of Fr.2.4 yielded compound **13** (90 mg). Fraction Fr.2.7 was separated on silica gel (dichloromethane:methanol, 1:0 to 0:1) to obtain Fr.2.7.1–Fr.2.7.5. Subfractions Fr.2.7.2 and Fr.2.7.4 were purified by preparative HPLC (acetonitrile:water, 20:80) to give compounds **7** (15 mg) and **10** (8 mg). Fraction Fr.2.8 was subjected to silica gel chromatography (petroleum ether:acetone), and subfraction Fr.2.8.8 was purified by MCI (methanol:water, 3:7), Sephadex LH-20 (chloroform:methanol, 1:1), and preparative HPLC (acetonitrile:water, 70:30 to 95:5) to afford compounds **11** (8 mg) and **14** (75 mg).

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3. Structure Identification

Compound 1 White powder; ESI-MS *m/z*: 627 $[M + Na]^+$. ^1H NMR (600 MHz, DMSO-d_6) δ: 0.38 (1H, d, J = 4.3 Hz, H-19), 0.56 (1H, d, J = 4.3 Hz, H-19), 0.85 (3H, d, J = 6.2 Hz, H-21), 0.88 (3H, s, H-30), 0.90 (3H, s, H-18), 0.98 (3H, s, H-29), 1.55 (3H, s, H-26), 1.63 (3H, s, H-27), 3.27–3.63 (H-2 , 3 , 4 , 5), 4.19 (1H, d, J = 5.6 Hz, H-1), 4.39 (1H, dd, J = 11.2, 3.4 Hz, H-3), 5.06 (1H, t, J = 7.2 Hz, H-24); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 70.8 (C-1), 40.1 (C-2), 78.8 (C-3), 51.7 (C-4), 35.3 (C-5), 20.0 (C-6), 27.7 (C-7), 47.4 (C-8), 21.9 (C-9), 32.4 (C-10), 24.4 (C-11), 36.6 (C-12), 44.8 (C-13), 48.5 (C-14), 35.1 (C-15), 28.7 (C-16), 52.9 (C-17), 18.0 (C-18), 28.9 (C-19), 36.1 (C-20), 19.1 (C-21), 36.0 (C-22), 25.0 (C-23), 124.9 (C-24), 130.3 (C-25), 25.5 (C-26), 17.5 (C-27), 177.9 (C-28), 9.3 (C-29), 18.1 (C-30), 103.3 (C-1), 72.2 (C-2), 70.6 (C-3), 66.8 (C-4), 63.9 (C-5). These data are consistent with those reported for mollic acid 3-O-α-L-arabinopyranoside (Rogers, 1989).

Compound 2 White powder; ESI-MS *m/z*: 657 $[M + Na]^+$. ^1H NMR (600 MHz, DMSO-d_6) δ: 0.38 (1H, d, J = 4.3 Hz, H-19), 0.56 (1H, d, J = 4.3 Hz, H-19), 0.85 (3H, d, J = 6.2 Hz, H-21), 0.89 (3H, s, H-30), 0.90 (3H, s, H-18), 0.98 (3H, s, H-29), 1.55 (3H, s, H-26), 1.63 (3H, s, H-27), 3.19–4.31 (H-2 , 3 , 4 ,

5 , 6), 4.40 (1H, dd, J = 12.1, 4.3 Hz, H-3), 4.59 (1H, d, J = 7.6 Hz, H-1), 5.06 (1H, t, J = 7.3 Hz, H-24); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 73.3 (C-1), 40.1 (C-2), 79.0 (C-3), 51.7 (C-4), 35.3 (C-5), 20.0 (C-6), 27.7 (C-7), 47.4 (C-8), 21.9 (C-9), 32.5 (C-10), 24.5 (C-11), 36.8 (C-12), 44.8 (C-13), 48.5 (C-14), 35.3 (C-15), 28.8 (C-16), 53.0 (C-17), 18.0 (C-18), 28.8 (C-19), 36.1 (C-20), 19.1 (C-21), 36.0 (C-22), 24.8 (C-23), 124.9 (C-24), 130.3 (C-25), 25.5 (C-26), 17.5 (C-27), 178.0 (C-28), 9.3 (C-29), 18.1 (C-30), 104.2 (C-1), 75.1 (C-2), 71.1 (C-3), 70.6 (C-4), 67.9 (C-5), 60.2 (C-6). These data are consistent with those reported for mollic acid 3-O-β-D-glucopyranoside (Rogers & Thevan, 1986).

