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Postprint: Chemical Constituents of Stauntonia obovata and Their Inhibitory Activity on Nitric Oxide Production

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Abstract

To identify the anti-inflammatory active constituents in Yangkaikou, this study employed Sephadex LH-20 column chromatography and reversed-phase high-performance liquid chromatography (RP-HPLC) to systematically separate and purify the ethanol extract of Yangkaikou. The structures of the obtained compounds were identified based on comprehensive analysis of mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopic data. Furthermore, the anti-inflammatory activity of the compounds was evaluated using a lipopolysaccharide (LPS)-induced nitric oxide (NO) release model in mouse macrophage RAW 264.7 cells. The results showed that: (1) Nineteen compounds were isolated and identified from Yangkaikou, namely 1,6-di-O-galloyl-β-D-glucoside (1), 3,5-dimethoxy-4-hydroxyphenylglycerol-9-O-β-D-glucopyranoside (2), (-)-epicatechin gallate (3), vanillic acid 4-O-β-D-glucoside (4), myricetin-3-O-β-D-glucoside (5), 6-O-galloylglucose (6), 3 -O-methylellagic acid-4-O-α-L-rhamnoside (7), 3,3 ,4-O-trimethylellagic acid-4-O-α-L-rhamnoside (8), 3,3 ,4-O-trimethylellagic acid-4 -O-rutinoside (9), 3,3 -O-dimethylellagic acid-4-O-β-D-glucoside (10), protocatechuic acid (11), protocatechuic aldehyde (12), β-D-glucopyranosyloxy phenylacetonitrile (13), 1-O-benzoyl inositol (14), 2α-hydroxyursolic acid (15), ursolic acid (16), friedelin (17), α-amyrin (18), and harprogenin-28-β-D-glucopyranosylester (19). Except for compounds 6, 10, and 18, all other compounds were isolated from this plant species for the first time. (2) Compound 12 exhibited significant inhibitory effect on LPS-induced NO release in RAW 264.7 cells, with an IC50 value of 18.2 mol · L-1. This study provides a scientific basis for further investigation of the anti-inflammatory effects of Yangkaikou.

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Full Text

Chemical Constituents from the Roots of *Melastoma normale* and Their Nitric Oxide Inhibitory Activities

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Abstract

To investigate the anti-inflammatory active constituents in Yangkaikou (the roots of *Melastoma normale*), this study systematically isolated and purified the ethanol extract of *M. normale* roots using Sephadex LH-20 column chromatography and reversed-phase high-performance liquid chromatography (RP-HPLC). The structures of the obtained compounds were identified through comprehensive analysis of mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopic data. Additionally, the anti-inflammatory activities of the compounds were evaluated using a lipopolysaccharide (LPS)-induced nitric oxide (NO) release model in murine macrophage RAW 264.7 cells. The results demonstrated that: (1) Nineteen compounds were isolated and identified as 1,6-di-O-galloyl-β-D-glucoside (1), syringylglycerol-9-O-β-D-glucopyranoside (2), (-)-epicatechin gallate (3), vanillic acid 4-O-β-D-glucoside (4), myricetin

3-O-β-D-glucopyranoside (5), 6-O-galloylglucose (6), 3 -O-methylellagic acid 4-O-α-L-rhamnoside (7), 3,3 ,4-tri-O-methylellagic acid 4-O-α-L-rhamnoside (8), 3,3 ,4-tri-O-methylellagic acid 4 -O-rutinoside (9), 3,3 -di-O-methylellagic acid 4-O-β-D-glucoside (10), protocatechuic acid (11), protocatechualdehyde (12), β-D-glucopyranosyloxyphenylacetonitrile (13), 1-O-benzoyl-myo-inositol (14), 2α-hydroxyursolic acid (15), ursolic acid (16), friedelin (17), α-amyrin (18), and harprogenin-28-β-D-glucopyranosylester (19). Except for compounds 6, 10, and 18, all other compounds were isolated from this plant species for the first time. (2) Compound 12 exhibited significant inhibitory activity against LPS-induced NO release in RAW 264.7 cells, with an IC_{50} value of 18.2 $mol \cdot L^{-1}$. This study provides a scientific basis for further investigation of the anti-inflammatory effects of *M. normale*.

Keywords: *Melastoma normale* roots, *Melastoma* L., nitric oxide inhibitory activities, chemical constituents, structural identification

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Introduction

Inflammation is a protective tissue response to infection or injury, during which nitric oxide (NO) acts as a key regulatory factor. However, excessive NO production contributes to the pathogenesis of numerous diseases, including arthritis, tumors, and cardiovascular disorders (Shi et al., 2024). Currently, the most commonly used anti-inflammatory drugs in clinical practice are non-steroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids, but long-term use of these medications can cause severe side effects such as gastrointestinal damage and immunosuppression. Therefore, the development of novel, highly effective, and low-toxicity anti-inflammatory agents holds significant clinical importance. Plant metabolites, with their structural diversity and broad pharmacological activities, represent an important source for drug discovery and lead compound identification.

The genus *Melastoma* (family Melastomataceae) comprises approximately 100 species worldwide, primarily distributed in southern Asia, northern Oceania, and Pacific islands. Chemical constituents of this genus mainly include flavonoids, tannins, organic acids (esters), and terpenoids, which exhibit anti-inflammatory, antimicrobial, antioxidant, hepatoprotective, and hypoglycemic activities (Zheng et al., 2021). *Melastoma normale*, one species within this genus, is widely distributed in Guangxi, Guangdong, Yunnan, Sichuan, and Taiwan provinces of China (Editorial Committee of the Flora of China of Chinese Academy of Sciences, 1984). Its dried roots constitute the traditional Chinese medicine "Yangkaikou." This medicinal material is warm in nature and possesses sweet, sour, and astringent tastes, with functions of astringency, hemostasis, and detoxification. In folk medicine, it is commonly used to treat stranguria, carbuncles, and ulcerated sores (Food and Drug Administration of Guangxi Zhuang Autonomous Region, 2014; Huang, 2015),

and clinically it is primarily employed for acute gastroenteritis, cervicitis, pyelonephritis, and urinary system infections (Jiang et al., 2010). Currently, few studies have reported on the chemical constituents of Yangkaikou, with identified components mainly including terpenoids, sterols, ellagic acids, and gallic acid derivatives (Zou et al., 2011; Tang et al., 2016; Wang, 2018; Zheng et al., 2021), which generally demonstrate promising pharmacological activities such as anti-inflammatory, antitumor, and antimicrobial effects (Wang, 2018; Zheng et al., 2021). Based on the traditional use of Yangkaikou in treating inflammation-related diseases and building upon our previous research (He et al., 2021), this study employed modern separation techniques and spectroscopic methods to isolate and identify chemical constituents from the ethanol extract of Yangkaikou, and evaluated their anti-inflammatory activities using an LPS-induced NO release model in RAW 264.7 cells. The study aimed to address two key questions: (1) What structurally unique constituents remain to be discovered in Yangkaikou? (2) Do these constituents possess anti-inflammatory activity? The findings are expected to further elucidate the pharmacological substance basis of Yangkaikou and provide a scientific foundation for subsequent anti-inflammatory drug development.

