

Two New Compounds from *Arctium lappa* RootsQingyu Yang ^{id}1,2#, Jun Chi ^{id}1,2#, Weijin Zhang ^{id}1,2, Lingxia Zhang ^{id}1,2,
Zhimin Wang ^{id}1,3* and Liping Dai ^{id}1,2*¹ Henan University of Chinese Medicine, Henan Collaborative Innovation Center for Research and Development on the Whole Industry Chain of Yu-Yao, Zhengzhou 450046, China² Engineering Technology Research Center for Comprehensive Development and Utilization of Authentic Medicinal Materials in Henan Province, Zhengzhou, 450046, China³ Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing 100700, China

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Abstract: A new caffeoylquinic acid, 1, 5-di-O-caffeoyl-3-O-(1-O-methoxy-2-O-caffeoyl-4-maloyl)-quinic acid (**1**), and a new 5-hydroxymethylfurfural dimer, 5-(((2-methoxymethyl) furan-5-methanol) methyl) furan-2-carbaldehyde (**2**), were discovered from *Arctium lappa* roots. A comprehensive HRMS, UV, IR, 1D and 2D NMR techniques were used for structural identification. These two compounds had Nitric Oxide (NO) inhibitory activity in lipopolysaccharide (LPS)-stimulated RAW 264.7 inflammatory cell in a dose-dependent manner.

Keywords: *Arctium lappa* roots; caffeoylquinic acid; 5-hydroxymethylfurfural derivative; anti-inflammatory activity. © 2024 ACG Publications. All rights reserved.

1. Introduction

Burdock roots, also known as Dali roots, are the roots of *Arctium lappa* L. (Compositae) [1], and it was first recorded in “*Mingyi Beilu*” of the Wei and Jin Dynasties as “rhizome, treat typhoid fever, cold and heat, sweating, stroke, long-term use, light body and anti-aging...”. It has abundant resources and was widely cultivated in Jiangsu, Henan and other provinces of China [2]. Burdock roots contained multiple functional ingredients with high nutritional value, making it an excellent tonic for both medicine and food [3]. In 2018, the National Health Commission of China listed burdock roots as a traditional Chinese medicine that could be utilized as health food [4]. Previous studies showed that burdock roots were rich in flavonoids, organic acid and polysaccharides, which had remarkable bioactivities, including anti-inflammatory, anti-oxidant, anti-diabetes and neuroprotective action [5-10]. In this study, phytochemical research of burdock roots was performed out, resulting in the separation of a new caffeoylquinic acid derivative, 1, 5-di-O-caffeoyl-3-O-(1-O-methoxy-2-O-caffeoyl-4-maloyl)-quinic acid (**1**), and a new 5-hydroxymethylfurfural dimer, 5-(((2-methoxy methyl) furan-5-methanol) methyl) furan-2-carbaldehyde (**2**) (Figure 1). Their anti-inflammatory capacities on LPS-stimulated RAW 264.7 cells were also assessed herein.

* Corresponding author: E-Mail: zzdai@163.com; Phone: +86-18703651652

These authors contributed to this work equally.

