Postprint: Chemical Constituents and Anti-inflammatory Activity of the Aerial Parts of Saposhnikovia divaricata

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Abstract

To investigate the anti-inflammatory active constituents from the aerial parts of Saposhnikovia divaricata, this study employed silica gel and ODS column chromatography methods along with high-performance liquid chromatography techniques to isolate and purify compounds from the 70% ethanol extract. Structural identification of the isolated compounds was performed by combining physicochemical properties with spectroscopic data including 1H-NMR, 13C-NMR, and MS. The inhibitory activity of the compounds against nitric oxide (NO) production was evaluated using a lipopolysaccharide (LPS)-induced RAW264.7 cell model. The results indicated that: (1) Fifteen compounds were isolated from the aerial parts of Saposhnikovia divaricata and identified as gingerglycolipid A (1), (E)-2-hexenyl-O-β-D-glucopyranoside (2), (Z)-3-hexenyl-O-β-D-glucopyranoside (3), n-hexyl-O-β-D-glucopyranoside (4), sachalinoside B (5), 5β,6α-dihydroxy-3β-(β-D-glucopyranosyloxy)-7-megastigmen-9-one (6), phenethyl-β-D-glucopyranoside (7), ethyl gallate (8), vanillic acid (9), grasshopper ketone (10), 2-ethoxy-2-(4-hydroxyphenyl)ethanol (11), 2-methoxy-2-(4 -hydroxyphenyl)ethanol (12), 1,2,3,4,6-penta-O-gally -β-D-glucopyranose (13), (-)-angelicidol-2-O-β-D-apiofuranosyl-(1$→6)— $-D-glucopyranoside (14), and (9Z,12Z)-N-(2-hydroxyethyl)octadeca-9,12-dienamide (15). Among these, compounds 1-5 and 7-10 were isolated from Apiaceae plants for the first time, while compounds 11-15 were discovered from plants of the genus Saposhnikovia for the first time. (2) In vitro anti-inflammatory activity assays of compounds 1-15 demonstrated that compounds 1, 3, 4, 7, 9, 12, and 14 exhibited varying degrees of inhibitory effects on NO release in LPS-induced RAW 264.7 cells.

Full Text

Preamble

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Investigation on Chemical Components and Anti-inflammatory Activities from the Aerial Parts of Saposhnikovia divaricata

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Abstract: To investigate the anti-inflammatory active constituents from the aerial parts of Saposhnikovia divaricata, this study employed silica gel, ODS column chromatography, and high-performance liquid chromatography to isolate and purify compounds from the 70% ethanol extract. The structures of the isolated compounds were identified based on their physicochemical properties and spectroscopic data, including ^{1}H-NMR, ^{13}C-NMR, and MS. Their inhibitory effects on nitric oxide (NO) production were evaluated using a lipopolysaccharide (LPS)-induced RAW264.7 cell model. The results showed that: (1) Fifteen compounds were isolated from the aerial parts of Saposhnikovia divaricata and identified as gingerglycolipid A (1), (E)-2-hexenyl-O-β-D-glucopyranoside (2), (Z)-3-hexenyl-O-β-D-glucopyranoside (3), hexyl-O-β-D-glucopyranoside (4), sachalinoside B (5), 5β,6α-dihydroxy-3β-(β-D-glucopyranosyloxy)-7-megastigmen-9-one (6), phenethyl-β-D-glucopyranoside (7), ethyl gallate (8), vanillic acid (9), grasshopper ketone (10), 2-ethoxy-2-(4-hydroxyphenyl)ethanol (11), 2-methoxy-2-(4 -hydroxyphenyl)ethanol (12), 1,2,3,4,6-penta-O-gally-β-D-glucopyranose (13), (-)-angelica angellinol-2-O-β-D-furan apiosyl-(1$→6)— $-D-glucopyranoside (14), and (9Z,12Z)-N-(2-hydroxyethyl)octadeca-9,12-dienamide (15). Among these, compounds 1–5 and 7–10 were isolated from the Umbelliferae family for the first time, while compounds 11–15 were discovered from the Saposhnikovia genus for the first time. (2) In vitro anti-inflammatory activity assays revealed that compounds 1, 3, 4, 7, 9, 12, and 14 exhibited varying degrees of inhibitory activity against NO release from LPS-induced RAW 264.7 cells.

Keywords: Saposhnikovia divaricata, aerial part, chemical constituents, extraction and separation, structural identification, anti-inflammatory activity

Saposhnikovia divaricata is the dried root of unflowered plants harvested in spring and autumn from the Umbelliferae family. It is mainly distributed in Heilongjiang, Liaoning, Inner Mongolia, and other provinces in China. Accord-