Materials and Methods

1.1 Plant Material

The plant material was collected in September 2022 from Yanshan Town, Guilin City, Guangxi, and identified as the roots of *Melastoma normale* by Researcher Huang Yusong. A voucher specimen (No. 20220912) was deposited at the Natural Products Research Center, Guangxi Institute of Botany.

1.2 Instruments and Reagents

The following instruments were used: Avance III HD-500 MHz NMR spectrometer (Bruker, Germany); LC-MS/IT-TOF liquid chromatography-mass spectrometer (Shimadzu, Japan); semi-preparative HPLC system (Sepuruisi, Beijing, China); CO_2 incubator (MCO-15AC, Sanyo, Japan); Spark multifunctional microplate reader (TECAN, Switzerland); Agilent Zorbax SB-C_{18} columns (5 m, 9.4 × 250 mm, flow rate 2 $mL \cdot min^{-1}$ and 5 m, 4.6 × 250 mm, flow rate 0.8 $mL \cdot min^{-1}$, Agilent, USA); Sephadex LH-20 (25–100 m, GE Healthcare Bio-Science AB, Switzerland); MCI gel CHP 20P and Diaion HP20SS (Mitsubishi Chemical, Japan); ODS C_{18} (50 m, Merck, Germany); Toyopearl HW-40F (TOSOH, Japan); silica gel for column chromatography and thin-layer chromatography (Qingdao Marine Chemical Factory). Reagents including petroleum ether, dichloromethane, ethyl acetate, acetone, n-butanol, and methanol were all analytical grade (Xilong Chemical Factory). Lipopolysaccharide (LPS) was purchased from Beijing Solarbio Science & Technology Co., Ltd.; DMEM medium and PBS from Hyclone (USA); NO assay kit from Nan-

jing Jiancheng Bioengineering Institute; fetal bovine serum (FBS) from GIBCO (USA); MTT reagent, positive control dexamethasone, and trypsin from Sigma (USA).

1.3 Experimental Procedures

1.3.1 Extraction and Isolation Nine kilograms of dried sample powder were extracted three times with industrial ethanol at room temperature for seven days each. The filtrates were combined and concentrated under reduced pressure at 45 °C to obtain 560.0 g of crude extract. The extract was suspended in 2 L of hot water and sequentially partitioned three times with petroleum ether and ethyl acetate. After concentration, petroleum ether, ethyl acetate, and aqueous fractions were obtained. The aqueous fraction (205.2 g) was subjected to Sephadex LH-20 column chromatography (10 cm × 40 cm) eluted with CH_3OH-H_2O (0:100$→100 : 0, V/V)toyield11combinedfractions(Fr.1→Fr.11).FractionFr.1(40.0g)wasfurtherseparatedonaDiaionHP2
100→100 : 0, V/V)aseluenttoaffordninesubfractions(Fr.1.1→Fr.1.9).Compound13(tR =
12.5min, 2.2mg)wasobtainedfromFr.1.3(1.5g)byreversed − phasesemi −
preparativeHPLCusingCH{3}OH−H{2}O(10 : 90→20 : 80, V/V)asmobilephase.FractionFr.1.5(12.2g)waso
*phaseC{18}$ column eluted with CH_3OH-H_2O (0:100$→$40:60, V/V) to yield compounds 2 (5.6 mg), 4 (6.1 mg), and 7 (16.3 mg). Fraction Fr.2 (62.0 g) was subjected to Sephadex LH-20 column chromatography (10 cm × 40 cm) with CH_3OH-H_2O (0:100$→*100 : 0, V/V)togivetenfractions(Fr.2.1→Fr.2.10).FractionFr.2.7(10.0g)waspurified
phasesilicagelcolumnchromatographyusingCH{3}OH−H{2}O(0 : 100→50 :
50, V/V)followedbySephadexLH − 20withCH{3}OH−H{2}O(10 : 90→60 :
40, V/V)toobtaincompounds1(7.6mg), 3(6.0mg), 5(6.4mg), 11(5.4mg), and14(5.8mg).FractionFr.2.9(7.8g)was
0→50 : 50, V/V)andthenonSephadexLH − 20withCH{2}Cl{2}−CH_{3}$OH (50:50, V/V) to yield compounds 15 (4.2 mg), 16 (5.4 mg), 17 (4.3 mg), and 18 (5.4 mg).

The petroleum ether fraction (200 g) was subjected to MCI column chromatography eluted with CH_3OH-H_2O (0:100$→100 : 0, V/V)toafford13fractions(Fr.PE1→Fr.PE13).FractionFr.P
*phaseC{18}$ column (6 cm × 25 cm) with CH_3OH-H_2O (0:100$→*100 :
0, V/V)togive16subfractions(Fr.PE6.1→Fr.PE6.16).SubfractionFr.PE6.3(1.54g)waschromatographedon
100→70 : 30, V/V)followedbySephadexLH − 20withCH{3}OH−H{2}O(0 :
100→50 : 50, V/V)toyieldcompounds6(7.0mg), 10(16mg), and12(5.1mg).SubfractionFr.PE6.5(3.3g)waspuri
40FcolumnwithCH{3}OH−H{2}O(0 : 100→70 : 30, V/V)toobtaincompounds8(18.1mg)and9(40.3mg).Subfr
650CcolumnwithCH{3}OH−H_{2}O(0 : 100→$70:30, V/V) to afford compound 19 (29.5 mg).