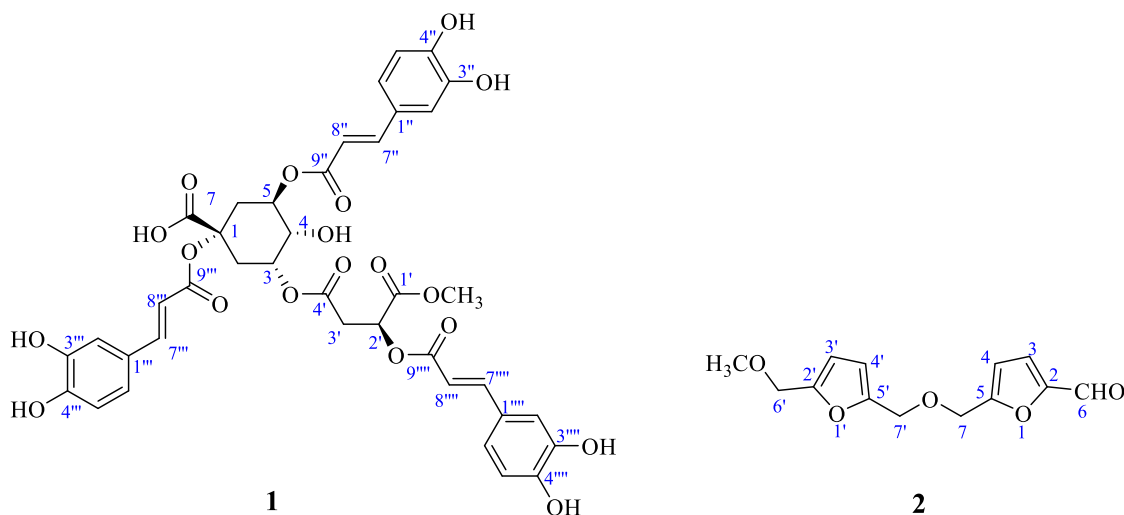


Figure 1. Chemical structures of compounds **1** and **2**

2. Materials and Methods

2.1. Apparatus and Reagents

An Anton paar MCP 5100 polarimeter (Anton Paar GmbH, Austria) were used for optical rotation measurements. A Thermo UPLC/Orbitrap-Exploris-120 instrument (Thermo Fisher Scientific Inc., USA, the same below) were used to obtain HR-ESIMS data. A Thermo Evolution 220 spectrophotometer was used to acquire UV spectra. A Thermo Nicolet iS5 spectrometer were used to get IR spectra. A Bruker Avance III 500-NMR device (Bruker BioSpin AG, Germany) were used to obtain NMR spectra, and TMS (teramethyl silane) was used as internal standard. The ECD spectrum was obtained on Applied Photophysics Chirascan V100 spectrometer. Column chromatography separations were performed with silica gel (100–200 mesh, Qingdao Marine Chemical Inc., Qingdao, China) and ODS-C₁₈ (40-63 μ m, Fuji, Japan). Semi-preparative HPLC was conducted on a Shimadzu LC-20AT series instrument (Shimadzu Corporation, Japan) with a YMC-pack ODS-A (250 \times 10 mm, I.D. 5 μ m) column and a Shimadzu SPD-M20A detector.

2.2. Plant Material

The roots of *Arctium lappa* L. (burdock roots) were procured in October 2021 from Xuzhou City of Jiangsu Province, China. Identification of the species were carried out by Professor Liping Dai from Henan University of Chinese Medicine. The voucher sample is stored at the Henan Collaborative Innovation Center for Research and Development on the Whole Industry Chain of Yu-Yao under the reference number HECM 0011085.

2.3. Extraction and Isolation

After cutting the 50.0 kg of dried burdock roots into pieces, they were extracted by refluxing with 70% ethanol (2 \times 150 L \times 2 h). Non-alcoholic crude extract was dispersed into water, followed by sequential extraction using EtOAc and n-butanol. A silica gel column (100 – 200 mesh) was selected to separate EtOAc fraction (360.0 g), using a dichloromethane-acetone solutions (1:0 \rightarrow 0:1). Eight fractions (Fr. 1~8) were obtained, and TLC was used to monitor the process. An ODS column chromatography with ACN-H₂O (10% \rightarrow 80%) was further used to separate Fr. 3 (13.4 g), to afford Fr. 3-1~4. Compound **1** (4.2 mg, 31 min) was obtained by semi-preparative HPLC purification of Fr. 3-4 (100.2 mg) using 11% ACN-H₂O (3 mL/min). An ODS column with MeOH-H₂O (5% \rightarrow 100%)

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was used for separation of Fr. 8 (50.6 g), to yield Fr. 8-1~12. Compound **2** (7.0 mg, 58 min) was obtained through semi-preparative HPLC purification of Fr. 8-11 (2.4 g) using 25% ACN-H₂O.

2.4. Spectral Data

Compound **1**: White amorphous powder; $[\alpha]_D^{25} + 28.0$ (*c* 0.1, MeOH); UV (MeOH) λ_{\max} (log ϵ): 330 (3.34), 300 (3.23) and 244 (3.09) nm; ECD (MeOH, $\Delta\epsilon$) λ_{\max} 209 (-0.78), 342 (0.96) nm; IR (KBr) λ_{\max} : 3300, 1698, 1600, 1519, 1275 and 970 cm⁻¹; HR-ESIMS at *m/z* 807.1794 [M-H]⁺ (calcd for C₃₉H₃₅O₁₉, 807.1787); ¹H and ¹³C NMR (500 and 125 MHz, methanol-*d*₄) data in Table 1.

