

## Research Article

# Anti-Inflammatory Phytoconstituents of *Origanum Majorana*

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*Origanum majorana* belongs to the Lamiaceae family as a famous spice plant in Egypt, which is used to treat arthritis, allergies, fever, flu, hypertension, respiratory infections, migraine, and asthma. In our studies, it was found that the ethanol extract of *O. marjoram* could significantly reduce NO release at 200 ( $P < 0.001$ ), 100 ( $P < 0.001$ ) and 50 ( $P < 0.01$ )  $\mu\text{g}/\text{mL}$  compared with the model group. Therefore, the chemical constituents were further studied to obtain the bioactive compounds. As a result, ten compounds were isolated and identified from the 70% ethanol extract of *O. marjoram*, including six flavonoids (3–8), two terpene derivatives (9–10), one lignan (2), and one phenolic glycoside (1). Among them, compounds 1–3, 5, and 9–10 were isolated from this genus for the first time. Compounds 1, 9, and 10 could significantly decrease the content of NO at the concentration of 100  $\mu\text{m}$  ( $P < 0.001$ ) in RAW264.7 cells induced by LPS. Furthermore, compounds 9 and 10 were more effective than compound 1 to lower the content at 50  $\mu\text{m}$  ( $P < 0.001$ ).

## 1. Introduction

Inflammation is the normal protective reaction in response to infection, tissue injury, trauma, or noxious stimuli, and it is the most common pathological process in many diseases [1, 2]. Activated macrophages directly resist pathogens through phagocytosis or indirectly resist pathogens by producing related factors, such as nitric oxide (NO), interleukin (IL), and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and produce inflammatory responses [3, 4]. Local skin inflammation is usually manifested as red, swollen, hot pain. Respiratory inflammation is usually characterized by cough, expectoration, and dyspnea. Besides fever and vomiting, brain inflammation also has obvious headache. Accumulating evidence suggest that inflammation is an important pathological process of various kinds of human chronic diseases including cardiovascular diseases, neurological disorders, and cancer. Therefore, it is determined as an important treatment strategy to inhibit the inflammatory

response [5–7]. Thus, it is particularly important to search for compounds with antiinflammatory activity.

Medical food is popular, and the market has grown in the whole world [8]. *Origanum majorana* is a medicinal plant, belonging to the Lamiaceae family, and it can be used as medical food. It is native to North Africa and southwest Asia and grown in France, Greece, Hungary, Egypt, and other Mediterranean countries [9]. *O. marjoram* is widely used in traditional medicine to treat a variety of diseases, including allergies, fever, flu, hypertension, respiratory infections, migraine, and asthma [10]. In Egypt, *O. marjoram* has been used as an antiseptic, insect repellent, and expectorant and for arthritis, muscle pain, and rheumatism for thousands of years. In our studies, it was found that the ethanol extract of *O. marjoram* significantly reduced NO release at 200 ( $P < 0.001$ ), 100 ( $P < 0.001$ ), and 50 ( $P < 0.01$ )  $\mu\text{g}/\text{mL}$  compared with the model group.

Therefore, in order to find the compounds with anti-inflammatory activity, the chemical constituents and the

antiinflammation activity were investigated. As a result, 10 compounds were isolated and identified as robust aside B (**1**) [11], syringaresinol- $\beta$ -D-glucoside (**2**) [12], (-)-(2S)-5,3',4'-trihydroxy-7-methoxy-dihydroflavone (**3**) [13], luteolin (**4**) [14], luteolin-7-O- $\beta$ -D-apiofuranosyl-(1 $\rightarrow$ 6)- $\beta$ -D-glucopyranoside (**5**) [15], apigenin-4'-O- $\alpha$ -D-glucopyranoside (**6**) [16], apigenin-8-C- $\beta$ -D-glucopyranoside (**7**) [17], 5,6,4'-trihydroxy-7,8,3'-trimethoxyflavone (**8**) [18], (6R, 7E, 9R)-9-hydroxy-4,7-megastigma-4, 7-dien-3-one-9-O- $\beta$ -D-glucopyranoside (**10**) [20] (Figure 1). The bioactivity assay showed that compounds **1**, **9**, and **10** could significantly decrease the content of NO in RAW264.7 cells induced by LPS.

## 2. Materials and Methods

**2.1. Reagents and Instruments.** The following technologies were used in this research. NMR spectra were performed on Bruker AM-400 instruments with TMS as the internal standard (Bruker, Bremerhaven, Germany). Column chromatography was performed by using silica gel (200–300 and 300–400 mesh, Qingdao Marine Chemical Inc., Qingdao, China), Rp-18 gel (40–63  $\mu$ m, Merck, Darmstadt, Germany), and Sephadex LH-20 (20–150  $\mu$ m, Amersham Biosciences, Uppsala, Sweden).

