Postprint: Chemical Constituents of *Pteris ensiformis* from Guizhou

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Abstract

To investigate the chemical constituents of Pteris ensiformis. This study employed silica gel, gel, MCI, C18 and other column chromatography for separation and purification, combined with 1H-NMR, 13 C-NMR, MS, IR and other spectroscopic data to identify the structures of the compounds; through MTS and APTT, PT and TT and other methods, antitumor and anticoagulant activities of some of the isolated monomeric compounds were screened. The results showed that: (1) 15 compounds were isolated from Pteris ensiformis, namely 2-hydroxy-acetylpyrrole (1), N-(3-carboxypropyl)-2-acetylpyrrole (2), 3-hydroxy-2-methylpyridine (3), N-methylhydroxylamine (4), pterosin S 13 13-O-glucoside (5), obtupterosin C C (6), entent-11 α-hydroxyhydroxy-15-oxokauranoxokauran-19-oic acid acid (7), entent-11 α-hydroxyhydroxy-15-oxokauroxokaur-16-en-19-oic acid acid (8), β-sitosterol (9), entent-11 α-hydroxyhydroxy-15-oxo-kaurkaur-16-en-19-oic acid acid-O-glucopyranosideglucopyranoside (10), 5, 5' 5'-dibutoxy-2, 2'-bifuran (11), 5, 5'-di(2-ethyl-hexyloxy)-2, 2'-bifuran (12), loliolide (13), succinic acid 14), fumaric acid 15). Compound 1 is a new pyrrole alkaloid natural product, compounds 1-7, 10-15 were isolated from Pteris ensiformis for the first time, compounds 1, 3, 4 were isolated from the genus Pteris for the first time; (2) Activity test results showed that compounds 1, 2, 3, 5, 6, 10 at a concentration of 40 mol/L had inhibitory effects on the in vitro tumor growth of tumor cells HL-60, A549, SMMCSMMC-7721, MDA-MB-231 and SW480; when the sample concentration was 2.0 mmol/L, compounds 1, 2, 3, 6 had shortening effects on APTT, compounds 1, 5, 6 had prolonging effects on PT. These research results enrich the chemical constituents of Pteris ensiformis from Guizhou and provide a basis for antitumor drug

Full Text

Chemical Constituents of *Pteris ensiformis* from Guizhou

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Abstract: This study investigated the chemical constituents of *Pteris ensiformis*. Silica gel, gel, MCI, and C18 column chromatography were employed for separation and purification, and compound structures were identified using spectroscopic data including ^{1}H-NMR, ^{13}C-NMR, MS, and IR. Antitumor and anticoagulant activities of selected monomeric compounds were evaluated using MTS, APTT, PT, and TT assays. The results showed that: (1) Fifteen compounds were isolated from *P. ensiformis*, namely 2-hydroxy-acetylpyrrole (1), *N*-(3-carboxypropyl)-2-acetylpyrrole (2), 3-hydroxy-2-methylpyridine (3), *N*-methylhydroxylamine (4), pterosin S 13-O-glucoside (5), obtupterosin C (6), ent-11α-hydroxy-15-oxokauran-19-oic acid (7), ent-11α-hydroxy-15-oxokaur-16-en-19-oic acid (8), β-sitosterol (9), ent-11α-hydroxy-15-oxo-kaur-16-en-19-oic acid-O-glucopyranoside (10), 5,5'-dibutoxy-2,2'-bifuran (11), 5,5'-di(2-ethylhexyloxy)-2,2'-bifuran (12), loliolide (13), succinic acid (14), and fumaric acid (15). Compound 1 is a new pyrrole alkaloid natural product. Compounds 1–7 and 10–15 were isolated from *P. ensiformis* for the first time, while compounds 1, 3, and 4 were isolated from the genus *Pteris* for the first time. (2) Bioactivity testing revealed that compounds 1, 2, 3, 5, 6, and 10 inhibited the in vitro growth of tumor cells HL-60, A549, SMMC-7721, MDA-MB-231, and SW480 at a concentration of 40 mol/L. At a sample concentration of 2.0 mmol/L, compounds 1, 2, 3, and 6 shortened APTT, while compounds 1, 5, and 6 prolonged PT. These findings enrich the chemical constituent profile of Guizhou *P. ensiformis* and provide a material basis for antitumor drug development.