Compound **2**: Yellow amorphous oil; $[\alpha]_D^{25} + 4.0$ (*c* 0.1, MeOH); UV (MeOH) λ_{\max} (log ϵ): 276 (2.48) and 223 (2.93) nm; IR (KBr) λ_{\max} : 3128, 2927, 1736, 1678, 1521, 1369 and 1089 cm⁻¹; HR-ESIMS at *m/z* 273.0732 [M+Na]⁺ (C₁₃H₁₄O₅Na, 273.0733); ¹H and ¹³C NMR (500 and 125 MHz, methanol-*d*₄) data in Table 2.

2.5. ECD Calculation for Compound **1**

ECD calculation of compound **1** were performed using Gaussian16. The 3D structures were first established on the basis of the ROESY correlations and coupling constants. Their energy minimized conformers were generated via the Molecular Mechanics field in Spartan 14. Then, the conformation optimization and ECD calculation for compound **1** was performed at the b3lyp/6-31g(d) level and the solvent effect of methanol was considered by IEFPCM solvent model using the Gaussian 16 package. Their ECD curve was produced via SpecDic software and OriginPro 8 with UV correction.

2.6. Bioassays for Anti-inflammation

2.6.1. Cell Culture and Viability

After being grown on 96-well plates over 24 hours, RAW 264.7 cells (1×10⁵ cells in 100 μL) were exposed to different doses of isolated compounds (6.25 – 100 μM). The media was removed after 24 hours, followed by a 4-hour treatment of the cells with 20 μL of MTT at a temperature of 37 °C. To dissolve the formazan crystals inside the cells, each well received 150 μL of DMSO, followed by agitating the plate on an oscillator for 10 minutes. Around 490 nm, the absorbance was finally detected, and cell survival rates were calculated [11,12].

2.6.2. NO Levels on LPS-Stimulated RAW 264.7 Cells

After being grown on 96-well plates over 24 hours, RAW 264.7 cells (8×10⁴ cells in 100 μL) were treated with compounds **1** and **2** (6.25 – 100 μM) for 2 hours. Subsequently, the cells underwent a 24-hour exposure to 1.0 μg/mL of LPS. A volume of 100 μL from the supernatants of the cell culture medium was gathered, and 100 μL of Griess reagent was added for analysis. Following a 10-minute incubation period, the measurement of absorbance at 540 nm was conducted. Rates of NO inhibition were calculated [11].

3. Results and Discussion

3.1. Structure Elucidation

The white amorphous powder of compound **1** was obtained. Its UV spectrum (Figure S4 in supporting information) displayed the characteristic absorptions of caffeoylquinic acids at 245, 300 and 330 nm [13]. IR (KBr disc) absorptions (Figure S5) indicated the existence of hydroxyl (3300 cm⁻¹), carbonyl (1698 cm⁻¹), aromatic double bond (1600, 1519 cm⁻¹) and *trans* double bond (1275, 970 cm⁻¹) groups. The molecular formula of **1** was predicted as C₃₉H₃₆O₁₉ in view of a pseudo molecular ion at *m/z* 807.1794 [M – H]⁻ (calcd for C₃₉H₃₅O₁₉, 807.1787) in its HR-ESIMS spectrum (Figure S3).

