Chemical Constituents of the Necroptosis-Inhibiting Active Fraction from Jiangxi Xanthium strumarium (I): Postprint

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Abstract

Xanthium sibiricum is a traditional folk medicinal plant. Our research group previously found that the 50% ethanol elution fraction from macroporous resin of Xanthium sibiricum from Jiangxi exhibits significant inhibitory activity against necroptosis. To identify the active constituents of Xanthium sibiricum, the active fraction was separated and purified using various methods including repeated silica gel column chromatography, Sephadex LH-20 gel column chromatography, preparative high-performance liquid chromatography, and recrystallization. The structures of the isolated compounds were identified using spectroscopic methods such as NMR and MS in conjunction with literature data. Fourteen compounds were isolated from this active fraction and identified as hydroxydihydrobovolide (1), raspberry ketone (2), salicyl alcohol (3), p-hydroxyacetophenone (4), p-hydroxybenzaldehyde (5), ethyl caffeate (6), feruloldehyde (7), isoscopoletin (8), 3,3'-bis(3,4-dihydro-4-hydroxy-6-methoxy-2H-1-benzopyran) (9), axillarin (10), quercetin (11), (+)-pinoresinol (12), β-sitosterol (13), and palmitic acid (14). Compounds 1, 2, 3, 4, 7, 8, 9, and 10 were isolated from Xanthium sibiricum for the first time.

Full Text

Chemical Constituents of the Necroptosis-Inhibiting Active Fraction from *Xanthium mongolicum* in Jiangxi ( )

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Abstract

Xanthium is a traditional folk medicinal plant. Our previous research demonstrated that the 50% ethanol elution fraction from macroporous resin of Jiangxi *Xanthium mongolicum* exhibits significant necroptosis-inhibiting activity. To identify the active constituents, the active fraction was isolated and purified using repeated silica gel column chromatography, Sephadex LH-20 gel column chromatography, preparative high-performance liquid chromatography, and recrystallization. The structures of isolated compounds were identified by spectroscopic methods including NMR and MS, combined with literature data. Fourteen compounds were isolated from this active fraction and identified as hydroxydihydrobovolide (1), raspberry ketone (2), salicyl alcohol (3), 4-hydroxyacetophenone (4), 4-hydroxybenzaldehyde (5), ethyl caffeate (6), ferulaldehyde (7), isoscopletin (8), 3,3 -bis(3,4-dihydro-4-hydroxy-6-methoxy-2H-1-benzopyran) (9), axillarin (10), quercetin (11), (+)-pinoresinol (12), β-sitosterol (13), and palmitic acid (14). Compounds 1, 2, 3, 4, 7, 8, 9, and 10 were isolated from *Xanthium mongolicum* for the first time.

Keywords: *Xanthium mongolicum*, necroptosis, active fraction, chemical constituents, Jiangxi

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Introduction

Necroptosis, also known as programmed necrosis or necrotic apoptosis, is a newly discovered form of cell death that is regulated by death signals yet exhibits necrotic structural characteristics. Numerous studies have shown that necroptosis is associated with pathological and organic damage in various diseases, including tumor metastasis, chemotherapy resistance, drug-induced liver injury, neurodegenerative disorders, myocardial infarction, stroke, pancreatitis, enteritis, and dermatitis (Andress et al., 2014; Dongshi et al., 2016). Intervening in necroptosis may clinically reduce damage caused by these diseases (Galluzzi L et al., 2012).

Traditional Chinese medicine has a history of thousands of years in treating inflammatory and immune diseases. *Xanthium* is an annual herb belonging to the family Compositae and genus *Xanthium* L. As a traditional folk medicinal plant, *Xanthium* is commonly used worldwide to treat rhinitis, rheumatoid arthritis, fever, vitiligo, lymphoid tuberculosis, herpes, and cancer (Huang et al., 2011; Nibret et al., 2011; Ma et al., 2009). According to the *Chinese Materia Medica*, *Xanthium* is cold in nature, bitter and pungent in taste, slightly toxic, and possesses wind-dispelling, cold-dispersing, detoxifying, and insecticidal effects (Chinese Medicine Dictionary, 1985). Modern pharmacological studies have demonstrated that *Xanthium* exhibits anti-inflammatory, anti-tumor,