Keywords: *Pteris ensiformis*; chemical constituents; structural identification; antitumor activity

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Pteris ensiformis, a species of the genus *Pteris*, is also known as slender-leaf brake fern, phoenix tail grass, well-rail grass, phoenix grass, or well-side madder. It is distributed in South China, Southwest China, East China, and Taiwan, and is used medicinally as whole herb or rhizome (Zhang et al., 2016; Shi et al., 2017). *Pteris ensiformis* possesses heat-clearing and dampness-removing, blood-activating and stasis-dispelling, and swelling-detoxifying effects (Liva et al., 2009; Pan et al., 2012; Guan et al., 2018), and is used to treat dysentery, jaundice, stranguria, and traumatic injuries (Guan et al., 2018). Previous studies have shown that *Pteris ensiformis* contains diterpenoids, sesquiterpenoids, flavonoids, phenolic acids, and other compounds (Zhang et al., 2016; Hou et al., 2020), among which diterpenoids and sesquiterpenoids exhibit certain

antitumor activities, with diterpenoids showing better antitumor activity, particularly those with an ent-kaurane skeleton such as pterisolic acid C, which effectively inhibits human gastric cancer BGC-823 cells, human colon cancer HCT-116 cells, and liver cancer Hep G2 cells (Zhang et al., 2016). However, no reports have been found on the chemical constituents and biological activities of *Pteris ensiformis* from Guizhou. Therefore, to further investigate the chemical constituents of Guizhou *Pteris ensiformis* and obtain more natural compounds with higher content, stronger activity, and lower toxicity, this study isolated fifteen compounds from the ethanol extract of Guizhou *Pteris ensiformis*. Spectroscopic analysis identified them as 2-hydroxy-acetylpyrrole (1), *N*-(3-carboxypropyl)-2-acetylpyrrole (2), 3-hydroxy-2-methylpyridine (3), *N*-methylhydroxylamine (4), pterosin S 13-O-glucoside (5), obtupterosin C (6), ent-11α-hydroxy-15-oxokauran-19-oic acid (7), ent-11α-hydroxy-15-oxokaur-16-en-19-oic acid (8), β-sitosterol (9), ent-11α-hydroxy-15-oxo-kaur-16-en-19-oic acid-O-glucopyranoside (10), 5,5'-dibutoxy-2,2'-bifuran (11), 5,5'-di(2-ethylhexyloxy)-2,2'-bifuran (12), loliolide (13), succinic acid (14), and fumaric acid (15). Compound 1 is a new natural product, and all other compounds except compounds 8 and 9 were isolated from *Pteris ensiformis* for the first time, with compounds 1, 3, and 4 being isolated from the genus *Pteris* for the first time. Preliminary investigations of the antitumor and anticoagulant activities of some compounds showed that compounds 1, 2, 3, 5, 6, and 10 inhibited the in vitro growth of tumor cells HL-60, A549, SMMC-7721, MDA-MB-231, and SW480 at 40 mol/L. At a sample concentration of 2.0 mmol/L, compounds 1, 2, 3, and 5, 6 exhibited varying degrees of anticoagulant effects.

1. Instruments and Materials

Instruments: Bruker Avance NEO 600 MHz NMR spectrometer, Bruker Daltonics Compact mass spectrometer (Bruker, Germany); ICAN Fourier transform infrared spectrometer (Tianjin Nengpu Technology Co., Ltd.); X-5 micro melting point apparatus (Gongyi Yuhua Instrument Co., Ltd.); WFH-203B three-purpose UV analyzer (Shanghai Jingke Industrial Co., Ltd.); column chromatography silica gel (100–200 mesh, 200–300 mesh, 300–400 mesh, Qingdao Marine Chemical Factory Branch); CHP 20P 75–150 m MCI GEL (Mitsubishi Chemical, Japan); 30–60 mesh polyamide (Zhejiang Taizhou Lujia Sijia Biochemical Plastic Factory); petroleum ether, dichloromethane, ethyl acetate, acetone, methanol, glacial acetic acid (Guizhou Xianghui Instrument Co., Ltd., AR grade).

Bioactivity Testing Equipment: Cellometer mini cell counter (Nexelom, USA); 370 CO_2 incubator (Thermo, USA); MULTISKAN FC microplate reader (Thermo, USA); MC-4000 coagulation analyzer (German Meichuang); 093UK-K272A coagulation control plasma (TECO, Germany), APTT reagent (TECO, Germany), PT reagent (TECO, Germany), TT reagent (TECO, Germany); HL-60 leukemia cells (ATCC, USA), A549 lung cancer cells (ATCC, USA), SMMC-7721 liver cancer cells (ATCC, USA), MDA-MB-231 breast

cancer cells (ATCC, USA), SW480 colon cancer cells (ATCC, USA); N1001A cisplatin (Meilun Biotechnology), D1106A paclitaxel (Meilun Biotechnology); MTS assay kit (Promega, USA).

