Study on the Chemical Constituents of *Trillium tschonoskii* and Screening of Their Anti-Breast-Cancer Activity

2026-06-26

ChinaRxiv

Abstract

To investigate the chemical constituents of Trillium tschonoskii and their effects on breast cancer cells, this study isolated and purified extracts of Trillium tschonoskii using silica gel and gel chromatography, identified the structures of the compounds based on physicochemical properties and spectral data, and evaluated the inhibitory activity of the compounds against the breast cancer cell line MDA-MB-231 using the MTT assay. The results showed that: (1) twenty-four compounds were identified from Trillium tschonoskii, namely lysicamine (1), hydroxyframoside (2), goniothalamusin (3), pyrocatechol (4), 6-hydroxy-7,8-dimethoxycoumarin (5), norisoboldine (6), 4,6-dihydroxy-2-methoxyacetophenone (7), hinokiresinol (8), sedanolide (9), rostratamine (10), cinnamicacid (11), magnolioside (12), platypterophathalide (13), transtorine (14), capillasterolide (15), globularin (16), formononetin (17), ethyl p-hydroxycinnamate (18), cirsilineol (19), chilenolide (20), acetoisovanillone (21), rubrosterone (22), didehydroconicol (23), and dehydrovomifoliol (24). All compounds were isolated and identified from Trillium tschonoskii for the first time. (2) Compounds 1, 4, 8, 9, 13, 14, 16, 19, and 23 exhibited varying degrees of inhibitory effects on MDA-MB-231 cells, among which compound 9 showed an inhibitory effect on MDA-MB-231 cells comparable to that of cyclophosphamide ($P>0.05$). The results of this study revealed the chemical constituents of Trillium tschonoskii, among which compound 9 has potential anti-breast cancer activity.

Full Text

DOI:10.11931/guihaia.gxzw202504022

Study on the Chemical Constituents of *Trillium tschonoskii* and Screening of Their Anti-Breast-Cancer Activity

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Abstract: To investigate the chemical constituents of *Trillium tschonoskii* and their effects on breast cancer cells, the extract of *T. tschonoskii* was separated and purified using silica gel and Sephadex gel. The structures of the compounds were identified on the basis of physicochemical properties and spectral data, and the inhibitory activities of the compounds against the breast cancer cell line MDA-MB-231 were evaluated by the MTT assay. The results showed that: (1) Twenty-four compounds were identified from *T.*

tschonoskii, namely lysicamine (**1**), hydroxyframoside (**2**), goniothalamusin (**3**), pyrocatechol (**4**), 6-hydroxy-7,8-dimethoxycoumarin (**5**), norisoboldine (**6**), 4,6-dihydroxy-2-methoxyacetophenone (**7**), hinokiresinol (**8**), sedanolide (**9**), rostratamine (**10**), cinnamic acid (**11**), magnolioside (**12**), platypterophathalide (**13**), transtorine (**14**), capillasterolide (**15**), globularin (**16**), formononetin (**17**), ethyl *p*-hydroxycinnamate (**18**), cirsilineol (**19**), [[unclear: compound name]] lactone (**20**), acetoisovanillone (**21**), rubrosterone (**22**), didehydroconicol (**23**), and dehydrovomifoliol (**24**). All compounds were isolated and identified from *T. tschonoskii* for the first time. (2) Compounds **1**, **4**, **8**, **9**, **13**, **14**, **16**, **19**, and **23** exhibited varying degrees of inhibitory effects on MDA-MB-231 cells; among them, the inhibitory effect of compound **9** on MDA-MB-231 cells was comparable to that of cyclophosphamide (P>0.05). These findings reveal the chemical constituents of *T. tschonoskii*, among which compound **9** has potential anti-breast-cancer activity.

Keywords: *Trillium tschonoskii*; chemical constituents; isolation and identification; breast cancer; MDA-MB-231 cells

Chinese Library Classification No.: Q946 **Document code:** A

Chemical constituents and their effects on breast cancer cells

of *Trillium tschonoskii*

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Abstract: In order to investigate the chemical components of *Trillium tschonoskii* and its effects on breast cancer cells. The extract of *T. tschonoskii* was isolated and purified by silica gel and Sephadex LH-20 and the structures of the compounds were identified based on physicochemical properties and spectral data. The inhibitory activity of the compound against the breast cancer cell

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Funding project: Science and Technology Research Project of Henan Province (252102311028).
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The MDA-MB-231 cell line was evaluated by the MTT method. The results were as follows: (1) Twenty-four compounds were identified from *T. tschonoskii*: lysicamine (**1**), hydroxyframoside (**2**), goniothalamusin (**3**), pyrocatechol (**4**), 6-hydroxy-7,8-dimethoxycoumarin (**5**), norisoboldine (**6**), 4,6-dihydroxy-2-methoxyacetophenone (**7**), hinokiresinol (**8**), sedanolide (**9**), rostratamine (**10**), cinnamic acid (**11**), magnolios (**12**), platypterophathalide (**13**), transtorin (**14**), capillasterolide (**15**), globularin (**16**), ononin (**17**), ethyl *p*-hydroxycinnamate (**18**), cirsilineol (**19**), loliolide (**20**), acetoisovanillone (**21**), rubrosterone (**22**), didehydroconicol (**23**), and dehydrovomifoliol (**24**). All compounds were isolated and identified from *T. tschonoskii* for the first time. (2) Compounds **1**, **4**, **8**, **9**, **13**, **14**, **16**, **19**, and **23** showed inhibitory effects of varying degrees on MDA-MB-231 cells. Among them, the inhibitory effect of Compound **9** on MDA-MB-231 cells was comparable to that of cyclophosphamide ($P > 0.05$). This study revealed the chemical composition of *T. tschonoskii*, among which Compound **9** has potential anti-breast-cancer activity.

