# Postprint: Study on Anthraquinone Constituents from Rubia oncotricha

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## Abstract

To investigate the chemical constituents of Rubia oncotricha, a plant endemic to China, the 70% ethanol extract of Rubia oncotricha was subjected to separation and purification by silica gel, ODS, and gel column chromatography, and the obtained compounds were structurally identified. A total of fifteen anthraquinone compounds were isolated from Rubia oncotricha, namely 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone-3-O-(6' -O-acetyl)-α-rhamnosyl(1$→2)− $-glucoside(1), 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone-3-O-$-rhamnosyl(1→2)− $-glucoside(2), 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone-3-O-(3'-O-acetyl)− $-rhamnosyl(1→2)− $-glucoside(3), 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone-3-O-$-glucoside(4), 1,3,6-trihydroxy-2-hydroxymethyl-9,10-anthraquinone-3-O-(6'-O-acetyl)− $-D-glucopyranoside(5), 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone-3-O-(6'-O-acetyl)− $-D-glucopyranoside(6), physcion-8-O− $-D-glucoside(7), emodin-8-O− $-D-glucoside(8), digiferruginol-11-O− $-gentiobioside(9), 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone-3-O-(6'-O-acetyl)− $-D-xylopyranosyl-(1→2)− $-Dglucopyranoside (10), 6-hydroxyrubiadin (11), 1,2-dihydroxyanthraquinone (12), chrysophanol (13), 6-hydroxyxanthopurpurin (14), and 1,3-dihydroxyanthraquinone (15). Compounds 7, 8, and 14 were isolated from the genus Rubia for the first time, while compounds 1-6, 9, 10, 12, and 13 were isolated from this medicinal material for the first time.

## Full Text

# Chemical Constituents of Anthraquinones from *Rubia oncotricha*

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**Abstract:** To investigate the chemical constituents of the Chinese endemic plant *Rubia oncotricha*, the 70% ethanol extract was subjected to separation and purification using silica gel, ODS, and gel column chromatography, followed by structural elucidation of the isolated compounds. Fifteen anthraquinone compounds were isolated from *R. oncotricha*: 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone-3-O-(6' -O-acetyl)-α-rhamnosyl(1$→2)− −*glucoside*(1), 2 − *methyl* − 1, 3, 6 − *trihydroxy* − 9, 10 − *anthraquinone* − 3 − *O*− −*rhamnosyl*(1→2)− −*glucoside*(2), 2 − *methyl* − 1, 3, 6 − *trihydroxy* − 9, 10 − *anthraquinone* − 3 − *O* − (3′ − *O* − *acetyl*)− −*rhamnosyl*(1→2)− −*glucoside*(3), 2−*methyl*−1, 3, 6−*trihydroxy*− 9, 10 − *anthraquinone* − 3 − *O*− −*glucoside*(4), 1, 3, 6 − *trihydroxy* − 2 − *hydroxymethyl* − 9, 10 − *anthraquinone* − 3 − *O* − (6′ − *O* − *acetyl*)− −*D* − *glucopyranoside*(5), 2 − *methyl* − 1, 3, 6 − *trihydroxy* − 9, 10 − *anthraquinone* − 3 − *O* − (6′ − *O* − *acetyl*)− −*D* − *glucopyranoside*(6), *physcion* − 8 − *O*− −*D* − *glucoside*(7), *emodin* − 8 − *O*− −*D* − *glucoside*(8), *digiferruginol* − 11 − *O*− −*gentiobioside*(9), 2−*methyl*−1, 3, 6−*trihydroxy*−9, 10−*anthraquinone*− 3 − *O* − (6′ − *O* − *acetyl*)− −*D* − *xylopyranosyl* − (1→2)− $-D-glucopyranoside (10), 6-hydroxyrubiadin (11), 1,2-dihydroxyanthraquinone (12), chrysophanol (13), 6-hydroxyxanthopurpurin (14), and 1,3-dihydroxyanthraquinone (15). Compounds 7, 8, and 14 were isolated from the genus *Rubia* for the first time, while compounds 1-6, 9, 10, 12, and 13 were isolated from this medicinal material for the first time.

