

Chemical Constituents and Antibacterial Activity of *Ailanthus altissima* Root Bark: Postprint

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Abstract

To investigate the material basis for the antibacterial activity of *Ailanthus altissima* root bark, this study employed silica gel, Sephadex LH-20, and other chromatographic methods to separate and purify the methanol extract of *Ailanthus altissima* root bark. The structures of the monomeric compounds were elucidated based on physicochemical properties and spectroscopic data analysis. The antibacterial activity of the compounds was evaluated using flow cytometry, with kanamycin as the control. The results showed that 22 compounds were isolated from *Ailanthus altissima* root bark and identified as pleuchiol (1), withastramonolide (2), 7-ketositosterol (3), betulin (4), methyl betulinate (5), 1, 2, 4-trimethoxybenzene (6), dimethyl maleate (7), sonderianol (8), dibutylphthalate (9), pinoresinol (10), ethyl p-hydroxybenzoate (11), avenalumic acid methyl ester (12), 5,3'-dihydroxy-3,7,4'-trimethoxy-flavone (13), spathulenol (14), 2-methyl-5-propionyl-7-hydroxychromone (15), 7,4'-dihydroxyflavone (16), annphenone (17), 3-hydroxy-4-methoxybenzoic acid (18), 5,3',4'-trihydroxy-7-methoxyflavanone (19), dibutyl phthalate (20), 4-O-methylgallic acid (21), and dioctyl terephthalate (22). All compounds were isolated from *Ailanthus altissima* root bark for the first time. Antibacterial activity assays revealed that compound 2 exhibited inhibitory activity against *Pseudomonas aeruginosa* and *Bacillus subtilis*, compound 3 showed inhibitory activity against *Bacillus subtilis*, compound 8 demonstrated inhibitory effects against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis*, and compound 17 had inhibitory activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Notably, the inhibitory effect of compound 2 against *Bacillus subtilis* showed no significant difference compared to kanamycin ($P > 0.05$).

Full Text

Preamble

Chemical Constituents from the Root Bark of *Ailanthus altissima* and Their Bacteriostatic Activity

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Abstract: To explore the material basis of the antibacterial activity of *Ailanthus altissima* root bark, this study employed silica gel and Sephadex LH-20 chromatography to separate and purify compounds from the methanol extract. The structures of isolated compounds were identified through physicochemical properties and spectroscopic data analysis. Antibacterial activity was evaluated using flow cytometry with kanamycin as the control. The results yielded 22 compounds from the root bark, identified as pleuchiol (1), withastramonolide (2), 7-ketositosterol (3), betulin (4), betulinic acid methyl ester (5), 1,2,4-trimethoxybenzene (6), dimethyl maleate (7), sonderianol (8), dibutyl phthalate (9), pinoresinol (10), p-hydroxybenzoic acid ethyl ester (11), avenalumic acid methyl ester (12), 5,3'-dihydroxy-3,7,4'-trimethoxyflavone (13), spathulenol (14), 2-methyl-5-acetonyl-7-hydroxychromone (15), 7,4'-dihydroxyflavone (16), annphenone (17), 3-hydroxy-4-methoxybenzoic acid (18), 5,3',4'-trihydroxy-7-methoxyflavanone (19), dibutyl phthalate (20), 4-O-methylgallic acid (21), and dioctyl terephthalate (22). All compounds were isolated from *A. altissima* root bark for the first time. Antibacterial assays revealed that compound 2 inhibited *Pseudomonas aeruginosa* and *Bacillus subtilis*, compound 3 inhibited *B. subtilis*, compound 8 inhibited *P. aeruginosa*, *Staphylococcus aureus*, and *B. subtilis*, and compound 17 inhibited *P. aeruginosa* and *S. aureus*. Notably, the inhibitory effect of compound 2 against *B. subtilis* showed no significant difference compared to kanamycin ($P > 0.05$).

Keywords: *Ailanthus altissima*; chemical constituents; separation and purification; structure identification; bacteriostatic activity

The root bark of *Ailanthus altissima*, also known as “Chun Gen Pi” or “Chou Chun Pi,” is the dried root bark or stem bark of the Simaroubaceae plant *Ailanthus altissima* (Mill.) Swingle (Compilation Group of National Chinese Herbal Medicine, 1975). It is primarily distributed in Henan, Guangxi, Hebei, Hubei, Anhui, Jiangxi, Zhejiang, and other regions, with traditional functions of clearing heat, drying dampness, astringing the intestines, and stopping diarrhea. Clinically, it is used to treat colitis, proctitis, vaginitis, hemorrhoids, endocervicitis, and bacterial dysentery caused by bacterial infections. Liu and Zhang (2003) treated radiation proctitis with a combination of *A. altissima* root bark powder and modified Zhenrenyangzang Decoction, achieving an 80% total effective rate with significant clinical improvement. Modern pharmacological studies

have demonstrated that *A. altissima* root bark exhibits antibacterial, antiviral, antimalarial, antitumor, antiplatelet aggregation, and antipyretic activities (Du et al., 2019; Yan et al., 2020). Zhu et al. evaluated the in vitro antibacterial activity of aqueous and ethanol crude extracts of *A. altissima* root bark, finding that the aqueous extract showed some inhibitory activity against *S. aureus* but no significant activity against *P. aeruginosa* or *Escherichia coli*, while the ethanol extract demonstrated significant inhibition against *S. aureus*, *P. aeruginosa*, and *E. coli*, with inhibition zones notably larger than those of the aqueous extract (Zhu et al., 2021). Reports on monomeric compounds from *A. altissima* root bark are limited; Qi et al. (2011) isolated canthin-6-one glycoside esters, aiantholide, shinjudilactone A, and 11-acetyl-aiantholide. Our research group previously isolated 15 compounds from 95% ethanol extracts of *A. altissima* root bark (Zhou et al., 2021). This study further investigates *A. altissima* root bark to clarify its antibacterial material basis and provide a reference for resource utilization and development of antibacterial agents.

