Chemical Constituents and Anti-inflammatory Activity of Pimpinella candolleana (Postprint)

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Abstract

Pimpinella candolleana is a traditional herb used by the Miao ethnic group in Guizhou for the treatment of jaundice-type hepatitis, acute cholecystitis, and related conditions. To investigate the chemical constituents and anti-inflammatory activity of Pimpinella candolleana, this study employed silica gel, gel, and ODS chromatographic techniques to separate and purify the 70% ethanol extract of the whole plant. Compound structures were identified based on spectroscopic data including NMR and MS, and the anti-inflammatory activities of individual compounds were evaluated using a lipopolysaccharide (LPS)-induced RAW264.7 macrophage inflammatory model. The results showed: (1) Twenty compounds were isolated and identified from Pimpinella candolleana, namely vanillin (1), sesamin (2), 2-methyl-2-hydroxy-5-methoxybenzo[d]furan-3-one (3), protocatechuic aldehyde (4), 1,5-dihydroxy-2,3-dimethoxyxanthone (5), isorhamnetin (6), kaempferol (7), 8-hydroxy-2-methylchromone (8), luteolin (9), quercetin (10), 1-O-β-D-glucopyranosyl-(2S,3S,4R,8E)-2-[(2 R)-2 -hydroxypalmitoylamino]-8-octadecene-1,3,4-triol (11), isorhamnetin-3-O-β-D-galactoside (12), isoquercitrin (13), norswertianolin (14), luteolin-6-C-α-L-arabinoside (15), kaempferol-3-O-β-D-galactoside (16), kaempferol-7-O-β-D-glucoside (17), luteolin-7-O-β-D-glucoside (18), isovitexin (19), and rutin (20). Among these, compounds 1, 3, 4, 6, 7, 10, 13, 16, 18, and 20 were isolated from this plant for the first time. (2) The anti-inflammatory results demonstrated that compounds 2-10, 12, 18, and 19 significantly inhibited LPS-induced NO release in RAW264.7 cells ($P<0.05$, $P<0.01$). Among them, compounds 4, 7, 10, and 18 at a concentration of 25 $\mathrm{mol \cdot L^{-1}}$ exhibited inhibition rates of 57.37%, 83.60%, 68.16%, and 81.14%, respectively. This study enriches the understanding of the chemical constituents of Pimpinella candolleana and identifies flavonoids as the active components responsible for its anti-inflammatory efficacy, providing a reference basis for further research and development of this medicinal plant.

Full Text

Chemical Constituents of Pimpinella candolleana and Their Anti-Inflammatory Activities

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Abstract

Pimpinella candolleana is a traditional Miao ethnic herbal medicine in Guizhou used for treating icteric hepatitis, acute cholecystitis, and other conditions. To investigate its chemical constituents and anti-inflammatory activities, we separated and purified compounds from the 70% ethanol extract of the whole plant using silica gel, gel, and ODS chromatography. Structures were identified by spectroscopic data including NMR and MS. Anti-inflammatory activity was evaluated using a lipopolysaccharide (LPS)-induced RAW264.7 macrophage cell model. The results showed that: (1) Twenty compounds were isolated and identified: vanillin (1), sesamin (2), 2-methyl-2-hydroxy-5-methoxybenz[d]hydrofuran-3-one (3), protocatechuic aldehyde (4), 1,5-dihydroxy-2,3-dimethoxyxanthone (5), isorhamnetin (6), kaempferol (7), 8-hydroxy-2-methylchromone (8), luteolin (9), quercetin (10), 1-O-β-D-glucopyranosyl-(2S,3S,4R,8E)-2-[(2' R)-2' -hydroxypalmitoylamino]-8-octadecene-1,3,4-triol (11), isorhamnetin-3-O-β-D-galactopyranoside (12), isoquercitrin (13), norswertianolin (14), luteolin-6-C-α-L-arabinoside (15), kaempferol-3-O-β-D-galactopyranoside (16), kaempferol-7-O-β-D-glucopyranoside (17), luteolin-7-O-β-D-glucopyranoside (18), isovitexin (19), and rutin (20). Among these, compounds 1, 3, 4, 6, 7, 10, 13, 16, 18, and 20 were isolated from this plant for the first time. (2) Anti-inflammatory results demonstrated that compounds 2–10, 12, 18, and 19 significantly inhibited LPS-induced NO release in RAW264.7 cells ($P<0.05$, $P<0.01$). Notably, compounds 4, 7, 10, and 18 at 25 $mol \cdot L^{-1}$ showed inhibition rates of 57.37%, 83.60%, 68.16%, and 81.14%, respectively. This study enriches the chemical profile of *P. candolleana* and identifies flavonoids as the active anti-inflammatory constituents, providing a reference basis for further research and development of this medicinal plant.

