doi.org/10.1002/cbdv.202300691 Research Article CHEMISTRY & BIODIVERSITY

www.cb.wiley.com

Special Collection

Three New Constituents with Anti-Inflammatory and Antimicrobial Activities *in Vitro* from the Roots of *Capsicum annuum* L.

Jiang Shi[+],[a, b] Lu-Lu Deng[+],[b] Jiang Li,[b] Min Wang,[b] Yan-Hua Fan,[b] Wei-Dong Pan,[a] Xiao-Jiang Hao,[b, c] and Shu-Zhen Mu*[b]

Three new compounds, including two new sesquiterpenes (**1**–**2**), named Annuumine E–F, and one new natural product, 3-hydroxy-2,6-dimethylbenzenemethanol (**3**), together with seventeen known compounds (**4**–**20**) were isolated from the ethanol extract of the roots of *Capsicum annuum* L. Among them, five compounds (**4**, **5**, **9**, **10** and **20**) were isolated from this plant for the first time. The structures of new compounds (**1**–**3**) were determined via detailed analysis of the IR, HR-ESI-MS and 1D and 2D NMR spectra. The anti-inflammatory activities of the isolated compounds were evaluated by their ability to reduce NO release by LPS-induced RAW 264.7 cells. Notably, compound **11** exhibited moderate anti-inflammatory activity (IC_{50} = 21.11 μM). Moreover, the antibacterial activities of the isolated compounds were also evaluated.

Introduction

Capsicum annuum L. (*C. annuum* L.) is an annual plant in the genus *Capsicum* and the family Solanaceae, and its fruit is a common vegetable and condiment. The roots of *C. annuum* L. are agricultural waste leftover after the harvest of *C. annuum* L fruits, and are traditionally treated through incineration. The incineration of large quantities of *C. annuum* L. roots not only pollutes the atmosphere, but also causes waste of plant resources. In recent years, the use of chemical and biological methods to analyze the chemical composition and possible biological functions of agricultural waste has expanded its applications in medicine and health,[1–4] which is of great importance in sustainable development.

Numerous studies on the fruits of *C. annuum* L. have resulted in the isolation of various classes of natural products, including terpenoids, alkaloids, steroids, flavonoids, polysaccharides and saponins, and many components were reported to have pharmacological effects, such as analgesic, anticancer, antioxidative, antiobesity and hypoglycemic, insecticidal and antibacterial activities.[5–9] In Chinese folk medicine, the roots of *C. annuum* L. have been used to treat cholecystitis and arthritis, but their chemical constituents are unclear. In addition, little research has focused on the roots of *C. annuum* L. To further clarify the chemical constituents of *C. annuum* L. roots, a chemical investigation was carried out. Here, three new compounds (**1**–**3**) were obtained from the ethanol extract, and their structures were determined on the basis of their physicochemical properties and spectral data. After evaluating their bioactivities *in vitro*, compound **11** was found to have moderate anti-inflammatory activity in RAW 264.7 cells, and compound **9** had some inhibitory activity against *Phytophthora cinnamomi* (*P. cinnamomi*), *Ralstonia solanacearum* (*R. solanacearum*) and *Bacillus subtilis* (*B. subtilis*) (Figure 1).

[a] *J. Shi,[+] W.-D. Pan*
College of Pharmacy
Guizhou University
South Section of Huaxi Road 2078
Guiyang 550025, P.R. China

[b] *J. Shi,[+] L.-L. Deng,[+] J. Li, M. Wang, Y.-H. Fan, X.-J. Hao, S.-Z. Mu*
State Key Laboratory of Functions and Applications of Medicinal Plants
Guizhou Medical University
3491 Baijin Road
Guiyang 550014, P. R. China,
E-mail: muzi0558@126.com

[c] *X.-J. Hao*
Kunming Institute of Botany
Chinese Academy of Sciences (CAS)
132 Lanhei Road
Kunming 650201, P. R. China

[+] *These authors contributed equally to this work.*

Supporting information for this article is available on the WWW under https://doi.org/10.1002/cbdv.202300691

Special Collection *Part of a Special Collection on Chemical Evidence/Interpretation to Diverse Effects of Ethnomedicines of China.*

Results and Discussion

Phytochemical Investigation

Annuumine E (**1**) was isolated as a colorless oil substance. Its molecular formula was determined to be $C_{15}H_{24}O_2$ according to excimer ion peak *m/z* 259.1661 $[M+Na]^+$ (calc. for $C_{15}H_{24}O_2Na$, 259.1669) in the HR-ESI-MS spectrometry. Its IR spectrum showed absorptions for hydroxy groups (3289, 1040 and 1005 cm^{-1}) and olefin group (1643 cm^{-1}). The ^{1}H-NMR spectrum (Table 1) displayed signals for three methyl protons at δ_H 1.74 (3H, s, H-13), 1.40 (3H, s, H-14) and 0.93 (3H, d, J = 6.9, H-15); two olefinic protons at δ_H 5.61 (1H, dd, J = 4.6, 2.8, H-1) and 4.70 (2H, s, H-12); two oxymethine protons at δ_H 4.38 (1H, dd, J = 8.8, 3.8, H-9) and 4.26–4.29 (1H, m, H-3). The ^{13}C-NMR spectrum (Table 1) exhibited 15 carbon signals, including three methyl carbons at δ_C 33.9 (C-14), 20.7 (C-13) and 8.7 (C-15); four