1.3.2 Acid Hydrolysis of Compounds The absolute configuration of sugars in the compounds was determined following the method of Takashi et al. (2007). Briefly, 1.0 mg of sample was hydrolyzed with 3 mL of 0.5 $mol \cdot L^{-1}$ HCl in a water bath for 1.5 h. The mixture was neutralized with IRA 400 anion exchange resin, filtered, and the filtrate was concentrated to dryness under reduced pressure. The residue was treated with 0.2 mL pyridine containing 1.0 mg L-

cysteine methyl ester hydrochloride at 60 °C for 1 h, followed by addition of 0.2 mL pyridine containing 1.0 mg o-tolyl isothiocyanate and incubation at 60 °C for another 1 h to obtain sugar derivatives. Standard sugar derivatives were prepared similarly. The derivatives were analyzed by HPLC at 40 °C column temperature with 25% acetonitrile–water as mobile phase at a flow rate of 0.8 $mL \cdot min^{-1}$. The absolute configuration was determined by comparing retention times with standard derivatives. Compounds 1, 2, 4, 5, 6, 9, 10, 13, and 19 were found to contain D-glucose (tR = 18.66 min, positive optical rotation), while compounds 7–9 contained L-rhamnose (tR = 29.88 min, negative optical rotation).

1.3.3 NO Inhibition Activity Assay **Cell Culture:** RAW 264.7 cells were cultured in complete medium consisting of high-glucose DMEM supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin-streptomycin at 37 °C in a humidified incubator with 5% CO_2. Cells were passaged every 2–3 days.

Cytotoxicity Assessment: Exponentially growing RAW 264.7 cells were seeded in 96-well plates at a density of 5×10^4 $cells \cdot mL^{-1}$ (100 L per well). After 24 h incubation, cells were treated with test compounds or vehicle control in quadruplicate. Following 24 h treatment, the medium was removed, cells were washed once with PBS, and 100 L fresh medium containing MTT (1 $mg \cdot mL^{-1}$) was added. After 4 h incubation, the supernatant was aspirated, 100 L DMSO was added to each well, and the plates were shaken until formazan crystals dissolved. Absorbance was measured at 570 nm using a microplate reader. Cell viability was calculated as: (average OD of treatment group / average OD of control group) × 100%. Experiments were performed in quadruplicate.

NO Inhibition Assay: Compounds showing no significant effect on cell viability at 10 and 40 $mol \cdot L^{-1}$ were selected for NO inhibition evaluation. RAW 264.7 cells were seeded in 96-well plates and treated with various concentrations of test compounds in the presence of LPS. Blank (DMEM only) and positive control (dexamethasone) groups were included, each with four replicate wells. After 5 h incubation, appropriate reagents were added according to group designations. Following 24 h treatment, the supernatant was collected and NO concentration was determined at 550 nm using a commercial NO assay kit according to the manufacturer' s instructions. NO inhibition rate was calculated as: [OD(LPS) -OD(drug)] / [OD(LPS) -OD(blank)] × 100% (He et al., 2021). Experiments were performed in quadruplicate.

Statistical Analysis: IC_{50} values were calculated using GraphPad Prism 5.0 software based on inhibition rates and drug concentrations. Data are expressed as mean ± standard deviation ($\bar{x} \pm SD$), and statistical significance was determined by t-test.

Results

2.1 Structural Identification

The structures of compounds 1–19 are shown in Figure 1. [Figure 1: see original paper]

Compound 1: Yellow amorphous powder. HR-ESI-MS showed a molecular ion peak at m/z 483.0783 $[M-H]^-$ (calculated molecular weight 483.0775), corresponding to the molecular formula $C_{20}H_{19}O_{14}{}^-$. ^1H NMR (acetone-d_6, 500 MHz) δ: 4.11–3.51 (3H, m, H-glc-2~4), 4.33 (1H, dd, J = 17.7, 5.5 Hz, H-glc-5), 4.56 (1H, dd, J = 12.2, 1.9 Hz, H-glc-6), 5.69 (1H, d, J = 7.9 Hz, H-glc-1), 7.11 (2H, s, H-galloyl-2, 6), 7.15 (2H, s, H-galloyl-2 , 6). ^{13}C NMR (acetone-d_6, 125 MHz) δ: 63.6 (C-glc-6), 69.9 (C-glc-4), 72.7 (C-glc-2), 74.9 (C-glc-5), 76.7 (C-glc-3), 94.8 (C-glc-1), 109.5 (C-galloyl-3, 3 , 5, 5), 120.2 (C-galloyl-1, 1), 138.7 (C-galloyl-4, 4), 145.2 (C-galloyl-2, 2 , 6, 6), 165.6 (C=O), 166.8 (C=O). Acid hydrolysis and HPLC analysis of the sugar derivative confirmed D-glucose by comparison with an authentic standard. These data are consistent with literature reports (Yuan and Sun, 1999; Liu et al., 2017), identifying compound 1 as 1,6-di-O-galloyl-β-D-glucoside.

Compound 2: White powder, $[\alpha]^{25}D$ -6.5 (c 0.55, MeOH). (+)HR-ESI-MS showed a molecular ion peak at m/z 429.1370 $[M+Na]^+$ (calculated molecular weight 429.1373), corresponding to the molecular formula $C_{17}H_{26}O_{11}Na^+$. ^1H NMR (DMSO-d_6, 500 MHz) δ: 6.62 (2H, s, H-2, 6), 4.48 (1H, d, J = 5.2 Hz, H-7), 3.64 (1H, m, H-8), 3.74 (1H, dd, J = 10.4, 3.6 Hz, H-9a), 3.12 (1H, dd, J = 10.4, 6.4 Hz, H-9b), 4.05 (1H, d, J = 7.6 Hz, H-1), 2.97 (1H, dd, J = 8.4, 7.6 Hz, H-2), 3.11 (1H, d, J = 8.4 Hz, H-3), 3.04 (1H, d, J = 8.4 Hz, H-4), 3.04 (1H, m, H-5), 3.62 (1H, m, J = 12.0 Hz, H-6 a), 3.40 (1H, dd, J = 12.0, 5.6 Hz, H-6 b), 3.72 (6H, s, 3,5-OCH_3). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 133.0 (C-1), 104.3 (C-2, 6), 147.4 (C-3, 5), 134.3 (C-4), 72.8 (C-7), 74.0 (C-8), 70.8 (C-9), 103.6 (C-1), 73.6 (C-2), 76.5 (C-3), 70.0 (C-4), 76.8 (C-5), 61.0 (C-6), 55.9 (3,5-OCH_3). Acid hydrolysis confirmed D-glucopyranose. These data are consistent with literature (Gan et al., 2010), identifying compound 2 as 3,5-dimethoxy-4-hydroxyphenylglycerol-9-O-β-D-glucopyranoside.