**Keywords:** *Rubia oncotricha*; chemical constituents; anthraquinone; isolation and purification; structure identification

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The genus *Rubia* (Rubiaceae) comprises approximately 70 species worldwide, with 36 species and 2 varieties distributed throughout China. Plants in this genus are primarily used medicinally for their roots. Previous phytochemical investigations have revealed diverse chemical constituents including quinones, terpenoids, and cyclopeptides, with anthraquinone derivatives being the predominant class. *Rubia* species exhibit various pharmacological activities such

as antimicrobial, anti-inflammatory, anticancer, antioxidant, immunomodulatory, hepatoprotective, and analgesic effects. *Rubia oncotricha* Hand.-Mazz., a member of this genus, is mainly distributed in Guangxi, Sichuan, Guizhou, and Yunnan provinces. The dried roots and rhizomes are used in traditional medicine to treat conditions including hematemesis, epistaxis, metrorrhagia, bronchitis, and traumatic injuries. This species is documented in the *Quality Standards of Traditional Chinese Medicine and Ethnic Medicine in Guizhou Province* (2003 edition) and represents a commonly used medicinal material in Guizhou. Additionally, *Flora of China* records that its roots are used as a substitute for *Rubia cordifolia* (called "small Rubia") in Yunnan's Wenshan region and serve as a post-illness tonic in Lingyun, Guangxi, indicating its significant folk medicinal value.

Previous studies on *R. oncotricha* have primarily isolated anthraquinone aglycones from methanol extracts, with most constituents derived from relatively low-polarity fractions. Anthraquinones are polycyclic compounds containing a quinone structure that exhibit broad-spectrum biological activities including antimicrobial, antiviral, antioxidant, and antitumor effects. Given the widespread ethnomedicinal use of *R. oncotricha* in Guizhou's minority regions and the promising bioactivities of anthraquinones, this study systematically investigated the anthraquinone constituents of *R. oncotricha* to provide a foundation for future research and development. Using modern separation techniques including silica gel and Sephadex LH-20 chromatography, we isolated and identified fifteen anthraquinone compounds, comprising both glycosides and aglycones, three of which represent first-time isolations from the genus *Rubia* and ten from this species.

## 1. Instruments and Materials

The following instruments were used: an ACQUITY-UPLC-TQD ultra-performance liquid chromatography-triple quadrupole tandem mass spectrometer (Waters, USA), an Agilent-QTOF mass spectrometer (Agilent, USA), a JEOL ECS 400 nuclear magnetic resonance spectrometer (JEOL, Japan), a BUCHI R-200 rotary evaporator (BUCHI, Switzerland), a ZF7 three-purpose UV analyzer (Gongyi Yuhua Instrument Co., Ltd.), and a KZ-20L ultra-pure water system (Shanghai Kezhi Environmental Protection Equipment Co., Ltd.). Chromatographic materials included silica gel (200–300 mesh, Qingdao Marine Chemical Co., Ltd.), thin-layer chromatography plates (Qingdao Marine Chemical Co., Ltd.), MCI CHP20/P120 gel (Mitsubishi, Japan), Sephadex LH-20 (Pharmacia Biotech, Switzerland), Toyopearl HW-40C and HW-40F (Tosoh, Japan). All reagents were of analytical grade.

*Rubia oncotricha* medicinal material was purchased from the traditional Chinese medicine market at Wandong Bridge, Nanming District, Guiyang, and authenticated by Associate Professor Long Qingde of Guizhou Medical University as the dried roots and rhizomes of *Rubia oncotricha* Hand.-Mazz. (Rubiaceae). Voucher specimens are deposited at the Guizhou Provincial Key Laboratory of

Pharmaceutics.

## 2. Extraction and Separation

Dried *R. oncotricha* roots and rhizomes (5 kg) were cut into 1–2 cm pieces and reflux-extracted three times with 70% ethanol. The combined filtrates were concentrated under reduced pressure to obtain a crude extract, which was dissolved in water and partitioned with organic solvents to yield petroleum ether (55 g), ethyl acetate (65 g), *n*-butanol (215 g), and water (257 g) fractions.