1.1 Materials, Instruments, and Reagents

1.1.1 Materials

Ailanthus altissima root bark was collected from Beihai City, Guangxi, and identified by Associate Professor WANG Jihong of Pingdingshan University as the root bark of *Ailanthus altissima* (Mill.) Swingle. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis* were obtained from Sichuan Rui Nuosai Biotechnology Co., Ltd.

1.1.2 Instruments and Reagents

Instruments included a Triple5600+ high-resolution mass spectrometer (Bruker, Germany), AVANCE NEO-600 NMR spectrometer (Waters, USA), ME188T analytical balance (Mettler Toledo, USA), Sephadex LH-20 dextran gel (Merck, Germany), LHY3100T electronic balance (Florenz Sartorius, Germany), column chromatography silica gel (Qingdao Marine Chemical Factory), and deuterated reagent DMSO-d₆ (Sigma-Aldrich, Germany).

1.2 Methods

1.2.1 Extraction and Separation

Dried *A. altissima* root bark (19.6 kg) was extracted by soaking in 95% methanol and 50% methanol. The solvent was recovered to obtain 2.3 kg of crude extract, which was suspended in distilled water and sequentially partitioned with equal volumes of petroleum ether, acetone, and n-butanol. Solvent recovery yielded petroleum ether fraction (151.3 g), acetone fraction (106.4 g), and n-butanol fraction (117.6 g).

The petroleum ether fraction was subjected to silica gel column chromatography with a petroleum ether-ethyl acetate gradient (80:20→50 : 50→20 :

80) to afford seven fractions (Fr.A1–Fr.A7). Fr.A2 (7.1 g) was further separated on silica gel using a petroleum ether–acetone gradient (70 : 30 → 30 : 70) to give five subfractions (Fr.A2–1–Fr.A2–5). Fr.A2–2 (206.8 mg) was purified by Sephadex LH–20 to yield compounds 8 (19 mg) and 23 (31 mg). Fr.A5 (8.3 g) was separated on silica gel using a dichloromethane gradient (60 : 40 → 20 : 80) to obtain six subfractions (Fr.A5–1–Fr.A5–6). Fr.A5–1 (224.1 mg) was eluted with petroleum ether–dichloromethane (50 : 50) on silica gel to give compound 22 (21 mg). Fr.A5–3 (95.3 mg) was purified by Sephadex LH–20 to afford compounds 4 (23 mg) and 15 (34 mg). Fr.A6 (6.8 g) was separated on silica gel with a petroleum ether–ethyl acetate gradient (50 : 50 → 20 : 80) to yield seven subfractions (Fr.A6–1–Fr.A6–7). Fr.A6–5 (102.4 mg) was purified by Sephadex LH–20 to give compounds 14 (28 mg) and 21 (26 mg).

The acetone fraction was subjected to silica gel column chromatography with a petroleum ether–dichloromethane gradient (70 : 30 → 50 : 50 → 30 : 70) to afford eight fractions (Fr.B1–Fr.B8). Fr.B1 (8.5 g) was separated on silica gel with a petroleum ether–ethyl acetate gradient (75 : 25 → 25 : 75) to give seven subfractions (Fr.B1–1–Fr.B1–7). Fr.B1–3 (192.3 mg) was purified by Sephadex LH–20 to yield compounds 1 (28 mg) and 3 (23 mg). Fr.B5 (253.4 mg) was eluted with petroleum ether–dichloromethane (60 : 40) on silica gel to afford compounds 13 (16 mg) and 14 (16 mg). Fr.B5–1 (161.5 mg) was eluted with petroleum ether–dichloromethane (55 : 45 → 15 : 75) to obtain six subfractions (Fr.B4–1–Fr.B4–6). Fr.B4–3 (181.5 mg) was eluted with petroleum ether–acetone (35 : 65) on silica gel to give compounds 7 (21 mg) and 12 (18 mg). Fr.B4–4 (73.5 mg) was purified by Sephadex LH–20 to yield compounds 2 (19 mg) and 19 (26 mg). Fr.B7 (10.2 g) was separated on silica gel with a petroleum ether–dichloromethane gradient (55 : 45 → 15 : 75) to afford eight subfractions (Fr.B7–1–Fr.B7–8). Fr.B7–2 (151.3 mg) was eluted with petroleum ether–dichloromethane (45 : 55) on silica gel to give compounds 9 (33 mg) and 16 (28 mg). Fr.B7–7 (131.4 mg) was eluted with petroleum ether–dichloromethane (35 : 65) on silica gel to afford compounds 11 (21 mg) and 18 (35 mg).

The n-butanol fraction was subjected to silica gel column chromatography with an ethyl acetate–methanol gradient (65 : 35 → 35 : 65 → 15 : 85) to give six fractions (Fr.C1–Fr.C6). Fr.C2 (8.4 g) was separated on silica gel with an acetone–methanol gradient (55 : 45 → 15 : 85) to yield seven subfractions (Fr.C2–1–Fr.C2–7). Fr.C2–2 (175.3 mg) was purified by Sephadex LH–20 to afford compounds 5 (33 mg), 6 (19 mg), and 17 (31 mg). Fr.C5–3 (95.3 mg) was purified by Sephadex LH–20 to yield compound 17 (31 mg).

