Postprint: Chemical Constituents and Anti-inflammatory Activity of Rosa roxburghii Roots and Rhizomes from Ethnomedicine

Authors: Liang Yong, Li Liangqun, Wang Li, Zhou Lang, Yang Xiaosheng

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Abstract

The roots and stems of Rosa roxburghii Tratt., an ethnic medicine, have been widely used in minority regions of Guizhou. To verify the anti-inflammatory activity of its chemical components, this study used fresh roots and stems of this ethnic medicine as raw material, employing silica gel column chromatography, Sephadex LH-20 column chromatography, and other methods to separate and purify the chemical constituents of its roots and stems, with compound structures identified through physicochemical properties and spectroscopic data such as NMR; using lipopolysaccharide (LPS)-induced mouse macrophage RAW264.7 cells as an inflammatory model to investigate the effects of chemical constituents from Rosa roxburghii roots and stems on NO inflammatory factor produced by macrophages after LPS stimulation, and to evaluate their anti-inflammatory activity. The results showed that: (1) A total of 15 compounds were isolated from the ethanol extract of Rosa roxburghii roots and stems, and their structures were identified as rosaside (1), rosamultin (2), euscaphic acid (3), β-D-glucopyranosyl-(2a\$→1b) − 2a − O− −L − $arabinopyranosyl$ − (2b→1c) − 2b − O− −L − $arabinopyranosyl$ − (2c→1d) − 2c − O− −L − $arabinopyranosyl$ − (2d→1e) − 2d − O− −L − $arabinopyranosyl$ − (2e→1f) − 2e − O− −L − $arabinopyranoside$(4), $catechin$(5), 3 − O − $methylellagicacid$ − 4′ − O− −D − $xylopyranoside$(6), 3 − O − $methylellagicacid$ − 4′ − O− \$-L-rhamnopyranoside (7), tormentic acid (8), betulinic acid (9), spinosic acid (10), arjunic acid (11), β-sitosterol (12), β-daucosterol (13), α-tocopherol (14), and n-hexacosane (15). Among them, compounds 4, 6, and 7 were isolated from this plant for the first time. (2) In vitro anti-inflammatory activity experiments were conducted on compounds 1-7, and the results revealed that compounds 1-7 significantly inhibited NO release from LPS-induced mouse macrophage RAW264.7 cells in a dose-dependent manner; compounds 1-7 exhibited good anti-inflammatory activity with IC50 values of 25.07, 24.56, 17.65, 9.80, 16.67, 40.83, and 34.98 mol · L-1, respectively (positive control dexamethasone

22.46 mol · L-1), among which compounds 3, 4, and 5 showed superior activity compared to dexamethasone. The experimental results elucidated that triterpenoids, ellagic acid derivatives, flavonoids, and oligosaccharides in Rosa roxburghii roots and stems are the main active constituents responsible for its anti-inflammatory effects, and validated the folk anti-inflammatory efficacy of Rosa roxburghii roots and stems.

Full Text

Chemical Constituents and Their Anti-Inflammatory Activities from the Rhizome of Ethnomedicine *Rosa roxburghii*

Yong Liang[1,2,3], Liangqun Li[1,2], Li Wang[1,2], Lang Zhou[1,2], Xiaosheng Yang[1,2*]

1. State Key Laboratory of Functions and Applications of Medicinal Plants, Guizhou Medical University, Guiyang 550002, China

2. The Key Laboratory of Chemistry for Natural Product of Guizhou Province and Chinese Academy of Sciences, Guiyang 550014, China

3. School of Pharmacy, Guizhou Medical University, Guiyang 550025, China

Abstract: The rhizome of *Rosa roxburghii*, an ethnic medicine widely used in minority regions of Guizhou, has long been valued for its anti-inflammatory properties. This study investigated the chemical constituents and anti-inflammatory activities of fresh *R. roxburghii* rhizome. Chemical constituents were isolated and purified using silica gel column chromatography and Sephadex LH-20 column chromatography, and their structures were identified through physicochemical properties and spectroscopic data including NMR. An in vitro inflammatory model was established using lipopolysaccharide (LPS)-induced mouse macrophage RAW264.7 cells to evaluate the effects of these constituents on NO production and assess their anti-inflammatory activity. The results demonstrated that: (1) Fifteen compounds were isolated from the ethanol extract of *R. roxburghii* rhizome and identified as kaji-ichigoside F1 (1), rosamultin (2), euscaphic acid (3), β-D-glucopyranosyl-(2a$\rightarrow$1b) $-$ 2a $-$ $O-$ $-L-$ *arabinopyranosyl* $-$ (2$b$$\rightarrow$1$c$) $-$ 2$b-O-$ $-L-$*arabinopyranosyl*$-$(2$c$$\rightarrow$1$d$)$-2c-O-$ $-L-$*arabinopyranosyl*$-$ (2$d$$\rightarrow$1$e$) $-$ 2d $-$ $O-$ $-L$ $-$ *arabinopyranosyl* $-$ (2$e$$\rightarrow$1$f$) $-$ 2e $-$ $O-$ $-L$ $-$ *arabinopyranoside*(4), *catechin*(5), $3-O-$*methylellagicacid*$-4'-O-$ $-D-$ *xylopyranoside*(6), $3-O-$*methylellagicacid*$-4'-O-$ $-$ $-L-rhamnopyranoside (7), tormentic acid (8), betulinic acid (9), spinosic acid (10), arjunic acid (11), β-sitosterol (12), β-daucosterol (13), α-tocopherol (14), and n-hexacosane (15). Among these, compounds 4, 6, and 7 were isolated from this plant for the first time. (2) In vitro anti-inflammatory activity assays of compounds 1-7 revealed that all seven compounds significantly inhibited LPS-induced NO