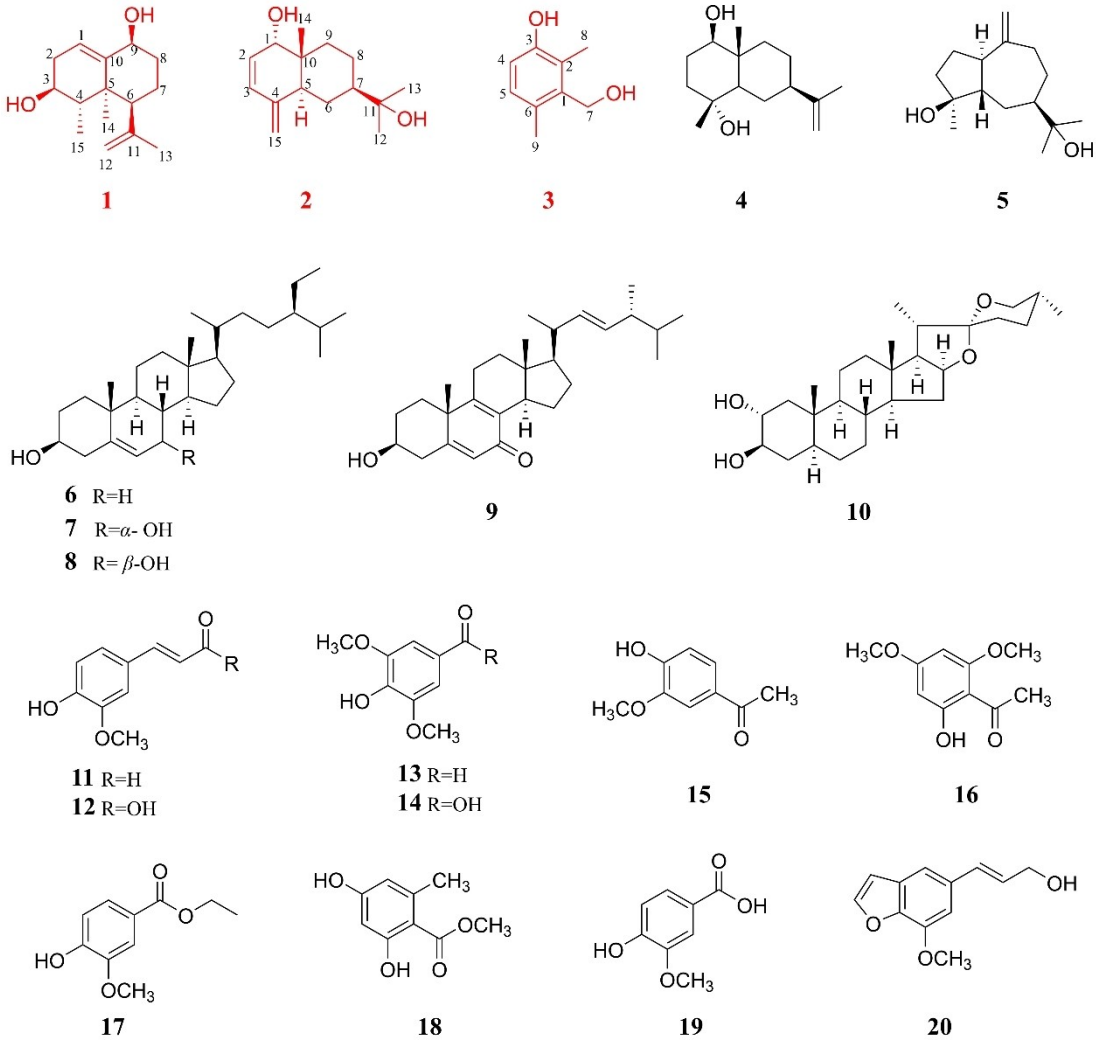

Figure 1. Chemical structures of compounds **1–20**.

Table 1. 1D-NMR data of compounds **1–2** in $CDCl_3$, **3** in CD_3OD (^1H for 600 MHz and ^{13}C for 150 MHz).

No.	**1** δ_H mult (*J* in Hz)	δ_C	**2** δ_H mult (*J* in Hz)	δ_C	**3** δ_H mult (*J* in Hz)	δ_C
1	5.61 dd (4.6, 2.8)	125.5	3.55 d (5.4)	72.6		138.6
2	*α*: 2.27–2.32 m *β*: 2.00–2.06 m	31.6	5.84 dd (9.5, 5.5)	127.0		125.3
3	4.26–4.29 m	67.5	6.21 d (9.7)	132.8		154.6
4	1.60–1.62 m	48.4		145.4	6.62 d (8.1)	115.2
5		39.8	2.32–2.36 m	38.0	6.81 d (8.1)	128.9
6	1.96-2.01 m	40.7	*α*: 2.04–2.07 m *β*: 1.12–1.16 m	24.4		129.2
7	*α*: 1.45–1.50 m *β*: 1.87 dt (13.5, 2.9)	42.4	1.34–1.39 m	48.7	4.64 s	59.4
8	*α*: 2.17–2.22 m *β*: 1.48-1.52 m	39.2	*α*:1.25–1.29 m *β*: 1.71–1.75 m	22.5	2.26 s	11.6
9	4.38 dd (8.8, 3.8)	74.6	*α*: 1.89–1.94 m *β*: 1.30–1.36 m	33.4	2.30 s	19.1
10		143.3		37.3		
11		150.6		72.9		
12	4.70 s	109.4	1.22 s	26.7		
13	1.74 s	20.7	1.23 s	27.4		
14	1.40 s	33.9	0.68 s	16.8		
15	0.93 d (6.9)	8.7	4.97 s	112.8		

methylene carbons, including one olefinic carbon at δ_C 109.4 (C-12), and three sp^3-hybridized methylene carbons at δ_C 42.4 (C-7), 39.2 (C-8) and 31.6 (C-2); three methine carbons, including one olefinic carbon at δ_C 125.5 (C-1), two oxygenated methine carbons at δ_C 74.6 (C-9) and 67.5 (C-3); three quaternary carbons, including an aliphatic carbon at δ_C 39.8 (C-5), two olefinic carbons at δ_C 143.3 (C-10) and 150.6 (C-11). Compound **1** had four degrees of unsaturation, and the removal of two degrees of unsaturation occupied by two olefins indicated that compound **1** has two more rings.