1.2.2 Antibacterial Activity Assay

The antibacterial activities of compounds 2, 3, 8, and 17 were evaluated using flow cytometry (Li YK, 1991; Li DX et al., 2013) with kanamycin as the positive control. Standard strains of *P. aeruginosa*, *S. aureus*, and *B. subtilis* were inoculated in MacConkey broth and cultured at 36 °C for 48 h, then diluted with physiological saline to 1×10^5 CFU · mL^{−1} and spread on agar plates. The control and test compounds were prepared at concentrations of 0.1, 0.5, 1.0, 4.0, 8.0, 12.0, 25.0, 50.0, 100.0, and 200.0 g · mL^{−1}. Sterile filter paper discs containing the samples were placed on culture media inoculated with each bacterial strain and incubated at 37 °C for 24 h. Inhibition zones and minimum

inhibitory concentrations (MIC) were determined to assess the antibacterial activity against *P. aeruginosa*, *S. aureus*, and *B. subtilis*.

2.1 Structure Identification of Compounds

Compound 1: White crystals. HR-ESI-MS m/z : 412.8731 $[M + H]^+$. ^1H -NMR (600 MHz, acetone- d_6) δ : 5.41 (1H, t, $J = 9.6$ Hz, H-6), 5.11 (1H, m, H-11), 5.02 (1H, d, $J = 9.6$ Hz, H-12), 3.54 (1H, m, H-3), 1.02 (3H, d, $J = 9.6$ Hz, H-21), 0.91 (3H, s, H-19), 0.83 (3H, d, $J = 9.6$ Hz, H-26), 0.81 (3H, d, $J = 6.3$ Hz, H-27), 0.79 (3H, t, $J = 9.6$ Hz, H-29), 0.68 (3H, s, H-18). ^{13}C -NMR-DEPT (150 MHz, acetone- d_6) δ : 39.7 (C-1, s), 33.4 (C-2, s), 74.1 (C-3, d), 42.5 (C-4, s), 140.9 (C-5, s), 123.2 (C-6, s), 25.3 (C-7, d), 52.4 (C-8, s), 51.3 (C-9, s), 35.7 (C-10, s), 129.4 (C-11, s), 136.4 (C-12, d), 42.3 (C-13, s), 58.1 (C-14, s), 24.2 (C-15, t), 30.4 (C-16, d), 56.1 (C-17, s), 12.3 (C-18, t), 21.5 (C-19, s), 36.1 (C-20, d), 19.2 (C-21, s), 41.2 (C-22, s), 25.8 (C-23, d), 47.1 (C-24, s), 29.1 (C-25, s), 21.4 (C-26, d), 19.3 (C-27, s), 24.3 (C-28, s), 12.9 (C-29, s). These data are consistent with literature values (Li N, 2021), identifying compound 1 as pleuchiol.

Compound 2: Yellow powder. HR-ESI-MS m/z : 486.7013 $[M + H]^+$. ^1H -NMR (600 MHz, acetone- d_6) δ : 6.71 (1H, ddd, $J = 9.6, 4.8, 2.2$ Hz, H-3), 5.81 (1H, dd, $J = 9.6, 4.8$ Hz, H-2), 4.03 (1H, br s, H-12), 2.97 (1H, d, $J = 9.6$ Hz, H-6). ^{13}C -NMR-DEPT (150 MHz, acetone- d_6) δ : 205.7 (C-1, s), 131.4 (C-2, s), 141.6 (C-3, s), 37.6 (C-4, d), 75.1 (C-5, s), 56.8 (C-6, s), 58.3 (C-7, s), 36.7 (C-8, s), 30.1 (C-9, d), 53.1 (C-10, t), 29.7 (C-11, s), 74.1 (C-12, d), 49.3 (C-13, s), 44.6 (C-14, s), 23.9 (C-15, d), 27.8 (C-16, s), 45.1 (C-17, s), 13.3 (C-18, d), 16.1 (C-19, t), 41.2 (C-20, d), 13.1 (C-21, s), 79.3 (C-22, s), 31.4 (C-23, s), 159.3 (C-24, s), 127.3 (C-25, s), 169.1 (C-26, q), 58.2 (C-27, s), 21.8 (C-28, s). These data are consistent with literature values (Kuang et al., 2010), identifying compound 2 as withastramonolide.

Compound 3: White powder. HR-ESI-MS m/z : 429.4239 $[M + H]^+$. ^1H -NMR (600 MHz, acetone- d_6) δ : 5.73 (1H, s, H-6), 3.81 (1H, m, H-3), 1.16 (3H, s, H-19), 0.87 (3H, d, $J = 9.6$ Hz, H-21), 0.83 (3H, t, $J = 9.6$ Hz, H-29), 0.81 (3H, d, $J = 9.6$ Hz, H-26), 0.73 (3H, d, $J = 4.8$ Hz, H-27), 0.68 (3H, s, H-18). ^{13}C -NMR-DEPT (150 MHz, acetone- d_6) δ : 37.1 (C-1, s), 30.6 (C-2, s), 69.2 (C-3, s), 40.8 (C-4, s), 164.7 (C-5, s), 125.3 (C-6, s), 201.4 (C-7, s), 46.1 (C-8, s), 50.7 (C-9, s), 39.1 (C-10, d), 20.6 (C-11, s), 39.1 (C-12, s), 42.7 (C-13, s), 50.3 (C-14, d), 27.6 (C-15, s), 29.1 (C-16, t), 55.2 (C-17, s), 12.1 (C-18, d), 18.4 (C-19, s), 35.7 (C-20, s), 20.1 (C-21, s), 34.1 (C-22, s), 25.8 (C-23, s), 46.1 (C-24, q), 30.2 (C-25, t), 20.5 (C-26, s), 20.1 (C-27, q), 22.8 (C-28, s), 12.1 (C-29, t). These data are consistent with literature values (Zhou et al., 2016), identifying compound 3 as 7-ketositosterol.