Plant Material: The experimental material was collected from Liping County, Qiandongnan Miao and Dong Autonomous Prefecture, Guizhou Province, and identified by Professor Zhao Junhua of Guizhou University of Traditional Chinese Medicine as *Pteris ensiformis* Burm. from the family Pteridaceae. Voucher specimens are deposited in the Pharmacognosy Laboratory of Guizhou University of Traditional Chinese Medicine.

2. Extraction and Separation

Dried *Pteris ensiformis* material (19.5 kg) was extracted by maceration with 95% industrial methanol at room temperature for 7 days each time, repeated 7 times (once with 95% methanol, three times with 90% methanol, and three times with 70% methanol). The extracts were combined and concentrated under reduced pressure to obtain approximately 7.8 kg of crude extract. The extract was dissolved in warm water and subjected to liquid-liquid extraction with equal volumes of petroleum ether, ethyl acetate, and water-saturated *n*-butanol successively. The solvents were recovered under reduced pressure to yield petroleum ether fraction (1,590 g), ethyl acetate fraction (340 g), and *n*-butanol fraction (2,550 g).

The ethyl acetate fraction (340 g) was initially separated by MCI resin (GEL CHP 20P) reversed-phase column chromatography using a methanol-water gradient system (3:7, 1:1, 3:2, 7:3, 4:1, 9:1, 10:0) to obtain seven fractions Fr.A–Fr.G. Fr.A (172 g) was subjected to silica gel column chromatography with a dichloromethane-ethyl acetate gradient (30:1$\rightarrow$0 : 1)$followed by ethyl acetate - methanol(20 : 1\rightarrow 1 : 1) to obtain compound 3(23mg) through recrystallization. The remaining fractions were combined based on TLC an$
1–$Fr.A-6.Fr.A-1(45g)$ was purified by silica gel column chromatography combined with gel chromatography and$
$methanol gradient(100 : 1\rightarrow 1 : 1)$ to yield $Fr.B-1–Fr.B-6.Fr.B-1$ and $Fr.B-$
2 were further purified by silica gel column chromatography and recrystallization to obtain compounds 13(76mg), 2$
$ethyl acetate(20 : 1\rightarrow$3:1) and recrystallization to obtain compound 7 (34 mg) and six subfractions Fr.C-1–Fr.C-6. Fr.C-3 and Fr.C-5 were further purified by repeated silica gel column chromatography, gel chromatography, and recrystallization to obtain compounds 9 (384 mg) and 12 (7 mg). The remaining portion of Fr.C was combined with Fr.D (12 g) and subjected to C18 reversed-phase column chromatography with methanol-water (1:1, 3:2, 7:3, 85:15, 10:0) to yield Fr.CD-1–Fr.CD-3. Fr.CD-1 was purified by silica gel column chromatography combined with gel chromatography and recrystallization to obtain compound 10 (64 mg).

The *n*-butanol fraction (2,550 g) was separated by silica gel column chromatography with an ethyl acetate-methanol gradient (1:0, 50:1, 30:1, 10:1, 5:1, 3:1, 1:1) to obtain seven fractions Fr.H–Fr.N. Fr.H (220 g)

was subjected to silica gel column chromatography with petroleum ether-ethyl acetate (15:1$→1 : 1)*toyieldFr.H* − 1–*Fr.H* − 5.*Fr.H* − 4(57*g*)*waspurifiedbysilicagelcolumnchromatographycombinedwithgelchromatographyandrecrystallizationt* *methanol*(30 : 1→3 : 1)*toyieldFr.I*−1–*Fr.I*−5.*Fr.I*−3*wasrepeatedlypurifiedbysilicagelcolumnchromatogra* *methanol*(50 : 1→$1:1) to yield Fr.L-1–Fr.L-7. Fr.L-4 (48 g) was purified by C18 reversed-phase column chromatography with methanol-water (1:1, 3:2, 7:3, 85:15, 10:0) to obtain Fr.L-4-1–Fr.L-4-5. Fr.L-4-2 was further purified by silica gel column chromatography and recrystallization to obtain compounds 6 (29 mg) and 5 (57 mg).