The key 2D-NMR correlations of **1** were shown in in Figure 2. The ^{1}H-^{1}H COSY correlations of H-1/H-2/H-3/H-4 and H-6/H-7-/H-8/H-9 indicated two fragments of CH(1)–CH_2(2)–CH(3)–CH(4) and CH(6)–CH(7)–CH_2(8)–CH_2(9). The HMBC cross-peaks from H-15 to C-3, C-4 and C-5; from H-14 to C-4, C-5, C-6 and C-10; and from H-13/H-12 to C-7 and C-11 indicated that two methyl groups connected to C-4 and C-5, respectively, and one isopropenyl group connected to C-6 (Figure 2). By comparing the NMR data of compound **1** (Table 1) with the known compound Kanshone D,[10] it was implied that the two compounds should had the same basic skeleton, they both belonged to the Lemnalane-type sesquiterpene. Compound **1** differed from Kanshone D in the lack of the bridge oxygen atom at the C-7 and C-8, the carbonyl group at the C-9 is reduced to a hydroxy group, while the other hydroxy group at the C-3 instead of the C-2 in Kanshone D. Then, the planar structure of compound **1** was determined. H-6, Me-15 and Me-14 are all α-configurations in Lemnalane-type sesquiterpenes.[10–12] The key NOESY correlations of H-15/H-3 and H-6/H-9 indicated that H-15, H-3, H-6 and H-9 were α-oriented. Therefore, the 3-OH and 9-OH was in the β-configuration. The absolute configuration of compound **1** was established by comparison of calculated and experimental electronic circular dichroism (ECD) spectra (Figure 3). Finally, **1**

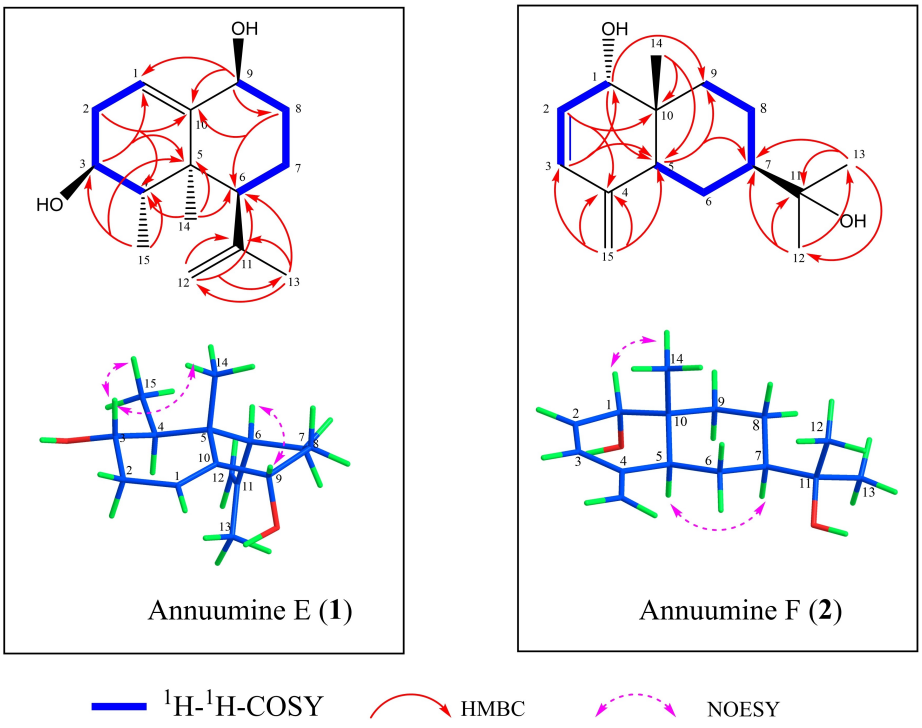

Figure 2. Key 2D-NMR correlations of Annuumine E (**1**) and Annuumine F (**2**).

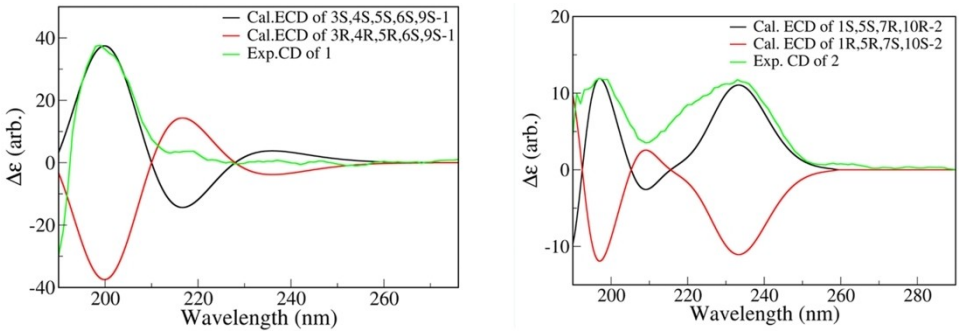

Figure 3. Comparison of the experimental and calculated ECD spectra of Annuumine E (**1**) and Annuumine F (**2**) in CH_3CN.

was assigned with a 3*S*, 4*S*, 5*S*, 6*S* and 9*S* absolute configuration. Hence, the structure of **1** was established and was named as Annuumine E.