Key words: *Trillium tschonoskii*, chemical composition, isolation and identification, breast cancer, MDA-MB-231 cells

Trillium refers to the roots and rhizomes of *Trillium tschonoskii*, *T. govanianum*, and *T. kamtschaticum*, plants of the genus *Trillium* in the family Liliaceae; it is also known as Sanjiaoqi, Tianzhugen, and Dizhu (Wuhan Institute of Botany, Chinese Academy of Sciences, 2003). *Trillium* is mainly produced in East Asia and North America, and in China it is distributed chiefly in Henan, Hebei, Jilin, Liaoning, Anhui, Shaanxi, and other regions. The *Supplement to Compendium of Materia Medica* states: "The root of *Trillium*, when pounded and applied to sores and toxins, can reduce swelling and dissipate nodules." *Trillium* is slightly warm in nature and sweet-bitter in taste; it is effective in detoxification and pain relief, sedation and calming of the mind, dispelling wind and relieving pain, and is used clinically for snake and insect bites, rheumatic arthralgia, sores, carbuncles, swelling and toxin, palpitations, neurasthenia, irregular menstruation, and other conditions (Khan et al., 2016). The chemical constituents of *Trillium* are mainly steroidal saponins, phenolic acids, sesquiterpenes, coumarins, phenylpropanoids, and polysaccharides (Zhong et al., 2013). Pharmacological studies have shown that *Trillium* has antitumor, anticoagulant, anti-inflammatory, analgesic, sedative-hypnotic, myocardial-protective, and immunomodulatory activities (Li Xiaopei et al., 2017). Studies have shown that the ethanol extract of *Trillium* can inhibit the proliferation of the breast cancer cell line MDA-MB-231 in nude mice and promote its apoptosis (Zeng Xiaocong et al., 2019).

Trillium is a commonly used medicine in the clinical treatment of breast cancer. To explore the material basis by which it treats and inhibits breast cancer cells, the authors separated and purified *Trillium* and identified 24 compounds; all compounds were isolated and identified from *Trillium* for the first time. Activity testing of the obtained compounds against the breast cancer cell line MDA-

MB-231 showed that several monomeric compounds inhibited the proliferation of MDA-MB-231 cells. Among them, sedanolide exhibited a relatively high inhibition rate against MDA-MB-231 cell proliferation and may have potential anti-breast-cancer activity.

1 Materials and Methods

1.1 Instruments and Reagents

ICP-MS mass spectrometer (Agilent, USA); Spinsolve nuclear magnetic resonance spectrometer (Magritek, New Zealand); PURA22 thermostatic electric water bath (Julabo, Germany); N-1300 rotary evaporator (Eyela, Japan); PBB8

model analytical balance (Prima, USA); HydroH24 electronic balance (Lauda, Germany); T200 ultrasonic cleaner (Telsonic, Switzerland); B2000 absorbance microplate reader (Branson, USA); Sephadex LH-20 gel (GE Healthcare, USA); silica gel for column chromatography (Qingdao Haiyang Chemical Plant); MTT (thiazolyl blue; Nanjing Lvhe Biotechnology Co., Ltd.); DMSO (dimethyl sulfoxide; Hunan Wokai Biotechnology Co., Ltd.); and the breast cancer cell line MDA-MB-231 (Chengdu Zero Six Biotechnology Co., Ltd.). All reagents used for extraction and separation were of analytical grade.

1.2 Plant material

Trillium tschonoskii was collected from Baofeng County, Henan Province, and its roots and rhizomes were identified as those of *Trillium tschonoskii* by Associate Professor Bai Xianguang of Pingdingshan University.

1.3 Research methods

1.3.1 Extraction and separation The plant material of *Trillium tschonoskii* (13.5 kg) was pulverized into coarse powder, soaked in 80% methanol (50 L) for 6 h, and extracted ultrasonically. Concentration under reduced pressure afforded 729.3 g of extract. The extract was dispersed in water and successively extracted with petroleum ether, dichloromethane, and *n*-butanol; after concentration under reduced pressure, extracts of the different solvent fractions were obtained.

The petroleum ether extract (31.4 g) was subjected to silica-gel column chromatography and eluted with an *n*-hexane–acetone gradient (75:25$\rightarrow$25 : 75)$togive Fr.AI–VI. Fr.AII was subjected to silica-gel column chromatography and eluted with an-n*-hexane–ethylacetate gradient(90 : 10\rightarrow10 : 90)togive Fr.AII-1–Fr.AII-6. Fr.AII-1 was subjected to silica-gel column chromatography and eluted with*n*-hexane–ethylacetate(35 : 65)to yield compound**1**(19mg); Fr.AII-3 was separated on Sephadex LH-20 and eluted with methanol to yield compound**9**(22mg); Fr.AII-6 was subjected to silica-gel column chromatography and eluted with-n*-hexane–ethylacetate(35 : 65)to yield compounds**2**(24mg) and**3**(21mg). Fr.AIII was subjected to silica-gel column chromatography and eluted with an-*

$n*-hexane–ethylacetategradient(90:10\rightarrow\$10:90)$ to give Fr. AIII-1–Fr. AIII-7. Fr. AIII-2 was subjected to silica-gel column chromatography and eluted with *n*-hexane–ethyl acetate (35:65) to yield compound **12** (24 mg); Fr. AIII-3 was recrystallized to yield compounds **4** (22 mg) and **24** (27 mg).