release from RAW264.7 macrophages in a dose-dependent manner. These compounds exhibited notable anti-inflammatory activity with IC_{50} values of 25.07, 24.56, 17.65, 9.80, 16.67, 40.83, and 34.98 $mol \cdot L^{-1}$, respectively (positive control dexamethasone: 22.46 $mol \cdot L^{-1}$). Notably, compounds 3, 4, and 5 demonstrated superior activity compared to dexamethasone. These findings elucidate that triterpenoids, ellagic acids, flavonoids, and oligosaccharides are the primary active constituents responsible for the anti-inflammatory effects of *R. roxburghii* rhizome, thereby validating its traditional use for treating inflammatory conditions.

Keywords: Ethnomedicine; *Rosa roxburghii* rhizome; chemical constituents; isolation and identification; anti-inflammatory activity

Introduction

Rosa roxburghii, a perennial deciduous shrub belonging to the family Rosaceae and genus *Rosa*, is known by various local names including "Silk-Reeling Flower," "Pineapple Rose," and "Wenxian Fruit." The plant is predominantly distributed in southwestern China, particularly in Guizhou Province. Historical records documenting its medicinal properties date back to 1690 in *Qian Shu*, which states: "Sweet with a slightly sour taste, it can relieve discomfort and eliminate food stagnation." As an ethnic medicinal material in Guizhou, *R. roxburghii* has been included in the *Quality Standards for Chinese Medicinal Materials and Ethnic Medicinal Materials in Guizhou Province* (1994 and 2003 editions), where it is indicated for improving digestion, strengthening the spleen, and arresting diarrhea, primarily for treating food stagnation, abdominal distension, diarrhea, and pain. The rhizome of *R. roxburghii* has been traditionally used to treat acute bacterial dysentery and chronic gastric ulcer.

In Guizhou' s minority regions, particularly Libo County, the Yao people commonly prepare decoctions from the rhizome to treat digestive system disorders and leukorrhea, as well as dysentery in livestock such as pigs, cattle, and sheep. The anti-inflammatory applications of *R. roxburghii* rhizome are documented across multiple ethnic groups: the Miao people use it for acute enteritis, the Tujia people for stomach pain, heatstroke, food stagnation, dysentery, and enteritis, the Buyi people combine it with other herbs for stomach pain, and the Gelao people use it with pomegranate peel for vomiting and diarrhea.

Beyond its high vitamin C content, *R. roxburghii* contains abundant bioactive compounds including polysaccharides, flavonoids, phenolic acids, and triterpenoids. Modern pharmacological studies have demonstrated its hypoglycemic, antibacterial, antioxidant, and anticancer properties. However, few reports have documented the chemical constituents of *R. roxburghii* rhizome, and no studies have investigated the anti-inflammatory activities of its chemical components. Based on the established therapeutic efficacy of this ethnic medicine in Guizhou'

s minority regions, this study was designed to elucidate the material basis of its anti-inflammatory effects. The findings provide scientific validation for the traditional use of *R. roxburghii* and establish a foundation for further development and utilization of its bioactive constituents.

Materials and Methods

1.1 Materials

Plant Material: Fresh *R. roxburghii* rhizomes were collected in Baiyun District, Guiyang City, Guizhou Province, and identified by Professor Sun Qingwen of Guizhou University of Traditional Chinese Medicine. Voucher specimens (CL201901) are deposited at the Functional Center of the Key Laboratory of Chemistry for Natural Product of Guizhou Province and Chinese Academy of Sciences.

Cell Line: Mouse monocyte-macrophage cells (RAW264.7) were purchased from Zhongqiaoxinzhou Biotechnology Company and cryopreserved at the Functional Center of the Key Laboratory of Chemistry for Natural Product of Guizhou Province and Chinese Academy of Sciences.

1.2 Instruments

The following instruments were used: Hewlett Packard 110 mass spectrometer (USA), Bruker AM-600 MHz NMR spectrometer (USA), inverted fluorescence microscope (Nikon), CO_2 incubator (ESCO), -80°C ultra-low temperature freezer (Thermo), and multimode microplate reader (PerkinElmer).

1.3 Reagents

Reagents included silica gel for column chromatography (60–100 mesh, 200–300 mesh, 300–400 mesh; Qingdao Marine Chemical Factory), Sephadex LH-20 (GE Healthcare), dimethyl sulfoxide (DMSO; Tianjin Zhiyuan Chemical Reagent Co.), PBS buffer (pH 7.4), fetal bovine serum (FBS), DMEM medium (Merck), trypsin-EDTA (Biological Industries), lipopolysaccharide (LPS), thiazolyl blue tetrazolium bromide (MTT) (Merck), dexamethasone (Merck), and NO detection kit (Beyotime Biotechnology).