Compound 3 White powder; ESI-MS m/z: 495 [M + Na]$^+$. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 0.29 (1H, d, J = 4.2 Hz, H-19), 0.47 (1H, d, J = 4.2 Hz, H-19), 0.79 (3H, d, J = 6.2 Hz, H-21), 0.82 (3H, s, H-30), 0.83 (3H, s, H-18), 0.84 (3H, s, H-29), 1.49 (3H, s, H-26), 1.56 (3H, s, H-27), 1.93 (1H, d, J = 7.9 Hz, H-8), 2.37 (1H, dd, J = 12.6, 4.5 Hz, H-5), 4.15 (1H, dd, J = 11.3, 3.0 Hz, H-3), 5.00 (1H, t, J = 7.2 Hz, H-24); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 32.5 (C-1), 29.0 (C-2), 71.0 (C-3), 51.6 (C-4), 40.4 (C-5), 35.3 (C-6), 69.1 (C-7), 53.8 (C-8), 19.8 (C-9), 24.5 (C-10), 27.8 (C-11), 35.5 (C-12), 44.8 (C-13), 47.6 (C-14), 37.5 (C-15), 25.0 (C-16), 48.5 (C-17), 17.5 (C-18), 25.5 (C-19), 35.9 (C-20), 18.1 (C-21), 36.5 (C-22), 22.3 (C-23), 125.0 (C-24), 130.4 (C-25), 17.9 (C-26), 25.0 (C-27), 178.3 (C-28), 8.7 (C-29), 19.0 (C-30). These data are consistent with those reported for cycloart-3,7-dihydroxy-24-en-28-oic acid (Milena et al., 2009).

Compound 4 White powder; ESI-MS m/z: 479 [M + Na]$^+$. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 0.64 (3H, s, H-27), 0.75 (3H, s, H-24), 0.86 (3H, s, H-25), 0.86 (3H, s, H-26), 0.92 (3H, s, H-23), 1.64 (3H, s, H-30), 2.96 (1H, dd, J = 10.7, 5.3 Hz, H-3α), 4.55 (1H, brs, H-29b), 4.68 (1H, brs, H-29a); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 38.3 (C-1), 27.2 (C-2), 76.9 (C-3), 38.6 (C-4), 55.0 (C-5), 18.0 (C-6), 34.0 (C-7), 40.3 (C-8), 50.0 (C-9), 36.8 (C-10), 20.5 (C-11), 25.2 (C-12), 37.7 (C-13), 42.1 (C-14), 30.2 (C-15), 31.8 (C-16), 55.5 (C-17), 46.7 (C-18), 48.6 (C-19), 150.4 (C-20), 29.3 (C-21), 36.4 (C-22), 28.2 (C-23), 15.8 (C-24), 15.9 (C-25), 16.0 (C-26), 14.4 (C-27), 177.4 (C-28), 109.7 (C-29), 19.0 (C-30). These data are consistent with those reported for betulinic acid (Wu et al., 2015).