The dichloromethane extract (31.6 g) was subjected to silica-gel column chromatography and eluted with an *n*-hexane–acetone gradient $(70:30\$\rightarrow0:100)togiveFr.BI–V.Fr.BIwassubjectedtosilica-gelcolumnchromatographyandelutedwithan*n*-hexane–ethylacetategradient(70:30\rightarrow0:100)togiveFr.BI-1–Fr.BI-6.Fr.BI-2wassubjectedtosilica-gelcolumnchromatographyandelutedwith*n*-hexane–ethylacetate(20:80)toyieldcompound**7**(22mg);Fr.BI-3wasrecrystallizedtoyieldcompound**8**(20mg);Fr.BI-5wasseparatedonSephadexLH-20andelutedwithmethanoltoyieldcompounds**10**(20mg)and**11**(23mg).Fr.BIIIwassubjectedtosilica-gelcolumnchromatographyandelutedwithan*n*-hexane–ethylacetategradient(70:30\rightarrow\$0:100)$ to give Fr. BIII-1–Fr. BIII-6. Fr. BIII-2 was recrystallized to yield compounds **16** (24 mg) and **17** (21 mg); Fr. BIII-4 was separated on Sephadex LH-20 and eluted with methanol to yield compounds **21** (22 mg) and **23** (28 mg).

The *n*-butanol extract (24.6 g) was subjected to silica-gel column chromatography and eluted with an *n*-hexane–methanol gradient $(85:15\$\rightarrow15:85)togiveFr.CI–IV.Fr.CIwassubjectedtosilica-gelcolumnchromatographyandelutedwithan*n*-hexane–methanolgradient(85:15\rightarrow\$15:85)$ to give Fr. CI-1–Fr. CI-5. Fr. CI-1 was subjected to silica-gel column chromatography and eluted with *n*-hexane–methanol (35:65) to yield compounds **5** (25 mg) and **6** (22 mg); Fr. CI-2 was subjected to silica-gel column chromatography and eluted with *n*-hexane–methanol (35:65) to yield compounds **13** (21 mg), **14** (22 mg), and **15** (24 mg); Fr. CI-4 was recrystallized to yield compound **18** (20 mg). Fr. CIII was subjected to silica-gel column chromatography using *n*-hexane

eluted with alkane–methanol (35:65) to give Fr. CIII-1–Fr. CIII-6. Fr. CIII-2 was subjected to silica-gel column chromatography and eluted with *n*-hexane–methanol (35:65) to afford compounds **19** (23 mg) and **20** (21 mg). Fr. CIII-3 was recrystallized to yield compound **22** (26 mg).

1.3.2 Assay of the inhibitory activity of the compounds against breast cancer cells

The inhibitory activity of the obtained compounds against the breast cancer cell line MDA-MB-231 was evaluated by the MTT method (Niu et al., 2002).

Step 1: MDA-MB-231 cells in the logarithmic growth phase were collected and prepared as a single-cell suspension at a concentration of 3×10^4 cells per milliliter. The suspension was seeded into 96-well plates (100 μL per well) and precultured in a constant-temperature incubator at 36.8 °C for 24 h. Compounds 1–24 to be tested were then added separately, with four concentration gradients set at 1.0, 10.0, 50.0, and 100.0 $\mu g \cdot mL^{-1}$, and an equal volume of DMSO culture medium was used as the blank control group. Culture was con-

tinued for 24 h. Then 10 μL of MTT solution was added to each well and incubated for 12 h, after which the absorbance was measured with a microplate reader. Step 2: Compounds showing inhibitory activity against MDA-MB-231 cells were selected, with cyclophosphamide used as the positive control group. A single-cell suspension at a concentration of 3×10^4 cells per milliliter was seeded into 96-well plates (100 μL per well) and cultured for 24 h. The compounds active against MDA-MB-231 cells were then added separately, with six concentration gradients set at 1.0, 5.0, 10.0, 25.0, 50.0, and 100.0 $\mu g \cdot mL^{-1}$, along with cyclophosphamide, and cultured for 24 h. Then 10 μL of MTT solution was added to each well and incubated for 12 h; after dissolution with DMSO solution, the supernatant was aspirated. Absorbance was measured with a microplate reader, and the IC_{50} values were calculated.

2 Results and Analysis

2.1 Structural Identification

The structures of the compounds are shown in Figure 1.

Figure 1. Chemical structures of compounds 1-24
Fig. 1 Chemical structures of compounds 1-24

Compound **1**: yellow powder; ESI-MS m/z: 316.1 $[M + Na]^+$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 8.91 (1H, d, $J = 11.4$ Hz, H-11), 8.62 (1H, d, $J = 11.4$ Hz, H-5), 8.27 (1H, d, $J = 11.4$ Hz, H-8), 8.13 (1H, dd, $J = 11.4, 4.2$ Hz, H-4), 7.83 (1H, d, $J = 11.4$ Hz, H-10), 7.64 (1H, dd, J

$= 11.4$, 4.2 Hz, H-9), 7.51 (1H, s, H-3), 3.97 (6H, s, 1-OCH_3), 3.82 (6H, s, 2-OCH_3).

^{13}C-NMR (150 MHz, DMSO-d_6) δ: 137.5 (C-1), 141.6 (C-2), 95.6 (C-3), 119.2 (C-4), 131.9 (C-5), 142.9 (C-6), 173.6 (C-7), 113.6 (C-8), 108.6 (C-9), 121.6 (C-10), 113.8 (C-11), 57.6 (1-OCH_3). The above data are basically consistent with the literature values for lysicamine (Sulaiman et al., 2024).