ing to the 2020 edition of the Chinese Pharmacopoeia, it is acrid and sweet in flavor, slightly warm in nature, and enters the Bladder, Liver, and Spleen meridians. It is used for treating common cold with headache, rheumatic arthralgia, rubella with pruritus, and tetanus (Chinese Pharmacopoeia Commission, 2020). Current research on Saposhnikovia divaricata has primarily focused on its root —the medicinal part—with studies identifying chromones, coumarins, polysaccharides, and volatile oils as its main chemical constituents. These compounds exhibit analgesic, anti-inflammatory, antipyretic (Yang et al., 2006), anti-allergic (Wu et al., 2016), anti-tumor (Ding et al., 2020), and antioxidant (Tai et al., 2007) pharmacological effects. However, as a traditional bulk medicinal material in China and a raw material for Chinese patent medicines such as Yupingfeng Powder and Fangfeng Tongsheng Granules, the expanding market demand has led to a sharp decline in resources. Meanwhile, the non-medicinal parts are often discarded as waste during harvesting, resulting in resource wastage. To fully develop and utilize this traditional Chinese medicine resource, enrich its anti-inflammatory substance basis, and alleviate market supply shortages, our research group conducted further studies on the chemical constituents of the aerial parts of Saposhnikovia divaricata. We isolated and identified 15 compounds and evaluated their anti-inflammatory activity using an LPS-induced mouse macrophage RAW264.7 inflammatory response model, with the inhibition rate of NO production in inflammatory cells as the evaluation index. The results showed that compounds 1, 3, 4, 7, 9, 12, and 14 inhibited LPS-induced NO release from RAW 264.7 cells to varying degrees, with compound 4 showing the highest activity. These findings provide a basis for the resource utilization of the non-medicinal parts of Saposhnikovia divaricata and further investigation into its anti-inflammatory mechanism of action.

1 Materials, Instruments, and Reagents

The aerial parts of Saposhnikovia divaricata were collected in Daqing City, Heilongjiang Province, and identified by Associate Professor Fan Ruifeng from the Department of Medicinal Botany, College of Pharmacy, Heilongjiang University of Chinese Medicine, as the dried aerial parts of Saposhnikovia divaricata (Umbelliferae). A voucher specimen (No. 20220812) was deposited in the Department of Chinese Medicinal Chemistry at Heilongjiang University of Chinese Medicine. Murine macrophage RAW 264.7 cells were purchased from the China Center for Type Culture Collection at Wuhan University.

Instruments and reagents included: 2424-2998 analytical HPLC (Waters, USA); CBM-20A refractive index detector (Shimadzu, Japan); Bruker-600 superconducting NMR spectrometer (Bruker, Germany); rotary evaporator (Tokyo Rikakikai, Japan); ultra-high-performance liquid chromatography system (Thermo Fisher Scientific, USA); UHPLC-Orbitrap-MS system (Thermo Fisher Scientific, USA); ELx 800 microplate reader (BioTek, USA); BT 25S electronic analytical balance (Sartorius, Germany); 150i CO_2 incubator (Thermo Fisher Scientific, USA); Vert-A1 fluorescence inverted microscope

(Carl Zeiss, Germany); TDL-4 low-speed centrifuge (Shanghai Anting Scientific Instrument Factory); WT-1ND clean bench (Beijing Wangtang Blue Wing Technology Co., Ltd.); analytical, semi-preparative, and preparative chromatography columns (Waters, USA); silica gel for column chromatography (Qingdao Marine Chemical Factory); TLC silica gel plates (Merck, Germany); dexamethasone (DXMS, Guangdong Nanguo Pharmaceutical Co., Ltd.); fetal bovine serum (FBS, Gibco, USA); DMEM medium (Gibco, USA); penicillin-streptomycin-amphotericin B triple antibiotic (Beyotime, Shanghai); dimethyl sulfoxide (DMSO, Sigma-Aldrich, USA); nitric oxide detection kit (Beyotime, Shanghai); lipopolysaccharide (LPS, Sigma-Aldrich, USA); 96-well plates (Corning, USA); and chromatography-grade chemical reagents (analytical grade, Tianjin Reagent No. 1 Factory).

2.1 Extraction and Separation

Dried aerial parts of Saposhnikovia divaricata (20.0 kg) were extracted twice with 70% ethanol (10-fold volume) by heating reflux for 2 hours each time. The extract was concentrated under reduced pressure using a rotary evaporator to recover the solvent, yielding 6.3 kg of extract with an extraction rate of 31.5%. A portion of the extract (1.3 kg) was dissolved in distilled water and separated using HP-20 macroporous resin to obtain four fractions: 10% ethanol fraction (147.0 g), 50% ethanol fraction (300.0 g), 70% ethanol fraction (330.0 g), and 95% ethanol fraction (23.2 g).

The 50% ethanol eluate (300.0 g) was subjected to silica gel column chromatography with a dichloromethane-methanol gradient (1:0$→0 : 1)$aseluent, yieldingsevenfractions(Fr.A–Fr.G).FractionDwasfurtherseparatedbyODSreversed−phasecolumnchromatographyusingamethanol − watergradient(1 : 9→1 : 0)toobtain15subfractions(Fr.D1–D15).SubfractionFr.D3waspurifiedbysilicagelcolumnchromatographywi methanolsystem(25 : 1→0 : 1)toyieldsevencomponents(Fr.D3 − 1–Fr.D3 − 7).Fr.D3 − 4wasseparatedbysemi − preparativeHPLC(MeOH/H{2}O = 42{2}$O=44%) to yield compounds 8 (5.8 mg) and 10 (10.6 mg).

Fraction F was separated by ODS reversed-phase column chromatography with a methanol-water gradient (1:9$→1 : 0)toobtain59subfractions(Fr.F1–F59).Fr.F12waspurifiedbysemi−preparativeHPLC(MeOH/H{2}O = 38{2}O = 60{2}O = 58→1 : 0)toobtain50subfractions(Fr.G1–G50).Fr.G12waspurifiedbysemi−preparativeHPLC(MeOH/H{2}$O=32 to yield compound 15 (8.6 mg).