1.4 Experimental Methods

1.4.1 Extraction and Isolation Fresh *R. roxburghii* rhizomes (40 kg) were washed, chopped, and extracted three times with 80% ethanol under heat reflux for 2 hours each. The extracts were filtered while hot, combined, and concentrated under reduced pressure until ethanol-free. The concentrate was further evaporated in a water bath at 60°C to yield 0.65 kg of crude extract.

The extract was subjected to silica gel column chromatography using chloroform:methanol gradients (10:1, 5:1, 1:1, 0:1) to obtain four fractions: A (11 g), B

(97 g), C (69 g), and D (263 g). Fraction D was eluted with chloroform:methanol (6:1) to yield subfractions D1 and D2. From D1, compound 5 (40 mg) was obtained by recrystallization from methanol after concentration. Subfraction D2 was purified using Sephadex LH-20 gel chromatography (methanol elution) and silica gel column chromatography (chloroform:methanol 6:1–4:1) to obtain compound 4 (200 mg), compound 6 (25 mg), and compound 7 (18 mg). Fraction C was subjected to isocratic elution with chloroform:methanol (30:1$→20 : 1→10 : 1)$toyieldsubfractionsC1, C2, andC3.Compounds1(65mg)and2(42mg)wereobtainedfromC1bychloroform : methanol(10 : 1)elutionandmethanolrecrystallization.Compound9(16mg)wasobtainedfromC3bygradientelu methanol(30 : 1–20 : 1)followedbyrecrystallization.SubfractionB2waspurifiedbygradientelutionwithchloro methanol(20 : 1–10 : 1)andrecrystallizationtoyieldcompounds3(35mg), 8(26mg), 10(15mg), 11(23mg), and13(ethylacetate(40 : 1→30 : 1→$20:1) to obtain compounds 15 (15 mg), 12 (25 mg), and 14 (18 mg).

1.4.2 Effects of Monomeric Compounds on RAW264.7 Cell Viability Cell viability was assessed using the MTT assay. RAW264.7 cells in logarithmic growth phase were seeded in 96-well plates at 2$×10^{4}$ cells · mL^{-1} (100 µL per well) and incubated at 37°C with 5% CO_2 until adherent. Various concentrations of test compounds were added, and cells were incubated for 24 h. MTT solution (20 µL, 5 mg · mL^{-1}) was added to each well, and after 4 h, the supernatant was removed and 150 µL DMSO was added. Following 10 min of dark oscillation, absorbance was measured at 570 nm.

1.4.3 Griess Assay for NO Release in RAW264.7 Cells The anti-inflammatory activity of monomeric compounds was evaluated using an LPS-induced RAW264.7 cell model, with NO release quantified by the Griess method. Cells in logarithmic growth phase were seeded in 96-well plates at 1$×10^{5}$ cells · mL^{-1}. After adherence, test compounds at various concentrations were added with LPS at a final concentration of 1 µg · mL^{-1}. Each concentration was tested in triplicate. Following 24 h incubation, culture supernatants were collected and NO levels were determined according to the kit instructions. Absorbance was measured at 540 nm using a microplate reader, and IC_{50} values were calculated using GraphPad Prism 7 software.

Results

2.1 Structural Identification

Compound 1 was obtained as a white powder. ESI-MS m/z: 673.3 $[M+Na]^+$, molecular formula $C_{36}H_{58}O_{10}$. ^{1}H NMR (600 MHz, CD_3OD) δ: 5.35 (1H, d, J=12.0 Hz, glc-1), 5.32 (1H, br s, H-12), 2.52 (1H, s, H-18), 2.65 (1H, m, H-3), 1.35 (3H, s, CH_3-27), 1.20 (3H, s, CH_3-29), 0.98 (3H, d, CH_3-25), 0.97 (3H, d, CH_3-23), 0.92 (3H, d, J=9.0 Hz, CH_3-30), 0.84 (3H, s, CH_3-23), 0.75 (3H, s, CH_3-24). ^{13}C NMR (150 MHz, CD_3OD) δ: 42.5 (C-1), 67.3 (C-2), 80.1 (C-3), 39.6 (C-4), 49.2 (C-5), 22.6 (C-6), 34.2 (C-7), 41.5 (C-8), 48.5 (C-9), 39.4 (C-10), 24.5 (C-11), 129.5 (C-12), 139.5 (C-13), 42.8 (C-14), 29.5 (C-15), 26.5 (C-16),

48.5 (C-17), 55.2 (C-18), 73.5 (C-19), 43.0 (C-20), 27.3 (C-21), 38.5 (C-22), 29.3 (C-23), 16.6 (C-24), 17.2 (C-25), 19.3 (C-26), 24.8 (C-27), 178.5 (C-28), 27.2 (C-29), 17.7 (C-30), 95.6 (C-1′), 73.5 (C-2′), 78.6 (C-3′), 71.3 (C-4′), 78.5 (C-5′), 62.5 (C-6′). These data are consistent with literature values (Yuan et al., 2019), identifying compound 1 as kaji-ichigoside F1.