Compound 2 White powder, ESI-MS m/z: 670.1 $[M+Na]^+$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 7.63 (1H, s, H-3), 6.93 (1H, d, $J = 11.4$ Hz, H-8″), 6.91 (1H, d, $J = 11.4$ Hz, H-4″), 6.71 (1H, overlap, H-5″), 6.68 (1H, overlap, H-7″), 6.71 (1H, overlap, H-4‴), 6.59 (1H, overlap, H-7‴), 6.51 (1H, dd, $J = 11.4$, 4.2 Hz, H-8‴), 5.94 (1H, m, H-8), 5.72 (1H, brs, H-1), 4.81 (1H, d, $J = 11.4$ Hz, H-1′), 4.36 (2H, m, H-1″), 4.17 (1H, m, H-1‴β), 4.03 (1H, m, H-5), 4.01 (1H, m, H-6′α), 3.97 (1H, dt, $J = 11.4$, 4.2 Hz, H-1‴α), 3.52 (1H, m, H-6′β), 3.47 (1H, m, H-2′), 3.32 (1H, m, H-3′), 3.27 (1H, m, H-4′), 3.24 (1H, m, H-5′), 2.74 (2H, t, $J = 11.4$ Hz, H-2″), 2.71 (2H, t, $J = 11.4$ Hz, H-2‴), 2.53 (1H, dd, $J = 11.4$, 4.2 Hz, H-6β), 2.21 (1H, dd, $J = 11.4$, 4.2 Hz, H-6α), 1.68 (3H, d, $J = 11.4$ Hz, H-10). ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 95.2 (C-1), 143.2 (C-2), 146.5 (C-3), 97.8 (C-4), 36.7 (C-5), 43.8 (C-6), 158.3 (C-7), 121.7 (C-8), 124.3 (C-9), 19.5 (C-10), 153.8 (C-11), 94.8 (C-1′), 72.6 (C-2′), 77.1 (C-3′), 70.3 (C-4′), 77.5 (C-5′), 61.3 (C-6′), 65.2 (C-1″), 36.1 (C-2″), 128.4 (C-3″), 127.4 (C-4″), 115.8

(C-5″), 156.4 (C-6″), 115.4 (C-7″), 130.4 (C-8″), 70.2 (C-1‴), 33.9 (C-2‴), 127.1 (C-3‴), 116.8 (C-4‴), 145.1 (C-5‴), 145.3 (C-6‴), 113.4 (C-7‴), 120.8 (C-8‴). The above data are basically consistent with the report by Feng Xiaotao et al. (2024); therefore, compound 1 was identified as hydroxyframoside.

Compound 3 Yellow oily substance, ESI-MS m/z: 413.2 $[M+Na]^+$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 5.79 (1H, ddt, J = 11.4, 4.2, 1.8 Hz, H-21), 5.13 (1H, dd, J = 11.4, 4.2 Hz, H-22), 5.07 (1H, d, J = 11.4 Hz, H-22), 4.62 (1H, m, H-24), 3.91 (1H, dd, J = 11.4, 4.2 Hz, H-25), 3.73 (1H, dd, J = 11.4, 4.2 Hz, H-25), 2.71 (1H, m, H-2), 2.34 (1H, m, H-23), 2.09 (2H, t, J = 11.4 Hz, H-12), 2.02 (2H, t, J = 11.4 Hz, H-15), 1.94 (2H, m, H-20), 1.87 (1H, m, H-23), 1.79 (1H, m, H-3), 1.51–1.32 (14H, m, H-5–11), 1.27–1.15 (8H, m, H-16–19), 1.09 (1H, m, H-3), 1.06 (2H, m, H-4). ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 171.3 (C-1), 41.5 (C-2), 32.9 (C-3), 28.9–29.7 (C-4–11), 19.1 (C-12), 76.8 (C-13), 79.1 (C-14), 20.4 (C-15), 27.3–28.5 (C-16–19), 34.1 (C-20), 140.2 (C-21), 109.2 (C-22), 30.2 (C-23), 79.1 (C-24), 65.3 (C-25). The above data are basically consistent with the literature values for goniothalamusin (Seidel, 1999).

Compound 4 White crystals, ESI-MS m/z: 133.0 $[M+Na]^+$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 7.62 (2H, d, J = 11.4 Hz, H-3, 4), 6.83 (2H, d, J = 11.4 Hz, H-2, 5). ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 143.5 (C-1), 113.8 (C-2), 127.5 (C-3), 127.5 (C-4), 113.8 (C-5), 43.5 (C-6). The above data are basically consistent with the literature values for pyrocatechol (Fu Xiaoya et al., 2024).

Compound 5 White powder, ESI-MS m/z: 245.1 $[M+Na]^+$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 7.62 (1H, d, J = 11.4 Hz, H-4), 6.81 (1H, s, H-5), 6.35 (1H, d, J = 11.4 Hz, H-3), 4.09 (3H, s, 7-OCH_3), 4.03 (3H, s, 8-OCH_3). ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 134.2 (C-1), 167.8

(C-2), 113.8 (C-3), 151.7 (C-4), 110.6 (C-5), 135.8 (C-6), 132.8 (C-7), 135.8 (C-8), 181.6 (C-9), 108.5 (C-10), 62.4 (8−OCH_3), 60.2 (7−OCH_3). The above data were basically consistent with the values reported in the literature (Fu Xiaoya et al., 2024) for 6-hydroxy-7,8-dimethoxycoumarin.