The 70% ethanol eluate (295.6 g) was separated by normal-phase silica gel column chromatography using a dichloromethane-methanol gradient (1:0$→0 : 1)asmobilephase.Afterconcentrationbyrotaryevaporation, fractionswerecombinedbasedonTLCanalysisand phasecolumnchromatographywithamethanol − watergradient(1 : 9→1 : 0)toobtaineightsubfractions(Fr.2A–Fr.2F).Fr.2Bwaspurifiedbypreparative HPLCtoyieldcompounds2(5.5m phasecolumnchromatographywithamethanol − watergradient(1 : 9→$1:0) to obtain three subfractions (Fr.3A–Fr.3C). Fr.3A was purified by semi-preparative HPLC to yield compounds 11 (5.2 mg) and 12 (3.9 mg). The structures of

compounds 1–15 are shown in Figure 1 [Figure 1: see original paper].

2.2.1 Preparation of Test Solutions

Compound Stock Solutions: Compounds 1–15 were dissolved in a small amount of DMSO to prepare stock solutions, which were diluted with DMEM medium to the required concentrations before use.

LPS Solution Preparation: In a clean bench sterilized under UV light for 30 minutes, 10 mg of LPS powder was accurately weighed and dissolved to prepare a 1 $mg \cdot mL^{-1}$ stock solution, which was aliquoted and stored at -20 °C. For experiments, the stock solution was diluted to a final concentration of 1 $g \cdot mL^{-1}$.

Positive Control Preparation: Dexamethasone (DXMS, 1.57 mg) was accurately weighed and dissolved in 2 mL DMEM medium containing 1% DMSO to prepare a 2 $mmol \cdot L^{-1}$ positive control stock solution, which was stored at -80 °C. Before use, it was diluted to a working concentration of 10 $mol \cdot L^{-1}$. A DMSO control solution was prepared using the same procedure.

2.2.2 Cell Culture

A frozen vial containing RAW 264.7 cells (1 mL) was thawed in a 37 °C water bath with gentle shaking. The cell suspension was then centrifuged at 1,000 $r \cdot min^{-1}$ for 5 minutes. The supernatant was discarded, and the cells were resuspended in 1 mL DMEM medium supplemented with 10% fetal bovine serum and 1% triple antibiotic (penicillin, streptomycin, amphotericin B). The entire cell suspension was transferred to a T-25 culture flask containing 4 mL pre-warmed medium and incubated overnight at 37 °C in a 5% CO_2 humidified incubator. Cells were passaged when they reached 80–90% confluence. The culture supernatant was discarded, and the cells were washed once with 2 mL sterile, pre-cooled PBS. After gently tapping the flask, 2 mL of cell culture medium was added, and the cells were pipetted to detach. One milliliter of the cell suspension was transferred to a new T-25 flask containing 4 mL pre-warmed medium for continued culture.

2.2.3 Compound Inhibition of NO Production in RAW 264.7 Cells

RAW 264.7 cells were cultured as described in Section 2.2.2 until they reached the logarithmic growth phase. The 96-well plate was divided into blank, model, treatment, and positive control groups, with four replicate wells per group. After seeding, the plate was incubated overnight (37 °C, 5% CO_2). The medium was then removed (except for the blank group), and 100 L DMEM medium containing 1 $g \cdot mL^{-1}$ LPS was added to all wells except the blank group. After 24 hours of incubation, a significant increase in NO secretion was observed, confirming successful establishment of the inflammatory cell model. The blank

and model groups received 100 L DMEM medium, while the treatment groups received 100 L DMEM medium containing compounds 1–15 at various final concentrations (100, 50, 25, 12.5, 6.25, and 3.125 $mol \cdot L^{-1}$). Dexamethasone (DXMS) served as the positive control. After an additional 24 hours of culture, 50 L of supernatant from each well was transferred to a new plate in the dark, and 50 L each of Griess reagent A and B were added. The optical density (OD) of each well was measured at 540 nm. The inhibition rate was calculated using the formula:

NO production inhibition rate = (OD_{model} -OD_{treatment}) / (OD_{model} -OD_{blank}) × 100%

IC_{50} values were calculated using GraphPad Prism Version 9.0 software.

2.2.4 Statistical Analysis

Statistical analysis was performed using GraphPad Prism Version 9.0 software with one-way ANOVA.