Compound 4: White powder. HR-ESI-MS m/z : 427.8159 $[M + H]^+$. ^1H -NMR (600 MHz, acetone- d_6) δ : 4.71 (1H, s, H-29a), 4.62 (1H, s, H-29b), 3.73 (1H, d, $J = 9.6$ Hz, H-3), 3.09 (1H, dd, $J = 9.6, 4.8$ Hz, H-3), 1.73 (3H, br s, H-30), 1.13

(3H, s, H-26), 1.02 (3H, s, H-23), 0.85 (3H, s, H-24), 0.79 (3H, s, H-25), 0.68 (3H, s, H-27). ^{13}C -NMR-DEPT (150 MHz, acetone- d_6) δ : 43.2 (C-1, s), 29.1 (C-2, s), 85.1 (C-3, d), 39.7 (C-4, s), 56.1 (C-5, s), 19.1 (C-6, s), 35.2 (C-7, s), 40.6 (C-8, d), 51.3 (C-9, s), 40.3 (C-10, s), 20.7 (C-11, s), 26.1 (C-12, s), 40.2 (C-13, s), 43.1 (C-14, s), 29.1 (C-15, t), 31.2 (C-16, s), 50.4 (C-17, s), 50.9 (C-18, s), 51.2 (C-19, d), 149.3 (C-20, s), 30.4 (C-21, s), 31.6 (C-22, s), 29.7 (C-23, d), 17.1 (C-24, s), 17.2 (C-25, s), 17.9 (C-26, s), 15.3 (C-27, s), 59.3 (C-28, t), 109.4 (C-29, q), 20.1 (C-30, q). These data are consistent with literature values (Omar et al., 2019), identifying compound 4 as betulin.

Compound 5: White needle crystals. HR-ESI-MS m/z : 471.1429 $[\text{M} + \text{H}]^+$. ^1H -NMR (600 MHz, acetone- d_6) δ : 4.91 (1H, d, $J = 4.8$ Hz, H-29a), 4.52 (1H, dd, $J = 9.6, 4.8$ Hz, H-29 β), 3.69 (3H, s, 3-OCH $_3$), 3.17 (1H, td, $J = 9.6, 4.8$ Hz, H-3), 2.93 (1H, td, $J = 9.6, 4.8$ Hz, H-19), 1.84 (3H, s, H-30), 1.57 (3H, s, H-27), 1.13 (3H, s, H-26), 0.87 (3H, s, H-25), 0.74 (3H, s, H-24), 0.65 (3H, s, H-23). ^{13}C -NMR-DEPT (150 MHz, acetone- d_6) δ : 39.1 (C-1, s), 26.8 (C-2, s), 80.1 (C-3, s), 40.3 (C-4, d), 56.7 (C-5, s), 19.1 (C-6, s), 35.1 (C-7, s), 41.3 (C-8, s), 52.4 (C-9, s), 36.8 (C-10, s), 21.7 (C-11, d), 26.2 (C-12, s), 39.1 (C-13, d), 43.5 (C-14, s), 30.2 (C-15, d), 31.8 (C-16, s), 57.2 (C-17, s), 51.2 (C-18, s), 46.8 (C-19, s), 151.4 (C-20, s), 31.1 (C-21, s), 36.9 (C-22, s), 29.1 (C-23, s), 16.1 (C-24, s), 15.8 (C-25, s), 15.6 (C-26, t), 15.3 (C-27, s), 175.3 (C-28, s), 108.4 (C-29, t), 20.1 (C-30, s), 50.2 (3-OCH $_3$, q). These data are consistent with literature values (Li et al., 2021), identifying compound 5 as betulinic acid methyl ester.

Compound 6: White crystals. HR-ESI-MS m/z : 169.3162 $[\text{M} + \text{H}]^+$. ^1H -NMR (600 MHz, acetone- d_6) δ : 7.61 (1H, d, $J = 9.6$ Hz, H-5), 7.45 (1H, s, H-2), 6.93 (1H, d, $J = 9.6$ Hz, H-6), 4.03 (9H, s, 4-OCH $_3$). ^{13}C -NMR-DEPT (150 MHz, acetone- d_6) δ : 151.3 (C-1, s), 113.4 (C-2, s), 146.2 (C-3, t), 148.1 (C-4, d), 124.1 (C-5, s), 113.2 (C-6, d), 55.8 (1-OCH $_3$, q), 55.8 (2-OCH $_3$, q), 55.0 (4-OCH $_3$, q). These data are consistent with literature values (Li et al., 2021), identifying compound 6 as 1,2,4-trimethoxybenzene.