Compound 3: Brownish-yellow powder, $[\alpha]D$ -162 (c 0.10, CH_3OH). (-)HR-ESI-MS showed a molecular ion peak at m/z 441.0817 $[M-H]^-$ (calculated molecular weight 441.0822), corresponding to the molecular formula $C_{22}H_{17}O_{10}{}^-$. ^1H NMR (DMSO-d_6, 500 MHz) δ: 5.95 (1H, d, J = 1.6 Hz, H-6), 5.82 (1H, d, J = 1.6 Hz, H-8), 6.87 (1H, d, J = 1.4 Hz, H-2), 6.66 (1H, d, J = 7.9 Hz, H-5), 6.73 (1H, dd, J = 7.9, 1.4 Hz, H-6), 6.84 (2H, s, H-2 , 6), 2.66 (1H, d, J = 17.1 Hz, H-4), 2.93 (1H, dd, J = 17.1, 4.6 Hz, H-4), 5.04 (1H, s, H-2), 5.33 (1H, s, H-3). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 166.3 (-COO-), 77.2 (C-2), 68.7 (C-3), 25.5 (C-4), 156.4 (C-5), 98.1 (C-6), 156.9 (C-7), 95.2 (C-8), 155.8 (C-9), 99.9 (C-10), 130.1 (C-1), 114.7 (C-2), 144.5 (C-3), 144.5 (C-4), 118.0 (C-5), 113.8 (C-6), 120.1 (C-1), 108.9 (C-2 , 6), 144.9 (C-3 , 5), 138.4 (C-4). These data are consistent with literature (Zeng et al., 2023), identifying compound 3

as (-)-epicatechin gallate.

Compound 4: Colorless needle crystals, m.p. 137–138 °C. (+)HR-ESI-MS showed a molecular ion peak at m/z 317.0872 $[M+H]^+$ (calculated molecular weight 317.0873), corresponding to the molecular formula $C_{13}H_{17}O_9^+$. ^{1}H NMR (CD_3OD, 500 MHz) δ: 7.63 (1H, d, J = 2.0 Hz, H-2), 7.60 (1H, dd, J = 8.3, 2.0 Hz, H-6), 7.20 (1H, d, J = 8.3 Hz, H-5), 5.02 (1H, d, J = 7.3 Hz, H-1), 3.38–3.55 (4H, m, H-2 , 3 , 4 , 5), 3.69 (1H, dd, J = 11.7, 5.5 Hz, H-6 a), 3.89–3.87 (1H, overlapped, H-6 b). ^{13}C NMR (CD_3OD, 125 MHz) δ: 158.6 (C-1), 113.7 (C-2), 118.8 (C-3), 151.3 (C-4), 119.1 (C-5), 126.9 (C-6), 173.1 (COOH), 103.6 (C-1), 78.1 (C-2), 77.9 (C-3), 71.3 (C-4), 74.9 (C-5), 62.4 (C-6). Acid hydrolysis confirmed D-glucopyranose. These data are consistent with literature (Sakushima et al., 1995; Zhang et al., 2020), identifying compound 4 as vanillic acid 4-O-β-D-glucoside.

Compound 5: Yellow crystals (MeOH), m.p. 194–195 °C. (-)HR-ESI-MS showed a molecular ion peak at m/z 479.0817 $[M-H]^-$ (calculated molecular weight 479.0826), corresponding to the molecular formula $C_{21}H_{19}O_{13}^-$. ^{1}H NMR (CD_3OD, 500 MHz) δ: 12.66 (1H, s, 5-OH), 6.91 (2H, s, H-12, 16), 6.38 (1H, d, J = 1.8 Hz, H-8), 6.20 (1H, d, J = 2.0 Hz, H-6), 5.46 (1H, d, J = 7.5 Hz, H-1). ^{13}C NMR (CD_3OD, 125 MHz) δ: 157 (C-2), 134.6 (C-3), 177.7 (C-4), 161.8 (C-5), 98.7 (C-6), 164.3 (C-7), 92.7 (C-8), 156.3 (C-9), 104.0 (C-10), 120.9 (C-11), 147.1 (C-12, 16), 145.0 (C-13, C-15), 137.2 (C-14), 101.3 (C-1), 74.3 (C-2), 77.2 (C-3), 78.2 (C-5), 61.2 (C-6). Acid hydrolysis confirmed D-glucopyranose. These data are consistent with literature (Zhao et al., 2012; Xia et al., 2019), identifying compound 5 as myricetin-3-O-β-D-glucoside.

Compound 6: White powder. (-)HR-ESI-MS showed a molecular ion peak at m/z 331.0668 $[M-H]^-$ (calculated molecular weight 331.0665), establishing the molecular formula as $C_{13}H_{16}O_{10}^-$. ^{1}H NMR (CD_3OD, 500 MHz) δ: 8.11 (2H, s, H-2, 6), 5.95 (1H, d, J = 3.7 Hz, α-H-glc-1), 3.02–3.13 (4H, m, α-H-glc-2~4, β-H-glc-3), 3.64 (1H, ddd, J = 7.2, 6.2, 3.2 Hz, α-H-glc-5), 5.38 (1H, dd, J = 11.9, 2.0 Hz, α-H-glc-6a), 4.41 (1H, dd, J = 11.9, 5.5 Hz, α-H-glc-6b), 5.15 (1H, d, J = 7.8 Hz, β-H-glc-1), 3.01 (1H, dd, J = 8.8, 7.8 Hz, β-H-glc-2), 3.67 (1H, t, J = 9.3 Hz, β-H-glc-4), 4.26 (1H, ddd, J = 10.0, 4.8, 2.1 Hz, β-H-glc-5), 5.10 (1H, dd, J = 11.9, 2.1 Hz, β-H-glc-6a), 4.87 (1H, dd, J = 11.9, 4.9 Hz, β-H-glc-6b). ^{13}C NMR (CD_3OD, 125 MHz) δ: galloyl: 169.4 (C=O), 147.3 (C-3, 5), 140.2 (C-4), 122.7 (C-1), 110.3 (C-2, 6); glucose: 93.9 (C-1α), 74.2 (C-2α), 75.1 (C-3α), 71.8 (C-4α), 76.6 (C-5α), 64.5 (C-6α), 98.6 (C-1β), 73.8 (C-2β), 78.5 (C-3β), 73.4 (C-4β), 78.2 (C-5β), 62.7 (C-6β). Acid hydrolysis confirmed D-glucopyranose. These data are consistent with literature (Subeki et al., 2005; Si et al., 2023), identifying compound 6 as 6-O-galloyl-D-glucose.