Compound 6 was a white powder. ESI-MS m/z: 336.1 $[M + Na]^+$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 7.56 (1H, s, H-11), 6.53 (1H, s, H-3), 6.41 (1H, s, H-8), 4.16 (1H, dd, J = 11.4, 4.2 Hz, H-7), 3.52 (3H, s, $2-OCH_3$), 3.49 (3H, s, $10-OCH_3$), 3.41–3.25 (2H, m, H-4), 3.14–2.92 (4H, m, H-5, 6). ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 146.2 (C-1), 138.2 (C-2), 103.5 (C-3), 27.4 (C-4), 41.9 (C-5), 56.9 (C-6), 35.1 (C-7), 113.8 (C-8), 142.6 (C-9), 137.9 (C-10), 109.5 (C-11), 59.7 ($2-OCH_3$), 57.1 ($10-OCH_3$). The above data were basically consistent with the values reported in the literature (Fu Xiaoya et al., 2024) for norisoboldine.

Compound 7 was a white needle crystal. ESI-MS m/z: 204.9 $[M + Na]^+$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 6.03 (1H, d, J = 2.1 Hz, H-3), 5.74 (1H, d, J = 11.4 Hz, H-5), 2.43 (3H, s, $2-COCH_3$). ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 105.3 (C-1), 167.4 (C-2), 97.1 (C-3), 153.9 (C-4), 93.6 (C-5), 148.6 (C-6), 34.3 ($2-COCH_3$). The above data were basically consistent with the val-

ues reported in the literature (Xu Haonan et al., 2024) for 4,6-dihydroxy-2-methoxyacetophenone.

Compound 8 was a yellow powder. ESI-MS m/z: 275.1 $[M+Na]^+$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 7.09 (2H, d, J = 11.4 Hz, H-2′, 6′), 6.93 (2H, d, J = 11.4 Hz, H-2, 6), 6.64 (4H, dd, J = 11.4, 4.2 Hz, H-3, 5, 3′, 5′), 6.58 (1H, m, H-8′), 6.32 (1H, d, J = 11.4 Hz, H-7′), 5.93 (1H, ddd, J = 11.4, 4.2, 1.8 Hz, H-8), 5.08 (2H, dd, J = 11.4, 4.2 Hz, H-9). ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 127.3 (C-1), 130.5 (C-2), 108.6 (C-3), 143.5 (C-4), 108.6 (C-5), 130.5 (C-6), 57.1 (C-7), 135.2 (C-8), 109.2 (C-9), 116.3 (C-1′), 128.5 (C-2′), 107.2 (C-3′), 137.2 (C-4′), 107.2 (C-5′), 128.5 (C-6′), 128.6 (C-7′), 121.5 (C-8′). The above data were basically consistent with the values reported in the literature (Cao Leilei et al., 2015) for hinokiresinol.

Compound 9 was a white powder. ESI-MS m/z: 275.1 $[M+Na]^+$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 6.53 (1H, dd, J = 11.4, 4.2 Hz), 4.02 (1H, m), 2.61-2.57 (2H, m), 2.51 (1H, m), 2.41 (2H, m), 2.03 (2H, m), 1.62 (2H, m), 1.03 (3H, t, J = 7.26 Hz). ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 153.6 (C-1), 126.4 (C-2), 87.1 (C-3), 26.3 (C-4), 26.1 (C-5), 36.7 (C-6), 133.1 (C-7), 26.9 (C-8), 23.1 (C-9), 21.7 (C-10). The above data were basically consistent with the values reported in the literature (Zhang Shuang et al., 2024) for sedanolide.

Compound 10 was a white powder. ESI-MS m/z: 275.1 $[M+Na]^+$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 9.61 (1H, brs, H-7′), 9.03 (1H, dd, J = 11.4, 4.2 Hz, H-5′), 8.64 (1H, d, J = 11.4 Hz, H-3′), 7.81 (1H, dd, J = 11.4, 4.2 Hz, H-4′), 5.42 (1H, dd, J = 11.4, 4.2 Hz, H-12), 4.96 (1H, brs, H-6), 4.03 (1H, m, H-3), 2.37 (3H, s, H-21), 1.96 (3H, s, H-18), 1.52 (3H, s, H-19). ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 39.6 (C-1), 32.4 (C-2), 70.3 (C-3), 41.7 (C-4), 136.8 (C-5), 120.5 (C-6), 34.7 (C-7), 75.1 (C-8), 46.2 (C-9), 38.1 (C-10), 26.1 (C-11), 74.9

(C-12), 60.3 (C-13), 87.2 (C-14), 35.6 (C-15), 33.7 (C-16), 95.1 (C-17), 12.3 (C-18), 20.7 (C-19), 176.3 (C-20), 157.6 (C-1′), 126.4 (C-2′), 137.6 (C-3′), 123.8 (C-4′), 147.3 (C-5′). The above data were generally consistent with the literature values for rostratamine (Zhao et al., 2016).

Compound 11 Yellow oil, ESI-MS m/z: 171.0 $[M+Na]^+$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 7.73 (1H, d, J = 11.4 Hz, H-7), 7.49 (2H, m, H-2, 6), 7.36 (3H, m, H-3, 4, 5), 6.28 (1H, d, J = 11.4 Hz, H-8). ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 126.9 (C-1), 126.4 (C-2), 127.3 (C-3), 129.6 (C-4), 128.1 (C-5), 127.6 (C-6), 146.4 (C-7), 118.1 (C-8), 173.4 (C-9). The above data were generally consistent with the literature values for cinnamic acid (Wang et al., 2009).