Keywords: *Pimpinella candolleana*; chemical constituents; isolation and identification; RAW264.7 cells; anti-inflammatory activity

Introduction

Pimpinella candolleana Wight et Arn. is a perennial herb belonging to the family Umbelliferae and genus *Pimpinella L.*, also known as *Xingyehuiqin*, *Shandanggui*, *Saoyanggu*, or *Zhizhuxiang*. It is a commonly used folk medicine in Guizhou and is recorded in the *Quality Standards for Chinese Medicinal Materials and Ethnic Medicinal Materials in Guizhou Province* (2003 edition). The plant is widely distributed in Guangxi and southwestern China. With pungent, slightly bitter taste and warm properties, it acts on the liver, lung, spleen, and stomach meridians. The whole plant is used medicinally to treat upper abdominal pain, indigestion, dysentery, and snakebites. It is documented in numerous local pharmacopeias: *Guiyang Folk Herbs* records its ability to "warm the middle, dispel cold, and relieve pain, treating cold deficiency, eruptions, stomach pain, and abdominal pain," while the *Sichuan Traditional Chinese Medicine Chronicle* notes its functions to "promote digestion, strengthen the spleen, and interrupt malaria; used for cold abdominal pain, hernia, rheumatic pain, spleen deficiency with food stagnation, and malaria; recently used for treating lymph node tuberculosis." In recent years, the herb has been developed into compound preparations for chronic hepatitis B and phlebitis caused by fat emulsion. Reported chemical constituents include flavonoids, sterols, and volatile oils. However, literature on the chemical constituents of *P. candolleana* remains limited, and even fewer studies have investigated their biological activities. Beyond reported α-glucosidase inhibitory, antioxidant, and antimicrobial activities, no other pharmacological effects have been documented, and the anti-inflammatory material basis remains unclear. Therefore, to deepen our understanding of its chemical constituents and explore its anti-inflammatory active substances, this study isolated and identified 20 compounds from the 70% ethanol extract of *P. candolleana* whole plant and evaluated the anti-inflammatory activity of 18 compounds to provide a scientific basis for further research and development.

1. Materials and Methods

1.1 Materials Plant Material: *Pimpinella candolleana* was collected in Gaopo, Huaxi, Guizhou Province, and identified by Professor Sun Qingwen of the School of Pharmacy, Guizhou University of Traditional Chinese Medicine, as the dried whole plant of *Pimpinella candolleana* (Umbelliferae). A voucher specimen (20190901) is deposited in the Guizhou Provincial Key Laboratory of Pharmaceutics.

Cell Line: Murine monocyte-macrophage RAW 264.7 cells were purchased from ATCC.

1.2 Instruments JEOL-ECS 400 MHz NMR spectrometer (JEOL, Japan); Bruker AV-600 superconducting NMR spectrometer (Bruker, Germany); ACQUITY-UPLC-TQD ultra-performance liquid chromatography-triple quadrupole tandem mass spectrometer (Waters, USA); CO_2 cell incubator (Thermo Scientific); Varioskan LUX multimode microplate reader (Thermo, USA); TS100 inverted microscope (Nikon, Japan).

1.3 Reagents D-101 macroporous resin (Tianjin Haiguang Chemical Co., Ltd.); column chromatography silica gel and TLC silica gel (Qingdao Marine Chemical Co., Ltd.); Sephadex LH-20 (Pharmacia Biotech, Switzerland); Toyopearl HW-40C and HW-40F gels (Tosoh, Japan); ODS (YMC, Japan). All reagents were analytical grade.

Fetal bovine serum (FBS) and DMEM high-glucose medium (Gibco, USA); lipopolysaccharide (LPS), penicillin-streptomycin solution, dimethyl sulfoxide, PBS buffer (Solarbio, Beijing); CCK-8 kit (Glpbio, USA); NO assay kit (Nanjing Jiancheng Bioengineering Institute); dexamethasone (DEX, Shanghai Zhenzhun Biotechnology Co., Ltd.).

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2. Experimental Procedures

2.1 Extraction and Isolation Dried *P. candolleana* whole plant (12 kg) was cut into coarse sections and extracted three times with 70% ethanol under heat reflux. The extracts were combined and concentrated under reduced pressure to obtain a crude extract (1.3 kg), which was passed through a D-101 macroporous adsorption resin and eluted sequentially with water (2 column volumes) and 80% ethanol (5 column volumes) to yield water-eluted (972 g) and 80% ethanol-eluted (530 g) fractions.

The 80% ethanol fraction was subjected to normal-phase silica gel column chromatography with dichloromethane-methanol (7:3$→6 : 4)*isocraticelution.Aftersolventrecoveryandconcentra*
phasesilicagelcolumnchromatographyusingpetroleumether−ethylacetate(10 : 0→0 : 10)*andethylacetate − methanol*(10 : 0→$7:3) gradient elution. Fractions were collected, detected by TLC, combined, and concentrated to obtain 10 fractions (Fr.1–10).

Fr.4 was subjected to normal-phase silica gel column chromatography with petroleum ether-dichloromethane (3:1$→0 : 1)*anddichloromethane − methanol*(70 : 1→$20:1) gradient elution. After TLC detection and concentration, 7 subfractions (Fr.4.1–4.7) were obtained. Fr.4.3 was repeatedly purified by Sephadex LH-20 (dichloromethane-methanol 1:1) and Toyopearl HW-40F (methanol) to yield compounds **1** (10.5 mg) and **2** (7.0 mg). Fr.4.5 was repeatedly purified by normal-phase silica gel, Sephadex LH-20 (dichloromethane-methanol 1:1), Sephadex LH-20 (methanol), and Toyopearl HW-40F (methanol) to yield compound **3** (30.0 mg).