3.1 Structural Identification of Compounds

Compound 1 was obtained as a white powder soluble in methanol. Its molecular formula was determined to be $C_{33}H_{56}O_{14}$ with m/z: 675.4 $[M-H]^-$. ^{1}H-NMR (600 MHz, C_5D_5N) δH: 2.31 (2H, t, J = 7.5 Hz, H-2), 1.26 (10H, m, H-3, 4, 5, 6, 7), 1.58 (2H, m, H-8), 5.46 (6H, m, H-9, 10, 12, 13, 15, 16), 2.92 (2H, t, J = 5.5 Hz, H-11), 2.89 (2H, t, J = 5.5 Hz, H-14), 2.06 (2H, m, H-17), 0.92 (3H, t, J = 7.6 Hz, H-18), 4.64 (2H, m, H-1), 3.54 (1H, m, H-2), 4.14 (2H, t, J = 6.7 Hz, H-3), 4.78 (1H, d, J = 7.1 Hz, H-1), 4.32 (1H, dd, J = 10.4, 5.9 Hz, H-2), 4.29 (1H, dd, J = 10.8, 5.7 Hz, H-3), 4.55 (1H, m, H-4), 4.49 (1H, overlap, H-5), 4.36 (1H, dd, J = 10.8, 6.2 Hz, H-6 a), 4.57 (1H, m, H-6 b), 4.78 (1H, d, J = 7.7 Hz, H-1), 4.03 (1H, dd, J = 10.0, 5.1 Hz, H-2), 4.46 (1H, t, J = 4.4 Hz, H-3), 4.52 (1H, overlap, H-4), 4.47 (1H, overlap, H-5), 3.43 (1H, d, J = 15.4 Hz, H-6 a), 4.40 (1H, m, H-6 b). ^{13}C-NMR (150 MHz, C_5D_5N) δC: 173.5 (C-1), 34.1 (C-2), 20.7 (C-3), 25.0 (C-4), 25.7 (C-5), 25.8 (C-6), 27.3 (C-7), 29.3 (C-8), 127.9 (C-9), 128.0 (C-10), 29.2 (C-11, 14), 128.5 (C-12, 13), 130.4 (C-15), 132.0 (C-16), 29.7 (C-17), 14.3 (C-18), 72.0 (C-1), 68.9 (C-2), 66.4 (C-3), 105.5 (C-1), 72.6 (C-2), 74.9 (C-3), 70.4 (C-4), 74.3 (C-5), 68.0 (C-6), 101.0 (C-1), 69.8 (C-2), 72.0 (C-3), 71.5 (C-4), 72.3 (C-5), 62.3 (C-6). These data were consistent with literature values (Lai et al., 2008), leading to the identification of compound 1 as gingerglycolipid A.

Compound 2 was obtained as a colorless syrupy substance soluble in methanol. Its molecular formula was determined to be $C_{12}H_{22}O_6$ with m/z: 261.1 $[M-H]^-$. ^{1}H-NMR (600 MHz, CD_3OD) δH: 4.09 (1H, dd, J = 11.9, 6.8 Hz, H-1a), 4.31 (1H, m, H-1b), 5.60 (1H, m, H-2), 5.74 (1H, m, H-3), 2.03 (2H, q, J = 7.0 Hz, H-4), 1.42 (2H, qt, J = 14.6, 7.3 Hz, H-5), 0.92 (3H, t, J = 7.3 Hz, H-6), 4.31 (1H, d, J = 7.8 Hz, H-1), 3.23 (1H, m, H-2), 3.34 (1H, m, H-3), 3.18 (1H, m,

H-4), 3.28 (1H, t, J = 9.6 Hz, H-5), 3.66 (1H, dd, J = 11.9, 5.7 Hz, H-6 a), 3.86 (1H, dd, J = 11.9, 2.3 Hz, H-6 b). ^{13}C-NMR (150 MHz, CD_3OD) δC: 71.0 (C-1), 127.5 (C-2), 136.0 (C-3), 35.6 (C-4), 23.5 (C-5), 14.1 (C-6), 103.1 (C-1), 75.2 (C-2), 78.3 (C-3), 71.8 (C-4), 78.1 (C-5), 62.9 (C-6). These data were consistent with literature values (Li et al., 2004), leading to the identification of compound 2 as (E)-2-hexenyl-O-β-D-glucopyranoside.

Compound 3 was obtained as colorless needle-like crystals soluble in methanol. Its molecular formula was determined to be $C_{11}H_{19}O_6$ with m/z: 285.1 $[M+Na]^+$. ^{1}H-NMR (600 MHz, C_5D_5N) δH: 3.69 (1H, dd, J = 16.4, 7.6 Hz, H-1a), 4.09 (1H, dd, J = 16.4, 7.6 Hz, H-1b), 2.44 (2H, q, J = 7.1 Hz, H-2), 5.41 (1H, m, H-3), 5.46 (1H, m, H-4), 1.93 (2H, m, H-5), 0.83 (3H, t, J = 7.5 Hz, H-6), 4.86 (1H, d, J = 7.7 Hz, H-1), 4.04 (1H, m, H-2), 4.27 (1H, m, H-3), 3.94 (1H, m, H-4), 4.24 (1H, m, H-5), 4.39 (1H, dd, J = 11.8, 5.3 Hz, H-6 a), 4.55 (1H, d, J = 11.8 Hz, H-6 b). ^{13}C-NMR (150 MHz, C_5D_5N) δC: 69.3 (C-1), 28.3 (C-2), 125.4 (C-3), 133.4 (C-4), 20.7 (C-5), 14.3 (C-6), 104.6 (C-1), 75.1 (C-2), 78.5 (C-3), 71.6 (C-4), 78.4 (C-5), 62.7 (C-6). These data were consistent with literature values (Lee et al., 2008), leading to the identification of compound 3 as (Z)-3-hexenyl-O-β-D-glucopyranoside.