Compound 7: White powder, readily soluble in methanol. (-)HR-ESI-MS showed a molecular ion peak at m/z 461.0725 $[M-H]^-$ (calculated molecular weight 461.0720), suggesting the molecular formula $C_{21}H_{17}O_{12}^-$. ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 7.73 (1H, s, H-5), 7.52 (1H, s, H-5), 4.05 (3H, s, 3-OCH_3), 5.48 (1H, d, J = 1.2 Hz, H-rha-1), 4.70 (1H, dd, J = 4.9 Hz, H-

rha-2), 4.01 (1H, m, H-rha-3), 3.86 (1H, m, H-rha-4), 3.55 (1H, dd, J = 9.3, 6.2 Hz, H-rha-5), 1.14 (3H, d, J = 6.2 Hz, H-rha-6). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 111.9 (C-1), 136.6 (C-2), 140.7 (C-3), 153.1 (C-4), 111.8 (C-5), 113.5 (C-6), 159.3 (C-7), 107.5 (C-1), 147.1 (C-2), 142.2 (C-3), 146.2 (C-4), 112.3 (C-5), 113.6 (C-6), 159.2 (C-7), 61.1 (3 -OCH_3), 100.7 (C-rha-1), 70.7 (C-rha-2), 70.5 (C-rha-3), 72.4 (C-rha-4), 70.4 (C-rha-5), 18.4 (C-rha-6), 61.1 (3 -OCH_3). Acid hydrolysis confirmed L-rhamnose. These data are consistent with literature (Qiu et al., 2021), identifying compound 7 as 3 -O-methylellagic acid 4-O-α-L-rhamnoside.

Compound 8: Light yellow powder. (–)HR-ESI-MS showed a molecular ion peak at m/z 489.1031 [M–H]$^-$ (calculated molecular weight 489.1033), establishing the molecular formula as $C_{23}H_{21}O_{12}^-$. ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 7.51 (1H, s, H-5), 7.78 (1H, s, H-5), 5.57 (1H, d, J = 1.2 Hz, H-1), 4.68 (1H, dd, J = 4.9 Hz, H-2), 4.06 (3H, s, 3-OCH_3), 4.04 (3H, s, 3 -OCH_3), 3.96 (3H, s, 4-OCH_3), 4.01 (1H, m, H-3), 3.86 (1H, m, H-4), 3.51 (1H, m, H-5), 1.13 (3H, d, J = 6.2 Hz, H-6). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 107.0 (C-1), 141.3 (C-2), 140.1 (C-3), 146.4 (C-4), 111.4 (C-5), 112.9 (C-6), 158.5 (C-7), 114.1 (C-1), 136.0 (C-2), 141.7 (C-3), 152.6 (C-4), 111.5 (C-5), 111.2 (C-6), 158.6 (C-7), 60.9 (C-3-OCH_3), 61.0 (C-4-OCH_3), 61.6 (C-3 -OCH_3), 100.1 (C-1), 69.9 (C-2), 70.1 (C-3), 71.8 (C-4), 69.9 (C-5), 17.8 (C-6). Acid hydrolysis confirmed L-rhamnose. These data are consistent with literature (Le et al., 2012), identifying compound 8 as 3,3 ,4-tri-O-methylellagic acid 4 -O-α-L-rhamnoside.

Compound 9: White powder. (+)HR-ESI-MS showed a molecular ion peak at m/z 653.1723 [M+H]$^+$ (calculated molecular weight 653.1718), establishing the molecular formula as $C_{29}H_{32}O_{17}^+$. ^{1}H NMR (Pyridine-d_5, 500 MHz) δ: 7.86 (1H, s, H-5), 8.46 (1H, s, H-5), 3.63 (3H, s, 4-OCH_3), 3.91 (3H, s, 3 -OCH_3), 4.12 (3H, s, 3-OCH_3), 6.34 (1H, d, J = 7.5 Hz, H-glc-1), 5.92 (1H, d, J = 7.5 Hz, H-rha-1), 4.18–4.89 (8H, m, glc-H-2~6, rha-H-2~5), 1.67 (3H, d, J = 6.2 Hz, rha-CH_3). ^{13}C NMR (Pyridine-d_5, 125 MHz) δ: 113.6 (C-1), 141.1 (C-2), 142.1 (C-3), 153.0 (C-4), 111.4 (C-5), 112.9 (C-6), 158.5 (C-7), 114.7 (C-1), 141.7 (C-2), 142.8 (C-3), 154.8 (C-4), 111.5 (C-5), 111.2 (C-6), 158.8 (C-7), 56.4 (C-3-OCH_3), 61.5 (C-4-OCH_3), 62.1 (C-3 -OCH_3), glucose: 102.8 (C-1), 74.7 (C-2), 78.9 (C-3), 73.5 (C-4), 78.3 (C-5), 72.4 (C-6), rhamnose: 101.4 (C-1), 69.9 (C-2), 70.9 (C-3), 71.6 (C-4), 67.7 (C-5), 18.4 (C-6). Acid hydrolysis confirmed D-glucose and L-rhamnose. These data are consistent with literature (Yoshida et al., 1991), identifying compound 9 as 3,3 ,4-tri-O-methylellagic acid 4 -O-rutinoside.

Compound 10: White powder. (+)HR-ESI-MS showed a molecular ion peak at m/z 493.0977 [M+H]$^+$ (calculated molecular weight 493.0982), establishing the molecular formula as $C_{22}H_{20}O_{13}^+$. ^{1}H NMR (Pyridine-d_5, 500 MHz) δ: 8.47 (1H, s, H-5), 8.07 (1H, s, H-5), 5.92 (1H, d, J = 6.1 Hz, H-1), 4.21 (3H, s, 3-OCH_3), 4.28 (3H, s, 3 -OCH_3). ^{13}C NMR (Pyridine-d_5, 125 MHz) δ: 115.4 (C-1), 142.9 (C-2), 143.5 (C-3), 153.2 (C-4), 113.4 (C-5), 113.8 (C-6), 159.6 (C-7), 114.5 (C-1), 141.9 (C-2), 142.4 (C-3), 155.0 (C-4), 112.3 (C-5),

113.6 (C-6), 159.7 (C-7), 62.5 (3-OCH_3), 61.9 (3 -OCH_3), glucose: 103.5 (C-1), 75.4 (C-2), 79.7 (C-3), 71.7 (C-4), 79.1 (C-5), 62.9 (C-6). Acid hydrolysis confirmed D-glucose. These data are consistent with literature (Li et al., 2008; Lü et al., 2020), identifying compound 10 as 3,3 -di-O-methylellagic acid 4-O-β-D-glucoside.

