Postprint: Chemical Constituents of Hypericum lagarocladum

Authors: Zhang Han, Deng Jingtong, Peng Yu, Han Qingdi, Zhou Xiandong, Yang Xinzhou

Date: 2022-07-05T00:00:00+00:00

Abstract

To investigate the material basis and search for bioactive compounds of the Yunnan-native plant Hypericum lagarocladum, the aerial parts were extracted by maceration with 80% ethanol in this study. The chemical constituents of Hypericum lagarocladum were separated and purified using chromatographic techniques including HP-20 macroporous adsorption resin, silica gel, Sephadex gel, and semi-preparative high-performance liquid chromatography, and the structures of the compounds were identified based on spectroscopic data. The results showed that fifteen compounds were isolated from Hypericum lagarocladum and identified as attenuatumione G (1), uralodin B (2), chipericumin C (3), 2,5-dihydroxy-1-methoxyxanthone (4), 1,7-dihydroxyxanthone (5), 1,7-dihydroxy-4-methoxyxanthone (6), quercitrin (7), apigenin-7-O-β-D-glucoside (8), apigenin-7-O-β-D-(6'' -O-acetyl)-glucoside (9), luteolin (10), quercetin (11), betulinic acid (12), methyl betulinate (13), betulonic acid (14), and β-sitosterol (15). Compounds 1–14 were isolated from this plant for the first time. The in vitro anti-breast cancer activity of compounds 1–14 was evaluated using the MTT assay, and the results revealed that only compounds 3, 6, and 13 exhibited certain inhibitory effects against two breast cancer cell lines, MCF-7 and MDA-MB-231, with IC50 values ranging from 48.6 to 123.5 g · mL−1. These findings hold theoretical and practical significance for the comprehensive development and utilization of Hypericum lagarocladum resources.

Full Text

Chemical Components of *Hypericum lagarocladum*

Han Zhang, Jingtong Deng, Yu Peng, Qingdi Han, Xiandong Zhou, Xinzhou Yang*

School of Pharmaceutical Sciences, South-Central Minzu University, Wuhan 430074, China

Abstract

This study investigated the chemical constituents of *Hypericum lagarocladum* to elucidate its material basis and identify bioactive compounds. The aerial parts of the plant were extracted with 80% ethanol, and the crude extract was isolated and purified using HP-20 macroporous adsorption resin column chromatography (CC), silica gel CC, Sephadex LH-20 CC, and semi-preparative HPLC. The structures of the isolated compounds were elucidated based on spectroscopic data and by comparison with literature values. Fifteen compounds were isolated from *H. lagarocladum* and identified as attenuatumione G (1), uralodin B (2), chipericumin C (3), 2,5-dihydroxy-1-methoxyxanthone (4), 1,7-dihydroxyxanthone (5), 1,7-dihydroxy-4-methoxyxanthone (6), quercitrin (7), apigenin-7-O-β-D-glucoside (8), apigenin-7-O-β-D-(6'' -O-acetyl)-glucoside (9), luteolin (10), quercetin (11), betulinic acid (12), betulinic acid methyl ester (13), betulonic acid (14), and β-sitosterol (15). Compounds 1–14 were isolated from this plant for the first time.

The isolated compounds 1–14 were evaluated for *in vitro* anti-breast cancer activity using the MTT assay. Only compounds 3, 6, and 13 exhibited moderate inhibitory effects against two breast cancer cell lines, MCF-7 and MDA-MB-231, with IC_{50} values ranging from 48.6 to 123.5 $g \cdot mL^{-1}$. These results provide a scientific foundation for the comprehensive development and utilization of *H. lagarocladum* resources.

Keywords: *Hypericum*, *Hypericum lagarocladum*, chemical constituents, polycyclic polyprenylated acylphloroglucinols (PPAPs), cytotoxic activity

---

Introduction

The genus *Hypericum* (family Clusiaceae) comprises approximately 460 species worldwide, with 64 species (33 endemic) found in China. These plants are widely distributed across the country, being most abundant in the southwestern regions and rarest in Xinjiang. *Hypericum* species have a long history of medicinal use in folk medicine both in China and internationally. For example, *Hypericum perforatum* is used to soothe the liver, relieve depression, clear heat and dampness, and reduce swelling and promote lactation, and is indicated for liver qi stagnation, emotional distress, chest tightness, joint swelling and pain, mastitis, and insufficient lactation. The chemical constituents of this genus primarily include phloroglucinols, flavonoids, dianthrones, triterpenoids, coumarins, and sterols, which exhibit diverse pharmacological activities. Notably, the phloroglucinol derivatives hyperjapones A–B and D demonstrate antitumor effects, while the xanthone neobractatin shows significant inhibitory activity against human breast cancer (MCF-7) and human hepatoma (HepG2) cells.