The ^1H NMR spectral data showed characteristic signals for three ABX-type aromatic rings at δ_{H} 7.10 (d, $J = 2.1$ Hz, H-2''), 7.06 (d, $J = 2.1$ Hz, 2'''), 7.05 (d, $J = 2.1$ Hz, 2'''), 6.98 (dd, $J = 8.2, 2.1$ Hz, H-6'''), 6.96 (dd, $J = 8.2, 2.1$ Hz, H-6''), 6.90 (dd, $J = 8.2, 2.1$ Hz, H-6'''), 6.73 – 6.80 (m, H-5'', H-5''', H-5'''); three (*E*)-olefinic bonds at δ_{H} 7.61 (d, $J = 15.9$ Hz, H-7'') and 7.56 (d, $J = 15.9$ Hz, H-7''', 7'''), 6.36 (d, $J = 15.9$ Hz, H-8'''), 6.32 (d, $J = 15.9$ Hz, H-8'') and 6.28 (d, $J = 15.9$ Hz, H-8''); four oxygenated methylene protons at δ_{H} 5.53 (ddd, $J = 10.6, 10.6, 4.5$ Hz H-5), 5.45 (q, $J = 3.6$ Hz, H-3), 5.36 (dd, $J = 7.3, 4.1$ Hz, H-2') and 3.92 (dd, $J = 10.0, 3.7$ Hz, H-4); a methoxy group at δ_{H} 3.56 (s); as well as two methylenes H₂-2 [δ_{H} 2.98 (m); 2.44 (dd, $J = 16.3, 3.4$ Hz)] and H₂-6 [δ_{H} 1.90 (m); 2.61 (dt, $J = 13.1, 3.8$ Hz)]. In the ^{13}C NMR spectrum (Table 1), there were six carbonyl carbons at δ_{C} 176.1 (C-7), 171.2 (C-1'), 170.5 (C-4'), 168.7 (C-9''), 167.8 (C-9''') and 167.8 (C-9'''); three sets of aromatic rings and double bonds at δ_{C} 149.8 (C-4'''), 149.6 (C-4'', 4'''), 148.2 (C-7'''), 147.2 (C-7'', 7'''), 146.8 (C-3'', 3''', C-3'''), 127.8 (C-1'''), 127.7 (C-1''), 127.6 (C-1'''), 123.4 (C-6'''), 123.1 (C-6'''), 123.0 (C-6''), 116.5 (C-5'', 5''', 5'''), 116.0 (C-8'''), 115.3 (C-8''), 114.0 (C-8'''), 115.2 (C-2'''), 115.1 (C-2'') and 114.0 (C-2''); one oxygenated quaternary carbon at δ_{C} 82.3 (C-1); four oxygenated methylenes at δ_{C} 74.2 (C-3), 72.4 (C-4), 71.3 (C-5) and 69.2 (C-2'); one methoxy group at δ_{C} 53.0 (-OCH₃); and three methylenes at δ_{C} 39.2 (C-6), 37.1 (C-3') and 32.8 (C-2). According to the information above, the structure of compound **1** should be a caffeoylquinic acid frequently in this species [14], and composed of one quinic acid, three caffeic acids, and one malic acid connected with ether other through ester bonds, as 1, 3-di-O-caffeoyl-5-O-(1-methoxyl-2-O-caffeoyl-4-maloyl)-quinic acid [14].