Annuumine F (**2**) was isolated as a light yellow oil substance. A molecular formula of $C_{15}H_{24}O_2$ was deduced based on the HR-ESI-MS spectrometry, which exhibited a quasimolecular ion peak $[M+H]^+$ at *m/z* 237.1844 (calc. for $C_{15}H_{25}O_2$, 237.1849), indicating four degrees of unsaturation. Its IR spectrum showed absorptions for hydroxy groups (3390, 1148 and 1031 cm^{-1}) and olefin groups (1682 and 1652 cm^{-1}). As shown in Table 1, the ^{1}H-NMR spectrum showed the three methyl protons at δ_H 1.23 (3H, s, H-13), 1.22 (3H, s, H-12) and 0.68 (3H, s, H-14); three olefinic protons at δ_H 6.21 (1H, d, J=9.7, H-3), 5.84 (1H, dd, J=9.5, 5.5, H-2) and 4.97 (2H, br. s, H-15); one oxymethine proton at δ_H 3.55 (1H, d, J=5.4, H, H-1). The ^{13}C-NMR spectrum (Table 1) exhibited 15 carbon signals, including three methyl groups at δ_C 27.4 (C-12), 26.7 (C-13), 16.9 (C-14); four methylene groups at δ_C 112.8 (C-15), 33.4 (C-9), 24.4 (C-6) and 22.5 (C-8); three methane groups at δ_C 127.0 (C-2), 132.8 (C-3), 72.6 (C-1), 48.7 (C-7), 38.0 (C-5); three quaternary carbons at δ_C 145.4 (C-4), 72.9 (C-11), 37.3 (C-10).

The characteristic carbon signals at δ_C 48.7 (CH, C-7), 38.0 (CH, C-5), 37.3 (C, C-10), 16.9 (CH_3, C-14) indicated that compound **2** was an eudesmane-type sesquiterpene. Compound **2** showed structural features similar to the known compound (1α-Hydroxyeudesma-2,4(15),1 l(13)-trien-5α,7αH-12-oic acid) by comparing their NMR data.[13] The differences is that compound **2** had no olefin double bond and carboxylic acid group at C-11, instead it had two methyl groups, which was supported by the key HMBC correlations from H-13/H-12 to C-11and C-7, and from H-12 to C-13 and from H-13 to C-12 as shown in Figure 2. The correlations in the NOESY spectrum indicated that compound **2** had the same conformation as 1α-Hydroxyeudesma-2,4(15),1 l(13)-trien-5α,7αH-12-oic acid, the key NOESY correlations of H-1/H-14 suggested that H-1 orientation were the same as the methyl group of Me-14, both are in the β configuration, indicating that the 1-OH is in the α configuration. Similarly, the key NOESY correlations of H-5/H-7 suggested that H-5 and H-7 were α configuration. In other words, it also indicated that the isopropenyl group at C-7 is the β conformation (Figure 2.). The absolute configuration of **2** was finally determined to be 1*S*, 5*S*, 7*R* and 10*R* by comparison of its experimental CD spectrum with the calculated ECD data of **2**. In summary, compound **2** is a new eucalyptane-type sesquiterpene and named Annuumine F.

Compound **3** was obtained as a white amorphous powder, and its molecular formula was established as $C_9H_{12}O_2$ based on the HR-ESI-MS ion peak at *m/z* 151.0753 $[M-H]^-$ (calc. for $C_9H_{11}O_2$, 151.0753). Its IR spectrum showed absorptions for phenolic hydroxy group (3126 and 1267 cm^{-1}) and hydroxy group (3396 and 1033 cm^{-1}) and aromatic ring (1490 and 810 cm^{-1})

According to the ^{1}H-NMR data in Table 1, compound **3** contained two methyl protons: δ_H 2.30 (3H, s, H-9) and 2.26 (3H, s, H-8); one hydroxymethyl proton: δ_H 4.64 (2H, s, H-7); two olefinic protons: δ_H 6.81 (1H, d, J=8.1, H-5) and 6.62 (1H, d, J=8.1, H-4). The ^{13}C-NMR spectrum in Table 1 revealed 9 carbon signals, including 6 carbons belonged to the aromatic ring at δ_C 154.6 (C-3), 138.6 (C-1), 128.8 (C-5) 128.2 (C-6), 125.3 (C-2) and 115.2 (C-4); one hydroxymethyl group at δ_C 59.4 (C-7); two methyl groups at δ_C 19.1 (C-9) and 11.6 (C-8).

The key 2D-NMR correlations of compound **3** were shown in in Figure 3. The ^{1}H-^{1}H COSY correlations of H-4/H-5 suggested that the two olefin protons on the aromatic ring were connected by a one-bond. The key HMBC correlations from H-7 to C-1, C-6 and C-2 indicated that a hydroxymethyl group connected to C-1. Furthermore, the HMBC correlations from H-8 to C-3, C-1 and C-2, and from H-9 to C-1 and C-6 and C-5 indicated that two methyl groups connected to C-2 and C-6, respectively. Then, the structure of compound **3** was determined as 3-hydroxy-2,6-dimethylbenzenemethanol. Compound **3** was a new natural product by searching the Scifinder database, and it was isolated for the first time from plants. Here its detailed IR, 1D/2D-NMR and HR-ESI-MS spectra were reported (Figure 4).

The other seventeen known compounds, including cyperusol C (**4**),[14] verianaterpene III (**5**),[15] β-sitosterol (**6**),[16] 7α-hydroxysitosterol (**7**) and 7β-hydroxysitosterol (**8**),[17] 3β-hydroxy-(22E,24R)-ergosta-5,8,22-trien-7-one (**9**),[18] gitogenin (**10**),[19] ferulic aldehyde (**11**),[20] ferulic acid (**12**),[21] syring aldehyde (**13**),[22] syringic acid (**14**),[23] 4-hydroxy-3-methoxyacetophenone (**15**),[24] xanthoxyline (**16**),[25] ethyl vanillate (**17**),[26] methyl orsellinate (**18**),[27] vanillic acid (**19**),[28] and 7-methoxywutaifuranol (**20**),[29] were identified according to their NMR and MS data by comparison with the literature.