The *n*-butanol fraction (215 g) was subjected to normal-phase silica gel column chromatography with a dichloromethane-methanol gradient (50:1 to 1:1) to afford seven fractions (Fr.1–Fr.7). Fraction 2 was further purified by silica gel column chromatography (ethyl acetate-methanol) and Toyopearl HW-40F column chromatography (methanol) to yield compounds **1** (1.0 g), **2** (1.4 g), and **9** (11.7 mg). Fraction 3 was separated by repeated silica gel column chromatography (ethyl acetate-methanol, dichloromethane-methanol), Sephadex LH-20 (chloroform-methanol), Toyopearl HW-40F (methanol), Toyopearl HW-40C (chloroform-methanol), and MCI column chromatography (methanol-water) to obtain compounds **3** (27.0 mg) and **10** (7.3 mg). Fraction 4 was processed through Sephadex LH-20 (methanol), Toyopearl HW-40F (methanol), MCI column chromatography (methanol-water), normal-phase silica gel (dichloromethane-methanol), and reversed-phase ODS column chromatography (methanol-water) to isolate compounds **4** (5.5 mg), **5** (16.0 mg), and **8** (7.7 mg). Fraction 6 was separated by normal-phase silica gel chromatography (ethyl acetate-methanol) and Sephadex LH-20 (chloroform-methanol) to yield compounds **6** (8.7 mg) and **7** (85.0 mg).

The ethyl acetate fraction (65 g) was separated by silica gel column chromatography using a dichloromethane-methanol gradient (50:1 to 0:1) to give four fractions (Fr.1–Fr.4). Fraction 1 was subjected to multiple silica gel column chromatography steps (petroleum ether-ethyl acetate, ethyl acetate-methanol, dichloromethane-acetone), Sephadex LH-20 (chloroform-methanol), and Toyopearl HW-40C (methanol) to obtain compound **14** (13.8 mg). Fraction 2 was purified by repeated silica gel column chromatography (chloroform-ethyl acetate, petroleum ether-ethyl acetate, dichloromethane-acetone) and Sephadex LH-20 (chloroform-methanol) to yield compound **13** (5.2 mg). Fraction 3 was separated by silica gel column chromatography (dichloromethane-acetone, petroleum ether-ethyl acetate) and Sephadex LH-20 (chloroform-methanol) to afford compound **15** (8.3 mg). Subfraction 3.6 was further purified by normal-phase silica gel (dichloromethane-acetone), Sephadex LH-20 (chloroform-methanol), and Toyopearl HW-40F (methanol), followed by crystallization and recrystallization to obtain compounds **11** (24.3 mg) and **12** (14.0 mg).

[Figure 1: see original paper] Chemical structures of compounds 1–15

## 3. Structure Identification

**Compound 1** was obtained as a yellow powder with ESI-MS m/z: 619 $[M-H]^-$ and molecular formula $C_{29}H_{32}O_{15}$. The $^1$H-NMR (400 MHz, DMSO-$d_6$) showed signals at $\delta$: 8.10 (1H, d, $J$ = 8.6 Hz, H-8), 7.47 (1H, d, $J$ = 2.5 Hz, H-5), 7.40 (1H, s, H-4), 7.23 (1H, dd, $J$ = 8.6, 2.6 Hz, H-7), 5.51 (1H, d, $J$ = 5.5 Hz, H-1 ), 5.28 (1H, d, $J$ = 1.7 Hz, H-1 ), 2.15 (3H, s, 2-$CH_3$), 1.93 (3H, s, H-2 ), and 1.09 (3H, d, $J$ = 6.1 Hz, 6 -$CH_3$). The $^{13}$C-NMR (100 MHz, DMSO-$d_6$) displayed resonances at $\delta$: 163.7 (C-1), 120.6 (C-2), 160.0 (C-3), 105.3 (C-4), 135.4 (C-4a), 112.7 (C-5), 161.3 (C-6), 121.6 (C-7), 129.8 (C-8), 124.5 (C-8a), 186.4 (C-9), 110.7 (C-9a), 181.7 (C-10), 132.0 (C-10a), 97.3 (C-1 ), 76.3 (C-2 ), 77.1 (C-3 ), 70.0 (C-4 ), 74.0 (C-5 ), 63.4 (C-6 ), 100.2 (C-1 ), 70.3 (C-2 ), 70.5 (C-3 ), 72.0 (C-4 ), 68.6 (C-5 ), 18.2 (C-6 ), 170.4 (C-1 ), 20.4 (C-2 ), and 8.8 (2-$CH_3$). These data were consistent with literature values (Bajpai et al., 2018), leading to the identification of **2-methyl-1,3,6-trihydroxy-9,10-anthraquinone-3-O-(6 -O-acetyl)-$\alpha$-rhamnosyl(1$\rightarrow$2)$-$ $-glucoside**.