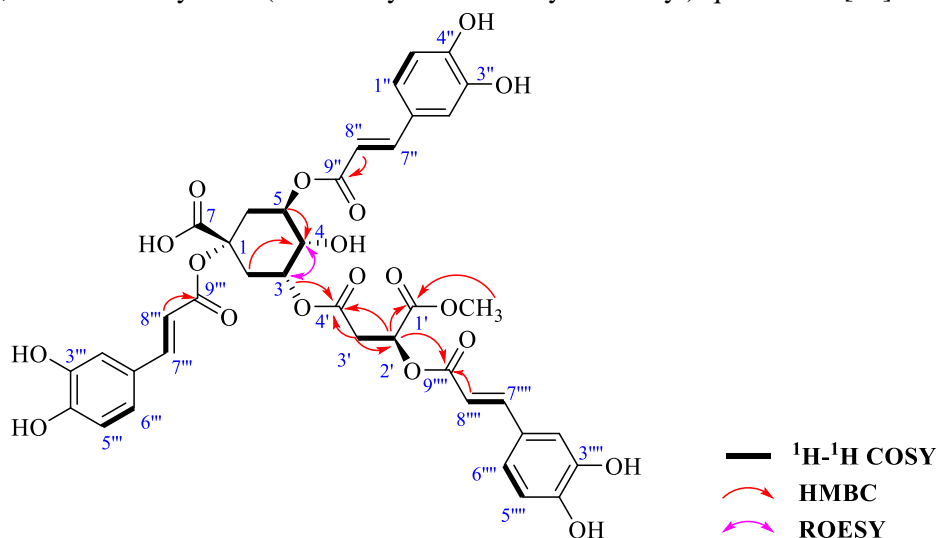


Figure 2. Key ^1H - ^1H COSY, HMBC and ROESY correlations of compound **1**

The obvious ^1H - ^1H COSY correlations (Figure 2) of H₂-2/H-3/H-4/H-5/H₂-6, and comparing their chemical shifts with those of literature [13], including the special down-fielded methines H-5 [δ_{H} 5.53 (ddd, $J = 10.6, 10.6, 4.5$ Hz)] and H-3 [δ_{H} 5.45 (q, $J = 3.6$ Hz)], the oxygenated methine H-4 [δ_{H} 3.92 (dd, $J = 10.0, 3.7$ Hz)] and the distinguishable methylenes H₂-2 [δ_{H} 2.98 (m); 2.44 (dd, $J = 16.3, 3.4$ Hz)] and H₂-6 [δ_{H} 1.90 (m); 2.61 (dt, $J = 13.1, 3.8$ Hz)], undoubtedly assigned the NMR data of quinic acid, while also indicating the existence of caffeoyl or maloyl groups at C-3 and C-5. The HMBC cross-peaks (Figure 2) from H-2' of malic acid to C-1' (δ_{C} 171.2), C-3' and C-4' (δ_{C} 170.5) as well as C-9'' (δ_{C} 167.8) of caffeoyl group, from the methoxy protons [δ_{H} 3.56 (s)] to C-1', and from H₂-3 to C-4', allowed the assignment of a caffeoyl and a methoxy group at C-2' and C-1' in the malic acid, respectively. Additionally, H-3 was obviously correlated with C-4' of malic acid, suggesting that the 1-O-methoxy-2-O-caffeoyl-maloyl fragment was linked at C-3 of quinic acid, which was the only difference between compound **1** and 1, 3-di-O-caffeoyl-5-O-(1-methoxyl-2-O-caffeoyl-4-maloyl)-quinic acid [13]. On the other words, the other two caffeoyl groups was respectively substituted at C-1 and C-5 of quinic acid based on the above results and the oxygenated quaternary carbon C-1 (δ_{C} 82.3). Therefore, the free hydroxyl and carboxyl groups were respectively linked to C-1 and C-4 of quinic acid, combined with the molecular formula C₃₉H₃₆O₁₉. In the NOESY (Figure 2) spectrum, the obvious correlations between H-3 and H-4 suggested H-3 and H-4 were on the same face. Furthermore,

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the good agreement of experimental and calculated ECD spectra (Figure 3) for 1*S*, 2*R*, 4*R*, 5*R*, 2'*S* confirmed the absolute configuration of compound **1** was 1*S*, 2*R*, 4*R*, 5*R*, 2'*S*. Ultimately, its structure was established as 1,5-di-*O*-caffeoyl-3-*O*-(1-*O*-methoxy-2-*O*-caffeoyl-4-maloyl)-quinic acid (**1**) (Figure 1).