Hypericum lagarocladum is a shrub (0.4–1.5 m tall) belonging to the genus *Hypericum*. It is mainly distributed in western Hunan, western Sichuan, southern Guizhou, and central to western Yunnan, growing in valleys, on hillsides, along roadsides, and in thickets at elevations of 1,500–2,700 m. To date, few studies have reported on the chemical constituents and biological activities of this species. Given that other *Hypericum* species have yielded polycyclic polyprenylated acylphloroglucinols (PPAPs) with novel skeletons and broad-spectrum activities, and considering the taxonomic specificity of secondary metabolites, we conducted a systematic phytochemical investigation of the aerial parts of *H. lagarocladum* from Yunnan. From the 80% ethanol extract, we isolated 15 compounds ([Figure 1: see original paper]), including three PPAPs with [3.3.1] and spirocyclic skeletons. All compounds except β-sitosterol were isolated from this plant for the first time and evaluated for *in vitro* anti-breast cancer activity.

Materials and Methods

1.1 Instruments and Reagents The following instruments were used: Finnigan MAT-95 mass spectrometer, Q-TOF Micro LC-MS-MS mass spectrometer, Bruker DRX-600 NMR spectrometer (Bruker, Germany), Waters preparative HPLC system (Waters, USA), COSMOSIL C_{18} column (250 mm × 10 mm, 5 m) and COSMOSIL 5PFP column (250 mm × 10 mm, 5 m) (COSMOSIL Ltd., Japan), HP-20 macroporous adsorption resin (Mitsubishi, Japan), silica gel GF_{254} TLC plates (200–300 and 300–400 mesh) (Yantai Jiangyou Silica Gel Development Co., Ltd.), Sephadex LH-20 (Amersham Biosciences Ltd., USA), and HPLC-grade methanol and acetonitrile (TEDIA Ltd., USA).

1.2 Plant Material *Hypericum lagarocladum* was collected in June 2019 from Liangwang Mountain, Kunming, Yunnan Province (102°52 45 E, 24°43 57 N). The plant was identified by Professor Wan Dingrong of South-Central Minzu University, and a voucher specimen (SC0869) was deposited in the herbarium of the School of Pharmaceutical Sciences, South-Central Minzu University.

1.3 Extraction and Isolation 1.3.1 Extraction and Partitioning

Dried aerial parts of *H. lagarocladum* (25.5 kg) were pulverized and extracted four times with 80% aqueous ethanol at room temperature (20 L each time, 3 days per extraction). The combined extracts were concentrated under reduced pressure to yield a crude extract (1.76 kg). The extract was suspended in hot water (5× volume) and successively partitioned with petroleum ether, ethyl acetate, and *n*-butanol to obtain petroleum ether (142 g), ethyl acetate (353 g), and *n*-butanol (693 g) fractions. The petroleum ether and ethyl acetate fractions were combined and subjected to HP-20 macroporous resin column chromatography, eluted with a water–ethanol gradient (20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, v/v). Based on TLC analysis, the eluates were combined into 11 fractions (A–K).

1.3.2 Separation and Purification

Fraction C was subjected to Sephadex LH-20 gel chromatography eluted with methanol (containing 0.1% formic acid) to yield nine subfractions (C1–C9). Subfraction C2 was purified by semi-preparative HPLC (acetonitrile–water, 30:70$→39 : 61, v/v, 22min, 0.1→50 : 50, v/v, 24min, 0.1→65 : 35, v/v, 16min, 0.1→$70:30, v/v, 18 min, 0.1% formic acid) to afford compounds **10** (9.2 mg) and **11** (7.2 mg).

Fraction D was separated by Sephadex LH-20 gel chromatography to yield ten subfractions (D1–D10). Subfraction D1 was purified by semi-preparative HPLC (acetonitrile–water, 60:40$→80 : 20, v/v, 25min, 0.1→60 : 40, v/v, 17min, 0.1→$65:35, v/v, 15 min, 0.1% formic acid) to afford compound **5** (4.6 mg).

Fraction E was subjected to silica gel column chromatography eluted with dichloromethane–methanol (8:1, v/v) to yield five subfractions (E1–E5). Subfraction E1 was further separated by Sephadex LH-20 gel chromatography into three subfractions (E1-1–E1-3). Subfraction E1-1 was purified by semi-preparative HPLC (acetonitrile–water, 10:90$→80 : 20, v/v, 14min, 0.1→$75:25, v/v, 18 min, 0.1% formic acid) to yield compound **15** (9.4 mg).

Fraction G was subjected to silica gel column chromatography eluted with a petroleum ether–ethyl acetate gradient (1:0$→0 : 1, v/v)toyieldsixsubfractions(G1–G6).SubfractionG3wasse
20gelchromatographyintofivesubfractions(G3 − 1–G3 − 5).SubfractionG3 − 1waspurifiedbysemi − preparativeHPLC(acetonitrile–water, 15 : 85→55 : 45, v/v, 19min, 0.1→76 : 24, v/v, 21min, 0.1→76 : 24, v/v, 23min, 0.1→$70:30, v/v, 26 min, 0.1% formic acid) to yield compound **13** (7.2 mg).