Compound 5 White powder; ESI-MS m/z: 481 [M + Na]$^+$. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.81 (3H, s, H-28), 0.82 (3H, s, H-26), 0.89 (3H, d, J = 6.6 Hz, H-29), 0.92 (3H, d, J = 6.5 Hz, H-30), 1.00 (3H, s, H-25), 1.08 (3H, s, H-27), 1.12 (3H, s, H-24), 1.20 (3H, s, H-23), 2.07 (1H, d, J = 11.4 Hz, H-18), 2.20 (1H, dt, J = 13.5, 3.6 Hz, H-1), 4.18 (1H, dd, J = 8.2, 5.5 Hz, H-11), 4.50 (1H, s, OH-11); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 70.9 (C-1), 28.9 (C-2), 78.8 (C-3), 38.3 (C-4), 55.4 (C-5), 18.5 (C-6), 33.4 (C-7), 39.8 (C-8), 54.7 (C-9), 39.3 (C-10), 70.4 (C-11), 116.2 (C-12), 145.2 (C-13), 43.1 (C-14), 27.7 (C-15), 27.3 (C-16), 34.0 (C-17), 51.1 (C-18), 41.2 (C-19), 41.1 (C-20), 31.4 (C-21), 41.8 (C-22), 27.8 (C-23), 16.7 (C-24), 15.8 (C-25), 16.9 (C-26), 24.4 (C-27), 28.5 (C-28), 18.1 (C-29), 21.4 (C-30). These data are consistent with those reported for

1β,3α,11α-trihydroxy-urs-12-ene (Topcu et al., 1999).

Compound 6 White powder; ESI-MS *m/z*: 495 $[M + Na]^+$. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 0.78 (3H, s, H-24), 0.88 (3H, s, H-25), 0.90 (3H, s, H-30), 0.98 (3H, s, H-29), 0.99 (3H, s, H-23), 1.14 (3H, s, H-26), 1.30 (3H, s, H-27), 1.72 (ddd, J = 13.0, 3.4, 3.4 Hz, H-1), 2.04 (1H, m, H-18), 2.13 (ddd, J = 13.4, 13.4, 5.9 Hz, H-16), 3.22 (1H, dd, J = 10.9, 5.1 Hz, H-3), 3.89 (1H, brs, H-12); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 39.0 (C-1), 27.6 (C-2), 78.9 (C-3), 39.0 (C-4), 55.3 (C-5), 17.9 (C-6), 34.1 (C-7), 42.3 (C-8), 44.8 (C-9), 36.7 (C-10), 29.0 (C-11), 76.7 (C-12), 90.8 (C-13), 42.4 (C-14), 28.2 (C-15), 21.3 (C-16), 44.8 (C-17), 51.3 (C-18), 39.7 (C-19), 31.8 (C-20), 34.4 (C-21), 27.3 (C-22), 28.2 (C-23), 15.5 (C-24), 16.5 (C-25), 18.8 (C-26), 18.7 (C-27), 180.0 (C-28), 33.4 (C-29), 24.0 (C-30). These data are consistent with those reported for oleanderolide (Fu et al., 2005).

Compound 7 White powder; ESI-MS *m/z*: 641 $[M + Na]^+$. ^{1}H NMR (600 MHz, CD_3OD) δ: 0.78 (3H, s, H-26), 0.85 (3H, s, H-23), 0.88 (3H, s, H-29), 0.95 (6H, s, H-24, 30), 0.99 (3H, s, H-25), 1.16 (3H, s, H-27), 2.82 (1H, m, H-18), 3.80 (1H, m, H-2), 4.54 (1H, d, J = 3.5 Hz, H-3), 5.22 (1H, t, J = 4.6 Hz, H-12), 5.81 (1H, d, J = 12.7 Hz, H-2), 6.71 (2H, d, J = 8.6 Hz, H-3 ,5), 6.84 (1H, d, J = 12.8 Hz, H-3), 7.60 (2H, d, J = 8.6 Hz, H-2 ,6); ^{13}C NMR (150 MHz, CD_3OD) δ: 47.7 (C-1), 67.5 (C-2), 85.2 (C-3), 40.6 (C-4), 56.4 (C-5), 19.4 (C-6), 33.6 (C-7), 40.4 (C-8), 48.5 (C-9), 39.5 (C-10), 24.0 (C-11), 124.3 (C-12), 144.8 (C-13), 43.1 (C-14), 28.8 (C-15), 24.6 (C-16), 48.2 (C-17), 42.8 (C-18), 47.3 (C-19), 31.5 (C-20), 35.0 (C-21), 33.9 (C-22), 29.6 (C-23), 17.7 (C-24), 17.0 (C-25), 18.2 (C-26), 26.4 (C-27), 181.0 (C-28), 33.8 (C-29), 24.0 (C-30), 168.6 (C-1), 117.4 (C-2), 140.1 (C-3), 129.4 (C-1), 133.6 (C-2), 115.8 (C-3), 159.8 (C-4), 115.8 (C-5), 133.6 (C-6). These data are consistent with those reported for (Z)-maslinic acid 3-O-p-coumarate (Xu et al., 2010).