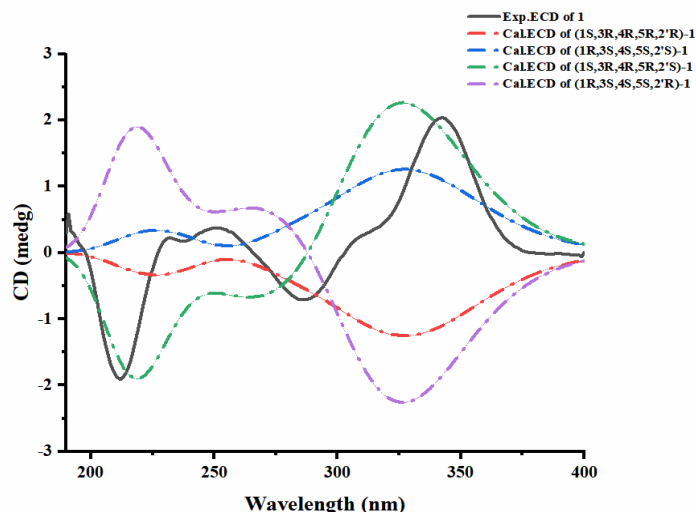


Figure 3. Experimental and calculated ECD spectra of compound **1**

A yellow amorphous oil of compound **2** was acquired. In its UV spectrum (Figure S25), two absorption peaks at 226 and 276 nm were observed. The clearly observed IR peak (Figure S26) at 1736 cm^{-1} suggested the carbonyl group in compound **2**. A molecular formula of $\text{C}_{13}\text{H}_{14}\text{O}_5$ was considered in view of the positive HR-ESIMS ion (Figure S24) at m/z 273.0732 $[\text{M}+\text{Na}]^+$ (calcd. for $\text{C}_{13}\text{H}_{14}\text{O}_5\text{Na}$, 249.0733). The ^1H NMR along with HSQC spectrograms (Table 2) of compound **2** showed the signals for an aldehyde proton at δ_{H} 9.56 (s, H-6), four olefinic protons at δ_{H} 7.38 (d, $J = 3.6$ Hz, H-3), 6.64 (d, $J = 3.6$ Hz, H-4), 6.39 (d, $J = 3.3$ Hz, H-3') and 6.37 (d, $J = 3.2$ Hz, H-4'), three oxygenated methylenes at δ_{H} 4.58 (s, H-7), 4.53 (s, H-7') and 4.38 (s, H-6'), as well as a methoxy group at δ_{H} 3.34 (s). The ^{13}C NMR and HSQC spectra showed 13 carbons, which included an aldehyde at δ_{C} 179.5 (C-6), four double bonds at δ_{C} 159.8 (C-5), 154.2 (C-2), 153.6 (C-2'), 152.9 (C-5'), 124.0 (C-3), 112.8 (C-4), 111.8 (C-4') and 111.3 (C-3'), three oxymethylenes at δ_{C} 67.2 (C-6'), 65.3 (C-7'), 64.5 (C-7), as well as a methoxy group at δ_{C} 57.9 (-OCH₃).

The H-3/H-4 and H-3'/H-4' fragments based on the ^1H - ^1H COSY analysis (Figure 4), along with a thorough examination of HMBC correlations (Figure 4), established the chemical structure of compound **2**. The olefinic protons H-3 and H-4 demonstrated obviously HMBC correlations with C-2 (δ_{C} 154.2) and C-5 (δ_{C} 159.8), and H-3' and H-4' were also clearly correlated with C-2' (δ_{C} 153.6) and C-5' (δ_{C} 152.9), which confirmed two difuran skeleton in compound **2**. The key correlations from H₂-7 (δ_{H} 4.58) to C-4, C-5, and from H₂-6 (δ_{H} 9.56) to C-2 indicate the presence of a 5-hydroxymethylfurfural in its structure. The cross-peaks of H₂-6' (δ_{H} 4.38) to C-2', C-3', from H-7' (δ_{H} 4.53) to C-4', C-5', as well as -OCH₃ (δ_{H} 3.34) to C-6' (δ_{C} 67.2), supported an additional presence of 5-hydroxymethylfurfural that C-6' was reduced and substituted by a methoxy group. The H₂-7' (δ_{H} 4.53) demonstrated a clear HMBC correlation with C-7 (δ_{C} 64.5), combined with the oxygenated methylenes C-7 and C-7', revealed that these two 5-hydroxymethylfurfural fragments were connected between C-7 and C-7' by an ether bond. As a result, compound **2** was elucidated as 5-(((2-methoxy methyl) furan-5-methanol) methyl) furan-2-carbaldehyde (Figure 1).