1.3.3 Anti-Breast Cancer Activity Assay

Human breast cancer cell lines MCF-7 and MDA-MB-231 were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA) and cultured in DMEM high-glucose medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin at 37°C in a humidified atmosphere containing 5% CO_2. Cells were passaged or fed as needed. For the assay, cells in logarithmic growth phase were seeded into 96-well plates at 1$×10^{{4}-5×10}{4}$ cells per well in 100 L medium and incubated overnight. Cell viability and cytotoxicity were measured using the MTT method as described by Xu et al. (2020). Test compounds were dissolved in DMSO to prepare stock solutions (50 mg · mL^{-1}), which were diluted with DMEM to final concentrations of 10, 20, 40, 60, 80, and 100 g · mL^{-1} (final DMSO concentration <0.1%). After removing the culture medium, 200 L of each test solution was added to the wells (five replicates per concentration), along with vehicle control and blank control (no cells). Following incubation for 12, 24, or 48 h, the medium was removed and 100 L of MTT solution (5 mg · mL^{-1} in DMEM) was added to each well. After 0.5 h incubation, the formazan crystals were dissolved in 150 L DMSO and the absorbance was measured at 562 nm. The inhibition rate was calculated as:

[1−(OD −OD)/(ODc −OD)]$×100_{50}$ values were determined according to the literature.

Results

2.1 Structural Elucidation of Compounds **Compound 1** was obtained as a yellow oil. ESI-MS: *m/z* 587 $[M+H]^+$, $C_{35}H_{55}O_7$. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 5.06 (1H, t, J = 7.1 Hz, H-27), 4.94 (1H, m, H-22), 4.57 (1H, dd, J = 10.9, 5.6 Hz, H-32), 3.27 (1H, d, J = 9.7 Hz, H-17), 3.15 (1H, dd, J = 14.0, 6.8 Hz, H-26α), 3.04 (1H, dd, J = 14.0, 6.8 Hz, H-26β), 2.67 (1H, dd, J = 12.8, 10.5 Hz, H-31α), 2.15 (1H, m, H-21α), 2.09 (1H, d, J = 12.3 Hz, H-15α), 2.05 (1H, dd, J = 13.2, 4.3 Hz, H-5α), 1.96 (1H, t, J = 6.4 Hz, H-11), 1.93 (1H, m, H-15β), 1.78 (2H, dd, J = 12.9, 5.3 Hz, H-21β, 31β), 1.71 (6H, s, H-24, 30), 1.67 (1H, m, H-4), 1.66 (3H, s, H-29), 1.61 (1H, m, H-16α), 1.57 (3H, s, H-25), 1.51 (2H, t, J = 13.4 Hz, H-5β, 16β), 1.38 (3H, s, H-34), 1.23 (3H, s, H-35), 1.18 (3H, s, H-19), 1.14 (3H, s, H-20), 1.09 (3H, d, J = 6.5 Hz, H-13), 1.07 (3H, s, H-14), 1.01 (3H, d, J = 6.5 Hz, H-12). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 209.7 (C-10), 204.4 (C-1), 193.8 (C-9), 174.0 (C-7), 133.9 (C-23), 132.8 (C-28), 122.1 (C-22), 121.1 (C-27), 117.0 (C-8), 90.5 (C-32), 82.9 (C-2), 78.7 (C-17), 73.1 (C-18), 71.0 (C-33), 59.7 (C-6), 48.4 (C-3), 42.4 (C-11), 41.9 (C-4), 37.9 (C-5), 33.1 (C-15), 30.3 (C-31), 27.5 (C-21), 27.4 (C-16), 27.1 (C-34), 26.4 (C-19), 26.1 (C-24), 25.8 (C-29), 24.2 (C-35), 23.6 (C-20), 22.3 (C-26), 21.6 (C-12), 20.6 (C-13), 18.2 (C-25), 18.0 (C-30), 15.1 (C-14). The ^{13}C-NMR and DEPT spectra displayed 35 carbon signals. Characteristic signals at δ 209.7 (C-10), 204.4 (C-1), 193.8 (C-9), 174.0 (C-7), 117.0 (C-8), 82.9 (C-2), 59.7 (C-6), 48.4 (C-3), 41.9 (C-4), and 37.9 (C-5) indicated a [3.3.1]-type polycyclic polyprenylated acylphloroglucinol skeleton. The ^{1}H-NMR data revealed an isobutyryl group, two prenyl groups, a 2-methyl-2,3-dihydroxybutyl group, and a tetrahydrofuran ring. These spectroscopic data were consistent with those reported by Zhou et al. (2016), leading to the identification of compound **1** as attenuatumione G.

Compound 2 was obtained as a yellow oil. ESI-MS: *m/z* 587 $[M+H]^+$, $C_{38}H_{50}O_5$. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.51 (2H, d, J = 7.5 Hz, H-12, 16), 7.38 (1H, t, J = 7.5 Hz, H-14), 7.25 (2H, t, J = 7.5 Hz, H-13, 15), 5.06 (1H, t, J = 7.2 Hz, H-35), 5.02 (2H, m, H-20, 25), 4.87 (1H, dd, J = 10.3, 7.6 Hz, H-30), 3.02 (1H, dd, J = 15.0, 10.4 Hz, H-29α), 2.98 (1H, dd, J = 15.0, 7.7 Hz, H-29β), 2.54 (2H, m, H-34), 2.18 (2H, m, H-19β, 24β), 2.07 (1H, m, H-5β), 2.05 (1H, m, H-18β), 2.02 (1H, m, H-19α), 2.00 (1H, m, H-4), 1.96 (1H, m, H-24α), 1.82 (1H, m, H-18α), 1.74 (3H, s, H-28), 1.68 (6H, s, H-37, 38), 1.67 (1H, m, H-5α), 1.65 (3H, s, H-23), 1.62 (3H, s, H-22), 1.58 (3H, s, H-27), 1.34 (3H, s, H-32), 1.22 (3H, s, H-33), 1.12 (3H, s, H-17). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 206.8 (C-1), 193.6 (C-10), 188.2 (C-9), 175.8 (C-7), 136.9 (C-11), 134.9 (C-36), 133.8 (C-26), 132.2 (C-14), 131.4 (C-21), 128.3 (C-12), 128.3 (C-16), 128.2 (C-13), 128.1 (C-15), 124.5 (C-20), 122.6 (C-25), 120.5 (C-35), 118.5 (C-8), 93.3 (C-30), 79.8 (C-2), 71.7 (C-31), 55.5 (C-6), 49.9 (C-3), 42.8 (C-4), 39.4 (C-5),