Fr.5 was passed through Sephadex LH-20 (dichloromethane-methanol 1:1). After TLC detection and concentration, 5 subfractions (Fr.5.1–5.5) were obtained. Fr.5.2 was repeatedly purified by Toyopearl HW-40F (methanol) and Sephadex LH-20 (methanol) to yield compounds **4** (10.0 mg) and **5** (24.4 mg). Fr.5.4 was purified by Toyopearl HW-40F (methanol) and Sephadex LH-20 (methanol) to yield compound **6** (10.0 mg). Fr.5.5 was purified by Toyopearl HW-40F (methanol) to yield compound **7** (17.0 mg).

Fr.6 was passed through Sephadex LH-20 (methanol). After TLC detection and concentration, 5 subfractions (Fr.6.1–6.5) were obtained. Fr.6.2 was purified by Toyopearl HW-40C (methanol), Toyopearl HW-40F (methanol), Sephadex LH-20 (50% acetone-water), and ODS column chromatography (20%–50% methanol-water) to yield compound **8** (11.0 mg). Fr.6.4 was purified by Toyopearl HW-40C (methanol), Toyopearl HW-40F (methanol), and Sephadex LH-20 (methanol) to yield compound **9** (80.0 mg). Fr.6.5 was purified by Toyopearl HW-40F (methanol) to yield compound **10** (87.0 mg).

Fr.8 was processed to obtain 6 subfractions (Fr.8.1–8.6). Fr.8.4 was purified by Toyopearl HW-40C (methanol), Toyopearl HW-40F (methanol), Toyopearl HW-40F (dichloromethane-methanol 1:1), ODS column chromatography (20%–40% methanol-water), Sephadex LH-20 (methanol), and Sephadex LH-20 (50% acetone-water) to yield compounds **11** (150.0 mg), **12** (44.0 mg), and **13** (36.4 mg). Fr.8.5 was purified by Toyopearl HW-40C (methanol), Toyopearl HW-40F (methanol), ODS column chromatography (20%–60% methanol-water), Sephadex LH-20 (methanol), and Sephadex LH-20 (50% acetone-water) to yield compounds **14** (5.2 mg), **15** (3.7 mg), **16** (7.0 mg), and **17** (2.7 mg).

Fr.9 was passed through Sephadex LH-20 (methanol) to obtain 2 subfractions (Fr.9.1–9.2). Fr.9.2 was purified by Toyopearl HW-40C (methanol), Toyopearl HW-40F (methanol), Sephadex LH-20 (50% acetone-water), normal-phase silica gel column, and dichloromethane-methanol (8.5:1.5) to yield compounds **18** (26.0 mg), **19** (25.0 mg), and **20** (87.0 mg).

2.2 Anti-Inflammatory Activity Evaluation Logarithmic-phase RAW264.7 cells were adjusted to 3$×10^{5}$ cells $\cdot$ mL^{-1} and seeded at 100 µL per well in 96-well plates, then incubated at 37 °C with 5% CO_2 for 24 h. Experiments were designed with blank, model, positive control, and treatment groups, each with 3 replicate wells. Dexamethasone (DEX) served as the positive control. Blank and model groups received complete medium, the positive control group received DEX at a final concentration of 25 mol $\cdot$ L^{-1}, and treatment groups received compounds within safe concentration ranges. After 3 h of incubation, all groups except the blank received LPS at a final concentration of 0.25 g $\cdot$ mL^{-1}. After 24 h, supernatants were collected and NO levels were measured using an NO assay kit according to the manufacturer's instructions. Experiments were repeated three times. NO content and inhibition rate were calculated using the following formulas:

NO content (mol·L^{-1}) = (OD_{sample} - OD_{blank})/(OD_{standard} - OD_{blank}) × standard concentration (20 mol·L^{-1}) × dilution factor (4) (1)

NO inhibition rate (%) = (NO_{LPS} - NO_{sample})/(NO_{LPS} - NO_{blank}) × 100% (2)

2.3 Statistical Analysis Data were analyzed using SPSS 22.0 and GraphPad Prism 8.0 software. Inter-group differences were compared using one-way ANOVA, with pairwise comparisons performed using the LSD method. Statistical significance was set at $P < 0.05$.

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3. Structure Identification

Compound 1: White needle crystals. ESI-MS m/z: 153 $[M+H]^+$, molecular formula $C_8H_8O_3$. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 9.80 (1H, s, H-7), 7.40 (1H, overlap, H-6), 7.40 (1H, overlap, H-2), 7.02 (1H, d, J = 8.4 Hz, H-5), 6.24 (1H, brs, -OH), 3.94 (3H, s, -OCH_3); ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 191.1 (C-7), 151.9 (C-3), 147.4 (C-4), 130.1 (C-1), 127.8 (C-6), 114.6 (C-5), 109.0 (C-2), 56.3 (-OCH_3). These data are consistent with literature values (Chen et al., 2020), identifying compound 1 as vanillin.