Compound 4 was obtained as a colorless syrupy substance soluble in methanol. Its molecular formula was determined to be $C_{12}H_{24}O_6$ with m/z: 263.2 $[M-H]^-$. ^{1}H-NMR (600 MHz, CD_3OD) δH: 3.53 (1H, dt, J = 9.5, 6.8 Hz, H-1a), 3.90 (1H, d, J = 9.5, 6.8 Hz, H-1b), 1.62 (2H, m, H-2), 1.39 (2H, m, H-3), 1.34 (2H, m, H-4), 1.31 (2H, m, H-5), 0.91 (3H, t, J = 6.8 Hz, H-6), 4.24 (1H, d, J = 7.8 Hz, H-1), 3.17 (1H, dd, J = 9.1, 7.8 Hz, H-2), 3.35 (1H, m, H-3), 3.30 (1H, m, H-4), 3.26 (1H, m, H-5), 3.67 (1H, dd, J = 11.9, 5.4 Hz, H-6 a), 3.86 (1H, dd, J = 11.9, 2.1 Hz, H-6 b). ^{13}C-NMR (150 MHz, CD_3OD) δC: 70.9 (C-1), 30.8 (C-2), 26.8 (C-3), 32.9 (C-4), 23.7 (C-5), 14.4 (C-6), 104.4 (C-1), 75.1 (C-2), 78.1 (C-3), 71.7 (C-4), 77.9 (C-5), 62.8 (C-6). These data were consistent with literature values (Li et al., 2004), leading to the identification of compound 4 as hexyl-O-β-D-glucopyranoside.

Compound 5 was obtained as a white powder soluble in methanol. Its molecular formula was determined to be $C_{16}H_{28}O_7$ with m/z: 331.2 $[M-H]^-$. ^{1}H-NMR (600 MHz, CD_3OD) δH: 4.98 (1H, dd, J = 10.9, 1.1 Hz, H-1a), 5.22 (1H, br.d, J = 17.4 Hz, H-1b), 6.00 (1H, dd, J = 17.4, 10.9 Hz, H-2), 4.08 (1H, t, J = 7.0 Hz, H-4), 1.88 (2H, m, H-5), 1.81 (2H, m, H-6), 1.23 (3H, s, H-8), 1.26 (3H, s, H-9), 1.29 (3H, s, H-10), 4.57 (1H, d, J = 7.8 Hz, H-1), 3.15 (1H, t, J = 7.8 Hz, H-2), 3.37 (1H, m, H-4), 3.65 (1H, dd, J = 11.9, 5.5 Hz, H-6 a), 3.82 (1H, dd, J = 11.9, 2.1 Hz, H-6 b). ^{13}C-NMR (150 MHz, CD_3OD) δC: 111.9 (C-1), 145.4 (C-2), 80.0 (C-3), 85.2 (C-4), 38.7 (C-5), 28.1 (C-6), 84.4 (C-7), 22.7 (C-8), 25.9 (C-9), 23.4 (C-10), 98.4 (C-1), 75.4 (C-2), 77.7 (C-3), 71.7 (C-4), 78.1 (C-5), 62.8 (C-6). These data were consistent with literature values (Fan et al., 2001), leading to the identification of compound 5 as sachalinoside B.

Compound 6 was obtained as a light yellow solid soluble in methanol. Its molecular formula was determined to be $C_{19}H_{32}O_9$ with m/z: 409.2 [M+Na-

$H_2O]^+$. ^{1}H-NMR (600 MHz, CD_3OD) δH: 1.40 (1H, dd, J = 13.1, 10.1 Hz, H-2a), 1.76 (1H, ddd, J = 13.1, 3.3, 1.2 Hz, H-2b), 3.93 (1H, m, H-3), 1.80 (1H, dd, J = 14.9, 8.5 Hz, H-4a), 2.42 (1H, ddd, J = 14.6, 5.0, 1.1 Hz, H-4b), 7.19 (1H, d, J = 15.8 Hz, H-7), 6.19 (1H, d, J = 15.8 Hz, H-8), 2.29 (3H, s, H-10), 0.96 (3H, s, H-11), 1.21 (3H, s, H-12), 1.19 (3H, s, H-13), 4.34 (1H, d, J = 7.8 Hz, H-1), 3.13 (1H, m, H-2), 3.36 (1H, m, H-3), 3.30 (2H, m, H-4 , 5), 3.67 (1H, dd, J = 11.9, 5.3 Hz, H-6 a), 3.90 (1H, dd, J = 11.9, 1.9 Hz, H-6 b). ^{13}C-NMR (150 MHz, CD_3OD) δC: 36.1 (C-1), 45.4 (C-2), 72.9 (C-3), 38.3 (C-4), 68.5 (C-5), 71.3 (C-6), 145.5 (C-7), 134.0 (C-8), 200.4 (C-9), 27.6 (C-10), 25.6 (C-11), 29.6 (C-12), 20.3 (C-13), 103.1 (C-1), 75.3 (C-2), 78.0 (C-3), 71.8 (C-4), 78.3 (C-5), 62.9 (C-6). These data were consistent with literature values (Zhang et al., 2012), leading to the identification of compound 6 as 5β,6α-dihydroxy-3β-(β-D-glucopyranosyloxy)-7-megastigmen-9-one.