Compound 2 was obtained as a white powder. ESI-MS m/z: 673.5 $[M+Na]^+$, molecular formula $C_{36}H_{58}O_{10}$. ^1H NMR (600 MHz, CD_3OD) δ: 5.35 (1H, d, J=12.0 Hz, glc-1), 5.32 (1H, br s, H-12), 2.50 (1H, s, H-18), 1.32 (3H, s, CH_3-27), 1.28 (3H, s, CH_3-29), 1.15 (3H, s, CH_3-25), 1.05 (3H, s, CH_3-23), 0.92 (3H, d, J=7.5 Hz, CH_3-30), 0.80 (3H, s, CH_3-26), 0.75 (3H, s, CH_3-24). ^{13}C NMR (150 MHz, CD_3OD) δ: 48.2 (C-1), 69.5 (C-2), 84.2 (C-3), 39.2 (C-4), 56.5 (C-5), 19.7 (C-6), 34.0 (C-7), 41.5 (C-8), 48.6 (C-9), 40.6 (C-10), 24.8 (C-11), 129.5 (C-12), 139.7 (C-13), 42.8 (C-14), 29.5 (C-15), 26.5 (C-16), 48.5 (C-17), 55.0 (C-18), 73.5 (C-19), 43.0 (C-20), 27.3 (C-21), 36.9 (C-22), 29.3 (C-23), 17.6 (C-24), 16.5 (C-25), 17.5 (C-26), 24.9 (C-27), 178.5 (C-28), 28.6 (C-29), 25.2 (C-30), 95.8 (C-1′), 73.6 (C-2′), 78.2 (C-3′), 71.3 (C-4′), 78.5 (C-5′), 62.3 (C-6′). These data are consistent with literature values (Li et al., 2008), identifying compound 2 as rosamultin.

Compound 3 was obtained as a white powder. ESI-MS m/z: 511.2 $[M+Na]^+$, molecular formula $C_{30}H_{48}O_5$. ^1H NMR (600 MHz, CD_3OD) δ: 5.30 (1H, br s, H-12), 3.91 (1H, br d, J=18.0 Hz, H-3), 3.31 (1H, overlapped, H-2), 2.50 (1H, s, H-18), 1.35 (3H, s, CH_3-27), 1.28 (3H, s, CH_3-29), 1.18 (3H, s, CH_3-25), 0.98 (3H, s, CH_3-23), 0.92 (3H, d, J=10.5 Hz, CH_3-30), 0.85 (3H, s, CH_3-26), 0.75 (3H, s, CH_3-24). ^{13}C NMR (150 MHz, CD_3OD) δ: 42.3 (C-1), 67.2 (C-2), 80.2 (C-3), 41.2 (C-4), 49.3 (C-5), 24.5 (C-6), 34.0 (C-7), 39.3 (C-8), 48.2 (C-9), 39.5 (C-10), 27.2 (C-11), 129.3 (C-12), 140.0 (C-13), 42.8 (C-14), 29.5 (C-15), 26.5 (C-16), 48.5 (C-17), 55.0 (C-18), 73.5 (C-19), 43.0 (C-20), 19.2 (C-21), 36.9 (C-22), 39.0 (C-23), 29.3 (C-24), 17.5 (C-25), 16.6 (C-26), 27.0 (C-27), 182.5 (C-28), 24.9 (C-29), 16.9 (C-30). These data are consistent with literature values (Liu et al., 2013), identifying compound 3 as euscaphic acid.

Compound 4 was obtained as a yellow semi-solid. ESI-MS m/z: 840.6 $[M-H]^-$, molecular formula $C_{31}H_{53}O_{26}$. ^1H NMR (600 MHz, CD_3OD) δ: 4.45 (d, H-1a), 3.96 (dd, H-2a), 3.48 (m, H-3a), 3.83 (m, H-4a), 3.95 (m, H-5a), 3.15 (d, H-6a), 5.08 (d, H-1b), 4.03 (dd, H-2b), 3.45 (m, H-3b), 3.85 (m, H-4b), 3.22 (d, H-5b), 4.63 (d, H-1c), 3.94 (m, H-2c), 3.46 (m, H-3c), 3.75 (m, H-4c), 3.25 (d, H-5c), 4.85 (br s, H-1d), 4.05 (dd, H-2d), 3.43 (m, H-3d), 3.73 (m, H-4d), 3.35 (d, H-5d), 4.83 (br s, H-1e), 4.05 (dd, H-2e), 3.46 (m, H-3e), 3.73 (m, H-4e), 3.30 (br s, H-5e), 4.50 (d, H-1f), 3.92 (dd, H-2f), 3.56 (m, H-3f), 3.60 (m, H-4f), 3.35 (d, H-5f). ^{13}C NMR (150 MHz, CD_3OD) δ: 99.5 (C-1a), 84.2 (C-2a), 69.8 (C-3a), 69.5 (C-4a), 76.5 (C-5a), 60.5 (C-6a), 94.2 (C-1b), 83.1 (C-2b), 76.3 (C-3b), 66.0 (C-4b), 62.5 (C-5b), 91.3 (C-1c), 83.1 (C-2c), 74.6 (C-3c), 64.9 (C-4c), 62.6 (C-5c), 98.5 (C-1d), 78.4 (C-2d), 73.8 (C-3d), 65.0 (C-4d), 62.6 (C-5d), 103.5 (C-1e), 78.1 (C-2e), 73.1 (C-3e), 72.0 (C-4e), 64.5 (C-5e), 105.5 (C-1f), 77.3 (C-2f), 71.2 (C-3f), 63.8