Compound 2: White needle crystals. Molecular formula $C_{20}H_{18}O_6$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 6.92 (2H, d, J = 1.6 Hz, H-2, 2'), 6.86 (2H, d, J = 8.0 Hz, H-5, 5'), 6.83 (2H, dd, J = 8.0, 1.6 Hz, H-6, 6'), 5.99 (4H, s, 2×OCH_2O), 4.64 (2H, d, J = 4.4 Hz, H-7, 7'), 4.11 (2H, m, H-9a, 9' a), 3.75 (2H, dd, J = 9.2, 4.4 Hz, H-9b, 9' b), 2.99 (2H, m, H-8, 8'); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 147.4 (C-4, 4'), 146.5 (C-3, 3'), 135.5 (C-1, 1'), 119.4 (C-5, 5'), 108.0 (C-6, 6'), 106.6 (C-2, 2'), 100.9 (2×OCH_2O), 84.9 (C-7, 7'), 71.0 (C-9, 9'), 53.8 (C-8, 8'). These data are consistent with literature values (Wu et al., 2021), identifying compound 2 as sesamin.

Compound 3: White powder. ESI-MS m/z: 193 $[M-H]^-$, molecular formula $C_{10}H_{10}O_4$. ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.31 (1H, dd, J = 9.2, 2.8 Hz, H-6), 7.07 (1H, d, J = 2.8 Hz, H-4), 7.00 (1H, d, J = 9.2 Hz, H-7), 3.79 (3H, s, 5-OCH_3), 1.52 (3H, s, CH_3); ^{13}C-NMR (100 MHz, CD_3OD) δ: 202.1 (C-3), 167.1 (C-9), 156.6 (C-5), 129.9 (C-6), 119.8 (C-8), 115.5 (C-7), 106.1 (C-4), 105.9 (C-2), 56.5 (5-OCH_3), 22.2 (-CH_3). These data are consistent with literature values (Shi et al., 1998), identifying compound 3 as 2-methyl-2-hydroxy-5-methoxybenz[d]hydrofuran-3-one.

Compound 4: White powder. ESI-MS m/z: 139 $[M+H]^+$, molecular formula $C_7H_6O_3$. ^{1}H-NMR (600 MHz, CD_3OD) δ: 9.67 (1H, s, H-7), 7.30 (1H, dd, J = 7.8, 1.8 Hz, H-6), 7.29 (1H, d, J = 1.8 Hz, H-2), 6.89 (1H, d, J = 7.8 Hz, H-5); ^{13}C-NMR (150 MHz, CD_3OD) δ: 193.2 (C-7), 154.7 (C-3), 147.5 (C-4), 130.6 (C-1), 126.8 (C-6), 116.5 (C-5), 115.3 (C-2). These data are consistent with

literature values (Yang et al., 2021), identifying compound 4 as protocatechuic aldehyde.

Compound 5: Yellow powder. ESI-MS m/z: 289 $[M+H]^+$, molecular formula $C_{15}H_{12}O_6$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 12.75 (1H, s, 1-OH), 10.51 (1H, brs, 5-OH), 7.54 (1H, dd, J = 7.8, 1.2 Hz, H-8), 7.31 (1H, dd, J = 7.8, 1.2 Hz, H-6), 7.25 (1H, t, J = 7.8 Hz, H-7), 6.75 (1H, s, H-4), 3.95 (3H, s, 3-OCH_3), 3.74 (3H, s, 2-OCH_3); ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 180.8 (C-9), 160.0 (C-3), 153.2 (C-1), 152.7 (C-4a), 146.3 (C-5), 145.0 (C-4b), 131.1 (C-2), 124.2 (C-7), 120.6 (C-8a), 120.5 (C-6), 114.4 (C-8), 103.2 (C-8b), 91.4 (C-4), 60.1 (2-OCH_3), 56.5 (3-OCH_3). These data are consistent with literature values (Yuan et al., 2006), identifying compound 5 as 1,5-dihydroxy-2,3-dimethoxyxanthone.

Compound 6: Yellow powder. ESI-MS m/z: 317 $[M+H]^+$, molecular formula $C_{16}H_{12}O_7$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 12.46 (1H, s, 5-OH), 7.75 (1H, d, J = 1.8 Hz, H-2'), 7.68 (1H, dd, J = 8.4, 1.8 Hz, H-6'), 6.94 (1H, d, J = 8.4 Hz, H-5'), 6.46 (1H, d, J = 1.8 Hz, H-8), 6.18 (1H, d, J = 1.8 Hz, H-6), 3.84 (3H, s, 3' -OCH_3); ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 175.9 (C-4), 164.3 (C-7), 160.7 (C-5), 156.2 (C-9), 148.8 (C-4'), 147.4 (C-3'), 146.5 (C-2), 135.9 (C-3), 122.0 (C-1'), 121.7 (C-6'), 115.5 (C-5'), 111.7 (C-2'), 102.9 (C-10), 98.3 (C-6), 93.6 (C-8), 55.8 (3' -OCH_3). These data are consistent with literature values (Dong et al., 2019), identifying compound 6 as isorhamnetin.