Anti-inflammatory Properties

To evaluate the anti-inflammatory effects of the compounds obtained from the roots of *C. annuum* L., the abilities of 20 compounds (**1**–**20**) to inhibit NO in LPS-induced RAW 264.7 cells were assessed. After screening for anti-inflammatory activity, it was found that compounds **9** and **11** inhibited NO release from LPS-induced RAW 264.7 cells with IC_{50} values of 50.65 and 21.11 μM, respectively. The other tested compounds showed weak inhibition or no effect in the same assay (Table 2). Additionally, compounds **9** and **11** reduced NO release from LPS-induced RAW 264.7 cells in a dose-dependent manner, with

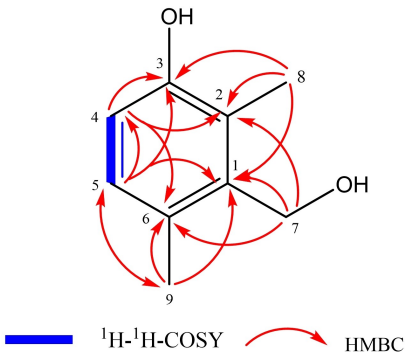

Figure 4. Key 2D-NMR correlations of 3-hydroxy-2,6-dimethylbenzenemethanol (**3**).

Table 2. Inhibitory effects of compounds **1**–**20** on NO production in LPS-stimulated RAW264.7 cells (means ± S.D.).

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
1	–	8	–	15	–
2	–	9	50.65 ± 0.53	16	–
3	–	10	–	17	–
4	–	11	21.11 ± 0.86	18	–
5	–	12	–	19	–
6	–	13	–	20	–
7	–	14	–	PDTC	5.37 ± 0.17

Note: these data represented the average values of three repeated experiments (mean ± S.D., n = 3); PDTC: positive control; –: no inhibition.

inhibition rates of 40.36% and 80.42% a concentration of 40 μM, respectively (Figure 5A/B). In addition, most compounds had no inhibitory effect on the proliferation of RAW 264.7 cells (Figure 6), and the cell survival rates between the control and test groups were not obviously different, except for compounds **10**, **11** and **20**.

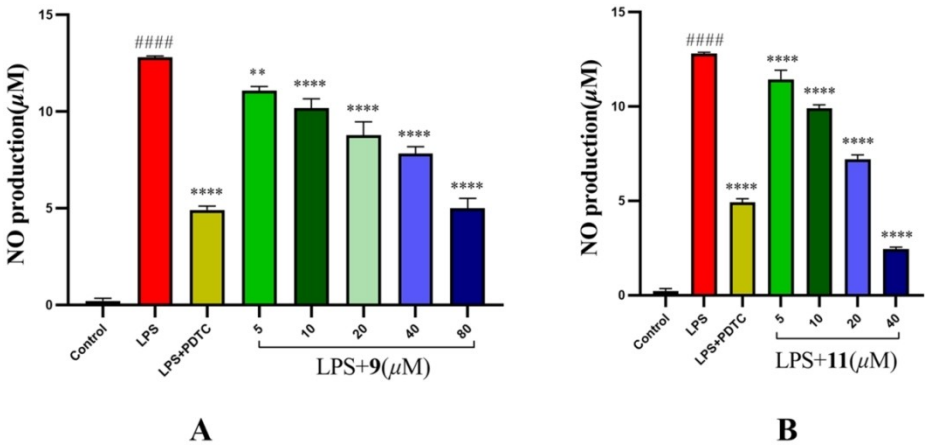

Figure 5. Compounds **9** and **11** inhibited the production of NO in LPS-stimulated RAW 264.7 cells. Note: Control: only contain normal cells, LPS: normal cells + LPS, LPS + PDTC: normal cells + LPS + PDTC (10 μM); Data are presented as means ± S.D., n = 3; ####p < 0.0001 *vs.* control; ****p < 0.0001 *vs.* LPS only.

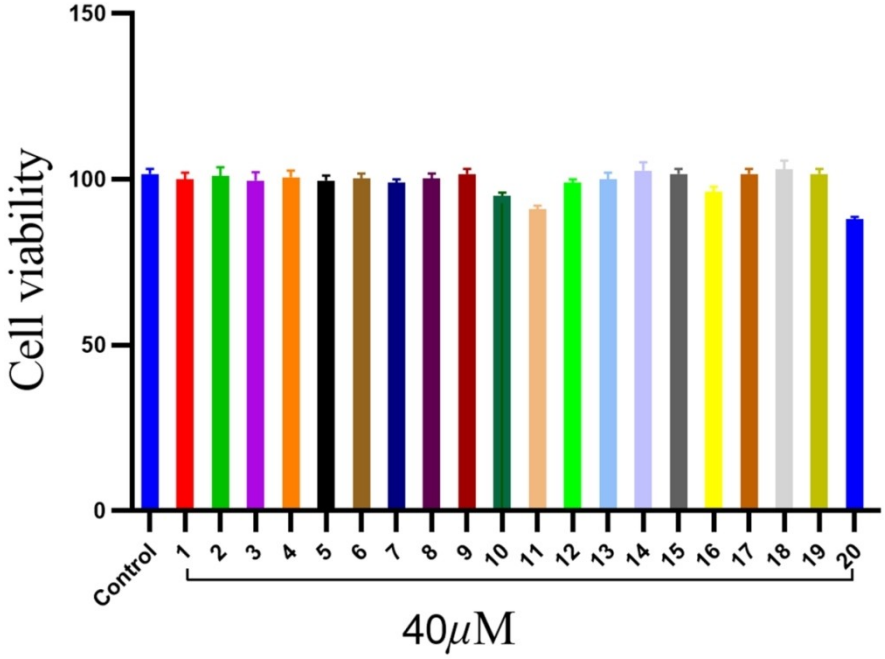

Figure 6. Effect of compounds **1**–**20** at 40 μM on the proliferation of RAW 264.7 cells (means ± S.D., n = 3).