Compound 8 Needle crystals (chloroform); ESI-MS *m/z*: 449 $[M + Na]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.72 (3H, s, H-24), 0.87 (3H, d, J = 6.4 Hz, H-25), 0.96 (3H, s, H-29), 1.01 (3H, s, H-30), 1.01 (3H, s, H-26), 1.05 (3H, s, H-27), 1.17 (3H, s, H-28); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 22.0 (C-1), 41.5 (C-2), 213.2 (C-3), 58.3 (C-4), 42.0 (C-5), 41.2 (C-6), 18.2 (C-7), 53.0 (C-8), 37.5 (C-9), 59.5 (C-10), 35.5 (C-11), 30.6 (C-12), 39.7 (C-13), 38.1 (C-14), 32.3 (C-15), 36.0 (C-16), 29.8 (C-17), 42.6 (C-18), 35.2 (C-19), 28.0 (C-20), 32.8 (C-21), 39.1 (C-22), 6.9 (C-23), 14.7 (C-24), 18.0 (C-25), 20.1 (C-26), 18.7 (C-27), 32.0 (C-28), 35.0 (C-29), 37.4 (C-30). These data are consistent with those reported for friedelin (Xu et al., 2014).

Compound 9 White powder; ESI-MS *m/z*: 495 $[M + Na]^+$. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 0.56 (3H, s, H-25), 0.58 (3H, s, H-23), 0.73 (3H, d, J = 6.0 Hz, H-30), 0.78 (3H, s, H-26), 0.97 (3H, s, H-24), 1.15 (3H, s, H-29), 1.47 (3H, s, H-27), 2.25 (1H, s, H-18), 3.62 (1H, m, H-3α), 5.04 (1H, s, H-12); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 38.5 (C-1), 27.0 (C-2), 77.1 (C-3), 38.3 (C-4), 55.0 (C-5), 18.2 (C-6), 32.8 (C-7), 41.2 (C-8), 47.0 (C-9), 36.7 (C-10), 23.2 (C-11), 126.9 (C-12), 138.7 (C-13), 41.5 (C-14), 28.3 (C-15), 25.3 (C-16), 46.8 (C-17),

53.4 (C-18), 71.8 (C-19), 37.5 (C-20), 25.9 (C-21), 38.2 (C-22), 28.2 (C-23), 15.2 (C-24), 16.1 (C-25), 16.8 (C-26), 24.1 (C-27), 179.0 (C-28), 26.6 (C-29), 16.5 (C-30). These data are consistent with those reported for pomolic acid (An et al., 2005).

Compound 10 White powder; ESI-MS *m/z*: 495 $[M + Na]^+$. ^1H NMR (600 MHz, DMSO-d_6) δ: 0.71 (3H, s, H-25), 0.75 (3H, s, H-24), 0.79 (3H, d, J = 6.5 Hz, H-30), 0.86 (3H, d, J = 6.5 Hz, H-29), 0.88 (3H, s, H-26), 0.89 (3H, s, H-27), 1.02 (3H, s, H-23), 2.09 (1H, d, J = 11.3 Hz, H-18), 3.74 (1H, d, J = 8.7 Hz, H-3β), 3.99 (1H, m, H-2β), 5.11 (1H, s, H-12); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 38.0 (C-1), 64.7 (C-2), 77.9 (C-3), 38.5 (C-4), 47.6 (C-5), 17.6 (C-6), 32.7 (C-7), 38.5 (C-8), 46.9 (C-9), 37.8 (C-10), 23.4 (C-11), 123.5 (C-12), 139.6 (C-13), 41.8 (C-14), 28.9 (C-15), 21.8 (C-16), 46.7 (C-17), 52.3 (C-18), 40.2 (C-19), 37.4 (C-20), 29.1 (C-21), 35.3 (C-22), 28.9 (C-23), 23.4 (C-24), 16.3 (C-25), 17.6 (C-26), 22.0 (C-27), 179.2 (C-28), 17.0 (C-29), 21.2 (C-30). These data are consistent with those reported for 2α,3α-dihydroxy-urs-12-en-28-oic acid (Wang et al., 2005).