Compound 7: White powder. HR-ESI-MS m/z : 143.1093 $[\text{M} + \text{H}]^+$. ^1H -NMR (600 MHz, acetone- d_6) δ : 6.91 (2H, s, H-2,3), 3.76 (6H, s, 1,2-OCH $_3$). ^{13}C -NMR-DEPT (150 MHz, acetone- d_6) δ : 169.2 (C-1, s), 133.1 (C-2, t), 133.1 (C-3, s), 169.2 (C-4, d), 51.6 (1-OCH $_3$, q), 51.6 (2-OCH $_3$, q). These data are consistent with literature values (Luo et al., 2021), identifying compound 7 as dimethyl maleate.

Compound 8: Yellow powder. HR-ESI-MS m/z : 298.6815 $[\text{M} + \text{H}]^+$. ^1H -NMR (600 MHz, acetone- d_6) δ : 6.71 (1H, s, H-11), 6.62 (1H, dd, $J = 9.6, 4.8$ Hz, H-15), 5.47 (1H, dd, $J = 9.6, 4.8$ Hz, H-16a), 5.09 (1H, dd, $J = 9.6, 4.8$ Hz, H-16 β), 2.91 (1H, $J = 9.6, 4.8, 2.2$ Hz, H-2 β), 2.37 (1H, ddd, $J = 9.6, 4.8, 2.2$ Hz, H-1), 2.23 (3H, s, H-17), 2.02 (1H, ddd, $J = 9.6, 4.8, 2.2$ Hz, H-1), 1.91 (2H, ddd, $J = 9.6, 4.8, 2.2$ Hz, H-6), 1.68 (2H, ddd, $J = 9.6, 4.8, 2.2$ Hz, H-7), 1.27 (3H, s, H-20), 1.21 (3H, s, H-18), 1.08 (3H, s, H-19). ^{13}C -NMR-DEPT (150 MHz, acetone- d_6) δ : 38.2 (C-1, s), 35.1 (C-2, t), 54.1 (C-3, d), 48.3 (C-4, s), 49.1 (C-5, d), 19.8 (C-6, t), 30.4 (C-7, s), 124.1 (C-8, d), 140.2 (C-9, s), 36.9

(C-10, d), 108.3 (C-11, q), 152.4 (C-12, t), 120.1 (C-13, s), 144.8 (C-14, t), 134.8 (C-15, s), 120.4 (C-16, q), 13.1 (C-17, d), 25.1 (C-18, s), 27.2 (C-19, s), 20.6 (C-20, q). These data are consistent with literature values (Craveiro & Silveira, 1982), identifying compound 8 as sonderianol.

Compound 9: White oil. HR-ESI-MS m/z : 279.2083 $[M + H]^+$. $^1\text{H-NMR}$ (600 MHz, acetone- d_6) δ : 7.82 (2H, dd, $J = 9.6, 4.8$ Hz, H-3,6), 7.63 (2H, dd, $J = 9.6, 4.8$ Hz, H-4,5), 4.27 (4H, t, $J = 9.6$ Hz, H-1), 1.83 (2H, m, H-2), 1.52 (4H, $J = 9.6$ Hz, H-3), 1.03 (6H, $J = 9.6$ Hz, H-4). $^{13}\text{C-NMR-DEPT}$ (150 MHz, acetone- d_6) δ : 131.6 (C-1, s), 131.6 (C-2, s), 127.3 (C-3, d), 128.6 (C-4, s), 128.6 (C-5, s), 127.3 (C-6, d), 64.9 (C-1, t), 29.1 (C-2, s), 20.8 (C-3, q), 14.2 (C-4, t). These data are consistent with literature values (Ma et al., 2021), identifying compound 9 as dibutyl phthalate.

Compound 10: White powder. HR-ESI-MS m/z : 384.3168 $[M + H]^+$. $^1\text{H-NMR}$ (600 MHz, acetone- d_6) δ : 7.02 (2H, d, $J = 4.8$ Hz, H-2,2'), 6.93 (2H, dd, $J = 9.6, 4.8$ Hz, H-6,6'), 6.81 (2H, d, $J = 9.6$ Hz, H-5,5'), 4.68 (2H, d, $J = 9.6$ Hz, H-7,7'), 4.17 (2H, m, H-9a, 9a'), 3.91 (6H, s, 3,3'-OCH₃), 3.73 (2H, m, H-9 β , 9 β '), 3.09 (2H, m, H-8,8'). $^{13}\text{C-NMR-DEPT}$ (150 MHz, acetone- d_6) δ : 140.1 (C-1, s), 108.7 (C-2, d), 151.2 (C-3, s), 146.8 (C-4, d), 115.8 (C-5, s), 119.4 (C-6, s), 88.2 (C-7, t), 56.1 (C-8, s), 73.1 (C-9, s), 140.2 (C-1, s), 108.7 (C-2, d), 151.2 (C-3, t), 146.8 (C-4, s), 115.8 (C-5, s), 119.4 (C-6, s), 88.2 (C-7, s), 56.1 (C-8, s), 73.1 (C-9, t), 57.1 (3-OCH₃, q), 57.1 (3'-OCH₃, q). These data are consistent with literature values (Zheng et al., 2020), identifying compound 10 as pinoresinol.