Structural Identification of Compounds

Compound 1: White crystals (methanol); melting point (mp): 111.9–131.1 °C; exhibited fluorescence under UV light at 254 nm. HR-ESI-MS data showed $[M+Na]^+$ at *m/z* 148.0364 (calculated for $C_6H_7NO_2Na$, 148.0369), establishing the molecular formula as $C_6H_7NO_2$ with 4 degrees of unsaturation. IR spectroscopy showed absorption at 1644.7 cm^{-1} suggesting a carbonyl group and at 3311.4 cm^{-1} indicating a hydroxyl group.

^{1}H-NMR (600 MHz, Methanol-d_4) δ: 7.09 (1H, dd, J = 2.4, 1.4 Hz), 7.00 (1H, dd, J = 3.9, 1.4 Hz), 6.24 (1H, dd, J = 3.9, 2.4 Hz) suggested two olefinic protons, while δ 4.63 (2H, s) indicated oxygen-bearing methylene protons attached to a quaternary carbon. ^{13}C-NMR (150 MHz, Methanol-d_4) revealed six carbon signals: δ 189.9 (carbonyl carbon), 65.2 (oxygen-bearing carbon), and 130.1, 126.6, 117.4, 111.2 (two double bond carbons). Combined with DEPT data, the compound contained 6 carbons: 1 methylene, 3 methines, and 2 quaternary carbons, suggesting a pyrrole alkaloid structure.

^{1}H-^{1}H COSY showed correlations between δ 6.24 (H-4) and δ 7.00 (H-3), 7.09 (H-5), establishing a C3-C4-C5 fragment. NOESY revealed correlation between δ 4.63 (H-7) and δ 7.00 (H-3), and HMBC showed correlation between δ 4.63 (H-7) and δ 189.9 (C-6), confirming the hydroxymethyl group at C-6. Additional HMBC correlations included δ 6.24 (H-4) with δ 117.4 (C-3), 126.6 (C-5), and 130.1 (C-2); δ 7.00 (H-3) with δ 111.2 (C-4), 126.6 (C-5), and 130.1 (C-2); and δ 7.09 (H-5) with δ 117.4 (C-3), 111.2 (C-4), and 130.1 (C-2). Based on these data, compound 1 was identified as a new natural product, 2-hydroxy-acetylpyrrole, with structure shown in [Figure 1: see original paper]. Key HMBC, ^{1}H-^{1}H COSY, and NOESY correlations are shown in [Figure 2: see original paper], and detailed NMR data are listed in .

Compound 2: $C_{10}H_{13}NO_3$, white crystals (methanol); HR-ESI-MS: $[M+Na]^+$ *m/z* 218.0787. ^{1}H-NMR (600 MHz, Methanol-d_4) δ: 7.11 (1H, dd, J = 4.1, 1.7 Hz, H-3), 7.04 (1H, t, J = 2.1 Hz, H-5), 6.16 (1H, dd, J = 4.1, 2.5 Hz, H-4), 4.36 (2H, t, J = 6.9 Hz, H-1), 2.42 (3H, s, H-7), 2.22 (2H, t, J = 7.4 Hz, H-3), 2.00–1.96 (2H, m, H-2); ^{13}C-NMR (150 MHz, Methanol-d_4) δ: 131.0 (C-2), 122.8 (C-3), 109.4 (C-4), 132.7 (C-5), 190.3 (C-6), 27.1 (C-7), 49.6 (C-1), 27.7 (C-2), 31.5 (C-3), 176.6 (C-4). These data matched literature values (Li et al.,

2019), identifying compound 2 as *N*-(3-carboxypropyl)-2-acetylpyrrole.

Compound 3: C_6H_7NO, white crystals (methanol); HR-ESI-MS: $[M+H]^+$ *m/z* 110.0600. ^{1}H-NMR (600 MHz, Methanol-d_4) δ: 7.85 (1H, dd, J = 4.9, 1.4 Hz, H-6), 7.16 (1H, dd, J = 8.2, 1.4 Hz, H-4), 7.08 (1H, dd, J = 8.2, 4.9 Hz, H-5), 2.39 (3H, s, H-7); ^{13}C-NMR (150 MHz, Methanol-d_4) δ: 147.5 (C-2), 153.8 (C-3), 123.4 (C-4), 123.7 (C-5), 139.2 (C-6), 18.4 (C-7). These data matched literature values (Duan et al., 2009), identifying compound 3 as 3-hydroxy-2-methylpyridine.

Compound 4: White powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 4.17 (1H, m, -NH), 3.17 (3H, d, J = 5.1 Hz, -CH_3); ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 48.8 (-NCH_3). These data matched literature values (Zhang et al., 2018), identifying compound 4 as *N*-methylhydroxylamine.