antibacterial, and antioxidant activities (Pharmacopoeia of the People' s Republic of China, 2020). The main *Xanthium* species in Jiangxi Province is *Xanthium mongolicum* (Fu et al., 2017). Our research group previously conducted activity screening on a self-established library of 100 Chinese herbal extracts from Jiangxi at the University of Texas Southwestern Medical Center and discovered that the ethanol extract of *Xanthium mongolicum* exhibited necroptosis-inhibiting activity in its macroporous resin ethanol elution fraction. To identify the active constituents responsible for this necroptosis inhibition, we systematically isolated and characterized its chemical components. This report describes the isolation of 14 compounds from the 50% ethanol elution fraction, with structures shown in Figure 1 [Figure 1: see original paper], eight of which were obtained from *Xanthium mongolicum* for the first time.

Materials and Instruments

The plant material was collected in Nanchang, Jiangxi in July 2017 and identified as the aerial parts of *Xanthium mongolicum* Kitag. (Compositae) by Professor Xianwang Liu of Jiangxi University of Chinese Medicine. A voucher specimen (ZY-20170701) is deposited in the Herbarium of Jiangxi University of Chinese Medicine.

Instruments: 2695 Alliance Separations Module HPLC and 1525 preparative HPLC [Lichrospher C18 preparative column (30 mm × 250 mm, 10 m)] (Waters, USA); Inova-600 NMR spectrometer (Varian, USA); AB Triple QUAD 4500 mass spectrometer (AB SCIEX, USA); WFH-203 (ZF-1) UV analyzer (Shanghai Jingke Industrial Co., Ltd.); AE100 electronic analytical balance (Mettler-Toledo, Switzerland). Sephadex LH-20 (GE Healthcare, Sweden); silica gel (200 mesh, Qingdao Marine Chemical Factory). All reagents for extraction and isolation were analytical grade; methanol for semi-preparative HPLC was chromatographic grade (Xilong Chemical Co., Ltd.); water was ultrapure.

Cell culture and reagents: The HT29 human colorectal cancer cell line was cultured in DMEM medium supplemented with 10% FBS and 1% P/S. Cell Titer-Glo assay kit was purchased from Promega. Tumor necrosis factor TNF-α was expressed and purified from an *E. coli* expression system. The pro-apoptotic compound Smac mimetic and caspase inhibitor z-VAD.fmk were synthesized by the Chemistry Center of Beijing Institute of Life Sciences.

Extraction and Separation

Ten kilograms of air-dried plant material were pulverized and sieved, then extracted by cold maceration with 95% ethanol (7 days each time, 3 times). The combined extracts were concentrated under reduced pressure to yield 800 g of crude extract. The crude extract was dissolved in ethanol, loaded onto a

pretreated macroporous resin column, and eluted with ethanol gradients of different concentrations to obtain five fractions: water eluate (Fraction A, 200 g), 30% ethanol eluate (Fraction B, 80 g), 50% ethanol eluate (Fraction C, 120 g), 70% ethanol eluate (Fraction D, 60 g), and 90% ethanol eluate (Fraction E, 150 g). Each fraction (20 mg) was dissolved in DMSO to prepare 20 mg/mL stock solutions, which were stored at -20 °C for screening.

Active Fraction Screening

The HT29 cell model was induced to undergo necroptosis by TSZ treatment (final concentrations: 20 ng · mL^{-1} TNF-α, 100 nM Smac, and 20 nM z-VAD) overnight. Samples prepared at 20 mg · mL^{-1} were then added to the TSZ-induced necroptotic HT29 cells and incubated at 37 °C overnight. Cell viability was determined by measuring ATP levels using the Cell Titer-Glo kit. Fractions showing higher cell viability indicated necroptosis-inhibiting activity.

The screening results for the different elution fractions are shown in Figure 2 [Figure 2: see original paper]. TSZ-induced HT29 cells showed 28% viability, which increased to 75% upon addition of Fraction C (50% ethanol eluate), confirming it as an active fraction.