36.7 (C-18), 29.2 (C-34), 27.7 (C-24), 27.3 (C-29), 26.2 (C-23), 26.1 (C-28), 26.1 (C-33), 25.9 (C-38), 25.2 (C-19), 23.2 (C-32), 18.3 (C-22), 18.2 (C-27), 17.9 (C-37), 14.2 (C-17). The ^{13}C-NMR and DEPT spectra displayed 38 carbon signals. Characteristic signals at δ 206.8 (C-1), 193.6 (C-10), 188.2 (C-9), 175.8 (C-7), 118.5 (C-8), 79.8 (C-2), 55.5 (C-6), 49.9 (C-3), 42.8 (C-4), and 39.4 (C-5) indicated a [3.3.1]-type polycyclic polyprenylated acylphloroglucinol skeleton. The ^{1}H-NMR data revealed a benzoyl group, three prenyl groups, and a tetrahydrofuran ring. These spectroscopic data matched those reported by Chen et al. (2010), leading to the identification of compound **2** as uralodin B.

Compound 3 was obtained as a yellow oil. ESI-MS: *m/z* 445 $[M-H]^-$, $C_{26}H_{38}O_6$. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 4.94 (1H, t, J = 7.8 Hz, H-18), 3.42 (1H, dt, J = 6.8 Hz, H-24), 2.74 (1H, dd, J = 14.0, 8.9 Hz, H-17a), 2.62 (1H, dd, J = 14.0, 6.6 Hz, H-17b), 2.22 (1H, d, J = 13.6 Hz, H-14b), 1.90 (1H, m, H-7a), 1.88 (1H, m, H-7b), 1.77 (1H, m, H-12), 1.76 (1H, m, H-11a), 1.75 (2H, m, H-10), 1.62 (3H, s, H-21), 1.58 (3H, s, H-20), 1.55 (1H, m, H-8), 1.41 (1H, d, J = 13.6 Hz, H-14a), 1.38 (1H, m, H-11b), 1.36 (3H, s, H-16), 1.33 (3H, s, H-22), 1.25 (3H, d, J = 6.7 Hz, H-26), 1.16 (3H, d, J = 6.7 Hz, H-25), 1.04 (3H, s, H-15). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 206.9 (C-5), 205.3 (C-23), 200.5 (C-1), 197.7 (C-3), 136.2 (C-19), 118.9 (C-18), 110.6 (C-2), 79.3 (C-9), 73.3 (C-13), 65.4 (C-4), 57.4 (C-6), 52.1 (C-12), 48.9 (C-14), 48.2 (C-8), 40.0 (C-10), 35.4 (C-17), 34.1 (C-24), 26.9 (C-16), 26.7 (C-7), 26.2 (C-22), 26.0 (C-21), 21.8 (C-11), 21.2 (C-15), 20.9 (C-26), 18.3 (C-25), 18.0 (C-20). The ^{13}C-NMR and DEPT spectra displayed 26 carbon signals. Characteristic signals at δ 206.9 (C-5), 200.5 (C-1), 197.7 (C-3), 110.6 (C-2), 79.3 (C-9), 73.3 (C-13), 65.4 (C-4), 57.4 (C-6), 52.1 (C-12), 48.9 (C-14), 48.2 (C-8), 40.0 (C-10), 26.7 (C-7), and 21.8 (C-11) indicated a spirocyclic polycyclic polyprenylated acylphloroglucinol skeleton. The ^{1}H-NMR data revealed an isopropionyl group and a prenyl group. These spectroscopic data were consistent with those reported by Abe et al. (2012), leading to the identification of compound **3** as chipericumin C.

Compound 4 was obtained as a yellow amorphous powder. ESI-MS: *m/z* 259 $[M+H]^+$, $C_{14}H_{10}O_5$. ^{1}H-NMR (600 MHz, CD_3OD) δ: 7.59 (1H, d, J = 7.5 Hz, H-8), 7.46 (1H, d, J = 7.8 Hz, H-3), 7.34 (1H, d, J = 7.8 Hz, H-4), 7.33 (1H, d, J = 7.5 Hz, H-6), 7.25 (1H, t, J = 7.5, 1.7 Hz, H-7), 3.90 (3H, s, 1-OCH_3). ^{13}C-NMR (150 MHz, CD_3OD) δ: 178.6 (C-9), 151.8 (C-4a), 148.0 (C-2), 147.5 (C-5), 146.5 (C-1), 145.3 (C-4b), 125.3 (C-3), 124.6 (C-7), 123.9 (C-8a), 120.7 (C-6), 117.1 (C-8b), 117.0 (C-8), 115.2 (C-4), 62.1 (1-OCH_3). These data matched those reported by Cheng et al. (2008), leading to the identification of compound **4** as 2,5-dihydroxy-1-methoxyxanthone.