Compound 5: White crystals (DMSO); HR-ESI-MS: $[M+Na]^+$ *m/z* 435.1618, $C_{20}H_{28}O_9$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 7.44 (1H, s, H-4), 5.03 (1H, d, J = 4.8 Hz, H-14), 4.87 (1H, d, J = 5.6 Hz, H-14), 4.61 (1H, t, J = 4.8 Hz, H-3), 4.18 (1H, d, J = 7.8 Hz, H-1), 3.81 (1H, d, J = 8.2 Hz, H-13), 3.65 (1H, d, J = 6.4 Hz, H-6), 3.61 (1H, d, J = 8.7 Hz, H-13), 3.42 (1H, s, H-6), 3.13 (1H, d, J = 4.5 Hz, H-5), 3.10 (2H, d, J = 7.8 Hz, H-12), 3.09 (1H, d, J = 3.7 Hz, H-3), 3.04 (1H, d, J = 9.1 Hz, H-4), 2.96 (1H, d, J = 8.3 Hz, H-2), 2.44 (3H, s, H-11), 2.43–2.40 (1H, m, H-2), 1.21 (3H, d, J = 7.3 Hz, H-10); ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 205.1 (C-1), 53.5 (C-2), 73.8 (C-3), 126.3 (C-4), 145.0 (C-5), 137.3 (C-6), 138.6 (C-7), 131.0 (C-8), 154.0 (C-9), 12.7 (C-10), 20.8 (C-11), 28.9 (C-12), 68.3 (C-13), 54.8 (C-14), 103.0 (C-1), 73.6 (C-2), 77.0 (C-3), 70.2 (C-4), 76.9 (C-5), 61.2 (C-6). These data matched literature values (Murakami et al., 1985), identifying compound 5 as pterosin S 13-O-glucoside.

Compound 6: White crystals (methanol); ^{1}H-NMR (600 MHz, Methanol-d_4) δ: 7.51 (2H, s, H-4,4), 5.11 (2H, d, J = 12.1 Hz, H-14,14), 5.00 (2H, d, J = 12.1 Hz, H-14,14), 4.74 (2H, d, J = 4.1 Hz, H-3,3), 4.25 (2H, d, J = 7.8 Hz, H-1), 4.06 (2H, d, J = 7.4 Hz, H-13,13), 3.84 (2H, s, H-6 ,6), 3.69 (2H, d, J = 9.6 Hz, H-13,13), 3.66 (2H, s, H-6 ,6), 3.31 (2H, s, H-3), 3.27 (2H, s, H-2), 3.26 (2H, s, H-5), 3.21 (4H, d, J = 7.9 Hz, H-12,12), 3.19 (2H, s, H-4), 2.52 (6H, s, H-11,11), 1.34 (6H, d, J = 7.3 Hz, H-10,10); ^{13}C-NMR (150 MHz, Methanol-d_4) δ: 207.8 (C-1,1), 54.9 (C-2,2), 76.1 (C-3,3), 127.7 (C-4,4), 147.3 (C-5,5), 138.5 (C-6,6), 139.6 (C-7,7), 132.9 (C-8,8), 155.3 (C-9,9), 13.0 (C-10,10), 21.5 (C-11,11), 29.9 (C-12,12), 70.2 (C-13,13), 57.8 (C-14,14), 104.6 (Glc-1,1), 71.6 (Glc-2,2), 78.0 (Glc-3,3), 75.1 (Glc-4,4), 78.0 (Glc-5,5), 62.7 (Glc-6,6). These data matched literature values (Peng et al., 2020), identifying compound 6 as obtupterosin C.

Compound 7: $C_{20}H_{30}O_4$, white crystals (methanol); HR-ESI-MS: $[M+Na]^+$ *m/z* 357.2036. ^{1}H-NMR (600 MHz, Methanol-d_4) δ: 3.88 (1H, d, J = 5.4 Hz, H-11), 2.43–2.37 (2H, m, H-6), 2.26 (1H, p, J = 6.8 Hz, H-2), 2.17–2.12 (1H, m, H-7), 1.95 (1H, d, J = 3.1 Hz, H-12), 1.92 (1H, s, H-16), 1.90 (1H, d, J = 3.1 Hz, H-1), 1.89 (1H, d, J = 2.7 Hz, H-12), 1.88–1.83 (1H, m, H-13),