Compound 7: Yellow powder. ESI-MS m/z: 287 $[M+H]^+$, molecular formula $C_{15}H_{10}O_6$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 12.47 (1H, s, 5-OH), 8.04 (2H, d, J = 9.0 Hz, H-2' , 6'), 6.93 (2H, d, J = 9.0 Hz, H-3' , 5'), 6.44 (1H, d, J = 2.4 Hz, H-8), 6.19 (1H, d, J = 2.4 Hz, H-6); ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 175.9 (C-4), 163.9 (C-7), 160.7 (C-5), 159.2 (C-4'), 156.2 (C-9), 146.8 (C-2), 135.7 (C-3), 129.5 (C-2' , 6'), 121.7 (C-1'), 115.4 (C-3' , 5'), 103.0 (C-10), 98.2 (C-6), 93.5 (C-8). These data are consistent with literature values (Jung et al., 2003), identifying compound 7 as kaempferol.

Compound 8: White powder. ESI-MS m/z: 177 $[M+H]^+$, molecular formula $C_{10}H_8O_3$. ^{1}H-NMR (400 MHz, C_5D_5N) δ: 7.98 (1H, dd, J = 8.0, 1.6 Hz, H-5), 7.44 (1H, dd, J = 8.0, 1.6 Hz, H-7), 7.29 (1H, t, J = 8.0 Hz, H-6), 6.28 (1H, s, H-3), 2.05 (3H, s, 2-CH_3); ^{13}C-NMR (100 MHz, C_5D_5N) δ: 178.4 (C-4), 166.3 (C-2), 148.6 (C-10), 147.5 (C-8), 126.0 (C-9), 125.8 (C-7), 120.2 (C-6), 115.5 (C-5), 111.1 (C-3), 20.4 (2-CH_3). These data are consistent with literature values (Wang et al., 2011), identifying compound 8 as 8-hydroxy-2-methylchromone.

Compound 9: Yellow powder. ESI-MS m/z: 287 $[M+H]^+$, molecular formula $C_{15}H_{10}O_6$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.33 (2H, m, H-2' , 6'), 6.84 (1H, d, J = 8.8 Hz, H-5'), 6.51 (1H, s, H-3), 6.35 (1H, d, J = 2.0 Hz, H-8), 6.10 (1H, d, J = 2.0 Hz, H-6); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 181.0 (C-4), 166.8 (C-2), 163.7 (C-7), 161.3 (C-5), 157.4 (C-9), 151.3 (C-4'), 146.3 (C-3'), 120.4 (C-1'), 118.7 (C-6'), 115.9 (C-5'), 112.6 (C-2'), 102.6 (C-10), 102.0 (C-3), 99.4 (C-6), 94.1 (C-8). These data are consistent with literature values (Chen et al., 2018), identifying compound 9 as luteolin.

Compound 10: Yellow powder. ESI-MS m/z: 303 $[M+H]^+$, molecular formula $C_{15}H_{10}O_7$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 12.50 (1H, s, 5-OH), 7.68 (1H, d, J = 2.4 Hz, H-2'), 7.54 (1H, dd, J = 8.4, 2.4 Hz, H-6'), 6.89 (1H, d, J = 8.4 Hz, H-5'), 6.41 (1H, d, J = 2.0 Hz, H-8), 6.19 (1H, d, J = 2.0 Hz, H-6); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 175.9 (C-4), 163.9 (C-7), 160.8 (C-9), 156.2 (C-5), 147.7 (C-4'), 146.8 (C-2), 145.1 (C-3'), 135.8 (C-3), 122.0 (C-1'), 120.0 (C-6'), 115.6 (C-2'), 115.1 (C-5'), 103.0 (C-10), 98.2 (C-6), 93.4 (C-8). These data are consistent with literature values (Wang et al., 2020), identifying compound 10 as quercetin.

Compound 11: White amorphous powder. ESI-MS m/z: 732 $[M+H]^+$, molecular formula $C_{40}H_{77}NO_{10}$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.54 (1H, d, J = 9.2 Hz, N-H), 5.34 (2H, m, H-8, 9), 4.13 (1H, d, J = 7.6 Hz, H-1' '), 4.08 (1H, m, H-2), 3.83 (1H, m, H-1b), 3.82 (1H, m, H-2'), 3.66 (1H, m, H-6' 'b), 3.64 (1H, m, H-1a), 3.42 (1H, m, H-6' 'a), 3.39 (2H, m, H-3, 4), 1.22 [s, (CH_2)n], 0.84 (6H, t, J = 6.8 Hz, 2×CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 173.8 (C-1'), 130.3 (C-8), 129.6 (C-9), 103.5 (C-1' '), 76.9 (C-5' '), 76.5 (C-3' '), 74.0 (C-3), 73.5 (C-2' '), 71.0 (C-2'), 70.5 (C-4), 70.0 (C-4' '), 69.1 (C-1), 61.1 (C-6''), 49.9 (C-2), 34.4, 32.4, 32.1, 31.6, 31.4, 29.2, 29.1, 29.0, 28.8, 28.7, 25.6, 24.5, 22.2 (all CH_2), 13.9 (Me). These data are consistent with literature values (Huang et al., 2005), identifying compound 11 as 1-O-β-D-glucopyranosyl-(2S,3S,4R,8E)-2-[(2' R)-2' -hydroxypalmitoylamino]-8-octadecene-1,3,4-triol.