Compound 5 was obtained as a yellow amorphous powder. ESI-MS: *m/z* 229 $[M+H]^+$, $C_{13}H_8O_4$. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.43 (1H, t, J = 7.3 Hz, H-3), 7.32 (1H, d, J = 3.0 Hz, H-8), 7.25 (1H, d, J = 9.0 Hz, H-5), 7.17 (1H, dd, J = 9.0, 3.0 Hz, H-6), 6.76 (1H, d, J = 8.4 Hz, H-4), 6.44 (1H, d, J = 8.4 Hz, H-2). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 182.2 (C-9), 161.9 (C-1), 156.5 (C-4a),

152.3 (C-7), 151.0 (C-4b), 136.9 (C-3), 124.9 (C-6), 121.2 (C-8a), 119.6 (C-5), 110.2 (C-2), 108.8 (C-8), 108.6 (C-8b), 107.2 (C-4). These data matched those reported by Wong et al. (2018), leading to the identification of compound **5** as 1,7-dihydroxyxanthone.

Compound 6 was obtained as a yellow amorphous powder. ESI-MS: *m/z* 259 $[M+H]^+$, $C_{14}H_{10}O_5$. ^{1}H-NMR (600 MHz, CD_3OD) δ: 7.52 (1H, d, J = 3.1 Hz, H-8), 7.49 (1H, d, J = 9.1 Hz, H-6), 7.36 (1H, d, J = 8.9 Hz, H-2), 7.32 (1H, dd, J = 9.1, 3.1 Hz, H-5), 6.66 (1H, d, J = 8.9 Hz, H-3), 3.93 (3H, s, 4-OCH_3). ^{13}C-NMR (150 MHz, CD_3OD) δ: 183.5 (C-9), 155.7 (C-7), 155.5 (C-1), 151.4 (C-4b), 147.4 (C-4a), 141.6 (C-4), 126.4 (C-6), 122.2 (C-8a), 121.4 (C-3), 120.5 (C-5), 109.9 (C-8b), 109.1 (C-8), 109.0 (C-2), 57.8 (4-OCH_3). These data matched those reported by Dao et al. (2012), leading to the identification of compound **6** as 1,7-dihydroxy-4-methoxyxanthone.

Compound 7 was obtained as a yellow amorphous powder. ESI-MS: *m/z* 449 $[M+H]^+$, $C_{21}H_{20}O_{11}$. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.30 (1H, d, J = 1.9 Hz, H-2), 7.25 (1H, dd, J = 8.6, 1.9 Hz, H-6), 6.89 (1H, d, J = 8.6 Hz, H-5), 6.40 (1H, d, J = 2.3 Hz, H-8), 6.19 (1H, d, J = 2.3 Hz, H-6), 5.25 (1H, d, J = 1.8 Hz, H-1), 3.96 (1H, m, H-2), 3.49 (1H, m, H-3), 3.29 (1H, m, H-5), 3.12 (1H, m, H-4), 0.79 (3H, d, J = 5.9 Hz, 5 -CH_3). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 180.0 (C-4), 166.0 (C-7), 163.1 (C-5), 159.4 (C-9), 158.8 (C-2), 149.9 (C-4), 146.4 (C-3), 136.4 (C-3), 123.1 (C-6), 122.8 (C-1), 117.0 (C-2), 116.0 (C-5), 105.8 (C-10), 103.1 (C-1), 99.5 (C-6), 94.6 (C-8), 73.3 (C-4), 72.1 (C-2), 72.1 (C-3), 71.9 (C-5), 17.7 (C-6). These data matched those reported by Zhong et al. (1997), leading to the identification of compound **7** as quercitrin.

Compound 8 was obtained as a yellow amorphous powder. ESI-MS: *m/z* 417 $[M+H]^+$, $C_{21}H_{20}O_{10}$. ^{1}H-NMR (600 MHz, CD_3OD) δ: 8.07 (2H, d, J = 8.8 Hz, H-2 , 6), 7.13 (2H, d, J = 8.8 Hz, H-3 , 5), 6.69 (1H, s, H-3), 6.86 (1H, d, J = 2.2 Hz, H-8), 6.46 (1H, d, J = 2.2 Hz, H-6), 5.08 (1H, d, J = 7.4 Hz, H-1). ^{13}C-NMR (150 MHz, CD_3OD) δ: 182.0 (C-4), 163.8 (C-2), 163.0 (C-7), 162.4 (C-5), 161.4 (C-4), 156.9 (C-9), 128.4 (C-2 , 6), 120.7 (C-1), 114.6 (C-3 , 5), 105.4 (C-10), 105.4 (C-1), 103.8 (C-3), 99.9 (C-2), 99.5 (C-6), 94.9 (C-8), 77.2 (C-4), 76.4 (C-6), 73.1 (C-3), 69.5 (C-5). These data matched those reported by Zhuang et al. (2009), leading to the identification of compound **8** as apigenin-7-O-β-D-glucoside.