Compound 11: Colorless needle crystals. HR-ESI-MS m/z : 167.3172 $[M + H]^+$. $^1\text{H-NMR}$ (600 MHz, acetone- d_6) δ : 8.04 (2H, d, $J = 9.6$ Hz, H-2,6), 6.93 (2H, d, $J = 9.6$ Hz, H-3,5), 4.41 (2H, q, $J = 9.6$ Hz, H-2), 1.37 (3H, t, $J = 9.6$ Hz, H-3). $^{13}\text{C-NMR-DEPT}$ (150 MHz, acetone- d_6) δ : 122.7 (C-1, d), 131.8 (C-2, s), 114.8 (C-3, s), 160.1 (C-4, d), 114.8 (C-5, d), 131.8 (C-6, d), 167.1 (C-1, t), 61.4 (C-2, q), 16.2 (C-3, q). These data are consistent with literature values (Li et al., 2020), identifying compound 11 as p-hydroxybenzoic acid ethyl ester.

Compound 12: Colorless oil. HR-ESI-MS m/z : 204.1046 $[M + H]^+$. $^1\text{H-NMR}$ (600 MHz, acetone- d_6) δ : 7.71 (1H, d, $J = 4.8$ Hz, H-4), 7.52 (1H, d, $J = 9.6$ Hz, H-3), 7.38 (2H, d, $J = 4.8$ Hz, H-2), 6.91 (2H, d, $J = 9.6$ Hz, H-3), 6.83 (1H, d, $J = 9.6$ Hz, H-5), 6.27 (1H, d, $J = 9.6$ Hz, H-2), 3.64 (3H, s, 3-OCH₃). $^{13}\text{C-NMR-DEPT}$ (150 MHz, acetone- d_6) δ : 171.2 (C-1, d), 115.1 (C-2, s), 147.2 (C-3, s), 159.4 (C-4, d), 116.2 (C-5, s), 127.1 (C-1, d), 130.8 (C-2, d), 117.4 (C-3, t), 134.1 (C-4, d), 117.4 (C-5, t), 130.8 (C-6, t), 51.8 (3-OCH₃, q). These data are consistent with literature values (Son et al., 2005), identifying compound 12 as avenalumic acid methyl ester.

Compound 13: Yellow powder. HR-ESI-MS m/z : 343.2064 $[M + H]^+$. $^1\text{H-NMR}$ (600 MHz, acetone- d_6) δ : 8.22 (1H, d, $J = 4.8$, H-2), 7.91 (1H, dd, $J = 9.6, 4.8$ Hz, H-6), 7.09 (1H, d, $J = 9.6$ Hz, H-5), 6.57 (2H, dd, $J = 4.8, 2.2$ Hz, H-7,9). $^{13}\text{C-NMR-DEPT}$ (150 MHz, acetone- d_6) δ : 137.2 (C-1, d), 151.2 (C-2,

s), 140.2 (C-3, d), 180.7 (C-4, s), 158.1 (C-5, d), 100.3 (C-6, s), 165.2 (C-7, s), 92.1 (C-8, s), 163.1 (C-9, t), 107.2 (C-10, t), 123.6 (C-1, s), 116.8 (C-2, s), 152.4 (C-3, d), 158.1 (C-4, t), 113.1 (C-5, d), 122.4 (C-6, t), 59.7 (3-OCH₃, q), 55.8 (7-OCH₃, q), 57.4 (4-OCH₃, q). These data are consistent with literature values (Yu, 2021), identifying compound 13 as 5,3-dihydroxy-3,7,4-trimethoxyflavone.

Compound 14: Colorless oil. HR-ESI-MS m/z : 220.2813 [M + H]⁺. ¹H-NMR (600 MHz, acetone-d₆) δ : 4.76 (1H, m, H-14 β), 4.71 (1H, m, H-14a), 2.36 (1H, dd, J = 9.6, 4.8 Hz, H-4 β), 2.17 (1H, m, H-6), 2.11 (1H, m, H-4a), 2.02 (1H, m, H-3 β), 1.83 (1H, m, H-7 β), 1.81 (1H, m, H-8 β), 1.72 (1H, m, H-7a), 1.63 (1H, m, H-8a), 1.44 (1H, m, H-10), 1.31 (3H, s, H-15), 1.14 (3H, s, H-12), 1.11 (3H, s, H-13), 0.98 (1H, m, H-3a), 0.83 (1H, m, H-2), 0.51 (1H, dd, J = 9.6, 4.8 Hz, H-1). ¹³C-NMR-DEPT (150 MHz, acetone-d₆) δ : 29.7 (C-1, s), 28.3 (C-2, d), 25.1 (C-3, s), 38.7 (C-4, s), 154.2 (C-5, d), 54.1 (C-6, t), 27.1 (C-7, s), 42.3 (C-8, d), 80.8 (C-9, t), 56.1 (C-10, s), 21.3 (C-11, s), 29.1 (C-12, s), 17.1 (C-13, t), 107.3 (C-14, s), 27.1 (C-15, t). These data are consistent with literature values (Yu, 2021), identifying compound 14 as spathulenol.

Compound 15: White crystals. HR-ESI-MS m/z : 233.1041 [M + H]⁺. ¹H-NMR (600 MHz, acetone-d₆) δ : 7.13 (1H, J = 9.6 Hz, d, H-8), 6.83 (1H, J = 9.6 Hz, d, H-6), 5.73 (1H, s, H-3), 4.36 (2H, s, H-11), 2.51 (3H, s, H-14), 2.42 (3H, s, H-13). ¹³C-NMR-DEPT (150 MHz, acetone-d₆) δ : 125.3 (C-1, t), 166.8 (C-2, s), 110.7 (C-3, d), 179.6 (C-4, s), 140.2 (C-5, d), 120.3 (C-6, s), 162.1 (C-7, s), 103.4 (C-8, t), 160.5 (C-9, d), 114.3 (C-10, s), 51.3 (C-11, t), 207.3 (C-12, s), 29.7 (C-13, t), 21.3 (C-14, d). These data are consistent with literature values (Zhang et al., 2021), identifying compound 15 as 2-methyl-5-acetonyl-7-hydroxychromone.