(C-4f), 63.5 (C-5f). These data are consistent with literature values (Ill et al., 2014), identifying compound 4 as β-D-glucopyranosyl-(2a$\rightarrow$1b)$-$2$a-O-$ $-L-$ $arabinopyranosyl-(2b\rightarrow1c)-2b-O-$ $-L-arabinopyranosyl-(2c\rightarrow1d)-2c-O-$ $-L-arabinopyranosyl-(2d\rightarrow1e)-2d-O-$ $-L-arabinopyranosyl-(2e\rightarrow1f)-2e-O-$ \$-L-arabinopyranoside.

Compound 5 was obtained as a yellow powder. ESI-MS m/z: 289.2 $[M-H]^-$, molecular formula $C_{15}H_{14}O_6$. ^{1}H NMR (600 MHz, CD_3OD) δ: 8.03 (4H, s, OH\$×4), $4.55(1H,d,J=10.5Hz,H-2)$, $5.95(1H,d,J=2.25Hz,H-6)$, $6.01(1H,d,J=2.25Hz,H-8)$, $6.91(1H,brs,H-2')$, $6.75(1H,brd,J=12Hz,H-5')$, $6.81(1H,d,J=12Hz,H-6')$, $4.05(1H,brs,OH\times\$3)$. ^{13}C NMR (150 MHz, CD_3OD) δ: 82.5 (C-2), 68.2 (C-3), 28.5 (C-4), 157.3 (C-5), 95.6 (C-6), 157.2 (C-7), 95.2 (C-8), 157.0 (C-9), 99.8 (C-10), 132.0 (C-1′), 115.5 (C-2′), 145.4 (C-3′), 145.3 (C-4′), 115.3 (C-5′), 119.3 (C-6′). These data are consistent with literature values (Yang et al., 2020), identifying compound 5 as catechin.

Compound 6 was obtained as yellow needle crystals. ESI-MS m/z: 919.5 $[2M+Na]^+$, molecular formula $C_{20}H_{16}O_{12}$. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 7.55 (1H, s, H-5), 7.72 (1H, s, H-5′), 3.95 (3H, s, -OCH_3), 5.00 (1H, d, J=14 Hz, H-1"). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 113.2 (C-1), 141.5 (C-2), 140.1 (C-3), 152.4 (C-4), 111.3 (C-5), 111.3 (C-6), 158.7 (C-7), 114.1 (C-1′), 141.7 (C-2′), 135.5 (C-3′), 146.5 (C-4′), 107.3 (C-5′), 111.4 (C-6′), 158.5 (C-7′), 60.9 (C3-OCH_3), 102.5 (C-1"), 72.5 (C-2"), 75.3 (C-3"), 69.2 (C-4"), 65.1 (C-5"). These data are consistent with literature values (Kong et al., 2009), identifying compound 6 as 3-O-methylellagic acid-4′-O-β-D-xylopyranoside.

Compound 7 was obtained as yellow needle crystals. ESI-MS m/z: 461.2 $[M-H]^-$, molecular formula $C_{21}H_{18}O_{12}$. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 7.48 (1H, s, H-5), 7.60 (1H, s, H-5′), 3.95 (3H, s, -OCH_3), 5.42 (1H, s, H-1"). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 107.3 (C-1), 140.1 (C-2), 136.2 (C-3), 146.5 (C-4), 111.3 (C-5), 111.5 (C-6), 158.7 (C-7), 114.1 (C-1′), 141.7 (C-2′), 141.6 (C-3′), 152.6 (C-4′), 111.6 (C-5′), 113.0 (C-6′), 158.5 (C-7′), 60.9 (C3-OCH_3), 100.3 (C-1"), 70.2 (C-2"), 70.5 (C-3"), 71.5 (C-4"), 69.8 (C-5"), 17.8 (C-6"). These data are consistent with literature values (Guan et al., 2007), identifying compound 7 as 3-O-methylellagic acid-4′-O-α-L-rhamnopyranoside.