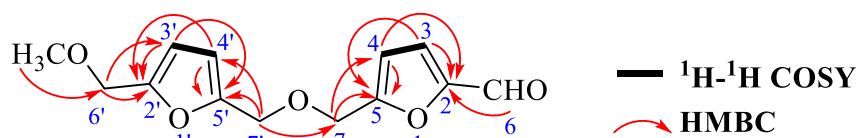


Figure 4. Key ^1H - ^1H COSY and HMBC correlations of compound **2**

Table 1. ^1H and ^{13}C NMR data (500 and 125 MHz, MeOD) for compound **1** (δ in ppm, J in Hz)

Position	δ_{H}	δ_{C}	Position	δ_{H}	δ_{C}
1		82.3	1'''		127.6
2a	2.98 (m)	32.8	2'''	7.05 (d, 2.1)	115.1
2b	2.44 (dd, 16.3, 3.4)		3'''		146.8
3	5.45 (q, 3.6)	74.2	4'''		149.6
4	3.92 (dd, 10.0, 3.7)	72.4	5'''	6.73-6.80 (m)	116.5
5	5.53 (ddd, 10.6, 10.6, 4.5)	71.3	6'''	6.98 (dd, 8.2, 2.1)	123.4
6a	1.90 (m)	39.2	7'''	7.56 (d, 15.9)	148.2
6b	2.61 (dt, 13.1, 3.8)		8'''	6.28 (d, 15.9)	114.0
7		176.1	9'''		167.8
1'		171.2	1''''		127.8
2'	5.36 (dd, 7.3, 4.1)	69.2	2''''	7.06(d, 2.1)	115.1
3'a	3.03 (m)	37.1	3''''		146.8
3'b	2.80 (m)		4''''		149.8
4'		170.5	5''''	6.73-6.80 (m)	116.5
1''		127.7	6''''	6.90 (dd, 8.2, 2.1)	123.1
2''	7.10 (d, 2.1)	115.2	7''''	7.56 (d, 15.9)	147.2
3''		146.8	8''''	6.36 (d, 15.9)	116.0
4''		149.6	9''''		167.8
5''	6.73-6.80 (m)	116.5	-OCH ₃	3.56 (3H, s)	53.0
6''	6.96 (dd, 8.2, 2.1)	123.0			
7''	7.61 (d, 15.9)	147.2			
8''	6.32 (d, 15.9)	115.3			
9''		168.7			

Table 2. ^1H and ^{13}C NMR data (500 and 125 MHz, MeOD) for compound **2** (δ in ppm, J in Hz)

Position	δ_{H}	δ_{C}
2		154.2
3	7.38 (d, 3.6)	124.0
4	6.64 (d, 3.6)	112.8
5		159.8
6	9.56 (s)	179.5
7	4.58 (s)	64.5
2'		153.6
3'	6.39 (d, 3.3)	111.3
4'	6.35 (d, 3.3)	111.8
5'		152.9
6'	4.38 (s)	67.2
7'	4.53 (s)	65.3
-OCH ₃	3.34 (s)	57.94

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3.2. Anti-Inflammatory Effects

According to cell viability tests, compounds **1** and **2** did not exhibit any cytotoxicity at concentration range of 6.25-100 μ M (Figure 5) against RAW 264.7 cells. Moreover, LPS-stimulated RAW 264.7 cells were used to investigate these two isolates' anti-inflammatory properties at 6.25, 12.5, 25, 50 and 100 μ M. According to the findings, each of them had anti-inflammatory activity and reduced the amount of NO *in vitro* (Figure 6).