The following reagents were used in this research. RAW264.7 cells were purchased from the Typical Culture Preservation Committee Cell Bank; fetal bovine serum (FBS) from Gibco, Grand Island, NE, USA; Dulbecco's Modified Eagle's Medium (DMEM) from Procell, Wuhan, China; LPS and L-NMMA from Sigma-Aldrich, St. Louis, MO, USA; and the NO kit from Shanghai Beyotime Biotechnology.

**2.2. Plant Material.** *O. majorana* was provided by the Department of Medicinal and Aromatic Plants, Horticultural Research Institute, Egyptian Agricultural Research Center. The samples were identified by Prof. Changqin Li (Henan University). A voucher specimen (20191008) was deposited in National R&D Center for Edible Fungus Processing Technology, Henan University.

**2.3. Extraction and Isolation.** The dried aerial parts of *O. majorana* (4.9 kg) were extracted with petroleum ether at room temperature for 72 h to remove the volatile oil. The filter residue was soaked and extracted three times (each time for 7 days) with 70% ethanol at room temperature and then filtered and evaporated under reduced pressure to obtain a crude extract (1360 g).

The crude extract was chromatographed on D101 macroporous resin column chromatography and eluted with water, 20% ethanol, 40% ethanol, 60% ethanol, and 95% ethanol, respectively, to obtain five fractions (Fr. A~Fr. E). Fr. B (115 g) was subjected to silica gel column chromatography, eluting with a gradient of CHCl<sub>3</sub>-MeOH (40:1~1:1) to obtain eight subfractions (Fr. B-1~Fr. B-8) by TLC plate analysis. Fr. C (70 g) was subjected to silica gel column chromatography, eluting with a gradient of CHCl<sub>3</sub>-MeOH

(50:1~5:1) to obtain five subfractions (Fr. C-1~Fr. C-5) by TLC plate analysis.

Fr. B-1 (1.57 g) was subjected to silica gel column chromatography and eluted with PE-EtOAc (3:1, v/v) to obtain fraction B-1-1 to fraction B-1-4. Fraction B-1-3 (167 mg) was subjected to silica gel column chromatography and eluted with CHCl<sub>3</sub>-MeOH (60:1, v/v) to obtain compound **9** (8.7 mg).

Fr. B-3 (1.59 g) was subjected to silica gel column chromatography and eluted with CHCl<sub>3</sub>-acetone (6:1, v/v) to obtain fraction B-3-1 to fraction B-3-7. Fraction B-3-1 (115 mg) was purified by Sephadex LH-20 (MeOH) to obtain compound **4** (24 mg).

Fr. B-4 (3.1 g) was separated by an automatic preparation of liquid chromatography (MeOH-H<sub>2</sub>O, 55:45, v/v) to obtain fraction B-4-1 to fraction B-4-4. Fraction B-4-1 (1.04 g) was subjected to silica gel column chromatography, eluted with a system of EtOAc-MeOH (10:1, v/v), and then purified by Sephadex LH-20 (MeOH) to obtain compound **2** (80 mg). Fraction B-4-3 (248 mg) was purified by Sephadex LH-20 (MeOH) to obtain compound **10** (35 mg).