1.80 (1H, ddd, J = 13.6, 11.7, 2.5 Hz, H-3), 1.73 (1H, td, J = 13.3, 3.5 Hz, H-9), 1.47–1.42 (1H, m, H-2), 1.42–1.38 (1H, m, H-7), 1.38–1.34 (1H, m, H-5), 1.23 (3H, d, J = 6.9 Hz, H-17), 1.21 (3H, s, H-20), 1.18 (2H, d, J = 12.4 Hz, H-14), 1.13–1.08 (1H, m, H-1), 1.06 (1H, dd, J = 13.5, 4.4 Hz, H-3), 0.96 (3H, s, H-18); ^{13}C-NMR (150 MHz, Methanol-d_4) δ: 40.6 (C-1), 20.0 (C-2), 39.0 (C-3), 44.6 (C-4), 57.5 (C-5), 21.3 (C-6), 36.3 (C-7), 52.5 (C-8), 62.3 (C-9), 39.7 (C-10), 65.7 (C-11), 34.4 (C-12), 35.9 (C-13), 38.3 (C-14), 224.8 (C-15), 50.6 (C-16), 11.5 (C-17), 29.4 (C-18), 181.3 (C-19), 16.0 (C-20). These data matched literature values (Maosong et al., 2016), identifying compound 7 as ent-11α-hydroxy-15-oxokauran-19-oic acid.

Compound 8: $C_{20}H_{28}O_4$, white crystals (methanol); HR-ESI-MS: $[M+Na]^+$ m/z 355.1879. ^{1}H-NMR (600 MHz, Methanol-d_4) δ: 5.72 (1H, s, H-17), 5.24 (1H, s, H-17), 4.01 (1H, d, J = 4.6 Hz, H-11), 3.03 (1H, d, J = 4.0 Hz, H-13), 2.43 (1H, d, J = 11.8 Hz, H-9), 1.22 (3H, s, H-19), 0.99 (3H, s, H-20); ^{13}C-NMR (150 MHz, Methanol-d_4) δ: 40.9 (C-1), 20.1 (C-2), 39.0 (C-3), 44.7 (C-4), 57.5 (C-5), 21.2 (C-6), 37.8 (C-7), 52.0 (C-8), 63.7 (C-9), 40.0 (C-10), 66.7 (C-11), 41.4 (C-12), 38.5 (C-13), 35.3 (C-14), 212.0 (C-15), 152.2 (C-16), 112.7 (C-17), 29.5 (C-18), 181.4 (C-19), 16.3 (C-20). These data matched literature values (Zhang et al., 1990), identifying compound 8 as ent-11α-hydroxy-15-oxokaur-16-en-19-oic acid.

Compound 9: White needle crystals (chloroform); ^{1}H-NMR (600 MHz, Chloroform-d) δ: 5.35 (1H, d, J = 4.5 Hz, H-6), 3.56–3.48 (1H, m, H-3), 1.00 (3H, s, CH_3-19), 0.92 (3H, d, J = 6.4 Hz, CH_3-21), 0.84 (3H, d, J = 2.5 Hz, CH_3-29), 0.82 (3H, d, J = 6.5 Hz, CH_3-26), 0.80 (3H, s, CH_3-27), 0.67 (3H, s, CH_3-18); ^{13}C-NMR (150 MHz, Chloroform-d) δ: 37.4 (C-1), 32.1 (C-2), 72.0 (C-3), 42.5 (C-4), 140.9 (C-5), 121.9 (C-6), 31.8 (C-7), 32.1 (C-8), 50.3 (C-9), 36.7 (C-10), 21.2 (C-11), 39.9 (C-12), 42.4 (C-13), 56.9 (C-14), 24.5 (C-15), 28.4 (C-16), 56.2 (C-17), 12.0 (C-18), 18.9 (C-19), 36.3 (C-20), 19.2 (C-21), 34.1 (C-22), 26.2 (C-23), 46.0 (C-24), 29.3 (C-25), 20.0 (C-26), 19.5 (C-27), 23.2 (C-28), 12.1 (C-29). These data matched literature values (Song et al., 2013), identifying compound 9 as β-sitosterol.