Compound 9 was obtained as a yellow amorphous powder. ESI-MS: *m/z* 473 $[M-H]^-$, $C_{23}H_{22}O_{11}$. ^{1}H-NMR (600 MHz, CD_3OD) δ: 7.85 (2H, d, J = 8.4 Hz, H-2 , 6), 6.89 (2H, d, J = 8.4 Hz, H-3 , 5), 6.72 (1H, d, J = 1.5 Hz, H-8), 6.61 (1H, s, H-3), 6.45 (1H, br.s, H-6), 5.04 (1H, d, J = 7.2 Hz, H-1), 4.47 (1H, dd, J = 11.3, 2.4 Hz, H-6b), 4.23 (1H, dd, J = 11.3, 4.6 Hz, H-6a), 3.77 (1H, s, H-5), 3.63 (1H, m, H-2), 3.56 (1H, m, H-3), 3.36 (1H, dd, J = 9.2, 9.2 Hz, H-4), 2.09 (3H, s, CO-CH_3-6). ^{13}C-NMR (150 MHz, CD_3OD) δ: 182.3 (C-4), 171.6 (C-1), 165.4 (C-7), 163.2 (C-2), 161.6 (C-5), 161.5 (C-4), 157.5 (C-9), 128.2 (C-2 , 6), 121.6 (C-1), 115.7 (C-3 , 5), 105.7 (C-10), 102.8 (C-3), 100.1 (C-1), 99.7 (C-2), 94.8 (C-8), 76.3 (C-3), 74.2 (C-5), 73.3 (C-2), 70.2 (C-7), 63.4 (C-6), 19.4

(C-2). These data matched those reported by Cheng et al. (2014), leading to the identification of compound **9** as apigenin-7-O-β-D-(6 -O-acetyl)-glucoside.

Compound 10 was obtained as a yellow amorphous powder. ESI-MS: *m/z* 287 $[M+H]^+$, $C_{15}H_{10}O_6$. ^{1}H-NMR (600 MHz, CD_3OD) δ: 7.38 (1H, d, J = 2.2 Hz, H-2), 7.37 (1H, dd, J = 8.5, 2.2 Hz, H-6), 6.88 (1H, d, J = 8.5 Hz, H-5), 6.53 (1H, s, H-3), 6.42 (1H, d, J = 2.1 Hz, H-8), 6.19 (1H, d, J = 2.1 Hz, H-6). ^{13}C-NMR (150 MHz, CD_3OD) δ: 183.7 (C-4), 166.4 (C-7), 166.1 (C-2), 163.2 (C-5), 159.3 (C-9), 151.0 (C-4), 147.1 (C-3), 123.7 (C-1), 120.3 (C-6), 116.8 (C-5), 114.1 (C-2), 105.3 (C-10), 103.9 (C-3), 100.1 (C-6), 95.0 (C-8). These data matched those reported by He et al. (2008), leading to the identification of compound **10** as luteolin.

Compound 11 was obtained as a yellow amorphous powder. ESI-MS: *m/z* 303 $[M+H]^+$, $C_{15}H_{10}O_7$. ^{1}H-NMR (600 MHz, CD_3OD) δ: 7.73 (1H, d, J = 2.2 Hz, H-2), 7.62 (1H, dd, J = 8.5, 2.2 Hz, H-6), 6.87 (1H, d, J = 8.5 Hz, H-5), 6.38 (1H, d, J = 2.1 Hz, H-8), 6.17 (1H, d, J = 2.1 Hz, H-6). ^{13}C-NMR (150 MHz, CD_3OD) δ: 177.3 (C-4), 165.6 (C-7), 162.5 (C-9), 158.2 (C-5), 148.8 (C-4), 148.0 (C-2), 146.2 (C-3), 137.3 (C-3), 124.1 (C-1), 121.7 (C-6), 116.2 (C-5), 115.9 (C-2), 104.5 (C-10), 99.2 (C-6), 94.4 (C-8). These data matched those reported by Wang et al. (2020), leading to the identification of compound **11** as quercetin.

Compound 12 was obtained as a white amorphous powder. ESI-MS: *m/z* 457 $[M+H]^+$, $C_{30}H_{48}O_3$. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 4.69 (1H, s, H-29a), 4.57 (1H, s, H-29b), 3.33 (1H, m, H-3), 1.68 (3H, s, H-23), 0.99 (3H, s, H-24), 0.95 (3H, s, H-25), 0.93 (3H, s, H-26), 0.84 (3H, s, H-27), 0.74 (3H, s, H-30). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 180.1 (C-28), 152.0 (C-20), 110.2 (C-29), 79.7 (C-3), 57.5 (C-17), 56.9 (C-5), 52.0 (C-9), 50.4 (C-18), 48.1 (C-19), 43.6 (C-14), 41.9 (C-8), 40.1 (C-1), 39.7 (C-4), 38.3 (C-13), 38.1 (C-10), 37.9 (C-22), 35.6 (C-7), 33.3 (C-16), 31.7 (C-15), 30.8 (C-21), 28.6 (C-23), 28.0 (C-2), 26.9 (C-12), 22.1 (C-11), 19.5 (C-6), 19.4 (C-30), 16.7 (C-26), 16.6 (C-25), 16.1 (C-24), 15.1 (C-27). These data matched those reported by Wang et al. (2021), leading to the identification of compound **12** as betulinic acid.