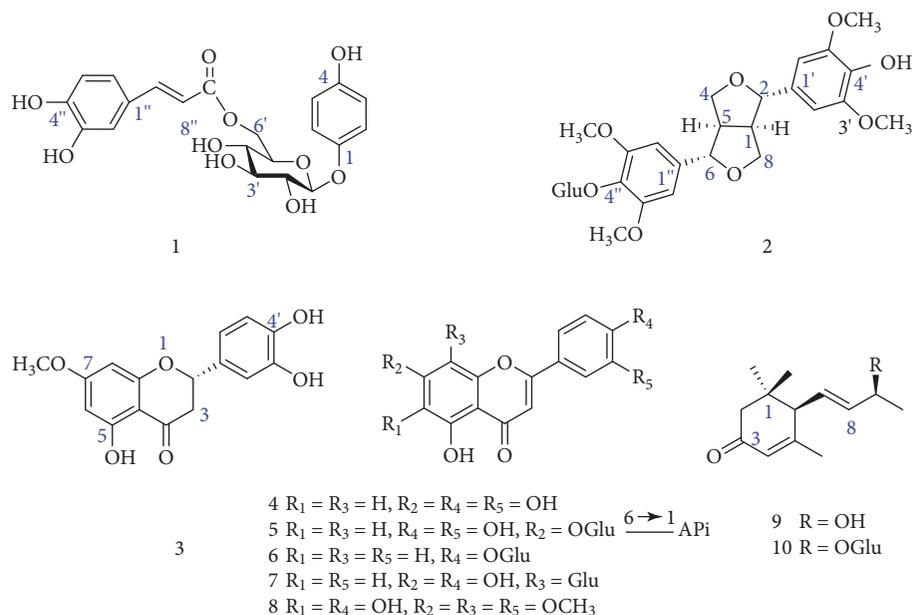
Fr. B-5 (8.0 g) was subjected to silica gel column chromatography and eluted with EtOAc-MeOH (20:1, v/v) to obtain fraction B-5-1 to fraction B-5-7. Fraction 1 (307 mg) was subjected to silica gel column chromatography, eluted with CHCl<sub>3</sub>-MeOH (40:1, v/v), and further purified by semipreparative HPLC (MeOH-H<sub>2</sub>O, 51:49 v/v) to obtain compound **5** (7.6 mg). Fraction B-5-4 (846 mg) was subjected to Sephadex LH-20 (MeOH) and further purified by semipreparative HPLC (MeOH-H<sub>2</sub>O, 48:52, v/v) to obtain compound **6** (5.2 mg).

Fr. B-6 (15 g) was performed by Rp-18 column chromatography (MeOH-H<sub>2</sub>O, 30:70 $\rightarrow$ 100:0, v/v) to obtain fraction B-6-1 to fraction B-6-5. Fraction B-6-1 (699 mg) was subjected to Sephadex LH-20 (MeOH) and further purified by semipreparative HPLC (MeOH-H<sub>2</sub>O, 51:49, v/v) to obtain compound **1** (10.8 mg). Fraction 6-4 (1.32 g) was repeated by crystallization to obtain compound **7** (32 mg).

Compound **8** (230 mg) was obtained by recrystallization from Fr. C-1-1 (1.06 g). Fr. C-1-2 (2.21 g) was subjected to silica gel column chromatography and eluted with CHCl<sub>3</sub>-MeOH (100:1, v/v) to obtain fraction C-1-2-1 to fraction C-1-2-3. Fraction C-1-2-3 (187 mg) was purified by Sephadex LH-20 (MeOH) to obtain compound **3** (33 mg).

## 2.4. Spectral Data of Compounds

**2.4.1. Robustaside B (1).** White amorphous powder, m/z: 434, molecular formula C<sub>21</sub>H<sub>22</sub>O<sub>10</sub>; <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 400 MHz)  $\delta_{\text{H}}$ : 3.36~3.50 (3H, m, H-2', 3', 4'), 4.35 (1H, dd, J = 11.8, 6.6 Hz, H-6'a), 4.53 (1H, d, J = 11.9 Hz, H-6'b), 4.73 (1H, d, J = 7.1 Hz, H-1'), 6.29 (1H, d, J = 15.9 Hz, H-7''), 6.65 (2H, d, J = 8.8 Hz, H-3, 5) 6.80 (1H, d, J = 8.1 Hz, H-5''), 6.94 (3H, m, H-6, 2, 6), 7.06 (1H, s, H-2''), 7.58 (1H, d, J = 15.9 Hz, H-8''); <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 100 MHz)  $\delta_{\text{C}}$ : 127.7 (C-1''), 115.0 (C-2''), 149.7 (C-3''), 146.8 (C-4''), 116.5 (C-5''), 123.1 (C-6''), 114.8 (C-7''), 147.2 (C-8''), 169.0 (C=O), 103.7 (C-1'), 74.9 (C-2'), 77.9 (C-3'), 71.8 (C-4'), 75.5 (C-5'), 64.7 (C-6'), 153.9 (C-1), 119.6 (C-2, 6), 116.6 (C-3, 5), and 152.3 (C-4).

FIGURE 1: Structures of compounds 1–10 isolated from *O. majorana*.