Compound 12 Pale-yellow solid, ESI-MS m/z: 377.0 $[M+Na]^+$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 7.63 (1H, d, J = 11.4 Hz, H-4), 7.21 (1H, s, H-5), 6.84 (1H, s, H-8), 6.25 (1H, d, J = 11.4 Hz, H-3), 3.91 (3H, s, 7-OCH_3). ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 138.2 (C-1), 156.4 (C-2), 108.5 (C-3), 143.7 (C-4), 108.6 (C-5), 141.5 (C-6), 153.4 (C-7), 97.6 (C-8), 138.3 (C-9), 107.5 (C-10), 96.5 (C-1′), 74.5 (C-2′), 68.2 (C-3′), 70.4 (C-4′), 77.1 (C-5′), 55.8 (7-OCH_3). The

above data were generally consistent with the literature values for magnolioside (Zhang Meiqin et al., 2024).

Compound 13 White needles, ESI-MS m/z: 398.0 $[M+Na]^+$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 7.73 (1H, s, 7-OH), 6.42 (1H, s, H-6), 5.37 (1H, dd, J = 11.4, 4.2 Hz, H-11), 5.26 (2H, s, H-3), 5.08 (1H, s, H-13), 5.02 (1H, s, H-13), 3.46 (1H, dd, J = 11.4, 4.2 Hz, H-10), 2.83 (1H, dd, J = 11.4, 4.2 Hz, H-10), 1.91 (3H, s, H-14). ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 161.5 (C-1), 132.5 (C-2), 68.7 (C-3), 109.1 (C-4), 141.6 (C-5), 102.4 (C-6), 154.3 (C-7), 103.1 (C-8), 137.1 (C-9), 31.7 (C-10), 87.2 (C-11), 137.5 (C-12), 113.1 (C-13), 18.6 (C-14). The above data were generally consistent with the literature values for platypterophathalide (Jakupovic et al., 1987).

Compound 14 White powder, ESI-MS m/z: 212.4 $[M+Na]^+$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 7.93 (1H, d, J = 11.4 Hz, H-5), 7.86 (1H, d, J = 11.4 Hz, H-8), 7.64 (1H, t, J = 11.4 Hz, H-7), 7.41 (1H, t, J = 11.4 Hz, H-6), 6.82 (1H, s, H-3). ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 132.5 (C-1), 147.3 (C-2), 117.2 (C-3), 175.2 (C-4), 124.2 (C-5), 120.5 (C-6), 131.6 (C-7), 123.5 (C-8), 126.2 (C-9), 134.2 (C-10). The above data were generally consistent with the literature values for transtorin (Hu Leshi et al., 2023).

Compound 15 White oil, ESI-MS m/z: 359.1 $[M+Na]^+$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 2.28 (2H, t, J = 11.4 Hz, H-14), 2.06 (3H, d, J = 11.4 Hz, 17-CH_3), 1.84 (1H, m, H-5a), 1.61 (3H, d, J = 11.4 Hz, 16-CH_3), 1.53 (1H, m, H-5β), 1.49 (2H, m, H-13), 1.31–1.27 (12H, m, H-7–12), 1.16 (1H, m, H-6a), 1.09 (1H, m, H-6β). ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 151.3 (C-1), 157.9 (C-2), 126.8 (C-3), 113.5 (C-4), 37.8 (C-5), 23.4 (C-6), 29.4 (C-7–12), 25.8 (C-13), 35.1 (C-14), 153.5 (C-15). The above data were generally consistent with the literature values for capillasterolide (Li Shaohua et al., 2024).

Compound 16 White powder, ESI-MS m/z: 515.1 $[M+Na]^+$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 7.71 (2H, m, H-2″, 6″), 7.53 (3H, m, H-3″, 4″, 5″), 6.74 (1H, d, J = 16.0 Hz, H-8″), 6.42 (1H,

d, J = 5.2 Hz, H-3), 5.18 (1H, m, H-1), 4.91 (1H, d, J = 11.4 Hz, H-1′), 3.92 (1H, m, H-6), 3.28 (1H, m, H-7). ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 103.5 (C-1), 125.9 (C-2), 136.8 (C-3), 98.6 (C-4), 37.1 (C-5), 76.8 (C-6), 60.3 (C-7), 63.1 (C-8), 42.7 (C-9), 67.3 (C-10), 100.3 (C-1′), 75.1 (C-2′), 78.1 (C-3′), 72.6 (C-4′), 79.1 (C-5′), 60.7 (C-6′), 126.8 (C-1″), 130.5 (C-2″), 127.3 (C-3″), 128.3 (C-4″), 127.3 (C-5″), 130.5 (C-6″), 109.2 (C-7″), 145.4 (C-8″). The above data were essentially consistent with the reported values for globularin in the literature (Li Shaohua et al., 2024).

Compound 17 Yellow powder, ESI-MS m/z: 291.1 $[M+Na]^+$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 8.29 (1H, s, H-2), 8.03 (1H, d, J = 11.4 Hz, H-5), 7.49 (2H, d, J = 11.4 Hz, H-2′, 6′), 7.08 (2H, d, J = 11.4 Hz, H-3′, 5′), 7.02 (1H, dd, J = 11.4, 4.2 Hz, H-6), 6.73 (1H, d, J = 11.4 Hz, H-8). ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 127.6 (C-1), 148.1 (C-2), 124.9 (C-3), 174.2 (C-4), 128.1 (C-5), 114.3 (C-6), 162.5 (C-7), 103.1 (C-8), 155.1 (C-9), 116.2 (C-10), 123.4 (C-1′),

128.6 (C-2′), 113.9 (C-3′), 142.8 (C-4′), 113.9 (C-5′), 128.6 (C-6′). The above data were essentially consistent with the reported values for formononetin in the literature (Liu Cuizhen et al., 2024).