Compound 16: Yellow powder. HR-ESI-MS m/z : 254.3246 [M + H]⁺. ¹H-NMR (600 MHz, acetone-d₆) δ : 8.52 (1H, d, J = 4.8 Hz, H-5), 8.02 (2H, d, J = 9.6 Hz, H-2, 6), 7.21 (1H, d, J = 9.6 Hz, H-8), 7.18 (3H, d, J = 9.6 Hz, H-6, 3, 5), 6.93 (1H, s, H-3). ¹³C-NMR-DEPT (150 MHz, acetone-d₆) δ : 135.6 (C-1, s), 164.1 (C-2, s), 105.3 (C-3, s), 176.3 (C-4, d), 128.5 (C-5, d), 115.4 (C-6, s), 165.3 (C-7, t), 104.6 (C-8, t), 161.1 (C-9, d), 118.3 (C-10, t), 125.2 (C-1, d), 130.4 (C-2, s), 118.1 (C-3, q), 163.1 (C-4, s), 118.1 (C-5, s), 130.4 (C-6, q). These data are consistent with literature values (Kitagawa et al., 1998), identifying compound 16 as 7,4-dihydroxyflavone.

Compound 17: Brown solid. HR-ESI-MS m/z : 345.1248 [M + H]⁺. ¹H-NMR (600 MHz, acetone-d₆) δ : 12.8 (1H, s, 6-OH), 6.34 (1H, br s, H-3), 6.09 (1H, d, J = 4.8 Hz, H-5), 5.13 (1H, d, J = 9.6 Hz, H-1), 3.92 (3H, s, 2-OCH₃), 3.83 (1H, m, H-6 a), 3.72 (1H, m, H-6 β), 3.39-3.44 (3H, m, H-2, 3, 5), 3.25 (1H, m, H-4). ¹³C-NMR-DEPT (150 MHz, acetone-d₆) δ : 102.4 (C-1, d), 164.3 (C-2, s), 91.8 (C-3, s), 164.1 (C-4, q), 95.8 (C-5, s), 163.1 (C-6, q), 202.6 (C-7, s), 33.1 (C-8, d), 102.3 (C-1, s), 74.2 (C-2, q), 76.8 (C-3, t), 70.1 (C-4, t), 77.1 (C-5, t), 61.2 (C-6, s), 55.8 (2-OCH₃, q). These data are consistent with literature values (Afshar et al., 2017), identifying compound 17 as annphenone.

Compound 18: White powder. HR-ESI-MS m/z : 169.2751 [M + H]⁺. ¹H-

NMR (600 MHz, acetone- d_6) δ : 7.53 (1H, dd, $J = 9.6, 4.8$ Hz, H-6), 7.41 (1H, s, H-2), 7.03 (1H, d, $J = 9.6$ Hz, H-5), 3.96 (3H, s, 4-OCH₃). ¹³C-NMR-DEPT (150 MHz, acetone- d_6) δ : 122.4 (C-1, t), 115.8 (C-2, d), 147.1 (C-3, d), 150.8 (C-4, s), 110.4 (C-5, d), 130.4 (C-6, t), 57.3 (4-OCH₃, q), 168.3 (7-COOH, q). These data are consistent with literature values (Liu et al., 2018), identifying compound 18 as 3-hydroxy-4-methoxybenzoic acid.

Compound 19: White powder. HR-ESI-MS m/z : 303.1043 $[M + H]^+$. ¹H-NMR (600 MHz, acetone- d_6) δ : 6.91–6.86 (3H, m, H-2,5,6), 5.93 (2H, m, H-6,8), 5.36 (1H, dd, $J = 4.8, 2.2$ Hz, H-2), 3.92 (3H, s, 7-OCH₃), 3.16 (2H, m, H-3). ¹³C-NMR-DEPT (150 MHz, acetone- d_6) δ : 63.2 (C-1, s), 79.1 (C-2, t), 43.6 (C-3, t), 196.4 (C-4, d), 161.7 (C-5, q), 95.1 (C-6, t), 166.8 (C-7, s), 94.1 (C-8, s), 163.2 (C-9, s), 103.1 (C-10, t), 130.2 (C-1, q), 113.8 (C-2, d), 146.1 (C-3, t), 146.3 (C-4, d), 114.1 (C-5, s), 117.2 (C-6, d), 56.1 (7-OCH₃, q). These data are consistent with literature values (Xie et al., 2021), identifying compound 19 as 5,3,4-trihydroxy-7-methoxyflavanone.

Compound 20: Yellow oil. HR-ESI-MS m/z : 279.3167 $[M + H]^+$. ¹H-NMR (600 MHz, acetone- d_6) δ : 7.81 (2H, m, H-3,6), 7.46 (2H, m, H-4,5), 4.29 (4H, t, $J = 9.6$ Hz, H-1,1), 1.83 (4H, m, H-2,2), 1.51 (4H, m, H-3,3), 1.03 (6H, t, $J = 9.6$ Hz, H-4,4). ¹³C-NMR-DEPT (150 MHz, acetone- d_6) δ : 133.1 (C-1, s), 133.1 (C-2, s), 130.2 (C-3, s), 132.6 (C-4, d), 132.6 (C-5, s), 130.2 (C-6, d), 66.4 (C-1, s), 31.2 (C-2, d), 20.1 (C-3, s), 14.6 (C-4, s), 66.4 (C-1, s), 31.2 (C-2, s), 20.1 (C-3, d), 14.6 (C-4, d). These data are consistent with literature values (Zou et al., 2019), identifying compound 20 as dibutyl phthalate.