**2.4.2. Syringaresinol- $\beta$ -D-glucoside (2).** White powder, *m/z*: 580, molecular formula C<sub>28</sub>H<sub>36</sub>O<sub>13</sub>; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>, 400 MHz)  $\delta_{\text{H}}$ : 3.00~3.19 (6H, *m*, H-2''', 6'''), 3.75 (6H, *s*, 3'-OCH<sub>3</sub>, 5'-OCH<sub>3</sub>), 3.74 (6H, *s*, 3''-OCH<sub>3</sub>, 5''-OCH<sub>3</sub>), 4.88 (1H, *brs*, H-1'''), 6.60 (2H, *s*, H-2', 6'), 6.66 (2H, *s*, H-2'', 6''); <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>)  $\delta_{\text{C}}$ : 152.6 (C-3'', C-5''), 147.9 (C-3', C-5'), 133.7 (C-1'''), 134.8 (C-4'), 137.2 (C-4''), 131.3 (C-1'), 104.1 (C-1'''), 103.6 (C-2'', 6''), 102.6 (C-2', 6'), 85.4 (C-2), 85.1 (C-6), 77.2 (C-5'''), 76.5 (C-3'''), 74.2 (C-2'''), 71.3 (C-4), 71.2 (C-8), 69.9 (C-4'''), 60.9 (C-6'''), 56.4 (3'-OCH<sub>3</sub>, 5'-OCH<sub>3</sub>), 56.0 (3-OCH<sub>3</sub>, 5-OCH<sub>3</sub>), 53.7 (C-1), and 53.6 (C-5).

**2.4.3. (-)-(2S)-5,3,4'-Trihydroxy-7-methoxy-dihydroflavone (3).** White powder, *m/z*: 302, molecular formula C<sub>16</sub>H<sub>14</sub>O<sub>6</sub>; <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta_{\text{H}}$ : 12.11 (1H, *s*, 5-OH), 9.06 (1H, *s*, H-3'), 6.89 (1H, *s*, H-2') 6.75 (2H, *t*, H-5', 6'), 6.07 (2H, *m*, H-8, H-6), 5.42 (1H, *dd*, *J* = 12.6, 3.0 Hz, H-2), 3.24 (1H, *dd*, *J* = 17.1, 12.6 Hz, H-3a), 2.72 (1H, *dd*, *J* = 17.1, 3.1 Hz, H-3b), 3.77 (3H, *s*, 7-OCH<sub>3</sub>), <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>)  $\delta_{\text{C}}$ : 196.9 (C-4), 167.4 (C-7), 163.2 (C-5), 162.9 (C-9), 145.8 (C-4'), 145.2 (C-3'), 129.3 (C-1'), 118.0 (C-6'), 115.4 (C-5'), 114.4 (C-2'), 102.7 (C-10), 94.6 (C-6), 93.8 (C-8), 78.7 (C-2), 55.9 (7-OCH<sub>3</sub>), and 42.2 (C-3).

**2.4.4. Luteolin (4).** Yellow powder, *m/z*: 286, molecular formula C<sub>15</sub>H<sub>10</sub>O<sub>6</sub>; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>, 400 MHz)  $\delta_{\text{H}}$ : 12.98 (1H, *s*, 5-OH), 7.42 (1H, *brd*, *J* = 7.6 Hz, H-6'), 7.39 (1H, *brs*, H-2'), 6.88 (1H, *d*, *J* = 7.6 Hz, H-5'), 6.67 (1H, *s*, H-3), 6.44 (1H, *d*, *J* = 1.6 Hz, H-8), 6.18 (1H, *d*, *J* = 1.6 Hz, H-6); <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>, 100 MHz)  $\delta_{\text{C}}$ : 164.2 (C-2), 102.9 (C-3), 181.7 (C-4), 161.5 (C-5), 98.9 (C-6), 164.2 (C-7), 93.9 (C-8), 157.3 (C-9), 103.7 (C-10), 121.5 (C-1'), 113.4 (C-2'), 145.8 (C-3'), 149.8 (C-4'), 116.0 (C-5'), 119.0 (C-6').

**2.4.5. Luteolin-7-O- $\beta$ -D-apiofuranosyl-(1 $\rightarrow$ 6)- $\beta$ -D-glucopyranoside (5).** Yellow powder, *m/z*: 580, molecular formula C<sub>26</sub>H<sub>28</sub>O<sub>15</sub>; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>, 400 MHz)  $\delta_{\text{H}}$ : 6.74 (1H, *s*, H-3), 6.47 (1H, *d*, *J* = 1.9 Hz, H-8), 6.39 (1H, *d*, *J* = 1.9 Hz, H-6), 5.18 (1H, *d*, *J* = 6.5 Hz, Glu-1); <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>)  $\delta_{\text{C}}$ : 162.3 (C-2), 103.0 (C-3), 181.7 (C-4), 156.8 (C-5), 99.2 (C-6), 164.5 (C-7), 94.3 (C-8), 161.1 (C-9), 105.4 (C-10), 121.0 (C-1'), 113.3 (C-2'), 145.8 (C-3'), 150.3 (C-4'), 116.0 (C-5'), 119.3 (C-6'), 98.5 (Glu-1), 73.0 (Glu-2), 75.7 (Glu-3), 69.4 (Glu-4), 76.6 (Glu-5), 65.6 (Glu-6), 108.8 (Api-1), 76.1 (Api-2), 79.2 (Api-3), 74.0 (Api-4), and 64.1 (Api-5).