**Compound 2** was isolated as a yellow powder with ESI-MS m/z: 577 $[M-H]^-$ and molecular formula $C_{27}H_{30}O_{14}$. The $^1$H-NMR (400 MHz, DMSO-$d_6$) showed $\delta$: 8.09 (1H, d, $J$ = 8.6 Hz, H-8), 7.47 (1H, d, $J$ = 2.5 Hz, H-5), 7.40 (1H, s, H-4), 7.23 (1H, dd, $J$ = 8.6, 2.6 Hz, H-7), 5.45 (1H, d, $J$ = 7.0 Hz, H-1 ), 5.38 (1H, d, $J$ = 1.7 Hz, H-1 ), 2.15 (3H, s, 2-$CH_3$), and 1.08 (3H, d, $J$ = 6.2 Hz, 6 -$CH_3$). The $^{13}$C-NMR (100 MHz, DMSO-$d_6$) exhibited $\delta$: 163.5 (C-1), 120.6 (C-2), 160.2 (C-3), 105.0 (C-4), 135.3 (C-4a), 112.7 (C-5), 161.4 (C-6), 121.5 (C-7), 129.7 (C-8), 124.6 (C-8a), 186.4 (C-9), 110.5 (C-9a), 181.7 (C-10), 132.0 (C-10a), 97.4 (C-1 ), 77.1 (C-2 ), 77.4 (C-3 ), 69.5 (C-4 ), 76.2 (C-5 ), 60.3 (C-6 ), 100.2 (C-1 ), 70.3 (C-2 ), 70.5 (C-3 ), 72.0 (C-4 ), 68.5 (C-5 ), 18.1 (C-2 ), and 8.8 (2-$CH_3$). These data matched literature reports (Itokawa et al., 1983; Itokawa et al., 1989), identifying the compound as **2-methyl-1,3,6-trihydroxy-9,10-anthraquinone-3-O-$\alpha$-rhamnosyl(1$\rightarrow$2)$-$ $-glucoside**.

**Compound 3** was obtained as a yellow powder with ESI-MS m/z: 619 $[M-H]^-$ and molecular formula $C_{29}H_{32}O_{15}$. The $^1$H-NMR (400 MHz, DMSO-$d_6$) showed $\delta$: 8.11 (1H, d, $J$ = 8.6 Hz, H-8), 7.49 (1H, d, $J$ = 2.5 Hz, H-5), 7.46 (1H, s, H-4), 7.24 (1H, dd, $J$ = 8.6, 2.5 Hz, H-7), 5.69 (1H, d, $J$ = 7.6 Hz, H-1 ), 4.79 (1H, d, $J$ = 1.7 Hz, H-1 ), 2.16 (3H, s, 2-$CH_3$), 2.10 (3H, s, H-2 ), and 0.98 (3H, d, $J$ = 6.4 Hz, 6 -$CH_3$). The $^{13}$C-NMR (100 MHz, DMSO-$d_6$) displayed $\delta$: 163.7 (C-1), 120.8 (C-2), 160.0 (C-3), 105.2 (C-4), 135.4 (C-4a), 112.7 (C-5), 161.4 (C-6), 121.5 (C-7), 129.7 (C-8), 124.5 (C-8a), 186.4 (C-9), 110.7 (C-9a), 181.7 (C-10), 132.0 (C-10a), 97.3 (C-1 ), 76.5 (C-2 ), 77.1 (C-3 ), 69.1 (C-4 ), 76.5 (C-5 ), 59.9 (C-6 ), 101.2 (C-1 ), 70.1 (C-2 ), 70.5 (C-3 ), 71.7 (C-4 ), 67.1 (C-5 ), 17.9 (C-6 ), 169.8 (C-1 ), 21.1 (C-2 ), and 8.7 (2-$CH_3$). These data were consistent with literature values (Itokawa et al., 1989), identifying the compound as **2-methyl-1,3,6-trihydroxy-9,10-anthraquinone-3-O-(3 -O-acetyl)-$\alpha$-rhamnosyl(1$\rightarrow$2)$-$ $-glucoside**.