Compound 12: Yellow powder. ESI-MS m/z: 479 $[M+H]^+$, molecular formula $C_{22}H_{22}O_{12}$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 12.61 (1H, brs, 5-OH), 8.03 (1H, d, J = 2.4 Hz, H-2'), 7.50 (1H, dd, J = 8.4, 2.4 Hz, H-6'), 6.91 (1H, d, J = 8.4 Hz, H-5'), 6.43 (1H, brs, H-8), 6.20 (1H, brs, H-6), 5.52 (1H, d, J = 7.8 Hz, H-1' '), 3.85 (3H, s, 3' -OCH_3), 3.36–3.69 (6H, sugar protons); ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 177.4 (C-4), 164.7 (C-7), 161.3 (C-5), 156.5 (C-9), 156.2 (C-2), 149.5 (C-3'), 147.1 (C-4'), 133.2 (C-3), 121.9 (C-6'), 121.1 (C-1'), 115.2 (C-2'), 113.6 (C-5'), 103.9 (C-10), 101.7 (C-1' '), 98.9 (C-6), 93.8 (C-8), 76.0 (C-5' '), 73.2 (C-3' '), 71.3 (C-2' '), 68.0 (C-4' '), 60.4 (C-6' '), 56.0 (3' -OCH_3). These data are consistent with literature values (Zhang et al., 2021), identifying compound 12 as isorhamnetin-3-O-β-D-galactopyranoside.

Compound 13: Yellow powder. ESI-MS m/z: 465 $[M+H]^+$, molecular formula $C_{21}H_{20}O_{12}$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 12.63 (1H, s, 5-OH), 7.58 (1H, dd, J = 9.0, 2.4 Hz, H-6'), 7.58 (1H, d, J = 2.4 Hz, H-2'), 6.84 (1H, d, J = 9.0 Hz, H-5'), 6.38 (1H, d, J = 1.8 Hz, H-8), 6.18 (1H, d, J = 1.8 Hz, H-6), 5.46 (1H, d, J = 7.2 Hz, H-1' '), 3.07–3.59 (6H, sugar protons); ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 177.4 (C-4), 164.8 (C-7), 161.3 (C-5), 156.4 (C-2), 156.1 (C-9), 148.6 (C-4'), 144.9 (C-3'), 133.3 (C-3), 121.6 (C-6'), 121.2 (C-1'), 116.2 (C-5'), 115.3 (C-2'), 103.8 (C-10), 101.0 (C-1' '), 98.9 (C-6), 93.6 (C-8), 77.6 (C-5' '), 76.6 (C-3' '), 74.2 (C-2' '), 70.0 (C-4' '), 61.0 (C-6' '). These data are consistent with literature values (Yu et al., 2021), identifying compound 13 as isoquercitrin.

Compound 14: Yellow powder. ESI-MS m/z: 421 $[M-H]^-$, molecular formula

$C_{19}H_{18}O_{11}$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.22 (1H, d, J = 9.2 Hz, H-6), 7.12 (1H, d, J = 9.2 Hz, H-7), 6.32 (1H, d, J = 2.0 Hz, H-4), 6.12 (1H, d, J = 2.0 Hz, H-2), 4.75 (1H, d, J = 7.6 Hz, H-1'), 3.17–3.76 (sugar protons); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 180.2 (C-9), 167.0 (C-3), 162.9 (C-1), 156.5 (C-4a), 149.3 (C-8), 144.8 (C-4b), 141.0 (C-5), 120.6 (C-6), 112.7 (C-7), 111.9 (C-8a), 103.6 (C-1'), 102.1 (C-8b), 98.5 (C-2), 93.8 (C-4), 77.4 (C-5'), 75.9 (C-3'), 73.5 (C-2'), 69.8 (C-4'), 60.9 (C-6'). These data are consistent with literature values (Sakamoto et al., 1982), identifying compound 14 as norswertianolin.

Compound 15: Yellow powder. ESI-MS m/z: 419 $[M+H]^+$, molecular formula $C_{20}H_{18}O_{10}$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.40 (2H, overlap, H-2' , 6'), 6.89 (1H, d, J = 8.0 Hz, H-5'), 6.64 (1H, s, H-8), 6.49 (1H, s, H-3), 4.55 (1H, d, J = 9.6 Hz, H-1' '), 3.39–4.17 (5H, sugar protons); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 181.7 (C-4), 163.7 (C-2), 163.1 (C-7), 159.9 (C-5), 156.2 (C-9), 149.8 (C-4'), 145.7 (C-3'), 121.3 (C-1'), 118.9 (C-6'), 115.9 (C-5'), 113.2 (C-2'), 108.9 (C-6), 103.3 (C-10), 102.7 (C-3), 93.9 (C-8), 74.5 (C-3' '), 74.0 (C-1' '), 70.2 (C-5' '), 68.9 (C-4' '), 68.5 (C-2''). These data are consistent with literature values (Liaw et al., 2022; Wang et al., 2011), identifying compound 15 as luteolin-6-C-α-L-arabinoside.