**2.4.6. Apigenin 4'-O- $\alpha$ -D-glucopyranoside (6).** Yellow powder, *m/z*: 432, molecular formula C<sub>21</sub>H<sub>20</sub>O<sub>10</sub>; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>, 400 MHz)  $\delta_{\text{H}}$ : 7.91 (2H, *d*, *J* = 8.7 Hz, H-2', H-6'), 6.88 (2H, *d*, *J* = 8.8 Hz, H-3', H-5'), 6.84 (*s*, H-3), 6.80 (*d*, *J* = 1.9 Hz, H-8), 6.40 (*d*, *J* = 1.9 Hz, H-6), 5.02 (2H, *d*, Ha-6'', Hb-6''); <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>, 100 MHz)  $\delta_{\text{C}}$ : 181.9 (C-4), 164.4 (C-2), 162.8 (C-5), 161.3 (C-4'), 156.8 (C-9), 128.6 (C-2'), 128.6 (C-6'), 121.0 (C-1'), 116.1 (C-3', 5'), 105.2 (C-10), 103.0 (C-3), 99.9 (C-1''), 99.5 (C-6), 95.0 (C-8), 77.2 (C-3''), 76.4 (C-5''), 73.1 (C-2''), 69.6 (C-4''), and 60.6 (C-6'').

**2.4.7. Apigenin-8-C- $\beta$ -D-glucopyranoside (7).** Yellow powder, *m/z*: 432, molecular formula C<sub>21</sub>H<sub>20</sub>O<sub>10</sub>; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>, 400 MHz)  $\delta_{\text{H}}$ : 13.17 (1H, *s*, 5-OH), 8.03 (2H, *d*, *J* = 8.7 Hz, H-2', H-6'), 6.89 (2H, *d*, *J* = 8.7 Hz, H-3', H-5'), 6.78 (1H, *s*, H-3), 6.27 (1H, *s*, H-6), 4.68 (1H, *d*, *J* = 9.3 Hz, H-1''), 3.83 (1H, *t*, *J* = 9.3 Hz, H-2''), 3.76 (1H, *d*, *J* = 10.8, H-6''), 3.49 (1H, *dd*, *J* = 10.8, 5.2 Hz, H-6''), 3.24 (3H, *m*, H-3'', H-4'', H-5''); <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>, 100 MHz)  $\delta_{\text{C}}$ : 182.1 (C-4), 164.0 (C-2), 162.6 (C-7), 161.1 (C-9), 160.4 (C-4'), 156.0 (C-5), 129.0 (C-2', C-6'), 121.6 (C-1'), 115.8 (C-3', C-5'), 104.6 (C-10), 104.0 (C-8), 102.5 (C-3), 98.2 (C-6), 81.9

(C-5''), 78.7 (C-3''), 73.4 (C-1''), 70.8 (C-2''), 70.5 (C-4''), and 61.3 (C-6'').

2.4.8. *5,6,4'-Trihydroxy-7,8,3'-trimethoxyflavone (8)*. Yellow powder, *m/z*: 360, molecular formula  $C_{18}H_{16}O_8$ ;  $^1H$ -NMR (DMSO- $d_6$ , 400 MHz)  $\delta_H$ : 3.89 (3H, s, 7-OCH<sub>3</sub>), 3.92 (3H, s, 8-OCH<sub>3</sub>), 3.95 (3H, s, 3'-OCH<sub>3</sub>), 6.94 (1H, s, H-3), 6.97 (1H, *d*, *J* = 8.3, H-5'), 7.56 (1H, *d*, *J* = 8.3, H-6'), 9.51 (1H, s, 6-OH), 10.13 (1H, s, 4'-OH), 12.51 (1H, s, 5-OH);  $^{13}C$ -NMR (DMSO- $d_6$ , 100 MHz)  $\delta_C$ : 163.8 (C-2), 102.7 (C-3), 182.6 (C-4), 145.4 (C-5), 134.2 (C-6), 148.0 (C-7), 132.9 (C-8), 143.1 (C-9), 106.1 (C-10), 121.7 (C-1'), 110.0 (C-2'), 148.1 (C-3'), 150.9 (C-4'), 115.9 (C-5'), 120.3 (C-6'), 55.9 (7-OCH<sub>3</sub>), 61.0 (8-OCH<sub>3</sub>), and 61.8 (3'-OCH<sub>3</sub>).