**Compound 4** was isolated as a yellow powder with ESI-MS m/z: 431 $[M-H]^-$

and molecular formula $C_{21}H_{20}O_{10}$. The $^{1}$H-NMR (400 MHz, DMSO-$d_6$) showed $\delta$: 8.07 (1H, d, $J$ = 8.6 Hz, H-8), 7.42 (1H, d, $J$ = 2.4 Hz, H-5), 7.41 (1H, s, H-4), 7.17 (1H, dd, $J$ = 8.6, 2.6 Hz, H-7), 5.10 (1H, d, $J$ = 7.3 Hz, H-1 ), and 2.16 (3H, s, 2-$CH_3$). The $^{13}$C-NMR (100 MHz, DMSO-$d_6$) exhibited $\delta$: 164.7 (C-1), 120.7 (C-2), 160.6 (C-3), 105.5 (C-4), 135.4 (C-4a), 113.1 (C-5), 161.3 (C-6), 121.8 (C-7), 129.7 (C-8), 123.8 (C-8a), 186.2 (C-9), 110.7 (C-9a), 181.9 (C-10), 132.1 (C-10a), 100.3 (C-1 ), 73.2 (C-2 ), 76.3 (C-3 ), 69.3 (C-4 ), 77.3 (C-5 ), 60.4 (C-6 ), and 8.5 (2-$CH_3$). These data matched literature reports (Itokawa et al., 1989), identifying the compound as **2-methyl-1,3,6-trihydroxy-9,10-anthraquinone-3-O-$\beta$-glucoside**.

**Compound 5** was obtained as a yellow powder with ESI-MS m/z: 489 $[M-H]^-$ and molecular formula $C_{23}H_{22}O_{12}$. The $^{1}$H-NMR (400 MHz, DMSO-$d_6$) showed $\delta$: 8.10 (1H, d, $J$ = 8.8 Hz, H-8), 7.47 (1H, d, $J$ = 2.4 Hz, H-5), 7.43 (1H, s, H-4), 7.23 (1H, dd, $J$ = 8.8, 2.4 Hz, H-7), 5.09 (1H, d, $J$ = 7.6 Hz, H-1 ), and 4.63 (1H, m, 2-$CH_2$OH), 4.54 (1H, m, 2-$CH_2$OH). The $^{13}$C-NMR (100 MHz, DMSO-$d_6$) displayed $\delta$: 161.7 (C-1), 123.7 (C-2), 161.5 (C-3), 106.1 (C-4), 133.8 (C-4a), 112.6 (C-5), 163.6 (C-6), 121.6 (C-7), 129.8 (C-8), 124.5 (C-8a), 186.4 (C-9), 111.2 (C-9a), 181.6 (C-10), 135.3 (C-10a), 100.6 (C-1 ), 73.2 (C-2 ), 75.8 (C-3 ), 70.0 (C-4 ), 74.2 (C-5 ), 63.5 (C-6 ), 170.5 (C-1 ), 20.5 (C-2 ), and 50.9 (2-$CH_2$OH). These data were consistent with literature values (Fan et al., 2011), identifying the compound as **1,3,6-trihydroxy-2-hydroxymethyl-9,10-anthraquinone-3-O-(6 -O-acetyl)-$\beta$-D-glucopyranoside**.