Compound 16: Yellow powder. ESI-MS m/z: 449 $[M+H]^+$, molecular formula $C_{21}H_{20}O_{11}$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.06 (2H, m, H-2' , 6'), 6.86 (2H, m, H-3' , 5'), 6.41 (1H, d, J = 2.0 Hz, H-8), 6.19 (1H, d, J = 2.0 Hz, H-6), 5.37 (1H, d, J = 7.6 Hz, H-1' '), 3.29–3.68 (sugar protons); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 177.4 (C-4), 164.6 (C-7), 161.1 (C-5), 159.9 (C-4'), 156.4 (C-2), 156.2 (C-9), 133.2 (C-3), 130.8 (C-2' , 6'), 120.8 (C-1'), 115.0 (C-3' , 5'), 103.7 (C-10), 101.8 (C-1' '), 98.7 (C-6), 93.6 (C-8), 75.7 (C-5' '), 73.1 (C-3' '), 71.2 (C-2' '), 67.8 (C-4' '), 60.1 (C-6' '). These data are consistent with literature values (Shi et al., 2019), identifying compound 16 as kaempferol-3-O-β-D-galactopyranoside.

Compound 17: Yellow powder. ESI-MS m/z: 449 $[M+H]^+$, molecular formula $C_{21}H_{20}O_{11}$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 12.50 (1H, s, 5-OH), 10.16 (1H, s, 3-OH), 9.56 (1H, s, 4' -OH), 8.08 (2H, d, J = 9.0 Hz, H-2' , 6'), 6.94 (2H, d, J = 9.0 Hz, H-3' , 5'), 6.80 (1H, d, J = 2.4 Hz, H-8), 6.42 (1H, d, J = 2.4 Hz, H-6), 5.07 (1H, d, J = 7.2 Hz, H-1' '), 3.16–3.72 (6H, sugar protons); ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 176.1 (C-4), 162.7 (C-7), 160.4 (C-5), 159.4 (C-4'), 155.8 (C-9), 147.5 (C-2), 136.0 (C-3), 129.7 (C-2' , 6'), 121.5 (C-1'), 115.5 (C-3' , 5'), 104.7 (C-10), 99.9 (C-1' '), 98.8 (C-6), 94.4 (C-8), 77.2 (C-3' '), 76.4 (C-5' '), 73.1 (C-2' '), 69.5 (C-4' '), 60.6 (C-6' '). These data are consistent with literature values (Li et al., 2018), identifying compound 17 as kaempferol-7-O-β-D-glucopyranoside.

Compound 18: Yellow powder. ESI-MS m/z: 449 $[M+H]^+$, molecular formula $C_{21}H_{20}O_{11}$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.45 (1H, dd, J = 8.0, 2.0 Hz, H-6'), 7.42 (1H, d, J = 2.0 Hz, H-2'), 6.90 (1H, d, J = 8.4 Hz, H-5'), 6.79 (1H, d, J = 2.4 Hz, H-8), 6.76 (1H, s, H-3), 6.44 (1H, d, J = 2.4 Hz, H-6), 5.09 (1H, d, J = 7.6 Hz, H-1' '), 3.15–3.72 (6H, sugar protons); ^{13}C-NMR (100 MHz,

DMSO-d_6) δ: 182.0 (C-4), 164.5 (C-2), 163.0 (C-7), 161.2 (C-5), 157.0 (C-9), 150.0 (C-4'), 145.8 (C-3'), 121.4 (C-1'), 119.2 (C-6'), 116.0 (C-5'), 113.6 (C-2'), 105.4 (C-3), 103.2 (C-10), 99.9 (C-1' '), 99.6 (C-6), 94.7 (C-8), 77.2 (C-4' '), 76.4 (C-3' '), 73.1 (C-2' '), 69.5 (C-5' '), 60.6 (C-6' '). These data are consistent with literature values (Xiao et al., 2019), identifying compound 18 as luteolin-7-O-β-D-glucopyranoside.

Compound 19: Yellow powder. ESI-MS m/z: 433 $[M+H]^+$, molecular formula $C_{21}H_{20}O_{10}$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.93 (2H, d, J = 8.8 Hz, H-2' , 6'), 6.92 (2H, d, J = 8.8 Hz, H-3' , 5'), 6.79 (1H, s, H-3), 6.51 (1H, s, H-8), 4.59 (1H, d, J = 10.0 Hz, H-1' '), 3.09–4.08 (6H, sugar protons); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 182.0 (C-4), 163.6 (C-2), 163.5 (C-7), 161.3 (C-9), 160.8 (C-4'), 156.3 (C-5), 128.6 (C-2' , 6'), 121.2 (C-1'), 116.1 (C-3' , 5'), 109.0 (C-6), 103.4 (C-10), 102.8 (C-3), 93.7 (C-8), 81.7 (C-5' '), 79.0 (C-1' '), 73.1 (C-2' '), 70.7 (C-3' '), 70.2 (C-4' '), 61.6 (C-6' '). These data are consistent with literature values (Ren et al., 2021), identifying compound 19 as isovitexin.