Compound 11: Brown amorphous powder. (–)HR-ESI-MS showed a molecular ion peak at m/z 153.0190 $[M-H]^-$ (calculated molecular weight 153.0188), suggesting the molecular formula $C_7H_5O_4{}^-$. ^1H NMR (DMSO-d_6, 500 MHz) δ: 6.88 (1H, d, J = 8.2 Hz, H-5), 7.39 (1H, dd, J = 8.2, 1.6 Hz, H-6), 7.46 (1H, d, J = 1.6 Hz, H-2). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 170.4 (COOH), 123.5 (C-1), 117.9 (C-2), 146.4 (C-3), 152.3 (C-4), 115.0 (C-5), 124.5 (C-6). These data are consistent with literature (Bai et al., 2024), identifying compound 11 as protocatechuic acid.

Compound 12: Dark yellow powder. (+)HR-ESI-MS showed a molecular ion peak at m/z 139.0394 $[M+H]^+$ (calculated molecular weight 139.0395), corresponding to the molecular formula $C_7H_7O_3{}^+$. ^1H NMR (DMSO-d_6, 500 MHz) δ: 9.65 (1H, s, CHO), 7.19 (1H, d, J = 2.0 Hz, H-2), 6.88 (1H, d, J = 8.0 Hz, H-5), 7.23 (1H, dd, J = 8.0, 2.0 Hz, H-6). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 191.6 (CHO), 129.3 (C-1), 114.9 (C-2), 146.4 (C-3), 152.6 (C-4), 116.0 (C-5), 124.9 (C-6). These data are consistent with literature (Su et al., 2021), identifying compound 12 as 3,4-dihydroxybenzaldehyde, i.e., protocatechualdehyde.

Compound 13: White powder, $[\alpha]D$ -26.0 °C (c 0.5, CH_3OH). (+)HR-ESI-MS showed a molecular ion peak at m/z 296.1125 $[M+H]^+$ (calculated molecular weight 296.1134), corresponding to the molecular formula $C_{14}H_{18}NO_6{}^+$. ^1H NMR (CD_3OD, 500 MHz) δ: 5.92 (1H, s, H-2), 7.5 (5H, m, aromatic protons H-4~8), 4.23 (1H, d, J = 7.3 Hz, H-1), 3.70 (1H, dd, J = 12.0, 5.8 Hz, H-6 α), 3.91 (1H, dd, J = 12.0, 1.8 Hz, H-6 β). ^{13}C NMR (CD_3OD, 125 MHz) δ: 119.4 (C-1), 68.4 (C-2), 134.9 (C-3), 130.1 (C-4, 8), 129.0 (C-5, 7), 131.0 (C-6), 102.0 (C-1), 74.8 (C-2), 78.4 (C-3), 71.5 (C-4), 77.8 (C-5), 62.8 (C-6). Acid hydrolysis confirmed D-glucose. These data are consistent with literature (Nahrstedt et al., 1993), identifying compound 13 as β-D-glucopyranosyloxyphenylacetonitrile.

Compound 14: White powder. (–)HR-ESI-MS showed a molecular ion peak at m/z 281.0816 $[M-H]^-$ (calculated molecular weight 283.0818), establishing the molecular formula as $C_{13}H_{15}O_7{}^-$. ^1H NMR (CD_3OD, 500 MHz) δ: 8.13 (2H, dd, J = 8.3, 1.2 Hz, H-2, 6), 7.60 (1H, m, H-1, 4), 7.48 (2H, t, J = 7.9 Hz, H-3, 5), 4.19 (1H, d, J = 2.3 Hz, H-1), 4.01 (1H, m, H-3), 3.71 (1H, dd, J = 9.6, 3.9 Hz, H-5), 3.50 (1H, d, J = 9.6 Hz, H-4). ^{13}C NMR (CD_3OD, 125 MHz) δ: 130.9 (C-1), 131.6 (C-2, C-6), 129.4 (C-3, C-5), 134.2 (C-4), 167.9 (C-7), 95.6 (C-1), 73.1 (C-2), 72.1 (C-3), 74.1 (C-4), 76.9 (C-5), 71.8 (C-6). These data are consistent with literature (Yutaka et al., 2013), identifying compound 14 as 1-O-benzoyl-myo-inositol.

Compound 15: White powder. Positive Liebermann-Burchard reaction indicated a steroid nucleus. (+)HR-ESI-MS showed a molecular ion peak at m/z

473.3632 $[M+H]^+$ (calculated molecular weight 473.3631), corresponding to the molecular formula $C_{30}H_{49}O_4^+$. ^1H NMR (DMSO-d_6, 500 MHz) δ: 11.92 (1H, brs, COOH), 4.22 (1H, m, H-2), 2.74 (1H, d, J = 9.4 Hz, H-3), 5.14 (1H, brs, H-12), 2.11 (1H, d, J = 10.8 Hz, H-18), 1.05 (3H, s, 23-CH_3), 0.75 (3H, s, 24-CH_3), 0.71 (3H, s, 25-CH_3), 0.88 (3H, s, 26-CH_3), 0.91 (3H, s, 27-CH_3), 0.92 (3H, s, 29-CH_3), 0.82 (3H, d, J = 6.4 Hz, 30-CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 47.4 (C-1), 67.5 (C-2), 82.6 (C-3), 39.2 (C-4), 55.0 (C-5), 18.3 (C-6), 32.9 (C-7), 39.4 (C-8), 47.3 (C-9), 37.9 (C-10), 23.2 (C-11), 124.8 (C-12), 138.5 (C-13), 42.0 (C-14), 27.8 (C-15), 24.1 (C-16), 47.1 (C-17), 52.7 (C-18), 38.8 (C-19), 38.7 (C-20), 30.5 (C-21), 36.6 (C-22), 29.1 (C-23), 16.7 (C-24), 17.2 (C-25), 17.3 (C-26), 23.6 (C-27), 178.5 (C-28), 17.4 (C-29), 21.4 (C-30). These data are consistent with literature (Chang et al., 2023), identifying compound 15 as 2α-hydroxyursolic acid.