Figure legend: Data represent ATP levels measured by Cell Titer-Glo kit, reflecting the number of surviving HT29 cells 12 h after necroptosis induction and sample treatment. DMSO, dimethyl sulfoxide; T, tumor necrosis factor TNF-α; S, Smac mimetic; Z, caspase inhibitor z-VAD; A, water eluted fraction; B, 30% ethanol eluted fraction; C, 50% ethanol eluted fraction; D, 70% ethanol eluted fraction; E, 90% ethanol eluted fraction.

Chemical Constituent Isolation

To further identify the active constituents, Fraction C (120 g) was subjected to silica gel column chromatography with a dichloromethane:methanol gradient (200:1 to 0:1). Fractions with similar Rf values were combined based on TLC analysis to yield subfractions C-1 through C-11.

Subfraction C-5 (15 g) was chromatographed on silica gel with petroleum ether:ethyl acetate (9:1 to 0:1) to give fractions C-5-1 through C-5-2. Fraction C-5-1 (5 g) was further separated by dichloromethane:methanol (15:1 to 0:1) to afford fractions C-5-1-1 through C-5-1-7. Fraction C-5-1-1 yielded white crystals that were recrystallized to obtain compound **13** (33 mg). Fraction C-5-1-5 (0.2 g) precipitated white crystals that were recrystallized from methanol to give compound **8** (9 mg). The remaining fractions were purified by preparative HPLC to yield compounds **6** (5 mg), **10** (4 mg), and **3** (5 mg).

Subfraction C-5-2 (7 g) was separated by dichloromethane:methanol (15:1

to 0:1) to give fractions C-5-2-1 through C-5-2-5. Each fraction was purified by preparative HPLC to obtain compounds **11** (6 mg), **4** (3 mg), **6** (50 mg), **9** (4 mg), and **2** (5 mg).

Subfraction C-6 (8 g) was chromatographed with dichloromethane:methanol (15:1 to 0:1) to afford fractions C-6-1 through C-6-8. Preparative HPLC purification of each fraction yielded compounds **7** (4 mg), **11** (7 mg), **5** (5 mg), **14** (10 mg), **1** (8 mg), and **12** (5 mg).

Structural Identification

Compound 1 was obtained as a yellow solid (methanol). ESI-MS m/z: 199 $[M+H]^+$, molecular formula $C_{11}H_{18}O_3$. ^{1}H-NMR (600 MHz, Methanol-d_4) δ: 1.81 (d, J = 0.85 Hz, 3H, H-12), 1.95 (d, J = 0.75 Hz, 3H, H-11), 1.32–1.01 (m, 8H, H-6,7,8,9), 0.91 (t, J = 6.8 Hz, 1H, H-10). ^{13}C-NMR (150 MHz, Methanol-d_4) δ: 173.16 (C-2), 124.28 (C-3), 159.11 (C-4), 107.73 (C-5), 35.50 (C-6), 31.30 (C-7), 22.37 (C-8), 22.10 (C-9), 12.96 (C-10), 9.31 (C-11), 6.91 (C-12). The ^{1}H-NMR and ^{13}C-NMR data matched those reported in the literature (Su et al., 2011), identifying compound 1 as hydroxydihydrobovolide. This compound was isolated from *Xanthium mongolicum* for the first time.

Compound 2 was isolated as a colorless powder (methanol). ESI-MS m/z: 163 $[M-H]^-$, molecular formula $C_{10}H_{12}O_2$. ^{1}H-NMR (600 MHz, Methanol-d_4) δ: 7.02 (2H, d, J = 8.5 Hz, H-2 /6), 6.70 (2H, d, J = 8.5 Hz, H-3 /5), 2.76 (4H, m, H-3,4), 2.14 (3H, s, 1-CH_3). ^{13}C-NMR (150 MHz, Methanol-d_4) δ: 28.6 (C-1), 210.0 (C-2), 28.6 (C-3), 44.9 (C-4), 131.8 (C-1), 128.8 (C-2 /6), 114.7 (C-3 /5), 155.2 (C-4). The ^{1}H-NMR and ^{13}C-NMR data matched those reported in the literature (Baek et al., 2011), identifying compound 2 as raspberry ketone (RK), also known as 4-(4-hydroxyphenyl)butan-2-one. This compound was isolated from *Xanthium mongolicum* for the first time.