Compound 10: $C_{26}H_{38}O_9$, white crystals (methanol); HR-ESI-MS: $[M+Na]^+$ m/z 517.2408. ^{1}H-NMR (600 MHz, Methanol-d_4) δ: 5.71 (1H, s, H-17), 5.41 (1H, d, J = 8.0 Hz, H-1), 5.23 (1H, s, H-17), 4.00 (1H, d, J = 4.3 Hz, H-11), 3.83 (1H, d, J = 11.9 Hz, H-13), 3.69 (1H, dd, J = 12.0, 4.1 Hz, H-9), 3.40 (1H, d, J = 9.1 Hz, H-3), 3.37 (1H, s, H-4), 3.36 (1H, d, J = 1.3 Hz, H-5), 3.35 (1H, s, H-2), 3.03–3.00 (2H, m, H-6), 2.47 (1H, d, J = 12.0 Hz, H-14), 2.21 (1H, d, J = 13.1 Hz, H-3), 2.10–2.06 (1H, m, H-2), 2.04–1.98 (1H, m, H-1), 1.97 (1H, s, H-2), 1.94 (2H, d, J = 3.2 Hz, H-6), 1.83–1.77 (1H, m, H-7), 1.48–1.44 (1H, m, H-14), 1.38–1.37 (1H, m, H-7), 1.35 (2H, s, H-12), 1.26 (3H, s, H-18), 1.23 (1H, d, J = 6.7 Hz, H-5), 1.14–1.11 (1H, m, H-1), 1.09 (1H, d, J = 4.1 Hz, H-3), 0.99 (3H, s, H-20); ^{13}C-NMR (150 MHz, Methanol-d_4) δ: 40.8 (C-1), 19.9 (C-2), 38.9 (C-3), 45.0 (C-4), 58.0 (C-5), 21.0 (C-6), 37.7 (C-7), 52.0 (C-8), 63.6 (C-9), 40.1 (C-10), 66.8 (C-11), 41.3 (C-12), 38.5 (C-13), 35.3 (C-14),

212.1 (C-15), 152.4 (C-16), 112.6 (C-17), 29.0 (C-18), 178.0 (C-19), 16.3 (C-20), 95.6 (C-1), 74.0 (C-2), 78.7 (C-3), 71.1 (C-4), 78.7 (C-5), 62.4 (C-6). These data matched literature values (Kazuo et al., 1977), identifying compound 10 as ent-11α-hydroxy-15-oxokaur-16-en-19-oic acid-O-glucopyranoside.

Compound 11: $C_{16}H_{22}O_4$, colorless oil; HR-ESI-MS: $[M+Na]^+$ *m/z* 301.1410. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 7.71 (2H, dd, J = 5.7, 3.4 Hz, H-3,3), 7.66 (2H, dd, J = 5.7, 3.4 Hz, H-4,4), 4.21 (4H, t, J = 6.6 Hz, H-6,6), 1.66–1.60 (4H, m, H-7,7), 1.39–1.33 (4H, m, H-8,8), 0.88 (6H, dt, J = 22.5, 7.5 Hz, H-9,9); ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 131.8 (C-2,2), 128.8 (C-3,3), 131.7 (C-4,4), 167.2 (C-5,5), 65.2 (C-6,6), 30.1 (C-7,7), 18.8 (C-8,8), 13.7 (C-9,9). These data matched literature values (Liu et al., 2010), identifying compound 11 as 5,5'-dibutoxy-2,2'-bifuran.

Compound 12: $C_{24}H_{38}O_4$, yellow oil; HR-ESI-MS: $[M+Na]^+$ *m/z* 413.2662. ^{1}H-NMR (600 MHz, Methanol-d_4) δ: 7.72 (2H, d, J = 9.1 Hz, H-3,3), 7.62 (2H, d, J = 9.1 Hz, H-4,4), 4.31–4.21 (4H, m, H-6,6), 1.71 (2H, dt, J = 20.5, 6.6 Hz, H-7,7), 1.49–1.43 (4H, m, H-12,12), 1.44–1.40 (4H, m, H-8,8), 1.39 (4H, m, H-10,10), 1.36–1.33 (4H, dd, J = 19.3, 7.4 Hz, H-9,9), 0.97 (6H, dd, J = 19.3, 7.4 Hz, H-13,13), 0.93 (6H, t, J = 7.4 Hz, H-11,11); ^{13}C-NMR (150 MHz, Methanol-d_4) δ: 133.6 (C-2,2), 129.9 (C-3,3), 132.4 (C-4,4), 169.3 (C-5,5), 69.1 (C-6,6), 40.2 (C-7,7), 31.6 (C-8,8), 30.1 (C-9,9), 24.0 (C-10,10), 14.4 (C-11,11), 25.0 (C-12,12), 11.4 (C-13,13). These data matched literature values (Zhi et al., 2015), identifying compound 12 as 5,5'-di(2-ethyl-hexyloxy)-2,2'-bifuran.