Compound 21: White solid. HR-ESI-MS m/z : 187.4162 $[M + H]^+$. ¹H-NMR (600 MHz, acetone- d_6) δ : 9.24 (1H, s, 3,5-OH), 9.02 (1H, s, 3-OH), 6.87 (1H, s, H-2,6), 3.18 (3H, s, 4-OCH₃). ¹³C-NMR-DEPT (150 MHz, acetone- d_6) δ : 120.1 (C-1, s), 146.4 (C-2, s), 109.1 (C-3, d), 139.1 (C-4, s), 109.1 (C-5, d), 146.4 (C-6, s), 59.4 (4-OCH₃, q). These data are consistent with literature values (Virginie et al., 2018), identifying compound 21 as 4-O-methylgallic acid.

Compound 22: Yellow oil. HR-ESI-MS m/z : 391.2037 $[M + H]^+$. ¹H-NMR (600 MHz, acetone- d_6) δ : 8.26 (4H, s, H-3,4,6,7), 4.34 (4H, m, H-1,1), 1.81 (2H, m, H-2,2), 1.03 (6H, t, $J = 9.6$ Hz, H-8,8), 0.87 (6H, t, $J = 9.6$ Hz, H-6,6). ¹³C-NMR-DEPT (150 MHz, acetone- d_6) δ : 165.4 (C-1, s), 136.3 (C-2, d), 129.4 (C-3, d), 129.4 (C-4, t), 136.3 (C-5, s), 129.4 (C-6, s), 129.4 (C-7, s), 165.4 (C-8, s), 67.8 (C-1, s), 40.3 (C-2, t), 32.8 (C-3, d), 30.2 (C-4, d), 24.5 (C-5, q), 15.6 (C-6, t), 25.1 (C-7, s), 12.3 (C-8, d), 67.8 (C-1, d), 40.3 (C-2, t), 32.8 (C-3, s), 30.2 (C-4, q), 24.5 (C-5, s), 15.6 (C-6, s), 25.1 (C-7, t), 12.3 (C-8, q). These data are consistent with literature values (Li et al., 2021), identifying compound 22 as dioctyl terephthalate.

2.2 Antibacterial Activity Test Results

As shown in Table 1, compound 2 exhibited inhibitory effects against *P. aeruginosa* and *B. subtilis*, compound 3 against *B. subtilis*, compound 8 against *P.*

aeruginosa, *S. aureus*, and *B. subtilis*, and compound 17 against *P. aeruginosa* and *S. aureus*. Notably, the inhibitory effect of compound 2 against *B. subtilis* showed no significant difference compared to kanamycin ($P > 0.05$).

Table 1 Antibacterial activities of compounds

	<i>Pseudomonas</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>
Sample name	<i>aeruginosa</i> Inhibition zone ($\text{mg} \cdot \text{mL}^{-1}$)	Inhibition zone ($\text{mg} \cdot \text{mL}^{-1}$)	Inhibition zone ($\text{mg} \cdot \text{mL}^{-1}$)
Compound 1	11.35 ± 0.73	20.16 ± 1.02	—
2	12.34 ± 0.89	—	17.01 ± 0.85
Compound 3	—	—	13.23 ± 0.91
Compound 8	11.67 ± 0.83	8.16 ± 0.56	—

Note: Compared with positive group, indicates $P > 0.05$. — indicates no antibacterial effect.*

3 Discussion and Conclusion

Ailanthus altissima root bark exhibits notable antibacterial and anti-inflammatory activities, yet reports on its chemical constituents remain limited. To investigate its antibacterial material basis, this study isolated and identified 22 compounds from the root bark, all reported for the first time from this source. The structural types primarily include phenols, flavonoids, sterols, and alkaloids. Previous reports on monomeric compounds from Simaroubaceae plants have mainly focused on quassinoids, alkaloids, lignans, flavonoids, triterpenoids, and anthraquinones, suggesting significant chemical diversity among species within the same family.

With increasing calls for “antibiotic prohibition and reduction” in the food and pharmaceutical industries, the development of natural antibacterial agents has become a research hotspot. Previous studies demonstrated that *A. altissima* root bark extracts possess good inhibitory activity against *P. aeruginosa*, *S. aureus*, *Candida albicans*, and *E. coli* (Wang et al., 2020), but did not elucidate the specific active constituents. Our antibacterial assays revealed that compound 2 produced an inhibition zone diameter exceeding 20 mm against *B. subtilis*, classified as extremely sensitive according to drug sensitivity criteria, with a MIC of only $1.0 \text{ mg} \cdot \text{mL}^{-1}$, suggesting it may be a major antibacterial component. Additionally, compounds 3, 8, and 17 also demonstrated good antibacterial activity, potentially broadening the antibacterial spectrum as candidate anti-inflammatory agents. The identified antibacterial compounds belong to diverse structural classes including flavonoids, phenols, and acetophenones, indicating that the antibacterial activity of *A. altissima* root bark results from synergistic effects of multiple compound types. This study enriches the chemical constituent database of *A. altissima* root bark and provides valuable insights for the development of novel antibacterial agents.

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