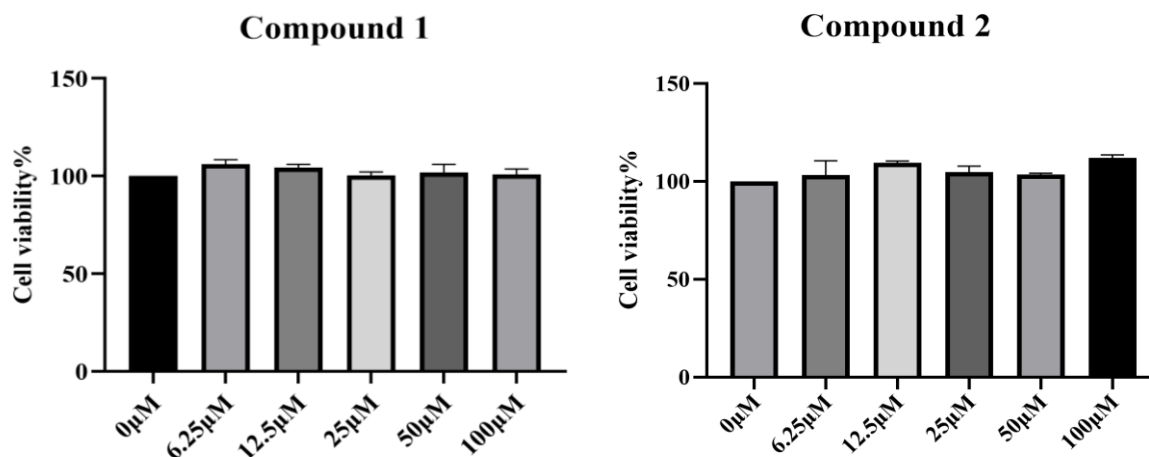


Figure 5. RAW 264.7 cell viabilities in the presence of compounds **1** and **2**

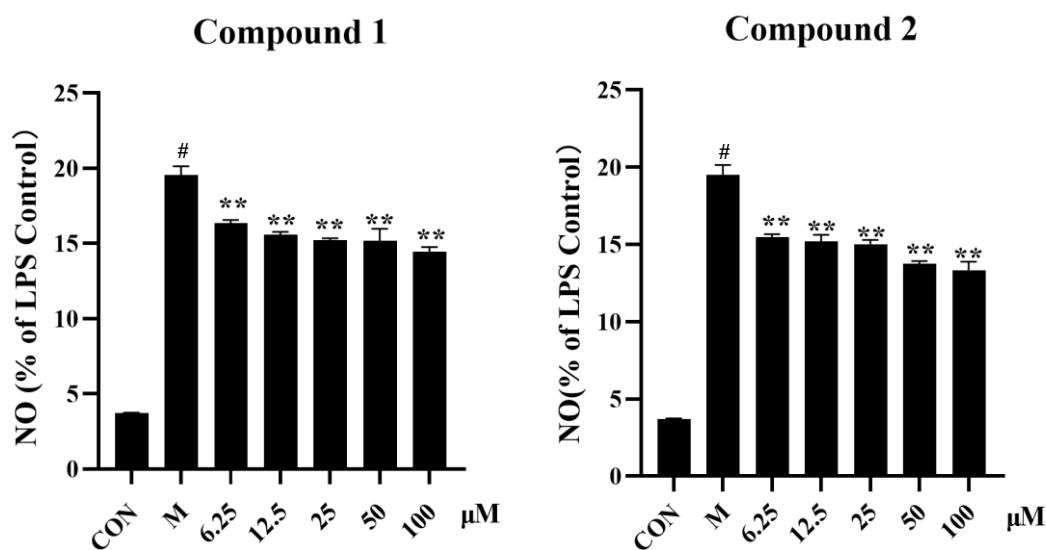


Figure 6. Effects of compounds **1** and **2** on NO release in LPS-stimulated RAW 264.7 cells. Compared with the control group, # $P < 0.05$, ## $P < 0.01$; Compared with the model group, * $P < 0.05$, ** $P < 0.01$

4. Conclusion

Through methodical chemical investigation, two new secondary metabolites **1** and **2**, were separated from the ethyl acetate fraction of *A. lappa* roots. Additionally, compounds **1** and **2** exhibited NO inhibition on LPS-stimulated RAW 264.7 cells. This research adds to understanding the chemical compositions of *A. lappa* roots and establish a scientific basis for their future use and development.

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Supporting Information

Supporting information accompanies this paper on <http://www.acgpubs.org/journal/records-of-natural-products>

ORCID

Qingyu Yang: [0009-0002-3183-8220](https://orcid.org/0009-0002-3183-8220)

Jun Chi: [0000-0002-7314-6583](https://orcid.org/0000-0002-7314-6583)

Weijing Zhang: [0009-0005-5907-6282](https://orcid.org/0009-0005-5907-6282)

Lingxia Zhang: [0000-0002-5562-6735](https://orcid.org/0000-0002-5562-6735)

Zhimin Wang: [0000-0003-4770-3814](https://orcid.org/0000-0003-4770-3814)

Liping Dai: [0000-0002-1910-7591](https://orcid.org/0000-0002-1910-7591)

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