Compound 13 was obtained as a white amorphous powder. ESI-MS: *m/z* 471 $[M+H]^+$, $C_{31}H_{50}O_3$. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 4.73 (1H, s, H-29a), 4.60 (1H, s, H-29b), 3.61 (3H, s, CO_2Me), 3.15 (1H, m, H-3), 2.88 (1H, m, H-19), 1.66 (3H, s, H-30), 0.97 (3H, s, H-24), 0.96 (3H, s, H-25), 0.93 (3H, s, H-26), 0.81 (3H, s, H-27), 0.75 (3H, s, H-23). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 180.8 (C-28), 150.6 (C-20), 109.9 (C-29), 79.2 (C-3), 56.4 (C-17), 55.5 (C-5), 51.1 (-CO_2Me), 50.6 (C-9), 49.4 (C-19), 47.0 (C-18), 42.3 (C-14), 40.8 (C-8), 39.0 (C-4), 38.8 (C-1), 38.5 (C-13), 37.3 (C-22), 37.2 (C-10), 34.4 (C-7), 32.3 (C-16), 30.7 (C-15), 29.8 (C-21), 28.1 (C-23), 27.5 (C-2), 25.6 (C-12), 21.0 (C-11), 19.5 (C-30), 18.4 (C-6), 16.3 (C-25), 16.2 (C-26), 15.5 (C-24), 14.8 (C-27). These data matched those reported by Kojima et al. (1987), leading to the identification of compound **13** as betulinic acid methyl ester.

Compound 14 was obtained as a white amorphous powder. ESI-MS: *m/z* 453 $[M-H]^-$, $C_{30}H_{46}O_3$. ^{1}H-NMR (600 MHz, CD_3OD) δ: 4.75 (1H, s, H-29a), 4.59 (1H, s, H-29b), 2.99 (1H, m, H-19), 2.50 (1H, m, H-2β), 2.39 (1H, m, H-2α), 2.30 (1H, m, H-16β), 2.20 (1H, m, H-13), 2.00 (2H, m, H-15β, 22β), 1.90 (1H, m, H-1β), 1.69 (1H, m, H-12β), 1.65 (3H, s, H-30), 1.60 (1H, m, H-18), 1.55 (1H, m, H-21β), 1.50 (2H, m, H-6), 1.45 (1H, m, H-22α), 1.43 (4H, m, H-7, 11β, 16α), 1.40 (1H, m, H-15α), 1.39 (2H, m, H-1α, 9), 1.36 (1H, m, H-5), 1.36 (1H, m, H-11α), 1.21 (1H, m, H-21α), 1.07 (3H, s, H-23), 1.04 (1H, m, H-12α), 0.99 (3H, s, H-24), 0.97 (3H, s, H-27), 0.95 (3H, s, H-26), 0.91 (3H, s, H-25). ^{13}C-NMR (150 MHz, CD_3OD) δ: 218.7 (C-3), 182.3 (C-28), 150.4 (C-20), 110.0 (C-29), 56.4 (C-17), 54.9 (C-5), 50.0 (C-9), 49.1 (C-18), 47.6 (C-4), 46.9 (C-19), 42.5 (C-14), 40.8 (C-8), 40.0 (C-1), 39.1 (C-13), 37.5 (C-22), 36.9 (C-10), 34.2 (C-2), 34.0 (C-7), 32.1 (C-16), 30.5 (C-15), 30.0 (C-21), 26.8 (C-23), 25.7 (C-12), 21.6 (C-11), 21.2 (C-24), 20.0 (C-6), 19.6 (C-30), 16.4 (C-25), 16.0 (C-26), 14.8 (C-27). These data matched those reported by Barthel et al. (2008), leading to the identification of compound **14** as betulonic acid.

Compound 15 was obtained as a white amorphous powder. EI-MS: *m/z* 397 $[M+H]^+$, $C_{29}H_{50}O$. TLC analysis in multiple solvent systems showed identical Rf values to an authentic standard, leading to the identification of compound **15** as β-sitosterol.

2.2 Cytotoxic Activity Results The cytotoxic activities of compounds **1–14** against MCF-7 and MDA-MB-231 breast cancer cell lines were evaluated using the MTT assay. Among the 14 compounds tested, only compounds **3**, **6**, and **13** showed moderate cytotoxic activity. The IC_{50} values against MCF-7 cells were $123.5\$\pm5.8, 52.7\pm2.7, and 99.4\pm5.3 \mu g \cdot mL^{\{-1\}}, respectively, while those against MDA-MB-231 cells were 112.8\pm6.2, 48.6\pm1.9, and 105.1\pm7.0 \mu g \cdot mL^{\{-1\}}, respectively. The remaining compounds showed no activity at concentrations up to 200 \mu g \cdot mL\hat{}\{-1\}\$$.