Compound 3 was obtained as a light yellow powder (water). ESI-MS m/z: 126 $[M-H]^-$, molecular formula $C_7H_8O_2$. ^{1}H-NMR (600 MHz, D_2O) δ: 7.24 (1H, dd, J = 7.5, 1.7 Hz, H-3), 7.18 (1H, m, H-5), 6.89 (1H, m, H-4), 6.84 (1H, d, J = 8.1 Hz, H-6), 4.57 (2H, s, H-7). ^{13}C-NMR (150 MHz, D_2O) δ: 153.8 (C-1), 126.5 (C-2), 129.6 (C-3), 120.5 (C-4), 129.5 (C-5), 115.5 (C-6), 59.6 (C-7). The ^{1}H-NMR and ^{13}C-NMR data matched those reported in the literature (Jensen et al., 1979), identifying compound 3 as salicyl alcohol. This compound was isolated from *Xanthium mongolicum* for the first time.

Compound 4 was isolated as a colorless powder (methanol). ESI-MS m/z: 134 $[M-H]^-$, molecular formula $C_8H_8O_2$. ^{1}H-NMR (600 MHz, Methanol-d_4) δ: 7.87 (2H, d, J = 8.5 Hz, H-2,6), 6.80 (2H, d, J = 8.6 Hz, H-3,5), 2.53 (3H, s, 8-CH_3). ^{13}C-NMR (150 MHz, Methanol-d_4) δ: 129.2 (C-1), 132.2 (C-2,6), 116.3 (C-3,5), 165.9 (C-4), 199.4 (C-7), 26.2 (C-8). The ^{1}H-NMR and ^{13}C-NMR data matched those reported in the literature (Dawa et al., 2008), identifying compound 4 as

4-hydroxyacetophenone (4-HAP). This compound was isolated from *Xanthium mongolicum* for the first time.

Compound 5 was obtained as a light yellow powder (methanol). ESI-MS m/z: 123 $[M+H]^+$, molecular formula $C_7H_6O_2$. ^{1}H-NMR (600 MHz, Methanol-d_4) δ: 9.7 (1H, s, -CHO), 7.7 (2H, d, J = 8.6 Hz, H-3,5), 6.9 (2H, d, J = 8.5 Hz, H-2,6). ^{13}C-NMR (150 MHz, Methanol-d_4) δ: 191.3 (CHO), 165.1 (C-4), 132.1 (C-2,6), 128.3 (C-1), 115.8 (C-3,5). The ^{1}H-NMR and ^{13}C-NMR data matched those reported in the literature (Xu et al., 2019), identifying compound 5 as 4-hydroxybenzaldehyde (4-HBd).

Compound 6 was isolated as light yellow clusters (methanol). ESI-MS m/z: 209 $[M+H]^+$, molecular formula $C_{11}H_{12}O_4$. ^{1}H-NMR (600 MHz, Methanol-d_4) δ: 7.52 (1H, d, J = 15.9 Hz, H-7), 7.02 (1H, d, J = 2.1 Hz, H-2), 6.93 (1H, dd, J = 8.2, 2.1 Hz, H-6), 6.76 (1H, d, J = 8.1 Hz, H-5), 6.24 (1H, d, J = 15.9 Hz, H-8), 4.20 (2H, q, J = 7.1 Hz, H-10), 1.30 (3H, t, J = 7.1 Hz, H-11). ^{13}C-NMR (150 MHz, Methanol-d_4) δ: 122.9 (C-1), 115.1 (C-2), 146.7 (C-3), 149.5 (C-4), 116.5 (C-5), 127.7 (C-6), 146.8 (C-7), 115.3 (C-8), 169.3 (C-9), 61.4 (C-10), 14.6 (C-11). The ^{1}H-NMR and ^{13}C-NMR data matched those reported in the literature (Zhu et al., 2018), identifying compound 6 as ethyl caffeate. This compound was isolated from *Xanthium mongolicum* for the first time.