Compound 18 White powder, ESI-MS m/z: 215.2 $[M+\mathrm{Na}]^+$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 7.61 (d, J = 11.4 Hz, 1H, H-7), 7.39 (d, J = 11.4 Hz, 2H, H-2, 6), 6.82 (q, J = 11.4 Hz, 2H, H-3, 5), 6.31 (d, J = 11.4 Hz, 1H, H-8), 4.19 (q, J = 11.4 Hz, 1H, H-1′), 1.28 (t, J = 11.4 Hz, 3H, H-2′). ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 131.7 (C-1), 127.5 (C-2), 117.7 (C-3), 153.2 (C-4), 117.7 (C-5), 127.5 (C-6), 135.2 (C-7), 114.6 (C-8), 158.2 (C-9), 62.6 (C-1′), 18.1 (C-2′). The above data were essentially consistent with the reported values for ethyl *p*-hydroxycinnamate in the literature (Cruz et al., 2022).

Compound 19 Yellow powder, ESI-MS m/z: 367.1 $[M+\mathrm{Na}]^+$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 7.51 (1H, dd, J = 11.4, 4.2 Hz, H-6′), 7.36 (1H, s, H-2′), 6.87 (1H, d, J = 11.4 Hz, H-5′), 6.62 (1H, s, H-8), 6.47 (1H, s, H-3), 4.02 (3H, s, H-3′), 3.91 (3H, s, H-6). ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 116.5 (C-1), 157.3 (C-2), 104.2 (C-3), 176.2 (C-4), 153.1 (C-5), 133.1 (C-6), 159.1 (C-7), 96.4 (C-8), 153.5 (C-9), 104.6 (C-10), 124.1 (C-1′), 111.2 (C-2′), 150.1 (C-3′), 150.6 (C-4′), 115.6 (C-5′), 22.4 (C-6′), 61.3 (6-$\mathrm{OCH_3}$), 57.1 (3′-$\mathrm{OCH_3}$). The above data were essentially consistent with the reported values for cirsilineol in the literature (He Feifan et al., 2024).

Compound 20 White crystals, ESI-MS m/z: 219.1 $[M+\mathrm{Na}]^+$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 5.81 (1H, s, H-3), 4.45 (1H, m, H-6), 2.37 (1H, td, J = 11.4 Hz, H-7a), 2.04 (1H, ddd, J = 11.4, 4.2, 1.8 Hz, H-5a), 1.81 (3H, s, H-10), 1.68 (1H, d, J = 11.4 Hz, H-7β), 1.43 (1H, d, J = 11.4 Hz, H-5β), 1.39 (3H, s, H-8), 1.31 (3H, s, H-9). ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 132.5 (C-1), 173.2 (C-2), 112.5 (C-3), 38.1 (C-4), 48.3 (C-5), 66.7 (C-6), 45.1 (C-7), 26.9 (C-8), 30.8 (C-9), 26.9 (C-10). The above data were essentially consistent with the reported values for loliolide in the literature (He Feifan et al., 2024).

Compound 21 White needles, ESI-MS m/z: 219.1 $[M+\mathrm{Na}]^+$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 7.52 (1H, dd, J = 11.4, 4.2 Hz, H-6), 7.43 (1H, d, J = 11.4 Hz, H-2), 7.02 (1H, dd, J = 11.4 Hz, H-5), 2.53 (3H, s, H-8), 3.94 (3H, s, 4-$\mathrm{OCH_3}$). ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 128.4

(C-1), 116.0 (C-2), 149.3 (C-3), 154.1 (C-4), 110.8 (C-5), 122.1 (C-6), 176.2 (C-7), 27.1 (C-8), 57.1 (4-$\mathrm{OCH_3}$). The above data are basically consistent with the values reported in the literature for acetovanillone (Lu Caixia et al., 2024).

Compound **22** was a white powder. ESI-MS m/z: 357.1 [M+Na]$^+$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 5.91 (1H, s, H-7), 4.06–4.04 (1H, m, H-2), 3.97–3.95 (1H, m, H-3), 3.13–3.09 (1H, m, H-15), 2.45–2.41 (1H, m, H-5), 2.19–2.17 (1H, m, H-15), 1.43–1.41 (1H, m, H-1), 1.77–1.74 (1H, m, H-1), 1.03 (3H, s, 19-$\mathrm{CH_3}$), 0.94 (3H, s, 18-$\mathrm{CH_3}$). ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 38.1 (C-1), 68.2 (C-2), 68.1 (C-3), 33.1 (C-4), 52.3 (C-5), 182.3 (C-6), 123.5 (C-7), 162.6 (C-8), 36.1 (C-9), 40.3 (C-10), 21.7 (C-11), 30.2 (C-12), 54.1 (C-13), 80.2 (C-14), 34.1 (C-15), 25.2 (C-16), 163.5 (C-17), 18.6 (C-18), 25.4 (C-19). The above data are

basically consistent with the values reported in the literature for rubrosterone (Xu Hongpan et al., 2024).