Compound 20: Yellow powder. ESI-MS m/z: 611 $[M+H]^+$, molecular formula $C_{27}H_{30}O_{16}$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.54 (1H, dd, J = 8.0, 2.4 Hz, H-6'), 7.53 (1H, d, J = 2.4 Hz, H-2'), 6.85 (1H, d, J = 8.8 Hz, H-5'), 6.39 (1H, d, J = 2.0 Hz, H-8), 6.19 (1H, d, J = 2.0 Hz, H-6), 5.33 (1H, d, J = 7.6 Hz, H-1' '), 4.38 (1H, d, J = 1.6 Hz, H-1' ' '), 0.98 (3H, d, J = 6.4 Hz, H-6' ' '), 3.05–3.71 (sugar protons); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 177.2 (C-4), 164.5 (C-7), 161.1 (C-5), 156.4 (C-2, 9), 148.4 (C-4'), 144.7 (C-3'), 133.3 (C-3), 121.5 (C-6'), 121.0 (C-1'), 116.2 (C-5'), 115.2 (C-2'), 103.7 (C-10), 101.2 (C-1' '), 100.6 (C-1' ' '), 98.7 (C-6), 93.5 (C-8), 76.5 (C-3' '), 75.8 (C-5' '), 74.0 (C-2' '), 71.9 (C-4' ' '), 70.6 (C-3' ' '), 70.2 (C-4' '), 70.0 (C-2' ' '), 68.1 (C-5' ' '), 66.9 (C-6' '), 17.5 (C-6' ' '). These data are consistent with literature values (Zhu et al., 2020), identifying compound 20 as rutin.

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4. Anti-Inflammatory Activity Screening Results

Cell viability under different compound concentrations was assessed using the CCK-8 assay to evaluate cytotoxicity. Based on these results, test concentrations were established: compounds 2, 3, 14, 16, and 20 at 100 $mol \cdot L^{-1}$; compounds 1, 5, 6, 8, 9, 11–13, and 19 at 50 $mol \cdot L^{-1}$; and compounds 4, 7, 10, and 18 at 25 $mol \cdot L^{-1}$. All compounds showed >90% cell viability at these concentrations, indicating no cytotoxicity.

After 24 h of LPS stimulation, NO secretion in the model group increased significantly compared to the blank group ($P < 0.01$), confirming successful model establishment. Compared with the model group, compounds 4, 7, 10, and 18 at 25 $mol \cdot L^{-1}$; compounds 5, 6, 8, 9, 12, and 19 at 50 $mol \cdot L^{-1}$; and compounds 2 and 3 at 100 $mol \cdot L^{-1}$ significantly reduced NO secretion ($P < 0.05$, $P < 0.01$). Six compounds (1, 11, 13, 14, 16, and 20) showed no significant effect on NO production. Results are summarized in Table 1 .

5. Discussion and Conclusion

This study isolated and identified 20 compounds from the 70% ethanol extract of *P. candolleana* whole plant, including 15 flavonoids (5–10, 12–20), 2 phenolic compounds (1, 4), 1 lignan (2), 1 phenylpropanoid (3), and 1 amide (11). Among these, compounds 2, 5, 8, 11, 12, 14, 15, and 17 were isolated from the genus *Pimpinella* for the first time, while compounds 1, 3, 4, 6, 7, 10, 13, 16, 18, and 20 were isolated from *P. candolleana* for the first time.

Inflammation is a common pathological condition when homeostasis is disturbed, often described as "nine out of ten diseases involve inflammation." Inflammatory processes involve multiple mediators, cytokines, and signaling pathways. Nitric oxide (NO) is a bioactive substance with both pro- and anti-inflammatory effects that plays a key regulatory role in inflammatory cascades, particularly in the initiation and signal transduction of inflammatory responses (Cao et al., 2021; Li et al., 2021; Yang et al., 2016). Therefore, this study used an LPS-induced RAW264.7 cell model to evaluate NO production.

The results showed that the lignan (2), phenylpropanoid (3), flavonoids (5–10, 12, 18–19), and phenolic compound (4) significantly inhibited LPS-induced NO production at safe concentrations, with inhibition rates of 78.36%, 76.51%, 80.82%, 64.88%, 83.60%, 61.21%, 79.80%, 68.16%, 62.14%, 81.14%, 71.26%, and 57.37%, respectively. Notably, compounds 5 at 50 $\mathrm{mol \cdot L^{-1}}$ and compounds 7 and 18 at 25 $\mathrm{mol \cdot L^{-1}}$ exhibited NO inhibition comparable to the positive control dexamethasone at 25 $\mathrm{mol \cdot L^{-1}}$.

Current pharmacological research on folk medicinal plants in the genus *Pimpinella* has primarily focused on crude extracts, with limited studies on individual compounds, particularly regarding anti-inflammatory activity. Only quinic acid derivatives from *Pimpinella brachycarpa* methanol extract have been reported for anti-inflammatory activity in LPS-induced BV-2 cells (Lee et al., 2013). This study on the chemical constituents and anti-inflammatory activity of *P. candolleana* enriches its chemical profile, identifies flavonoids as the active anti-inflammatory constituents, and establishes a foundation for further pharmacological research and development. It also provides an important reference for expanding chemical and biological studies of medicinal plants in the genus *Pimpinella*.

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Figure 1. Chemical structures of compounds 1–20 [Figure 1: see original paper]

Table 1. Inhibition ratio of constituents on production of NO in LPS-induced RAW 264.7 cells (n=3)

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