Compound 16: Colorless needle crystals (ethyl acetate), m.p. 290–291 °C. (+)HR-ESI-MS showed a molecular ion peak at m/z 457.3686 $[M+H]^+$ (calculated molecular weight 457.3682), establishing the molecular formula as $C_{30}H_{49}O_3^+$. ^1H NMR (DMSO-d_6, 500 MHz) δ: 4.30 (1H, d, J = 4.7 Hz, 3-OH), 2.99 (1H, m, H-3), 5.12 (1H, s, H-12), 2.10 (1H, d, J = 10.8 Hz, H-18), 1.04 (3H, s, 23-CH_3), 0.74 (3H, s, 24-CH_3), 0.67 (3H, s, 25-CH_3), 0.86 (3H, s, 26-CH_3), 0.89 (3H, s, 27-CH_3), 0.91 (3H, s, 29-CH_3), 0.81 (3H, d, J = 6.4 Hz, 30-CH_3), 11.95 (1H, s, COOH). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 38.3 (C-1), 27.1 (C-2), 76.9 (C-3), 38.6 (C-4), 54.8 (C-5), 18.1 (C-6), 32.8 (C-7), 46.9 (C-9), 36.4 (C-10), 22.9 (C-11), 124.6 (C-12), 138.3 (C-13), 41.7 (C-8, 14), 27.6 (C-15), 23.9 (C-16), 47.1 (C-17), 52.4 (C-18), 38.5 (C-19), 38.4 (C-20), 30.2 (C-21), 36.6 (C-22), 28.3 (C-23), 15.3 (C-24), 16.2 (C-25), 17.1 (C-26), 23.3 (C-27), 178.4 (C-28), 16.9 (C-29), 21.1 (C-30). These data are consistent with literature (Zhang and Chuo, 2020), identifying compound 16 as ursolic acid.

Compound 17: White needle crystals, m.p. 264–265 °C, positive Liebermann-Burchard reaction. (+)HR-ESI-MS showed a molecular ion peak at m/z 427.3931 $[M+H]^+$ (calculated molecular weight 427.3940), corresponding to the molecular formula $C_{30}H_{51}O^+$. ^1H NMR ($CDCl_3$, 500 MHz) δ: 1.96 (1H, m), 1.20–1.80 (23H, m), 0.88 (3H, d, J = 6.5 Hz, CH_3-23), 0.72 (3H, s, CH_3-24), 0.87 (3H, s, CH_3-25), 1.00 (6H, s, CH_3-26, 29), 1.05 (3H, s, CH_3-27), 1.18 (3H, s, CH_3-28), 0.95 (3H, s, CH_3-30). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 22.3 (C-1), 41.6 (C-2), 213.4 (C-3), 58.2 (C-4), 42.1 (C-5), 41.2 (C-6), 18.6 (C-7), 53.0 (C-8), 37.4 (C-9), 59.4 (C-10), 35.3 (C-11), 30.0 (C-12), 39.2 (C-13), 38.2 (C-14), 32.4 (C-15), 35.9 (C-16), 30.4 (C-17), 42.7 (C-18), 35.6 (C-19), 28.1 (C-20), 32.7 (C-21), 39.6 (C-22), 14.6 (C-23), 6.8 (C-24), 18.2 (C-25), 17.9 (C-26), 20.2 (C-27), 31.7 (C-28), 35.0 (C-29), 32.0 (C-30). These data are consistent with literature (Hu et al., 2023), identifying compound 17 as friedelin.

Compound 18: White powder. (+)HR-ESI-MS showed a molecular ion peak at m/z 427.3931 $[M+H]^+$ (calculated molecular weight 427.3940), corresponding to the molecular formula $C_{30}H_{51}O^+$. ^1H NMR ($CDCl_3$, 500 MHz) δ: 5.13 (1H, t, J = 3.6 Hz, H-12), 3.22 (1H, dd, J = 11.0, 5.2 Hz, H-3), 1.04 (3H, s, CH_3-23),

1.02 (3H, s, CH_3-24), 1.07 (3H, s, CH_3-25), 0.80 (3H, s, CH_3-26), 0.91 (3H, s, CH_3-27), 0.79 (3H, s, CH_3-28), 0.99 (3H, d, J = 7.0 Hz, CH_3-29), 0.95 (3H, d, J = 7.0 Hz, CH_3-30). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 38.8 (C-1), 27.2 (C-2), 79.0 (C-3), 39.2 (C-4), 55.3 (C-5), 18.4 (C-6), 32.9 (C-7), 40.0 (C-8), 47.7 (C-9), 36.9 (C-10), 23.2 (C-11), 124.4 (C-12), 139.6 (C-13), 42.1 (C-14), 28.5 (C-15), 26.6 (C-16), 33.8 (C-17), 59.1 (C-18), 39.7 (C-19), 39.6 (C-20), 31.3 (C-21), 41.5 (C-22), 28.2 (C-23), 15.6 (C-24), 15.7 (C-25), 16.9 (C-26), 23.3 (C-27), 28.8 (C-28), 17.5 (C-29), 21.4 (C-30). These data are consistent with literature (Liu et al., 2021), identifying compound 18 as α-amyrin.