Compound 8 was obtained as a white powder. ESI-MS m/z: 511.3 $[M+Na]^+$, molecular formula $C_{30}H_{48}O_5$. ^{1}H NMR (600 MHz, CD_3OD) δ: 5.20 (1H, br s, H-12), 4.38 (1H, m, H-2), 3.41 (1H, overlapped, H-3), 2.48 (1H, s, H-18), 1.28 (3H, s, CH_3-27), 1.06 (3H, s, CH_3-29), 0.92 (3H, s, CH_3-25), 0.88 (3H, s, CH_3-23), 0.68 (3H, s, CH_3-26), 0.82 (3H, d, J=10.5 Hz, CH_3-30), 0.65 (3H, s, CH_3-24). ^{13}C NMR (150 MHz, CD_3OD) δ: 47.5 (C-1), 67.2 (C-2), 82.5 (C-3), 38.9 (C-4), 55.2 (C-5), 18.4 (C-6), 32.4 (C-7), 40.0 (C-8), 46.8 (C-9), 38.7 (C-10), 23.5 (C-11), 127.0 (C-12), 138.9 (C-13), 41.5 (C-14), 28.3 (C-15), 25.6 (C-16), 47.3 (C-17), 53.5 (C-18), 71.8 (C-19), 41.6 (C-20), 26.1 (C-21), 37.5 (C-22), 29.2 (C-23), 16.5 (C-24), 16.4 (C-25), 18.4 (C-26), 24.2 (C-27), 179.2 (C-28), 26.7 (C-29), 17.4 (C-30). These data are consistent with literature values (Yang and

Zhao, 2003), identifying compound 8 as tormentic acid.

Compound 9 was obtained as a white powder. ESI-MS m/z: 479.4 $[M+Na]^+$, molecular formula $C_{30}H_{48}O_3$. ^1H NMR (600 MHz, $CDCl_3$) δ: 4.65 (1H, br s, H-29a), 4.52 (1H, s, H-29b), 3.10 (1H, dd, J=11.2 Hz, H-3), 1.65 (3H, s, H-30), 0.95 (3H, s, H-23), 0.86 (3H, s, H-26), 0.78 (3H, s, H-25), 0.68 (3H, s, H-24). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 38.5 (C-1), 27.2 (C-2), 79.3 (C-3), 38.5 (C-4), 55.2 (C-5), 17.9 (C-6), 34.5 (C-7), 40.5 (C-8), 50.3 (C-9), 37.2 (C-10), 22.5 (C-11), 25.7 (C-12), 37.3 (C-13), 43.0 (C-14), 27.2 (C-15), 32.2 (C-16), 57.8 (C-17), 45.5 (C-18), 49.6 (C-19), 149.7 (C-20), 29.0 (C-21), 37.5 (C-22), 27.5 (C-23), 15.2 (C-24), 16.0 (C-25), 16.2 (C-26), 15.2 (C-27), 181.2 (C-28), 109.2 (C-29), 19.5 (C-30). These data are consistent with literature values (Simin et al., 2007), identifying compound 9 as betulinic acid.

Compound 10 was obtained as a white powder. ESI-MS m/z: 495.3 $[M+Na]^+$, molecular formula $C_{30}H_{48}O_4$. ^1H NMR (600 MHz, CD_3OD) δ: 5.33 (1H, br s, H-12), 3.15 (1H, overlapped, H-3), 3.10 (1H, s, H-18), 1.28 (3H, s, CH_3-27), 1.06 (3H, s, CH_3-29), 0.92 (3H, s, CH_3-25), 1.02 (3H, s, CH_3-23), 0.98 (3H, s, CH_3-30), 0.78 (3H, s, CH_3-26), 0.82 (3H, s, CH_3-24). ^{13}C NMR (150 MHz, CD_3OD) δ: 38.5 (C-1), 26.8 (C-2), 78.6 (C-3), 38.5 (C-4), 55.5 (C-5), 18.4 (C-6), 32.8 (C-7), 39.5 (C-8), 47.6 (C-9), 37.2 (C-10), 23.9 (C-11), 123.5 (C-12), 143.5 (C-13), 41.5 (C-14), 28.3 (C-15), 27.5 (C-16), 45.5 (C-17), 44.2 (C-18), 81.3 (C-19), 34.9 (C-20), 28.5 (C-21), 32.8 (C-22), 27.6 (C-23), 15.1 (C-24), 14.5 (C-25), 16.6 (C-26), 24.2 (C-27), 181.2 (C-28), 27.5 (C-29), 24 (C-30). These data are consistent with literature values (Xiao et al., 2011), identifying compound 10 as spinosic acid.

Compound 11 was obtained as a white powder. ESI-MS m/z: 511.1 $[M+Na]^+$, molecular formula $C_{30}H_{48}O_5$. ^1H NMR (600 MHz, CD_3OD) δ: 5.30 (1H, br s, H-12), 3.95 (1H, br d, J=16.5 Hz, H-3), 3.63 (1H, m, H-2), 2.48 (1H, s, H-18), 1.28 (3H, s, CH_3-27), 1.15 (3H, s, CH_3-29), 1.01 (3H, s, CH_3-25), 0.96 (3H, s, CH_3-23), 0.92 (3H, d, J=10.5 Hz, CH_3-30), 0.78 (3H, s, CH_3-26), 0.76 (3H, s, CH_3-24). ^{13}C NMR (150 MHz, CD_3OD) δ: 47.5 (C-1), 69.5 (C-2), 84.2 (C-3), 40.5 (C-4), 56.5 (C-5), 19.3 (C-6), 33.8 (C-7), 40.5 (C-8), 48.1 (C-9), 39.0 (C-10), 23.9 (C-11), 124.5 (C-12), 140.1 (C-13), 42.5 (C-14), 29.2 (C-15), 29.4 (C-16), 46.1 (C-17), 45.3 (C-18), 82.4 (C-19), 34.0 (C-20), 29.2 (C-21), 36.0 (C-22), 28.7 (C-23), 17.6 (C-24), 16.8 (C-25), 17.4 (C-26), 24.9 (C-27), 182.4 (C-28), 28.5 (C-29), 24.6 (C-30). These data are consistent with literature values (Zhang et al., 2005), identifying compound 11 as arjunic acid.