Compound 7 was obtained as a white powder soluble in methanol. Its molecular formula was determined to be $C_{14}H_{20}O_6$ with m/z: 329.1 $[M+COOH]^-$. ^{1}H-NMR (600 MHz, CD_3OD) δH: 7.26 (4H, m, J = 4.4 Hz, H-2, 3, 5, 6), 7.17 (1H, m, H-4), 2.94 (2H, m, H-7), 3.75 (1H, m, H-8a), 4.09 (1H, m, H-8b), 4.30 (1H, d, J = 8.0 Hz, H-1), 3.18 (1H, t, J = 8.0 Hz, H-2), 3.28 (2H, m, H-3 , 5), 3.30 (1H, overlap, H-4), 3.66 (1H, dd, J = 12.0, 5.2 Hz, H-6 a), 3.86 (1H, dd, J = 12.0, 1.7 Hz, H-6 b). ^{13}C-NMR (150 MHz, CD_3OD) δC: 140.1 (C-1), 130.0 (C-2, 6), 129.3 (C-3, 5), 127.2 (C-4), 37.2 (C-7), 71.7 (C-8), 104.4 (C-1), 75.1 (C-2), 78.1 (C-3), 71.7 (C-4), 78.0 (C-5), 62.8 (C-6). These data were consistent with literature values (Zhang et al., 2013), leading to the identification of compound 7 as phenethyl-β-D-glucopyranoside.

Compound 8 was obtained as white needle-like crystals soluble in methanol, with a melting point of 140–142 °C. Its molecular formula was determined to be $C_9H_{10}O_5$ with m/z: 197.1 $[M-H]^-$. ^{1}H-NMR (600 MHz, CD_3OD) δH: 7.05 (2H, s, H-6), 4.28 (2H, q, J = 7.2 Hz, H-8), 1.34 (3H, t, J = 7.2 Hz, H-9). ^{13}C-NMR (150 MHz, CD_3OD) δC: 168.5 (C-1), 146.5 (C-2, 3), 139.7 (C-4), 121.8 (C-5), 110.0 (C-6, 7), 61.7 (C-8), 14.6 (C-9). These data were consistent with literature values (Zhou et al., 2007), leading to the identification of compound 8 as ethyl gallate.

Compound 9 was obtained as white needle-like crystals soluble in methanol, with a melting point of 210–212 °C. Its molecular formula was determined to be $C_8H_8O_4$ with m/z: 167.0 $[M-H]^-$. ^{1}H-NMR (600 MHz, CD_3OD) δH: 7.55 (1H, d, J = 1.9 Hz, H-2), 6.84 (1H, d, J = 8.7 Hz, H-5), 7.56 (1H, dd, J = 8.7, 1.9 Hz, H-6), 3.89 (3H, s, OCH_3). ^{13}C-NMR (150 MHz, CD_3OD) δC: 123.0 (C-1), 113.8 (C-2), 152.6 (C-3), 148.6 (C-4), 115.8 (C-5), 125.2 (C-6), 56.4 (OCH_3), 170.1 (CO). These data were consistent with literature values (Prachayasittikul et al., 2009), leading to the identification of compound 9 as vanillic acid.

Compound 10 was obtained as a white amorphous powder soluble in methanol. Its molecular formula was determined to be $C_{13}H_{20}O_3$ with m/z: 247.1 $[M+Na]^+$. ^{1}H-NMR (600 MHz, CD_3OD) δH: 1.93 (1H, m, H-2a), 1.41 (1H, m, H-2b), 4.21 (1H, m, H-3), 2.21 (1H, overlap, H-4a), 1.34 (1H, m, H-4b),

5.82 (1H, s, H-8), 2.19 (3H, s, H-10), 1.15 (3H, s, H-11), 1.38 (6H, s, H-12, 13). ^{13}C-NMR (150 MHz, CD_3OD) δC: 37.0 (C-1), 49.9 (C-2), 64.4 (C-3), 49.7 (C-4), 72.4 (C-5), 119.9 (C-6), 211.5 (C-7), 101.1 (C-8), 200.8 (C-9), 26.5 (C-10), 29.3 (C-11), 32.3 (C-12), 30.8 (C-13). These data were consistent with literature values (Ren et al., 2013), leading to the identification of compound 10 as grasshopper ketone.

Compound 11 was obtained as a colorless oily substance soluble in methanol, with a melting point of 99–100 °C. Its molecular formula was determined to be $C_{10}H_{14}O_3$ with m/z: 181.1 $[M-H]^-$. ^{1}H-NMR (600 MHz, CD_3OD) δH: 3.62 (1H, dd, J = 11.6, 8.1 Hz, H-1b), 3.49 (1H, J = 11.6, 4.0 Hz, H-1a), 4.27 (1H, dd, J = 8.1, 4.0 Hz, H-2), 6.76 (2H, d, J = 8.4 Hz, H-4, 8), 7.13 (2H, d, J = 8.4 Hz, H-5, 7), 3.40 (2H, d, J = 7.0 Hz, H-1), 1.16 (3H, t, J = 7.0 Hz, H-2). ^{13}C-NMR (150 MHz, CD_3OD) δC: 67.8 (C-1), 84.0 (C-2), 131.6 (C-3), 116.2 (C-4, 8), 131.6 (C-5), 158.2 (C-6), 129.2 (C-7), 65.1 (C-1), 15.5 (C-2). These data were consistent with literature values (Wei et al., 2016), leading to the identification of compound 11 as 2-ethoxy-2-(4-hydroxyphenyl)ethanol.