Compound 19: White powder. (+)HR-ESI-MS showed a molecular ion peak at m/z 687.3711 $[M+Na]^+$ (calculated molecular weight 687.3720), establishing the molecular formula as $C_{36}H_{56}O_{10}Na^+$. ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 1.43 (2H, dd, J = 13.2, 3.0 Hz, H-1), 2.55 (1H, d, J = 13.0 Hz, H-1), 4.48 (2H, dd, J = 8.9, 2.7 Hz, H-2), 4.22 (1H, d, J = 9.0 Hz, H-3), 1.93 (1H, m, H-5), 5.05 (1H, m, H-6), 1.80 (1H, d, J = 13.0 Hz, H-7), 2.34 (1H, m, H-9), 5.94 (1H, d, J = 10.0 Hz, H-11), 6.59 (1H, dd, J = 10.0, 2.3 Hz, H-12), 0.95 (2H, d, J = 12.5 Hz, H-15), 2.67 (2H, m, H-16), 2.19 (2H, m, H-19), 2.22 (1H, m, H-21), 2.69 (1H, m, H-21), 1.28 (1H, m, H-22), 1.67 (1H, m, H-22), 4.12 (1H, d, J = 11 Hz, H-23), 4.40 (1H, d, J = 11 Hz, H-23), 1.74 (3H, s, H-24), 1.64 (3H, s, H-25), 1.61 (3H, s, H-26), 0.96 (3H, s, H-27), 0.84 (3H, s, H-29), 0.81 (3H, s, H-30), 6.20 (1H, d, J = 8.6 Hz, Glc-1), 4.10 (1H, m, Glc-2), 4.24 (1H, t, J = 8.5 Hz, Glc-3), 4.15 (1H, t, J = 8.4 Hz, Glc-4), 3.95 (1H, m, Glc-5), 4.21 (1H, m, J = 9.1 Hz, Glc-6), 4.35 (1H, m, Glc-6). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 36.1 (C-1), 69.2 (C-2), 78.4 (C-3), 44.0 (C-4), 49.1 (C-5), 66.8 (C-6), 41.0 (C-7), 42.6 (C-8), 55.2 (C-9), 38.2 (C-10), 127.6 (C-11), 126.2 (C-12), 137.4 (C-13), 47.5 (C-14), 25.6 (C-15), 49.0 (C-16), 48.0 (C-17), 132.4 (C-18), 32.5 (C-19), 33.0 (C-20), 41.5 (C-21), 37.4 (C-22), 66.4 (C-23), 17.2 (C-24), 21.5 (C-25), 17.9 (C-26), 20.3 (C-27), 176.0 (C-28), 32.1 (C-29), 24.6 (C-30), 96.6 (C-1), 74.4 (C-2), 78.9 (C-3), 71.6 (C-4), 79.7 (C-5), 62.7 (C-6). Acid hydrolysis confirmed D-glucose. These data are consistent with literature (Qi et al., 2010), identifying compound 19 as harprogenin-28-β-D-glucopyranosylester.

Anti-inflammatory Activity

As shown in Table 1, compounds 6–10, 12, and 15 did not affect RAW 264.7 cell viability at test concentrations (10 and 40 $\text{mol} \cdot \text{L}^{-1}$), with survival rates exceeding 95%. Further investigation revealed that compound 12 significantly inhibited LPS-induced NO release in RAW 264.7 cells, with an IC_{50} value of (18.2 ± 0.1) $\text{mol} \cdot \text{L}^{-1}$ [positive control dexamethasone $IC_{50} = (2.5 \pm 0.2)$ $\text{mol} \cdot \text{L}^{-1}$], while other compounds showed no inhibitory activity ($IC_{50} > 40$ $\text{mol} \cdot \text{L}^{-1}$).

Table 1. Inhibitory activity on NO production of compounds 6–10, 12, and 15 in LPS-induced RAW 264.7 cells

Compound	IC_{50} (µmol · L^{-1})	Cell Viability
12	18.2 ± 0.1*	96.2 ± 1.1
Dexamethasone	2.5 ± 0.2**	95.4 ± 1.6
6	>40	96.8 ± 2.6
7	>40	99.1 ± 2.3
8	>40	96.9 ± 1.9
9	>40	97.3 ± 1.5
10	>40	97.6 ± 1.7
15	>40	98.5 ± 1.2

Note: a vs LPS control group: * indicates $P < 0.05$ and ** indicates $P < 0.01$; dexamethasone was used as positive control.

Discussion and Conclusion

This study capitalized on the characteristic abundance of acidic constituents in the *Melastoma* genus by employing separation materials with minimal irreversible adsorption, including Sephadex LH-20, Diaion HP20SS, Toyopearl HW-40F, Toyopearl Butyl-650C, and MCI-gel CHP20, to systematically fractionate the ethanol extract of Yangkaikou. This approach led to the isolation and identification of 12 phenolic compounds, 5 triterpenoids, and 2 other compounds. Among these, compounds 1–5, 7–9, 11–17, and 19 were reported from this plant for the first time, while compounds 6 and 9 represent the first isolation from the *Melastoma* genus. The phenolic constituents identified are consistent with previous HPLC-DAD-ESI-MS/MS analyses of Yangkaikou aqueous extracts, which reported major components including gallic acid derivatives and (epi)catechin derivatives (Tang et al., 2016). These findings expand the compound library for Yangkaikou and provide a material basis for the deep development and utilization of this medicinal resource.

Yangkaikou is widely used in clinical practice for various inflammation-related diseases, which correlates closely with its content of anti-inflammatory active constituents. Previous studies have demonstrated that compound 12 (protocatechualdehyde) possesses notable anti-inflammatory activity, which was further confirmed in this investigation. Its anti-inflammatory mechanism may involve inhibition of interleukin-6 (IL-6), interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) secretion, and NO release (Qiu et al., 2022). Additionally, compound 3 has been reported to exert anti-inflammatory effects primarily by suppressing secretion of inflammatory cytokines including IL-6, IL-1β, and TNF-α; regulating expression of inflammation-related enzymes such as iNOS, NF- B, and COX-2 at both gene and protein levels; and modulating Toll-like receptor (TLR) downstream pathways, Notch signaling, and mitogen-activated protein kinase (MAPK) signaling pathways that mediate inflammatory responses (Wen et al.,

2017). Compound 11 has been shown to inhibit TNF-α, IL-1β, and IL-6 mRNA expression and NO secretion, and to block TNF signaling and its downstream NF- B pathway (Zhang, 2023). Compound 16 can exert anti-inflammatory effects by regulating inflammatory cytokine secretion and modulating TLR4, MAPK, and NF- B signaling pathways, as well as mediating inflammasome formation (Zhang et al., 2024). Compound 17 has been reported to display anti-inflammatory activity through inhibition of NO release (Zhao et al., 2017). The presence of these anti-inflammatory active constituents validates the scientific basis for the traditional and modern clinical applications of Yangkaikou. Although the ellagic acid glycosides 7-10 showed no anti-inflammatory activity in this study, the aglycone ellagic acid has demonstrated anti-inflammatory activity by reducing levels of TNF-α, IL-6, IL-1β, and IL-18, and suppressing expression of inducible nitric oxide synthase (iNOS), cyclooxygenase, NO, and prostaglandin E2 (PGE2) (Xia et al., 2023), suggesting that the sugar moieties do not enhance the anti-inflammatory activity of these compounds. This finding provides direction for future drug development based on this class of constituents.

In summary, this study has further elucidated the chemical constituents and biological activities of Yangkaikou, providing both a material basis and theoretical foundation for the discovery of lead compounds and the development of anti-inflammatory agents.

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