Compound 12 was obtained as white needle crystals. ESI-MS m/z: 437.5 $[M+Na]^+$, molecular formula $C_{29}H_{50}O$. ^1H NMR (600 MHz, $CDCl_3$) δ: 5.15 (1H, s, 6-H), 6.81 (1H, d, J=12.0 Hz, H-3), 0.85 (7H, d, J=6.9 Hz, H-2, H-26, H-9), 0.75 (1H, d, J=6.4 Hz, H-27). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 37.4 (C-1), 29.8 (C-2), 71.5 (C-3), 42.2 (C-4), 141.1 (C-5), 121.7 (C-6), 31.3 (C-7), 32.5 (C-8), 50.3 (C-9), 36.5 (C-10), 21.3 (C-11), 39.5 (C-12), 42.5 (C-13), 56.6 (C-14), 24.4 (C-15), 28.5 (C-16), 56.2 (C-17), 12.1 (C-18), 19.5 (C-19), 36.3 (C-20), 18.5 (C-21), 34.1 (C-22), 26.2 (C-23), 46.0 (C-24), 29.2 (C-25), 19.6 (C-26), 19.1 (C-

27), 23.2 (C-28), 11.8 (C-29). These data are consistent with literature values (Huang et al., 2020), identifying compound 12 as β-sitosterol.

Compound 13 was obtained as a white powder. ESI-MS m/z: 599.7 $[M+Na]^+$, molecular formula $C_{35}H_{60}O_6$. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 5.35 (1H, br s, H-6), 4.56 (1H, d, J=15 Hz, H-1´). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 37.6 (C-1), 30.3 (C-2), 78.3 (C-3), 39.2 (C-4), 141.1 (C-5), 122.2 (C-6), 32.3 (C-7), 32.1 (C-8), 50.3 (C-9), 36.5 (C-10), 21.3 (C-11), 39.5 (C-12), 42.6 (C-13), 56.5 (C-14), 24.6 (C-17), 12.3 (C-18), 19.2 (C-19), 36.5 (C-20), 19.0 (C-21), 34.3 (C-22), 26.3 (C-23), 46.3 (C-24), 29.5 (C-25), 19.6 (C-26), 19.6 (C-27), 23.5 (C-28), 12.3 (C-29), 102.5 (C-1´), 75.3 (C-2´), 78.6 (C-3´), 71.8 (C-4´), 78.2 (C-5´), 62.5 (C-6´). These data are consistent with literature values (Zhan and Xia, 2005), identifying compound 13 as β-daucosterol.

Compound 14 was obtained as an oily liquid. ESI-MS m/z: 429.3 $[M-H]^-$, molecular formula $C_{29}H_{50}O_2$. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 2.58 (2H, t, J=10.2 Hz, H-4), 2.12 (3H, s, H-7a), 2.06 (6H, s, H-5a, H-8a), 1.73 (2H, m, H-3), 1.25 (3H, s, H-2a), 0.86 (3H, d, J=10.2 Hz, H-12´a), 0.86 (3H, d, J=10.2 Hz, H-13´), 0.83 (3H, d, J=9.6 Hz, H-4´a), 0.82 (3H, d, J=9.6 Hz, H-8´a). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 145.6 (C-9), 144.5 (C-6), 122.5 (C-8), 121.2 (C-7), 118.5 (C-5), 117.3 (C-10), 74.5 (C-2), 39.6 (C-1´), 39.4 (C-11´), 37.5 (C-3´), 37.4 (C-5´), 37.4 (C-7´), 37.3 (C-9´), 32.6 (C-4´), 32.6 (C-8´), 31.5 (C-3), 27.9 (C-12´), 24.5 (C-10´), 24.4 (C-6´), 23.8 (C-2a), 22.5 (C-12´a), 22.6 (C-13´), 21.2 (C-2´), 20.5 (C-4), 19.7 (C-4´a), 19.6 (C-8´a), 12.3 (C-7a), 11.5 (C-8a), 11.2 (C-5a). These data are consistent with literature values (Kyeong et al., 2013), identifying compound 14 as α-tocopherol.