Compound 7 was obtained as a light yellow oil (methanol). HR-ESI-MS m/z: 179 $[M+H]^+$, molecular formula $C_{10}H_{10}O_3$. ^{1}H-NMR (600 MHz, Acetone-d_6) δ: 9.64 (1H, d, J = 7.7 Hz, H-9), 7.55 (1H, s, H-2), 8.14 (1H, d, J = 15.8 Hz, H-7), 7.20 (1H, dd, J = 8.3, 2.0 Hz, H-6), 7.04 (1H, d, J = 8.3 Hz, H-5), 6.59 (1H, dd, J = 15.8, 7.7 Hz, H-8), 3.91 (3H, s, 3-OCH_3). ^{13}C-NMR (150 MHz, Acetone-d_6) δ: 126.6 (C-1), 111.4 (C-2), 150.5 (C-3), 147.0 (C-4), 122.0 (C-5), 126.6 (C-6), 153.9 (C-7), 114.0 (C-8), 193.1 (C-9), 55.4 (3-OCH_3). The ^{1}H-NMR and ^{13}C-NMR data matched those reported in the literature (Haruna et al., 1982), identifying compound 7 as ferulaldehyde (FRA), also known as coniferaldehyde. This compound was isolated from *Xanthium mongolicum* for the first time.

Compound 8 was isolated as a white powder (methanol). ESI-MS m/z: 193 $[M+H]^+$, molecular formula $C_{10}H_8O_4$. ^{1}H-NMR (600 MHz, Methanol-d_4) δ: 7.86 (1H, d, J = 9.5 Hz, H-4), 7.12 (1H, s, H-8), 6.78 (1H, s, H-5), 6.21 (1H, d, J = 9.4 Hz, H-3), 3.91 (3H, s, H-11). ^{13}C-NMR (150 MHz, Methanol-d_4) δ: 111.22 (C-3), 144.73 (C-4), 102.56 (C-5), 106.54 (C-8), 55.41 (C-11). The ^{1}H-NMR and ^{13}C-NMR data matched those reported in the literature (Shu et al., 2010), identifying compound 8 as isoscopletin. This compound was isolated from *Xanthium mongolicum* for the first time.

Compound 9 was obtained as a light yellow oil (methanol). HR-ESI-MS m/z: 335 $[M+H]^+$, molecular formula $C_{20}H_{22}O_6$. ^{1}H-NMR (600 MHz, Methanol-d_4) δ: 6.97 (2H, d, J = 1.9 Hz, H-5,5), 6.83 (2H, dd, J = 8.2, 2.0 Hz, H-7,7), 6.79 (2H, d, J = 8.1 Hz, H-8,8), 4.73 (2H, d, J = 4.2 Hz, H-4,4), 4.25 (2H, dd, J = 9.1, 6.9 Hz, H-2a,2a), 3.88 (6H, s, 6,6 -OCH_3), 3.86 (2H, dd, J = 9.5,

3.9 Hz, H-2b,2b), 3.17 (2H, m, H-3,3). ^{13}C-NMR (150 MHz, Methanol-d_4) δ: 71.2 (C-2,2), 54.0 (C-3,3), 86.1 (C-4,4), 114.7 (C-5,5), 147.7 (C-6,6), 118.6 (C-7,7), 109.5 (C-8,8), 145.9 (C-9,9), 132.4 (C-10,10), 55.0 (OCH_3). The ^{1}H-NMR and ^{13}C-NMR data matched those reported in the literature (Saleem et al., 1997), identifying compound 9 as 3,3 -bis(3,4-dihydro-4-hydroxy-6-methoxy-2H-1-benzopyran). This compound was isolated from *Xanthium mongolicum* for the first time.