Compound 12 was obtained as an amorphous powder soluble in methanol. Its molecular formula was determined to be $C_9H_{12}O_3$ with m/z: 191.1 $[M+Na]^+$. ^{1}H-NMR (600 MHz, CD_3OD) δH: 3.49 (1H, dd, J = 11.6, 3.8 Hz, H-1a), 3.62 (1H, dd, J = 11.6, 8.3 Hz, H-1b), 4.16 (1H, dd, J = 8.3, 3.8 Hz, H-2), 7.12 (2H, d, J = 8.5 Hz, H-2 , 6), 6.77 (2H, d, J = 8.5 Hz, H-3 , 5), 3.23 (3H, s, 2-OCH_3). ^{13}C-NMR (150 MHz, CD_3OD) δC: 67.8 (C-1), 86.0 (C-2), 130.9 (C-1), 129.3 (C-2), 116.8 (C-3), 158.4 (C-4), 116.2 (C-5), 129.3 (C-6), 56.8 (2-OCH_3). These data were consistent with literature values (Matsumura et al., 2002), leading to the identification of compound 12 as 2-methoxy-2-(4 -hydroxyphenyl)ethanol.

Compound 13 was obtained as a light brown powder soluble in methanol, with a melting point of 203–204 °C. Its molecular formula was determined to be $C_{41}H_{32}O_{26}$ with m/z: 963.1 $[M+Na]^+$. ^{1}H-NMR (600 MHz, CD_3OD) δH: 6.24 (1H, d, J = 8.3 Hz, H-1), 5.59 (1H, overlap, H-2), 5.62 (1H, t, J = 9.6 Hz, H-3), 5.62 (1H, overlap, H-4), 4.41 (1H, overlap, H-5), 4.52 (1H, br.d, J = 10.9 Hz, H-6), 5.91 (1H, t, J = 10.9 Hz, H-6), 7.12, 7.06, 6.99, 6.96, 6.91 (10H, s, H-2 , 6 , 2 , 6 , 2 , 6 , 2 , 6 , 2 , 6). ^{13}C-NMR (150 MHz, CD_3OD) δC: 167.9 (1 -CO), 167.3 (1 -CO), 167.0 (1 -CO), 166.9 (1 -CO), 166.2 (1 -CO), 121.0 (C-1), 120.4 (C-1), 120.2 (C-1), 120.2 (C-1), 119.7 (C-1), 110.6 (C-2 , 6), 110.5 (C-2 , 6), 110.4 (C-2 , 6), 110.4 (C-2 , 6), 110.4 (C-2 , 6), 146.5 (C-3 , 5 , C-3 , 5), 146.5 (C-3 , 5), 146.4 (C-3 , 5), 146.3 (C-3 , 5), 140.8 (C-4), 140.3 (C-4), 140.3 (C-4), 140.1 (C-4), 140.0 (C-4), 93.8 (C-1), 72.2 (C-2), 74.1 (C-3), 69.8 (C-4), 74.4 (C-5), 63.1 (C-6). These data were consistent with literature values (Cui et al., 2002), leading to the identification of compound 13 as 1,2,3,4,6-penta-O-gallyl-β-D-glucopyranose.

Compound 14 was obtained as a white solid soluble in methanol. Its molecular formula was determined to be $C_{21}H_{36}O_{11}$ with m/z: 465.2 $[M+H]^+$. ^{1}H-NMR (600 MHz, CD_3OD) δH: 4.00 (1H, m, H-2), 1.03 (1H, dd, J = 13.6, 2.6 Hz, H-3),

2.22 (1H, m, H-3), 1.71 (1H, d, J = 4.9 Hz, H-4), 3.87 (1H, dd, J = 7.6, 2.9 Hz, H-5), 2.49 (1H, dd, J = 13.2, 8.0 Hz, H-6), 1.34 (1H, brd, J = 13.5 Hz, H-6), 0.87 (3H, s, H-8), 1.10 (3H, s, H-9), 0.95 (3H, s, H-10), 4.21 (1H, d, J = 7.8 Hz, H-1), 3.16 (1H, t, J = 8.1 Hz, H-2), 3.30 (1H, m, H-3), 3.25 (1H, m, H-4), 3.26 (1H, m, H-5), 3.95 (1H, m, H-6), 3.62 (1H, dd, J = 11.2, 6.1 Hz, H-6), 5.02 (1H, d, J = 1.8 Hz, H-1), 3.90 (1H, d, J = 1.8 Hz, H-2), 3.77 (1H, d, J = 9.6 Hz, H-4), 3.95 (1H, d, J = 9.6 Hz, H-4), 3.59 (2H, s, H-5). ^{13}C-NMR (150 MHz, CD_3OD) δC: 48.8 (C-1), 83.5 (C-2), 34.5 (C-3), 53.6 (C-4), 75.9 (C-5), 39.7 (C-6), 51.1 (C-7), 20.4 (C-8), 21.4 (C-9), 13.4 (C-10), 103.1 (C-1), 75.1 (C-2), 76.8 (C-3), 71.8 (C-4), 78.2 (C-5), 68.4 (C-6), 110.9 (C-1), 78.0 (C-2), 80.6 (C-3), 75.0 (C-4), 65.8 (C-5). These data were consistent with literature values (Liu et al., 2021), leading to the identification of compound 14 as (-)-angelica angellinol-2-O-β-D-furan apiosyl-(1$→6)− $-D-glucopyranoside.