Antibacterial Activity

Antibiotic resistance is an urgent problem in antimicrobial drug research. Natural plants, as an important source of antimicrobial agents, might be effective against multidrug resistant strains without causing side effects or harming environment.[30] The *in vitro* antibacterial activities of **1–20** were evaluated against three phytopathogenic bacteria, including *P. cinnamomi*, *R. solanacearum* and *B. subtilis*, at concentrations ranging from 6.25–800 μg/mL, using benzocillin sodium as the positive control. The results (see *Supporting Information* Table S1) indicated that compound **9** had some antibacterial effects against all three plant pathogenic bacteria, with a minimum inhibitory concentration (MIC) of 400 μg/mL. Compound **17** also showed some inhibitory effects on *R. solanacearum* and *B. subtilis* (MIC = 400 μg/mL). The other compounds did not inhibit the proliferation of the three plant pathogenic bacteria in the concentration range tested.

Conclusions

In this study, three new compounds (**1–3**) and seventeen known compounds (**4–20**) were obtained, which enriches the chemical composition of *C. annuum* L. When screened in an LPS-stimulated RAW 264.7 cell model, compound **11** displayed significant anti-inflammatory activity at 40 μM. Moreover, these findings will be helpful in developing and utilizing the roots of *C. annuum* L. in the future.

Experimental Section

General Experimental Procedures

One-dimensional (1D) and two-dimensional (2D) nuclear magnetic resonance (NMR) spectra were measured on a Bruker AM-600 spectrometer. High resolution electrospray ionization mass spectroscopy (HR-ESI-MS) data were obtained by Q EXACTIVE FOCUS (Thermo Fisher Technologies Co., Ltd., Waltham, MA, USA) spectrometer. Electrospray ionization (ESI) data were obtained by an HP 1100SMD. Infrared (IR) spectra were recorded on a iCAN9 FT/IR spectrometer. The extract was obtained through a 300 L extraction tank (JF21060, Jiangsu Jufeng Machinery Co., Ltd., Huaian, China). Column chromatography (CC) was performed on silica gel (40–80 mesh, 200–300 mesh, and 300–400 mesh, Qingdao Haiyang Chem. Ind. Ltd., Qingdao, China), silica gel H (40–80 μM mesh, Qingdao, China), Sephadex LH-20 (40–70 μM, Amersham Pharmacia Biotech AB, Uppsala, Sweden), and C-18 reversed-phase silica gel (20–45 μM, Merck, Darmstadt, Germany). Thin-layer chromatography (TLC) plates were precoated with silica gel GF254 (Qingdao Haiyang Chem. Ind. Ltd., Qingdao, China). Semipreparative HPLC (Sp-HPLC) was also performed on the SPD-16 set, (SHIMADZU Co., Ltd., Shanghai, China). Optical density (OD) was measured by microplate reader (Thermo Fisher, Waltham, MA, USA). Centrifuge tubes and 96 cell culture plates were purchased from NEST Biotechnology Co. Ltd. (Beijing, China). Dimethyl sulfoxide (DMSO), phosphate buffer solution (PBS), pyrrolidinedithiocarbamate (PDTC) and benzocillin sodium were purchased from Sigma–Aldrich (Shanghai, China). Lipopolysaccharide (LPS), Griess reagent and Cell Counting Kit-8 (CCK-8) were purchased from Beyotime Biotechnology (Shanghai, China). HPLC grade acetonitrile, methanol and water were purchased from the manufacturer CICN High Purity Solvents Co., Ltd. (Shanghai, China). All the chemicals and solvents used for extraction and isolation were of analytical grade (Sinopharm Chemical Reagent Co. Ltd.).

Plant Materials

Roots of *C. annuum* L. were collected from Xiazi town of Guizhou province, China in September 2021, and its specimen (Number: GNRC-20210925) was identified as the roots of *C. annuum* L. by professor Qingwen Sun from Guizhou University of Traditional Chinese Medicine.

Extraction and Isolation

The dry powdered roots of *C. annuum* L. (170 kg) were refluxed 3 times with 95% ethanol, 3 h each time. The combined extract was filtered and concentrated with a rotary evaporator under reduced pressure to yield the crude extract (50 L), which was then extracted with petroleum ether (PE), ethyl acetate (EA), and *n*-butanol. The EA extract (250.8 g) was separated by silica gel CC and eluted with a gradient of CH_2Cl_2/MeOH (50:1, 20:1, 10:1, 5:1, 1:1) to give five fractions (Fr. A–Fr. E) based on TLC analysis. Fr. A (2.86 g) was separated by silica gel H CC using a gradient system of CH_2Cl_2/EA(10:1) to obtain compound **11** (10 mg), compound **13** (8 mg) and compound **15** (13 mg), and separated with Sephadex LH-20 CC eluting with MeOH/$CHCl_3$ (1:1) to afford compound **20** (3 mg). Fr. B (7.56 g) was separated with Sephadex LH-20 CC eluting with MeOH to afford two subfractions (Fr. B1–Fr. B2), Fr. B1 (2.11 g) purified by recrystallization (MeOH/$CHCl_3$) to afford compound **6** (20 mg) and compound **10** (22 mg). The Fr. B2 (450 mg) was further purified via Sp-HPLC with an XBridge BEH–C18-OBD Prep Column (10 mm i.d.× 250 mm) by using a mobile phase of CH_3CN/H_2O (78:22, 1.5 mL/min) to obtain compound **7** (8 mg), compound **8** (3 mg) and compound **9** (8 mg). Fr. C (2.72 g) was separated by silica gel CC with a elution of cyclohexane/acetone (10:1) to give compound **16** (7 mg), compound **17** (6 mg) and compound **18** (6 mg). Fr. D (13.67 g) was separated by C-18 reversed-phase silica gel CC by using a elution of CH_3OH/H_2O (50:50 to 100:0, 1.5 mL/min) to afford two subfractions (Fr. D1–Fr. D2). Fr. D1 (480 mg) was further purified via Sp-HPLC with a YMC-Pack-Ph Prep Column (10 mm i.d.× 250 mm) by using a mobile phase of CH_3OH/H_2O (54:46, 2.0 mL/min) to obtain compound **1** (10 mg) and compound **2** (15 mg), and Fr. D2 (400 mg) was separated with silica gel CC eluting with CH_2Cl_2/ acetone /EA (20:1:1) to yield compounds **4** (8 mg) and **5** (5 mg). Fr. E (23.68 g) was separated by epeated silica gel CC with a elution of PE/EA (3:1) to give compound **3** (107 mg), compound **12** (38 mg), compound **14** (62 mg) and compound **19** (15 mg).