Compound **23** was a white powder. ESI-MS m/z: 249.0 $[M+Na]^+$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 7.51 (1H, brs, H-2′), 7.18 (1H, d, J = 11.4 Hz, H-3), 7.09 (bs, 1H, H-4′), 6.91 (1H, d, J = 11.4 Hz, H-6′), 6.86 (1H, dd, J = 11.4, 4.2 Hz, H-5), 2.41 (3H, s, H-10′), 1.57 (6H, s, H-8′ and H-9′). ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 135.6 (C-1), 121.5 (C-2), 112.6 (C-3), 143.6 (C-4), 114.9 (C-5), 119.1 (C-6), 127.1 (C-1′), 123.1 (C-2′), 136.2 (C-3′), 126.4 (C-4′), 122.9 (C-5′), 136.2 (C-6′), 76.9 (C-7′), 26.9 (C-8′), 26.9 (C-9′), 20.9 (C-10′). The above data are basically consistent with the values reported in the literature for didehydroconicol (Wang Man et al., 2024).

Compound **24** was a white oily substance. ESI-MS m/z: 245.1 $[M+Na]^+$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 7.02 (1H, d, J = 11.4 Hz, H-4), 6.31 (1H, d, J = 11.4 Hz, H-3), 5.71 (1H, t, J = 11.4 Hz, H-3′), 2.47 (1H, d, J = 11.4 Hz, H-5′), 2.35 (3H, s, H-1), 2.08 (1H, d, J = 11.4 Hz, H-5′), 1.91 (3H, d, J = 4.2 Hz, H-9), 1.02 (3H, s, H-7), 0.97 (3H, s, H-8). ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 25.1 (C-1), 173.2 (C-2), 127.5 (C-3), 136.4 (C-4), 50.6 (C-5), 43.4 (C-6), 29.3 (C-7), 24.1 (C-8), 19.7 (C-9), 79.3 (C-1′), 154.3 (C-2′), 127.1 (C-3′), 181.3 (C-4′). The above data are basically consistent with the values reported in the literature for dehydrovomifoliol (Li Zhenxing et al., 2024).

2.2 Results of the inhibitory activity of the compounds against breast cancer cells

The results in Table 1 show that, compared with the blank control group, compounds **1**, **4**, **8**, **9**, **13**, **14**, **16**, **19**, and **23** exhibited different degrees of inhibitory effects on MDA-MB-231 cells, while the other compounds did not show significant inhibitory activity against MDA-MB-231 cells ($IC_{50} > 50\ \mu mol \cdot L^{-1}$). The IC_{50} value of compound **9** for cell proliferation was (19.02$±$5.34) $\mu mol \cdot L^{-1}$, comparable to that of the positive control group, (18.62$±$5.16) $\mu mol \cdot L^{-1}$ ($P > 0.05$).

Table 1 Inhibitory activity of the compounds against MDA-MB-231 cells ($\bar{x} \pm s$, n=3)

Table 1 Inhibitory activity of the compound on MDA-MB-231 cells ($\bar{x} \pm s$, n=3)

Compound	IC_{50} ($\mu mol \cdot L^{-1}$)
1	37.51$±6.28 * || * *4 * *|29.16±5.71 * || * *8 * *|43.58±$6.81*

9	19.02$±5.34||**13**|32.64±6.17
	||**14**|41.26±6.53||**16**|31.58±6.03
	||**19**|35.56±6.18||**23**|38.43±6.42
	$\|\|Positivecontrolgroup$|18.62±$5.16

Note: Data = mean ± standard deviation ($\bar{x} \pm s$), $n = 3$. Compared with the positive control group, $*P < 0.05$.

3 Discussion and Conclusion

Breast cancer is one of the most common malignant tumors among women worldwide and poses a serious threat to women' s life and health. Although current treatment modalities for breast cancer are diverse, including surgery, chemotherapy, radiotherapy, endocrine therapy, and targeted therapy, many challenges remain (Pearce et al., 2025). While Western-medicine chemotherapy kills tumor cells, it often also causes considerable toxicity to normal cells, leading to severe adverse reactions and reduced quality of life; some patients develop resistance to endocrine therapy or targeted therapy, resulting in poor therapeutic outcomes. Therefore, the search for safe and effective new anticancer drugs or adjuvant therapeutic agents is of important clinical significance (Zerang et al., 2025). Traditional Chinese medicine, owing to its multicomponent and multitarget modes of action, shows unique advantages in tumor therapy, and screening anticancer active constituents from Chinese medicines has become one of the research hotspots. As a traditional Chinese medicine, Trillium has been shown in existing studies to possess certain anticancer activity, and in-depth research on it is expected to provide new ideas and drug sources for breast cancer treatment.

In this study, 24 compounds were isolated and identified from Trillium, all of which were isolated and identified from Trillium for the first time; among them, compounds **1**, **3**, **6**, **8**, **9**, **13**, **14**, and **23** were isolated and identified from plants of the genus *Trillium* for the first time. The inhibitory activity of the obtained compounds against breast cancer MDA-MB-231 cells was evaluated. The experimental results showed that multiple individual compounds in the Trillium extract inhibited the proliferation of MDA-MB-231 cells to varying degrees. The calculated IC_{50} values indicated that the inhibitory abilities of different individual compounds against MDA-MB-231 cells differed, but compounds **1**, **4**, **8**, **9**, **13**, **14**, **16**, **19**, and **23** all effectively inhibited cell proliferation. Among them, compound **9** showed a relatively high inhibition rate against cell proliferation, with an IC_{50} value of $(19.02 \pm 5.34)\ \mu\text{mol} \cdot \text{L}^{-1}$, suggesting that compound **9** may have potential anti-breast-cancer activity.

This study enriches the material basis for the pharmacological effects of Trillium and provides certain assistance for the research and development of new drugs for breast cancer.

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