Compound 15 was obtained as a colorless oily substance soluble in methanol, with a melting point of 38–40 °C. Its molecular formula was determined to be $C_{20}H_{37}NO_2$ with m/z: 322.3 $[M-H]^-$. ^{1}H-NMR (600 MHz, CD_3OD) δH: 2.39 (2H, t, J = 8.8 Hz, H-2), 1.80 (2H, m, H-3), 1.24 (2H, m, H-4), 1.25 (2H, m, H-5), 1.26 (2H, m, H-6), 1.28 (2H, m, H-7), 2.06 (4H, m, H-8, 14), 5.32 (1H, m, H-9), 5.34 (1H, m, H-10), 2.90 (2H, m, H-11), 5.47 (2H, m, H-12, 13), 1.30 (2H, m, H-15), 1.32 (2H, m, H-16), 1.33 (2H, m, H-17), 0.89 (3H, t, J = 7.0 Hz, H-18), 3.47 (2H, m, H-1), 3.75 (2H, m, H-2). ^{13}C-NMR (150 MHz, CD_3OD) δC: 173.4 (C-1), 26.2 (C-2), 36.6 (C-3), 29.3 (C-4), 29.5 (C-5), 29.5 (C-6), 29.8 (C-7), 27.4 (C-8), 130.3 (C-9), 128.3 (C-10), 25.9 (C-11), 128.3 (C-12), 130.3 (C-13), 27.4 (C-14), 29.6 (C-15), 31.6 (C-16), 22.7 (C-17), 14.1 (C-18), 43.0 (C-1), 61.6 (C-2). These data were consistent with literature values (Yang et al., 2021), leading to the identification of compound 15 as (9Z,12Z)-N-(2-hydroxyethyl)octadeca-9,12-dienamide.

3.2 Anti-inflammatory Activity

After LPS stimulation, NO production in RAW 264.7 cells increased significantly compared to the unstimulated blank group, confirming successful establishment of the inflammatory cell model. When the isolated compounds were administered, those with IC_{50} values greater than 50 $mol \cdot L^{-1}$ were considered to have no NO production inhibitory activity. The screening results demonstrated that compounds 1, 3, 4, 7, 9, 12, and 14 exhibited varying degrees of inhibitory activity against LPS-induced NO release from RAW 264.7 cells, showing promising in vitro anti-inflammatory effects. Among them, compound 4 showed the strongest inhibitory effect on inflammatory factors. The detailed experimental results are presented in Table 1 .

4 Discussion and Conclusion

Saposhnikovia divaricata is a traditional Chinese medicinal herb with functions of dispelling wind and relieving exterior symptoms, overcoming dampness, and

stopping pain and convulsions. Current research has primarily focused on its medicinal parts, with coumarins, polysaccharides, and chromones identified as the main active components responsible for its analgesic, anti-inflammatory, antipyretic, and anti-allergic pharmacological effects (Cao et al., 2021). However, studies on the chemical constituents of its non-medicinal parts remain limited. This study investigated the chemical components of the aerial parts of Saposhnikovia divaricata, isolating and identifying 15 compounds. Among them, compounds 1–4 are fatty glycosides, compounds 8, 9, 11, 12, and 13 are phenolics, compounds 5, 6, 10, and 14 are monoterpenoids, compound 7 is an aromatic glycoside, and compound 15 is an alkaloid. Compounds 1–5 and 7–10 were isolated from the Umbelliferae family for the first time, while compounds 11–15 were discovered from the Saposhnikovia genus for the first time.

Inflammation is a defensive response that occurs when animal tissues are damaged by various inflammatory factors, representing a pathological process where damage and anti-damage coexist. The pathogenesis of inflammation is associated with excessive secretion of pro-inflammatory factors such as NO, IL-6, and IL-1β, and inhibiting their production represents an important therapeutic strategy. RAW 264.7 cells are a commonly used cell line widely applied in studies evaluating the activity of various compounds, extracts, and drugs, as well as investigating signal pathway regulation mechanisms. This study employed an LPS-induced RAW 264.7 cell model to evaluate the in vitro anti-inflammatory activity of the isolated compounds. The results showed that compounds 1, 3, 4, 7, 9, 12, and 14 inhibited LPS-induced NO release from RAW 264.7 cells to varying degrees, with compound 4 (a fatty glycoside) exhibiting the highest activity ($IC_{50} = 9.48 \pm 1.68$ $mol \cdot L^{-1}$). These findings suggest that the aerial parts of Saposhnikovia divaricata hold promise for treating inflammatory diseases, though the underlying anti-inflammatory mechanisms require further investigation. Additionally, previous studies have reported that ethyl gallate (8) exhibits weak antibacterial activity against Bacillus subtilis with a MIC of 1.00 $mg \cdot mL^{-1}$ (Zhou et al., 2007), and compound (9Z,12Z)-N-(2-hydroxyethyl)octadeca-9,12-dienamide (15) possesses strong antifungal activity (Yang et al., 2021).

In summary, this systematic chemical investigation elucidates the material basis of the aerial parts of Saposhnikovia divaricata and expands our understanding of its chemical constituents. The pharmacological activity studies provide a foundation for further investigation into the anti-inflammatory mechanisms of these compounds and offer scientific reference for exploring and developing the medicinal value of Saposhnikovia divaricata.

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