2.4.9. *(6R, 7E, 9R)-9-Hydroxy-4,7-megastigmadien-3-one (9)*. Colorless oil, *m/z*: 208, molecular formula  $C_{13}H_{20}O_2$ ;  $^1H$ -NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta_H$ : 5.89 (1H, s, H-4), 5.67 (1H, *dd*, *J* = 15.3, 9.4 Hz, H-8), 5.54 (1H, *dd*, *J* = 15.3, 7.8 Hz, H-7), 4.35 (1H, *m*, H-9), 2.50 (1H, *d*, *J* = 8.8 Hz, H-6), 2.32 (1H, *d*, *J* = 18.1 Hz, H-2a), 2.07 (1H, *d*, *J* = 18.1 Hz, H-2b), 1.89 (3H, *m*, H-13), 1.29 (3H, *d*, *J* = 6.4 Hz, H-10), 1.02 (3H, s, H-11), 0.95 (3H, s, H-12);  $^{13}C$ -NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta_C$ : 36.3 (C-1), 47.6 (C-2), 199.4 (C-3), 125.9 (C-4), 162.1 (C-5), 55.6 (C-6), 126.7 (C-7), 138.7 (C-8), 68.4 (C-9), 23.8 (C-10), 27.3 (C-11), 28.0 (C-12), and 23.7 (C-13).

2.4.10. *(6R, 9S)-9-Hydroxy-megastigma-4,7-dien-3-one-9-O-β-D-glucopyranoside (10)*. White powder, *m/z*: 370, molecular formula  $C_{19}H_{30}O_7$ ;  $^1H$ -NMR (CD<sub>3</sub>OD, 400 MHz)  $\delta_H$ : 5.89 (1H, s, H-4), 5.76 (1H, *dd*, *J* = 15.4, 9.6 Hz, H-7), 5.60 (1H, *dd*, *J* = 15.4, 7.4 Hz, H-8), 4.48 (1H, *dd*, *J* = 7.4, 6.4 Hz, H-9), 4.29 (1H, *d*, *J* = 7.7 Hz, H-1'), 2.70 (1H, *d*, *J* = 16.6 Hz, H-2), 2.48 (1H, *d*, *J* = 9.6 Hz, H-6), 2.06 (1H, *d*, *J* = 16.7 Hz, H-2), 1.98 (3H, s, H-13), 1.29 (3H, *d*, *J* = 6.4 Hz, H-10), 1.03 (3H, s, H-11), 0.99 (3H, s, H-12);  $^{13}C$ -NMR (CD<sub>3</sub>OD, 100 MHz)  $\delta_C$ : 202.0 (C-3), 165.7 (C-5), 137.0 (C-8), 131.2 (C-7), 126.2 (C-4), 101.2 (C-1'), 78.3 (C-3'), 78.2 (C-5'), 74.9 (C-2'), 74.7 (C-9), 71.7 (C-4'), 62.8 (C-6'), 56.9 (C-6), 48.4 (C-2), 37.2 (C-1), 28.0 (C-12), 27.4 (C-11), 23.9 (C-13), and 22.2 (C-10).

## 2.5. Cell Culture and Treatment

2.5.1. *Cell Culture and Cell Viability Assay*. Cell treatment methods refer to the literature [21]. Cells were then treated with different concentrations (6.25, 12.5, 25, 50, 100, and 200  $\mu$ M) of the compounds **1**, **2**, and **9–10**, while the control group was given the same volume of DMED. 10  $\mu$ L MTT (0.5 mg/mL) was added to each well followed by further incubation for 4 h at 37°C. Then, the cell culture medium was discarded and added into 100  $\mu$ L DMSO. The absorbance was measured at 490 nm to calculate the survival rate of cells.

2.6. *Nitric Oxide Assay*. Cell treatment methods refer to the literature [22]. Cells were treated with nontoxic concentrations of the compounds, and the positive control group

was treated with 50  $\mu$ M L-NMMA stimulation for 1 h, followed by 1  $\mu$ g/mL LPS treatment. The control group did not add LPS. After further incubation for 24 h, the concentration of NO was determined according to production instructions.

## 3. Results and Analysis

3.1. *Effects of the Ethanol Extract of O. majorana on RAW264.7 Cell Viability and NO Production*. NO is an important inflammatory mediator because various inflammation-related diseases are caused by the excessive production of nitric oxide [23]. First, the effect of the ethanol extract of *O. majorana* was tested on cell viability in LPS-induced RAW 264.7 cells. The results showed that the ethanol extract had no effect on cell viability when the concentration was under 200  $\mu$ g/mL (Figure 2(a)).