Compound 11 White powder; ESI-MS *m/z*: 495 $[M + Na]^+$. ^1H NMR (600 MHz, DMSO-d_6) δ: 0.39 (3H, s, H-24), 0.45 (3H, s, H-25), 0.50 (3H, d, J = 6.5 Hz, H-30), 0.56 (3H, d, J = 6.5 Hz, H-29), 0.60 (3H, s, H-26), 0.61 (3H, s, H-27), 0.72 (3H, s, H-23), 4.12 (1H, d, J = 8.9 Hz, H-3α), 4.81 (1H, m, H-2α), 5.38 (1H, s, H-12); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 47.2 (C-1), 67.5 (C-2), 82.6 (C-3), 38.8 (C-4), 55.0 (C-5), 18.3 (C-6), 32.9 (C-7), 40.0 (C-8), 47.3 (C-9), 37.8 (C-10), 23.2 (C-11), 124.6 (C-12), 138.7 (C-13), 42.0 (C-14), 27.7 (C-15), 23.5 (C-16), 48.8 (C-17), 52.7 (C-18), 38.7 (C-19), 38.5 (C-20), 30.6 (C-21), 36.6 (C-22), 29.0 (C-23), 17.4 (C-24), 17.4 (C-25), 17.5 (C-26), 24.3 (C-27), 178.6 (C-28), 21.3 (C-29), 16.8 (C-30). These data are consistent with those reported for corosolic acid (Chen et al., 2008).

Compound 12 White powder; ESI-MS *m/z*: 479 $[M + Na]^+$. ^1H NMR (600 MHz, $CDCl_3$) δ: 0.74 (3H, s, H-24), 0.77 (3H, s, H-25), 0.90 (3H, s, H-29), 0.91 (3H, s, H-30), 0.92 (3H, s, H-23), 0.98 (3H, s, H-23), 1.13 (3H, s, H-26), 2.82 (1H, m, H-18), 3.22 (1H, m, H-3), 5.27 (1H, brs, H-12); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 38.7 (C-1), 27.5 (C-2), 79.4 (C-3), 39.1 (C-4), 55.6 (C-5), 18.6 (C-6), 32.9 (C-7), 39.6 (C-8), 48.0 (C-9), 37.4 (C-10), 23.2 (C-11), 123.0 (C-12), 144.0 (C-13), 41.2 (C-14), 27.9 (C-15), 23.6 (C-16), 47.0 (C-17), 42.0 (C-18), 46.2 (C-19), 31.0 (C-20), 34.1 (C-21), 32.8 (C-22), 28.4 (C-23), 15.7 (C-24), 15.9 (C-25), 17.5 (C-26), 26.3 (C-27), 184.0 (C-28), 33.4 (C-29), 23.9 (C-30). These data are consistent with those reported for oleanolic acid (Liu et al., 2006).

Compound 13 White powder; ESI-MS *m/z*: 479 $[M + Na]^+$. ^1H NMR (600 MHz, DMSO-d_6) δ: 0.66 (3H, s, H-24), 0.74 (3H, s, H-25), 0.80 (3H, d, J = 6.4 Hz, H-29), 0.86 (3H, d, J = 4.0 Hz, H-30), 0.88 (3H, s, H-23), 0.90 (3H, s, H-26), 1.03 (3H, s, H-27), 2.09 (1H, d, J = 11.3 Hz, H-18), 3.02 (1H, dd, J = 11.1, 5.0 Hz, H-3), 5.11 (1H, t, J = 3.7 Hz, H-12); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 36.5 (C-1), 27.0 (C-2), 79.2 (C-3), 38.2 (C-4), 54.8 (C-5), 18.0 (C-6), 32.7 (C-7), 40.1 (C-8), 47.0 (C-9), 38.5 (C-10), 23.3 (C-11), 124.6 (C-12),