Annuumine E (**1**). Colorless oily substance; $[\alpha]_D^{25} = -31.88$ (c = 0.224, MeOH); ^{1}H and ^{13}C-NMR spectral data see Table 1; IR (KBr) ν_{max} 3289, 2969, 2727, 2871, 2451, 1643, 1369, 1040, 888 cm^{-1}; HR-ESI-MS *m/z* 259.1661 $[M+Na]^+$ (calc. for $C_{15}H_{24}O_2Na$, 259.1669).

Annuumine F (**2**). Pale yellow oily substance; $[\alpha]_D^{25} = +87.31$ (c = 0.141, MeOH); ^{1}H and ^{13}C-NMR spectral data see Table 1; IR (KBr) ν_{max} 3390, 2969, 2940, 2868, 2358, 1716, 1682, 1652, 1457, 1380, 1148, 1031 cm^{-1}; HR-ESI-MS *m/z* 237.1844 $[M+H]^+$ (calc. for $C_{15}H_{25}O_2$, 237.1849).

3-Hydroxy-2,6-dimethylbenzenemethanol (**3**). White amorphous powder; ^{1}H and ^{13}C-NMR spectral data see Table 1; IR (KBr) ν_{max} 3396, 3126, 1490, 1380, 1267, 1033, 981, 810 cm^{-1}; HR-ESI-MS *m/z*. 151.0754 $[M-H]^-$ (calc. for $C_9H_{11}O_2$, 151.0736).

Cell Culture

The RAW 264.7 murine macrophage cell lines were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). RAW 264.7 cells were cultured at 37 °C in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) inactivated FBS and 1% penicillin/streptomycin in a water-saturated environment consisting of 5% CO_2.

Cytotoxicity Assay

A CCK-8 assay was used to determine the cell viability after RAW 264.7 cells were treated with the test compounds, as previously reported.[31] Briefly, The RAW264.7 cells were resuspended by adding a certain amount of complete culture medium and counted using a cell counting plate. The cells were inoculated in 96-well plates at a density of 5×10^4 cells/mL, and 100 μL/well cells were placed in an incubator at 37 °C with 5% CO_2 to grow the cells against the wall. The normal control group, different concentrations of compounds at 2.5, 5, 10, 20, 40 and 80 μM were set up in parallel replicate wells and added to the 96-well plate. After 24 h of action, 10 μL of CCK-8 solution was added to each well, and the incubation was continued in the incubator for 2–4 h. Finally, the absorbance at 450 nm was recorded using a microplate reader.

Anti-Inflammatory In Vitro Study

In brief, RAW264.7 cells were treated according to the above method, and the cell density was adjusted to 4×10^5 cells/mL. The cells were inoculated in 24-well plates with an addition volume of 500 μL/well and cultured overnight. The experiment was divided into three groups: (1) blank group (containing cells only); (2) model group (cells + LPS); and (3) experimental group (cells + compound + LPS). On the second day, the old culture medium was discarded and 500 μL of fresh culture medium was added directly to the blank and model groups, while compound solutions (500 μL) containing different concentrations (5, 10, 20, 40 and 80 μM) were added to each well of the experimental group. Two hours later, LPS was added to the model and experimental groups to stimulate the cells to obtain a final LPS concentration was 5 μg/mL. After 24 h, the cell supernatant was extracted, and the NO concentration in the cell supernatant was detected and calculated using the Griess reagent. The NO standard curve $y=0.0072x+0.0444$, $R^2=0.9998$.

Antimicrobial Experimental Research

P. cinnamomi, *R. solanacearum* and *B. subtilis* strains were used in this study. Three strains were grown at 37 °C either on Luria-Bertani (LB) solid medium agar for 12 h. Collecting the single colonies, based on their colony morphology, were harvested and suspended in liquid culture of Mueller-Hinton (MH) broth medium and grown at 37 °C for 24 h.[32] Cultures of each strain were suspended in distilled water and adjusted to 3×10^8 CFU/mL, and diluted 3000 times with MH broth medium to obtain a bacterial solution containing approximately 10^5 CFU/mL of bacteria. 100 μL of MH broth medium was added to the 96-well plate, and 100 μL of test compound (1.6 mg/mL) was added to the first row. Then the compound concentration was diluted twofold in turn and repeated until the last well, and 100 μL was aspirated and thrown away in the eighth row. At the last, 100 μL of diluted solution was added in each well. The 96-well plates were placed in a 37 °C incubator for 8–24 h and stained with triphenyltetrazolium chloride (TTC) (5 mg/mL) to observe the results. DMSO was used as a negative control and benzocillin sodium as a positive control, and each group was repeated three times.

Author Contributions

Jiang Shi and Min Wang performed the extraction, isolation. Jiang Shi performed the identification, manuscript writing. Lu-lu Deng performed the anti-inflammatory activity experiment. Jiang Li performed sample collection. Yan-hua Fan and Wei-dong Pan supervised the work of biology aspect. Xiao-jiang Hao and Shu-zhen Mu were the project leader and designed the experiment. Shu-zhen Mu performed Writing- Reviewing and Editing.

Acknowledgements

This study was supported financially by the National Natural Science Foundation of China (U1812403-3-3).

Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: anti-inflammatory · active constituent · roots *of Capsicum annuum* L. · sesquiterpenes · structural identification

[1] R. Kamel, N. A. El-Wakil, A. Dufresne, N. A. Elkasabgy, *Int. J. Biol. Macromol.* **2020**, *163*, 1579–1590.
[2] P. A. Milani, K. B. Debs, G. Labuto, E. Carrilho, *Environ. Sci. Pollut. Res. Int.* **2018**, *25*, 35895–35905.
[3] G. Viruthagiri, S. Sathiya priya, N. Shanmugam, A. Balaji, K. Balamurugan, E. Gopinathan, *Spectrochim. Acta Part A* **2015**, *149*, 468–475.
[4] A. Zamani, A. P. Marjani, Z. Mousavi, *Green Process. Synth.* **2019**, *8*, 421–429.
[5] M. Iorizzi, V. Lanzotti, G. Ranalli, S. De Marino, F. Zollo, *J. Agric. Food Chem.* **2002**, *50*, 4310–4316.
[6] Y. Kawaguchi, T. Ochi, Y. Takaishi, K. Kawazoe, K. H. Lee, *J. Nat. Prod.* **2004**, *67*, 1893–1896.
[7] S. Yahara, T. Ura, C. Sakamoto, T. Nohara, *Phytochemistry* **1994**, *37*, 831–835.
[8] C. Song, C. Yu, X. Zhu, X. Luo, Y. Zhang, L. Meng, Y. Luo, H. Chang, C. Qin, Y. Liu, *Nat. Prod. Res.* **2022**, *36*, 546–552.
[9] T. Ochi, Y. Takaishi, K. Kogure, I. Yamauti, *J. Nat. Prod.* **2003**, *66*, 1094–1096.
[10] A. Bagchi, Y. Oshima, H. Hikino, *Phytochemistry* **1988**, *27*, 3667–3669.
[11] A. Bagchi, Y. Oshima, H. Hikino, *Phytochemistry* **1988**, *27*, 1199–1201.
[12] A. Bagchi, Y. Oshima, H. Hikinot, *Phytochemistry* **1988**, *27*, 2877–2879.
[13] J. A. Marco, J. F. Sanz, P. del Hierro, *Phytochemistry* **1991**, *30*, 2403–2404.
[14] F. Xu, T. Morikawa, H. Matsuda, K. Ninomiya, M. Yoshikawa, *J. Nat. Prod.* **2004**, *67*, 569–576.
[15] T. Matsumoto, T. Kitagawa, D. Imahori, H. Yoshikawa, M. Okayama, M. Kobayashi, N. Kojima, M. Yamashita, T. Watanabe, *J. Nat. Med.* **2021**, *75*, 942–948.
[16] X. H. Wei, S. J. Yang, N. Liang, D. Y. Hu, L. H. Jin, W. Xue, S. Yang, *Molecules* **2013**, *18*, 1325–1336.
[17] Y. F., L. X., W. Y., B. Wu, *J. Chin. Pharm. Sci.* **2007**, 288–293.
[18] H. Y. Tang, Q. Zhang, H. Li, J. M. Gao, *Nat. Prod. Res.* **2015**, *29*, 345–348.
[19] D. J. Jin, M. C. Liao, C. X. Wang, L. J. Wang, *Central South Pharmacy* **2006**, *4*, 248–252.

[20] Y. X. Xian, H. L. Zhou, X. Wang, J. Q. Yu, Z. J. Zheng, B. T. Yang, *Asian J. Chem.* **2015**, *27*, 1063–10625.
[21] D. M. Jeong, H. A. Jung, H. S. Kang, J. S. Choi, *Nat. Prod. Sci.* **2008**, *14*, 1–15.
[22] Y. Ren, G. D. Anaya-Eugenio, A. A. Czarnecki, T. N. Ninh, C. Yuan, H. B. Chai, D. D. Soejarto, J. E. Burdette, E. J. C. de Blanco, A. D. Kinghorn, *Bioorg. Med. Chem.* **2018**, *26*, 4452–4460.
[23] F. A. Abbas, S. M. Al-Massarany, S. Khan, T. A. Al-Howiriny, J. S. Mossa, E. A. Abourashed, *Nat. Prod. Res.* **2007**, *21*, 383–391.
[24] M. I. Choudhary, H. Hussain, I. Ahmed, S. F. Asghar, A. Aziz, *Rec. Nat. Prod.* **2009**, *3*, 139–152.
[25] N. Chotsaeng, C. Laosinwattana, P. Charoenying, *Molecules* **2017**, *22*, 1841–1857.
[26] W. Wei, E. W. Hao, M. Zhang, X. L. Pan, J. F. Qin, J. L. Xie, J. Y. Zhou, X. T. Hou, J. G. Deng, *Nat. Prod. Res.* **2021**, *35*, 921–929.
[27] D. M. X. Donnelly, D. J. Coveney, N. Fukuda, J. Polonsky, *J. Nat. Prod.* **2004**, *49*, 111–116.
[28] S. Prachayasittikul, S. Suphapong, A. Worachartcheewan, R. Lawung, S. Ruchirawat, V. Prachayasittikul, *Molecules* **2009**, *14*, 850–867.
[29] H. Y. Huang, T. Ishikawa, C. F. Peng, I. L. Tsai, I. S. Chen, *J. Nat. Prod.* **2008**, *71*, 1146–1151.
[30] M. Mokhtar, F. Youcefi, S. Keddari, Y. Saimi, S. Otsmane Elhaou, F. Cacciola, *Chem. Biodiversity* **2022**, *19*, 545–565.
[31] N. Cai, W. Luo, L. Yao, X. Li, Z. Wang, H. Xu, H. Li, Z. Hu, W. Bao, X. Xu, *J. Funct. Foods* **2020**, *75*, 104229–104307.
[32] R. Abd-Elrahim, M. R. A. Tohamy, M. M. Atia, M. M. A. Elashtokhy, M. A. S. Ali, *Saudi J. Biol. Sci.* **2022**, *29*, 2163–2172.

Manuscript received: May 12, 2023
Accepted manuscript online: June 17, 2023
Version of record online: July 28, 2023