Compound 10 was isolated as a yellow powder (methanol). ESI-MS m/z: 347 $[M+H]^+$, molecular formula $C_{17}H_{14}O_8$. ^{1}H-NMR (600 MHz, Methanol-d_4) δ: 7.62 (1H, d, J = 2.2 Hz, H-2), 7.52 (1H, dd, J = 8.5, 2.2 Hz, H-6), 6.89 (1H, d, J = 8.5 Hz, H-5), 6.49 (1H, s, H-8), 3.88 (3H, s, 3 -OCH_3), 3.78 (3H, s, 6-OCH_3). ^{13}C-NMR (150 MHz, Methanol-d_4) δ: 156.72 (C-2), 137.83 (C-3), 178.89 (C-4), 152.28 (C-5), 131.18 (C-6), 157.40 (C-7), 93.58 (C-8), 152.37 (C-9), 104.89 (C-10), 121.53 (C-1), 115.02 (C-2), 145.07 (C-3), 148.58 (C-4), 115.07 (C-5), 120.94 (C-6), 59.55 (3-OCH_3), 59.11 (6-OCH_3). The ^{1}H-NMR and ^{13}C-NMR data matched those reported in the literature (Fadul et al., 2020), identifying compound 10 as axillarin. This compound was isolated from *Xanthium mongolicum* for the first time.

Compound 11 was obtained as yellow needle crystals (methanol). ESI-MS m/z: 303 $[M+H]^+$, molecular formula $C_{15}H_{10}O_7$. ^{1}H-NMR (600 MHz, Methanol-d_4) δ: 7.51 (1H, s, H-2), 7.37 (1H, d, J = 8.5 Hz, H-6), 6.72 (1H, d, J = 8.4 Hz, H-5), 6.24 (1H, s, H-8), 6.02 (1H, s, H-6). ^{13}C-NMR (150 MHz, Methanol-d_4) δ: 146.8 (C-2), 135.7 (C-3), 175.8 (C-4), 156.1 (C-5), 98.2 (C-6), 163.9 (C-7), 93.4 (C-8), 160.7 (C-9), 103.0 (C-10), 122.0 (C-1), 115.1 (C-2), 145.1 (C-3), 147.7 (C-4), 115.6 (C-5), 120.0 (C-6). The ^{1}H-NMR and ^{13}C-NMR data matched those reported in the literature (Zeng et al., 2017), identifying compound 11 as quercetin.

Compound 12 was isolated as yellow crystalline powder (methanol). ESI-MS m/z: 359 $[M+H]^+$, molecular formula $C_{20}H_{22}O_6$. ^{1}H-NMR (600 MHz, Methanol-d_4) δ: 6.97 (2H, d, J = 1.9 Hz, H-2,2), 6.93 (2H, dd, J = 8.2, 1.8 Hz, H-6,6), 6.79 (2H, d, J = 8.1 Hz, H-5,5), 4.73 (2H, d, J = 4.4 Hz, H-7,7), 4.26 (2H, dd, J = 9.0, 6.9 Hz, H-9a,9 a), 3.86 (2H, dd, J = 9.0, 3.2 Hz, H-9b,9 b), 3.88 (6H, s, 2×-CH_3O). ^{13}C-NMR (150 MHz, Methanol-d_4) δ: 132.3 (C-1,1), 109.5 (C-2,2), 147.7 (C-3,3), 145.9 (C-4,4), 114.6 (C-5,5), 118.6 (C-6,6), 86.1 (C-7,7), 53.9 (C-8,8), 71.2 (C-4,8), 54.9 (3 ,3 -OCH_3). The ^{1}H-NMR and ^{13}C-NMR data matched those reported in the literature (Zhang et al., 2020), identifying compound 12 as (+)-pinoresinol (PINO).

Compound 13 was isolated as colorless needle crystals (methanol), molecular formula $C_{32}H_{56}O$, showing purple spots with 10% sulfuric acid-ethanol solution. Co-TLC with an authentic sample showed identical Rf values in three different solvent systems without melting point depression, confirming it as β-sitosterol.

Compound 14 was obtained as white crystalline powder (methanol). ESI-MS m/z: 257 $[M+H]^+$, molecular formula $C_{16}H_{32}O_2$. ^{1}H-NMR (600 MHz,

Methanol-d_4) δ: 0.89 (3H, t, J = 5.9 Hz, H-2), 1.6 (24H, brs, H-4~15), 2.08 (2H, m, H-3), 2.29 (2H, t, J = 5.9 Hz, H-2). ^{13}C-NMR (150 MHz, Methanol-d_4) δ: 173.7 (C-1), 33.2 (C-2), 31.7 (C-14), 29.6 (C-7~12), 29.5 (C-6), 29.4 (C-13), 29.3 (C-4), 24.7 (C-3), 22.4 (C-15), 13.5 (C-16). The ^{1}H-NMR and ^{13}C-NMR data matched those reported in the literature (Zhu et al., 2018), identifying compound 14 as palmitic acid.