Compound 13: White crystals (methanol); ^{1}H-NMR (600 MHz, Methanol-d_4) δ: 5.75 (1H, s, H-7), 4.22 (1H, p, J = 3.5 Hz, H-3), 2.42 (1H, dt, J = 13.5, 2.6 Hz, H-4), 1.99 (1H, dt, J = 14.4, 2.7 Hz, H-2), 1.76 (3H, s, H-11), 1.74 (1H, d, J = 4.2 Hz, H-4a), 1.53 (1H, dd, J = 14.5, 3.8 Hz, H-2), 1.46 (3H, s, H-9), 1.28 (3H, s, H-10); ^{13}C-NMR (150 MHz, Methanol-d_4) δ: 174.5 (C-2), 113.3 (C-3), 185.7 (C-3a), 37.2 (C-4), 47.9 (C-5), 67.2 (C-6), 46.4 (C-7), 89.0 (C-7a), 27.0 (C-8), 31.0 (C-9), 27.4 (C-10). These data matched literature values (Yang et al., 2020), identifying compound 13 as loliolide.

Compound 14: White crystals (methanol); ^{1}H-NMR (600 MHz, Methanol-d_4) δ: 2.52 (4H, s, H-2,3); ^{13}C-NMR (150 MHz, Methanol-d_4) δ: 176.2 (C-1,4), 29.8 (C-2,3). These data matched literature values (Li et al., 2007), identifying compound 14 as succinic acid.

Compound 15: White crystals (methanol); ^{1}H-NMR (600 MHz, Methanol-d_4) δ: 6.76 (2H, s, H-2,3); ^{13}C-NMR (150 MHz, Methanol-d_4) δ: 168.0 (C-1,4), 135.2 (C-2,3). These data matched literature values (Tan et al., 2017), identifying compound 15 as fumaric acid.

The structures of compounds 1–15 are shown in [Figure 3: see original paper].

4.1 Antitumor Activity Screening Results

Compounds 1, 2, 3, 4, 5, 6, and 10 were selected for screening against HL-60 leukemia, A549 lung cancer, SMMC-7721 liver cancer, MDA-MB-231 breast cancer, and SW480 colon cancer cells using the MTS assay, with cisplatin (DDP) and paclitaxel (Taxol) as positive controls. The results showed that six compounds exhibited varying antitumor activities against HL-60, A549, SMMC-7721, MDA-MB-231, and SW480 cells. Detailed data are presented in .

4.2 Anticoagulant Activity Screening Results

The anticoagulant activities of compounds 1, 2, 3, 4, 5, 6, and 10 were evaluated by measuring their effects on activated partial thromboplastin time (APTT), prothrombin time (PT), and thrombin time (TT) in human control plasma, using low molecular weight heparin (LMWH) as positive control for APTT and TT, and heparin (HEP) for PT. Compared with the blank control, compounds 1, 2, 3, and 6 slightly shortened APTT, while compounds 1, 5, and 6 slightly prolonged PT at a sample concentration of 2.0 mmol/L. Detailed data are presented in .

Discussion

This study isolated fifteen compounds from *Pteris ensiformis*, among which compound 1 is a new natural product. Except for compounds 8 and 9, all other compounds were isolated from *Pteris ensiformis* for the first time, with compounds 1, 3, and 4 being isolated from the genus *Pteris* for the first time. Literature reports indicate that compound 6 exhibits cytotoxic activity against HCT-116 cells (Duan et al., 2009), compound 8 possesses antitumor, anti-inflammatory, and antiplatelet aggregation activities (Jiang et al., 1996), and compound 9 has cholesterol-lowering, hypoglycemic, antioxidant, anti-inflammatory, and antibacterial effects (Chen et al., 2021). This study screened compounds 1, 2, 3, 4, 5, 6, and 10 for antitumor and anticoagulant activities, revealing that six compounds inhibited the in vitro growth of tumor cells HL-60, A549, SMMC-7721, MDA-MB-231, and SW480 at 40 mol/L. At 2.0 mmol/L, compounds 1, 2, 3, and 6 shortened APTT, while compounds 1, 5, and 6 prolonged PT.

These results enrich the chemical constituent and bioactivity profile of Guizhou *Pteris ensiformis*, demonstrating its diverse chemical structures and potential antitumor applications, thus providing a material basis and scientific rationale for the medicinal development of this plant. However, this study did not investigate multiple cell lines for each tumor type, which warrants further research.

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