Therefore, the concentrations of 200, 100, and 50  $\mu$ g/mL were used to detect the NO release in LPS-induced RAW 264.7 cells. In Figure 2(b), the ethanol extract significantly reduced NO release compared with the LPS group at 200 ( $P < 0.001$ ), 100 ( $P < 0.001$ ), and 50 ( $P < 0.01$ )  $\mu$ g/mL.

3.2. *Effects of Compounds on RAW 264.7 Cell Viability*. To determine the nontoxic concentration of compounds, the effects of different concentrations of compounds on the viability of RAW 264.7 cells were detected by an MTT assay. In Figure 3, compared with the control group, compounds **1**, **9**, and **10** did not affect cell viability when the concentration was below 100  $\mu$ M. Therefore, the concentration of 100, 50, and 25  $\mu$ M was selected for activity screening.

3.3. *Effects of Compounds on NO Production*. Therefore, we evaluated the antiinflammatory activity efficacy of compounds **1**, **9**, and **10** by detecting NO content. In Figure 4, compared with the control group, NO content in the LPS group was significantly higher, indicating that the inflammatory model was successful. Compared with the LPS group, positive control group NO release decreased obviously ( $P < 0.001$ ), compounds **1**, **9**, and **10** significantly reduced LPS-induced NO production, suggesting that these compounds had significant antiinflammatory activity. Compound **1** inhibited NO release at 100, 50, and 25  $\mu$ M, and the effect was the best at 100  $\mu$ M ( $P < 0.001$ ). Compounds **9** and **10** significantly decreased NO at 100 and 50  $\mu$ M ( $\mu$ ), while compound **9** also inhibited the release of NO at 25  $\mu$ M ( $P < 0.01$ ).

## 4. Discussion

In this study, 10 compounds were extracted and separated from *O. marjoram*, including six flavonoids, two terpene derivatives, one lignan, and one phenolic glycoside. Among the six flavonoids, compounds **4–8** are flavones, while compound **3** is a flavanone. The skeleton of compound **2** belongs to furofuran lignans. Compounds **1–3**, **5**, and **9–10** were isolated from this genus for the first time.

In our studies, it was found that the ethanol extract of *O. marjoram* could significantly reduce NO release which

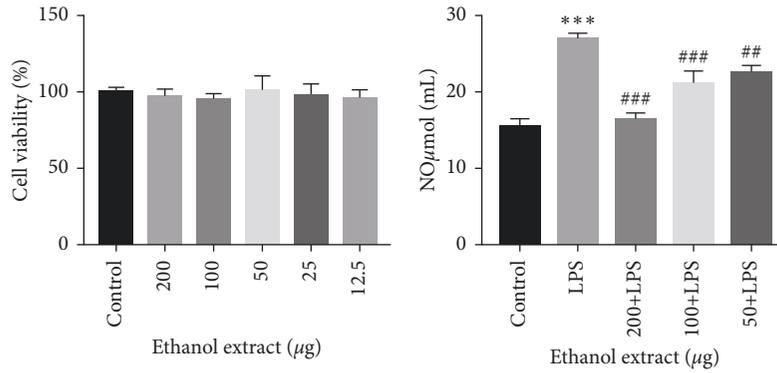


FIGURE 2: Effects of the ethanol extract on the viability of RAW 264.7 cells (a). Effects of the ethanol extract on NO production in LPS-induced RAW 264.7 cells (b). Data are mean  $\pm$  SD,  $n = 3$ ; compared with the control group, \*\*\* $P < 0.001$ , and compared with the LPS group, ### $P < 0.001$  and ## $P < 0.01$ .