Discussion and Conclusion

Polycyclic polyprenylated acylphloroglucinols (PPAPs) are characteristic constituents of *Hypericum* species with complex and unique structures, including [3.3.1]-, [3.2.1]-, [5.3.1]-, adamantane-type, adamantane-like, spirocyclic, and other skeletons. In this study, we isolated 15 compounds from *H. lagarocladum* collected in Yunnan, including PPAPs, flavonoids, triterpenoids, and sterols. Among these, three were PPAPs with [3.3.1] and spirocyclic skeletons. This work enriches the chemical diversity of the *Hypericum* genus and expands our understanding of the material basis of *H. lagarocladum*.

PPAPs exhibit diverse biological activities, including antitumor, antidepressant, antibacterial, and anti-inflammatory effects. However, our *in vitro* anti-breast cancer screening of the newly isolated compounds did not identify any with po-

tent activity against MCF-7 or MDA-MB-231 cells. Another major bioactivity of PPAPs is antidepressant activity; for instance, hyperforin is a non-competitive reuptake inhibitor of multiple neurotransmitters that exerts its antidepressant effects by competitively binding to transporter proteins (Müller et al., 1998). Therefore, further studies on the antidepressant activity of the isolated PPAPs are warranted. In summary, this study provides a foundation for investigating the chemical constituents and pharmacological activities of *H. lagarocladum* and offers a scientific basis for its rational development and utilization.

References

Abe S, Tanaka N, Kobayashi J, 2012. Prenylated acylphloroglucinols, chipericumins A—D, from *Hypericum chinense*. *J Nat Prod*, 75(3): 484-488.

Barthel A, Stark S, Csuk R, 2008. Oxidative transformations of betulinol. *Tetrahedron*, 64: 9225-9229.

Chinese Pharmacopoeia Commission, 2020. *Pharmacopoeia of the People' s Republic of China (Volume 1)*. Beijing: China Medical Science Press: 242.

Chen XQ, Li Y, Cheng X, et al., 2010. Polycyclic polyprenylated acylphloroglucinols and chromone O-glucosides from *Hypericum henryi* subsp. *uraloides*. *Chem Biodivers*, 7(1): 133-139.

Cheng WY, Zhong FF, Zhao YH, et al., 2008. Study on the antioxidant constituents from the barks of *Garcinia xanthochymus*. *Nat Prod Res Dev*, 20: 836-838, 895.

Cheng M, Meng LJ, Zhou XD, et al., 2014. Chemical constituents of flavonoids and their glycosides in *Melastoma dodecandrum*. *Chin J Chin Mat Med*, 39(17): 3301-3305.

Dao TT, Dang TT, Nguyen PH, et al., 2012. Xanthones from *Polygala karensium* inhibit neuraminidases from influenza A viruses. *Bioorg Med Chem Lett*, 22(11): 3688-3692.

He L, Shi QR, Liu RH, et al., 2008. Anti-inflammatory constituents from the stems of *Daphne genkwa*. *Acad J Second Mili Med Univ*, 29(10): 1221-1226.

Kojima H, Tominaga H, Sato S, et al., 1987. Pentacyclic triterpenoids from *Prunella vulgaris*. *Phytochemistry*, 26(4): 1107-1111.

Li XW, Norman KBR, 2007. *Flora of China*. Beijing: Science Press; St. Louis: Missouri Botanical Garden Press: 13: 2-6, 14-15.

Müller WE, Singer A, Wonnemann M, et al., 1998. Hyperforin represents the neurotransmitter reuptake inhibiting constituent of hypericum extract. *Pharmacopsychiatry*, 31(1): 16-21.

Wong KW, EE GCL, Ismail IS, et al., 2018. Xanthones from stem bark of *Garcinia rostrata*. *Chem Nat Comp*, 54(6): 1160-1163.

Wang XY, 2020. Chemical constituents from *Lobelia chinensis*. *Chin Tradit Pat Med*, 42(12): 3208-3210.

Wang JM, Liu SY, Yang Y, et al., 2021. Chemical constituents from *Poria cocos*. *Chin Tradit Pat Med*, 43(10): 2728-2732.

Xu C, Huang HQ, Lv YB, et al., 2020. Study on inhibiting hepatocarcinoma cells proliferation of petroleum ether extract from *Inula racemosa* by accelerated solvent extraction *in vitro*. *J Huazhong Norm Univ (Nat Sci Ed)*, 54(5): 833-840.

Yin HJ, 2020. Research on the chemical constituents and bioactivities of the fruits of *Garcinia bracteata*. Wuhan: South-Central Minzu University: 20-70.

Yang XW, Li YP, Su J, et al., 2016. Hyperjapones A-E, terpenoid polymethylated acylphloroglucinols from *Hypercium japonicum*. *Org Lett*, 18(8): 1876-1879.

Zhou ZB, Zhang YM, Luo JG, et al., 2016. Cytotoxic polycyclic polyprenylated acylphloroglucinol derivatives and xanthones from *Hypericum attenuatum*. *Phytochem Lett*, 15: 215-219.

Zhong XN, Qtsuka H, Ide T, et al., 1997. Three flavonol glycosides from leaves of *Myrsine seguinii*. *Phytochemistry*, 46(5): 943-946.

Zhuang PY, Fu WW, Tan CH, et al., 2009. Study on the chemical constituents of *Heterostemma alatum* Wight. *Nat Prod Res Dev*, 21: 963-965.

Note: Figure translations are in progress. See original paper for figures.