Compound 15 was obtained as an oily liquid. ESI-MS m/z: 389.4 $[M+Na]^+$, molecular formula $C_{26}H_{54}$. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 1.25 (54H, m, H-2-25), 0.88 (6H, t, J=8.4 Hz, H-1, 26). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 14.1 (C-1, 6), 22.7 (C-2, 25), 29.5 (C-5, 22), 29.5 (C-6–C-21), 31.9 (C-3, 4). These data are consistent with literature values (Ye et al., 2015), identifying compound 15 as n-hexacosane.

[Figure 1: see original paper] Chemical structures of compounds 1–14

2.2 Effects of Monomeric Compounds on RAW264.7 Cell Viability

As shown in [Figure 2: see original paper], cell viability exceeded 90% for all compounds 1–7 at concentrations \$ $50mol \cdot L$^{-1}\$ compared to the blank control group. These results indicate that compounds 1–7 exhibit no significant cytotoxicity to RAW264.7 macrophages at concentrations \$ $50mol \cdot L^{\{-1\}, justifying the use of 50mol \cdot L}${-1}\$ as the maximum concentration for subsequent experiments.

A-G represent compounds 1–7. Compared with the blank group: *P<0.05,* ***P<0.01,*** P<0.001. The same notation applies below.

[Figure 2: see original paper] Effects of compounds on the cell viability of mouse macrophages

2.3 Effects of Monomeric Compounds on NO Release in RAW264.7 Cells

An in vitro inflammatory model was established using LPS-stimulated macrophages, and NO release in cell supernatants was measured by the Griess method. It is essential to ensure that test concentrations do not affect cell viability to avoid false-positive results. While unstimulated RAW264.7 cells produce minimal NO, LPS stimulation triggers an "inflammatory cascade" with massive NO release. As shown in [Figure 3: see original paper], NO release following LPS stimulation was significantly higher than in the blank group ($P<0.001$), confirming successful model establishment. Compared with the LPS group, compounds 1–7 reduced NO release in a dose-dependent manner at concentrations of 3.125, 6.25, 12.5, 25, and 50 $\mu mol \cdot L^{-1}$.

H represents dexamethasone; final LPS concentration was 1 $\mu g \cdot mL^{-1}$. Compared with the blank group: ###$P<0.001$; compared with the model group: *$P<0.05$,* ***$P<0.01$,*** $P<0.001$.

[Figure 3: see original paper] Effects of monomeric compounds on NO expression in RAW264.7 cells

As shown in , compounds 3, 4, and 5 demonstrated superior in vitro anti-inflammatory activity compared to dexamethasone, while compounds 1, 2, 6, and 7 exhibited moderate activity.

IC_{50} values of compounds 1–7

Compound	IC_{50} ($\mu mol \cdot L^{-1}$)
1	25.07
2	24.56
3	17.65
4	9.80
5	16.67
6	40.83
7	34.98
Dexamethasone	22.46

Note: Compounds 1–7 were tested at concentrations of 3.125–50 $\mu mol \cdot L^{-1}$.

Discussion and Conclusion

This study isolated and identified 15 compounds from *R. roxburghii* rhizome, including seven pentacyclic triterpenoids, two ellagic acids, two sterols, one flavonoid, one oligosaccharide, one polyphenol, and one aliphatic hydrocarbon.

Among these, compounds 4, 6, and 7 were reported from this plant for the first time. The highest content compounds were the triterpenoid glycosides kajiichigoside F1 and rosamultin (compounds 1 and 2), which reached 0.075% in fresh material.

Ethnomedicines play important roles in preventing and treating inflammatory diseases through multi-component, multi-target, and multi-pathway synergistic regulation. *Rosa roxburghii* is particularly abundant in Guizhou and has a long history of folk application. The anti-inflammatory activity results demonstrate that triterpenoid aglycones exhibit superior activity compared to triterpenoid saponins, confirming that pentacyclic triterpenoids with free carboxylic acid at C-28 possess better activity—a finding consistent with previous reports (Xue, 2018). Compounds 1–7 significantly inhibited NO release from mouse macrophages in a dose-dependent manner, with compounds 3, 4, and 5 showing superior activity compared to dexamethasone, while compounds 1, 2, 6, and 7 demonstrated moderate activity. These results validate the anti-inflammatory efficacy of *R. roxburghii* rhizome in Guizhou' s minority regions and suggest similar mechanisms to other Rosaceae species such as *Rosa laevigata*, though further mechanistic studies are warranted.

Triterpenoids represent the major active constituents in *R. roxburghii* rhizome and exhibit various bioactivities including immune enhancement, anti-aging, anti-atherosclerotic, and digestive properties. The ellagic acid compounds 6 and 7 showed IC_{50} values of 40.83 and 34.98 $\mu mol \cdot L^{-1}$, respectively, with relatively weaker anti-inflammatory effects compared to triterpenoids. Their mechanism may involve downregulating inflammatory factor gene expression and inhibiting pro-inflammatory cytokine and mediator secretion. As natural polyphenols with skin-nourishing properties, ellagic acids warrant further investigation for cosmetic applications. This study demonstrates that triterpenoids, ellagic acids, flavonoids, and oligosaccharides are the primary anti-inflammatory constituents of *R. roxburghii* rhizome, validating its traditional use and providing a foundation for developing anti-intestinal inflammatory agents and veterinary medicines.

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