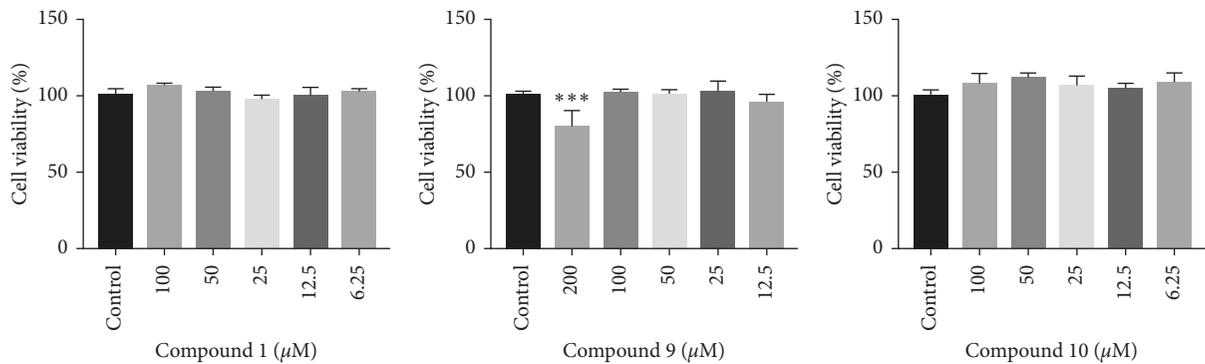


FIGURE 3: Effects of compounds **1**, **9**, and **10** on the viability of RAW 264.7 cells. Data presented are the mean  $\pm$  SD,  $n = 4$ .

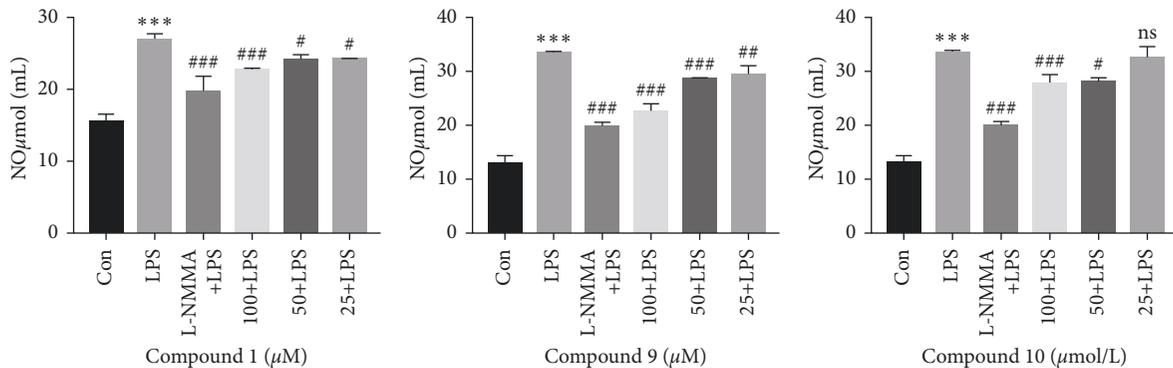


FIGURE 4: Effects of compounds **1**, **9**, and **10** on NO production in LPS-induced RAW 264.7 cells. Data presented are the mean  $\pm$  SD,  $n = 3$ . Compared with the control group, \*\*\* $P < 0.001$ . Compared with the LPS group, ### $P < 0.001$  and ## $P < 0.01$ .

showed good antiinflammatory activities. Therefore, the phytochemical constituents were studied to look for the bioactive molecules. Finally, 10 compounds were identified, and 3 compounds (**1**, **9**, and **10**) could significantly decrease LPS-induced NO production in RAW 264.7 cells. It has been reported that compound **9** exhibited an inhibition effect against leukotriene C 4 (LTC 4) synthesis and degranulation definitely in c-kit ligand (KL) induced mast cells. Mast cell LTC 4 and degranulation are the main initiating cells of type I allergic inflammation [24]. These also indicated that compound **9** had a better antiinflammatory effect. So, it can

be further studied for the antiinflammatory mechanism of compound **9** and to develop its application prospects.

Also, *O. marjoram* is rich in flavonoids. The flavonoids have, among others, pharmacological actions as neural protection, antimyocardial ischemia, blood pressure reduction, learning memory improvement, antigastric ulcer, reproductive tissue protection, antitumor, and lowering of blood sugar [25]. Flavones can improve the cognitive dysfunction of Alzheimer's disease (AD) model animals from multiple targets, alleviate AD-like pathological symptoms, and inhibit the pathological progress of AD [26]. Flavanones

can effectively block angiogenesis and dilate blood vessels [27]. There are abundant plant resources of *O. marjoram*, and the ethanol extract is rich in flavonoids, which has a good medicinal prospect.

At present, the research on *O. marjoram* mainly focuses on the constituents and pharmacological activities of the volatile oil, but its nonvolatile components and monomer compounds are lacking. In order to expand the application range of *O. marjoram* and make full use of its medicinal value, the chemical constituents and antiinflammatory activity of the ethanol extract of *O. marjoram* were preliminarily studied, which provided reasonable and reliable scientific basis for expanding the drug sources.

### Data Availability

All data used during the study appear in the submitted article.

### Conflicts of Interest

All authors declare that they have no conflicts of interest.

### Authors' Contributions

All authors contributed equally to this work.

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