Discussion

Xanthium mongolicum and *Xanthii Fructus* are medicinal materials derived from the same botanical source but different parts—the dried aerial parts and mature fruits with involucre, respectively—recorded in the *Jiangsu Provincial Standards for Chinese Medicinal Materials* (1989 edition) and *Pharmacopoeia of the People' s Republic of China* (2020 edition). While *Xanthii Fructus* is warm in nature and used to dispel wind-cold and unblock nasal passages, *Xanthium mongolicum* is slightly cold and used to clear wind-heat and detoxify (Liu, 2013). Our previous screening at the University of Texas Southwestern Medical Center revealed that *Xanthium mongolicum* from Jiangxi exhibited necroptosis-inhibiting activity, with the 50% ethanol elution fraction showing particularly significant activity.

Among the 14 compounds isolated from this active fraction, compound 1 (hydroxydihydrobovolide) is an α,β-unsaturated-γ-lactone obtained from *Xanthium mongolicum* for the first time, exhibiting notable anti-tumor (Su et al., 2011) and anti-HIV activities (Zhang et al., 2005). Compounds 2–7 are phenolic compounds: raspberry ketone shows hepatoprotective, cardioprotective, antioxidant, and anti-inflammatory effects (Hamdy et al., 2020; Mir et al., 2021) and is widely used as a flavoring agent in food products and a whitening agent in cosmetics (Lin et al., 2011); salicyl alcohol exhibits good anti-*Staphylococcus aureus* activity (Du, 2020); 4-HAP is commonly used as a preservative synergist (Fan et al., 2020); 4-HBd demonstrates anti-thrombotic effects (Shen et al., 2017), blood-brain barrier protection (Zhu et al., 2018), neuroinflammatory damage reduction (Xiang et al., 2017), anti-neuronal apoptosis activity (Zhou et al., 2017), and can induce autophagy in mouse bone marrow-derived macrophages (Jina et al., 2020); ethyl caffeate possesses anti-inflammatory, immunomodulatory, and anti-tumor activities as a specific NF- B signaling pathway inhibitor with promising clinical applications (Ma et al., 2012); ferulaldehyde (FRA), abundant in fruit and vegetable peels and plant leaves, exhibits anti-inflammatory and antioxidant activities (Zhao et al., 2008) and can suppress LPS-induced nitric oxide synthase expression and nitric oxide synthesis in RAW264.7 macrophages (Kim et al., 1999). Compound 9, 3,3 -bis(3,4-dihydro-4-hydroxy-6-methoxy-2H-1-benzopyran), is a polymer isolated from *Xanthium mongolicum* for the first time. Compounds 10 and 11 are flavonoids: axillarin shows strong anti-*Candida albicans* activity (Fatma et al., 2018) and DPPH/superoxide anion scavenging activity (Fadul et al., 2020); quercetin exhibits multiple bioactivities including free radical scavenging, antioxidant, antibacterial, anti-inflammatory, immunomod-

ulatory, antiviral, and anticancer effects, potentially beneficial for controlling or treating COVID-19 (Khazdair et al., 2021). Compound 12, pinoresinol, is a high-value plant lignan that significantly inhibits proliferation of human leukemia cells (Sepporta et al., 2013) and attenuates colon cancer progression by inducing cell cycle arrest and apoptosis (Fini et al., 2018).

Necroptosis is a cell death type closely associated with inflammatory responses and immune function. Except for compounds 8, 9, 13, and 14, all other isolated compounds have demonstrated anti-inflammatory and immunomodulatory activities. However, whether these activities correlate with the previously observed necroptosis-inhibiting effect of *Xanthium mongolicum*, and whether specific natural small-molecule inhibitors of necroptosis can be identified from these compounds, requires further systematic and in-depth investigation by our research group.

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