138.2 (C-13), 41.7 (C-14), 28.3 (C-15), 23.8 (C-16), 46.8 (C-17), 52.4 (C-18), 38.4 (C-19), 38.4 (C-20), 30.2 (C-21), 36.3 (C-22), 27.6 (C-23), 15.2 (C-24), 16.1 (C-25), 17.0 (C-26), 22.9 (C-27), 178.3 (C-28), 16.9 (C-29), 21.1 (C-30). These data are consistent with those reported for ursolic acid (Li et al., 2014).

Compound 14 White powder; ESI-MS *m/z*: 511 $[M + Na]^+$. ^1H NMR (600 MHz, DMSO-d_6) δ: 0.62 (3H, s, H-24), 0.64 (3H, s, H-25), 0.77 (3H, d, J = 6.4 Hz, H-29), 0.83 (3H, d, J = 4.0 Hz, H-30), 0.85 (3H, s, H-23), 1.01 (3H, s, H-26), 1.21 (3H, s, H-27), 2.30 (1H, s, H-18), 2.67 (1H, dd, J = 9.4, 3.8 Hz, H-3), 3.70 (1H, m, H-2), 5.10 (1H, s, H-12); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 46.8 (C-1), 67.2 (C-2), 82.4 (C-3), 39.0 (C-4), 54.9 (C-5), 18.2 (C-6), 32.7 (C-7), 40.4 (C-8), 47.0 (C-9), 37.7 (C-10), 23.3 (C-11), 126.8 (C-12), 138.7 (C-13), 41.5 (C-14), 28.1 (C-15), 25.2 (C-16), 47.0 (C-17), 53.2 (C-18), 71.7 (C-19), 41.2 (C-20), 26.0 (C-21), 37.3 (C-22), 28.9 (C-23), 16.4 (C-24), 16.4 (C-25), 17.2 (C-26), 24.0 (C-27), 177.7 (C-28), 26.4 (C-29), 16.7 (C-30). These data are consistent with those reported for tormentic acid (Zheng & Piao, 2012).

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4. Discussion and Conclusion

This study yielded fourteen triterpenes and their saponins from the ethanol extract of *L. ovalifolia* var. *elliptica* stems and leaves. Notably, the cycloartane triterpenes (1–3) represent new records for the *Lyonia* genus, while compounds 1–7 are isolated from this genus for the first time, and compounds 8–11 and 14 are reported from this plant species for the first time.

Previous chemical investigations of *Lyonia* species have identified lanostane triterpenes as the predominant triterpenoid type, exhibiting significant antiproliferative activities against malignant cells (Teng et al., 2018). However, this class of compounds was not isolated from *L. ovalifolia* var. *elliptica* in our study. Instead, we identified cycloartane triterpenes (1–3) as new additions to the genus, which have demonstrated potent inhibitory effects against breast, liver, and prostate cancer cell lines, as well as anti-osteoporotic and anti-complement activities (Yang et al., 2016; Li et al., 2017). Compound 1 was obtained in gram-scale quantity from this plant and has been shown to effectively inhibit the growth of Ca-Ski cervical cancer cells (Wong et al., 2012), suggesting its potential as a quality marker for this medicinal material and as an important chemotaxonomic reference. Additionally, compound 2 exhibits analgesic and anti-inflammatory effects against thermally and chemically induced nociception in mice and rat paw edema (Ojewole, 2008), while compound 3 shows antimicrobial activity against Gram-positive, Gram-negative bacteria, and pathogenic fungi (Milena et al., 2009). Compounds 11, 12, and 13 possess anti-inflammatory and antitumor properties (Ju et al., 2003; Chiang et al., 2005). In summary, our findings expand the diversity of triterpenoid constituents in the *Lyonia* genus and provide a material basis for further research and application of *L. ovalifolia* var. *elliptica*.

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