**Compound 6** was isolated as a yellow powder with ESI-MS m/z: 475 $[M+H]^+$ and molecular formula $C_{23}H_{22}O_{11}$. The $^{1}$H-NMR (400 MHz, DMSO-$d_6$) showed $\delta$: 8.10 (1H, d, $J$ = 8.6 Hz, H-8), 7.47 (1H, d, $J$ = 2.6 Hz, H-5), 7.43 (1H, s, H-4), 7.23 (1H, dd, $J$ = 8.6, 2.6 Hz, H-7), 5.11 (1H, d, $J$ = 7.6 Hz, H-1 ), 2.17 (3H, s, 2-$CH_3$), and 2.04 (3H, s, H-2 ). The $^{13}$C-NMR (100 MHz, DMSO-$d_6$) exhibited $\delta$: 163.7 (C-1), 120.9 (C-2), 160.6 (C-3), 105.8 (C-4), 135.4 (C-4a), 112.6 (C-5), 161.3 (C-6), 121.5 (C-7), 129.7 (C-8), 124.5 (C-8a), 186.5 (C-9), 110.7 (C-9a), 181.7 (C-10), 132.0 (C-10a), 100.2 (C-1 ), 73.2 (C-2 ), 76.0 (C-3 ), 69.9 (C-4 ), 74.2 (C-5 ), 63.5 (C-6 ), 170.5 (C-1 ), 20.5 (C-2 ), and 8.5 (2-$CH_3$). These data matched literature reports (Qiao et al., 1990), identifying the compound as **2-methyl-1,3,6-trihydroxy-9,10-anthraquinone-3-O-(6 -O-acetyl)-$\beta$-D-glucopyranoside**.

**Compound 7** was obtained as a yellow powder with ESI-MS m/z: 445 $[M-H]^-$ and molecular formula $C_{22}H_{22}O_{10}$. The $^{1}$H-NMR (400 MHz, DMSO-$d_6$) showed $\delta$: 7.48 (1H, d, $J$ = 1.7 Hz, H-4), 7.35 (1H, d, $J$ = 2.5 Hz, H-5), 7.17 (2H, d, $J$ = 2.5 Hz, H-2, 7), 5.17 (1H, d, $J$ = 6.4 Hz, H-1 ), 3.94 (3H, s, 3-$OCH_3$), and 2.41 (3H, s, 6-$CH_3$). The $^{13}$C-NMR (100 MHz, DMSO-$d_6$) displayed $\delta$: 161.7 (C-1), 124.3 (C-2), 147.2 (C-3), 119.4 (C-4), 136.4 (C-4a), 107.3 (C-5), 164.7 (C-6), 106.5 (C-7), 160.7 (C-8), 114.5 (C-8a), 186.5 (C-9), 114.4 (C-9a), 181.9 (C-10), 132.1 (C-10a), 100.6 (C-1 ), 73.2 (C-2 ), 76.6 (C-3 ), 69.8 (C-4 ), 77.5 (C-5 ), 60.8 (C-6 ), 56.1 (3-$OCH_3$), and 21.4 (6-$CH_3$). These data were consistent with literature values (Gao et al., 2013), identifying the compound

as **physcion-8-O-$\beta$-D-glucoside**.

**Compound 8** was isolated as a yellow powder with ESI-MS m/z: 431 [M-H]$^-$ and molecular formula $C_{21}H_{20}O_{10}$. The $^1$H-NMR (400 MHz, DMSO-$d_6$) showed $\delta$: 7.45 (1H, s, H-4), 7.27 (1H, d, $J$ = 2.0 Hz, H-5), 7.15 (1H, s, H-2), 6.98 (1H, d, $J$ = 2.4 Hz, H-7), 5.12 (1H, d, $J$ = 5.0 Hz, H-1 ), and 2.39 (3H, s, 3-$CH_3$). The $^{13}$C-NMR (100 MHz, DMSO-$d_6$) exhibited $\delta$: 161.1 (C-1), 124.2 (C-2), 146.9 (C-3), 119.3 (C-4), 132.1 (C-4a), 108.3 (C-5), 164.2 (C-6), 108.3 (C-7), 161.7 (C-8), 114.5 (C-8a), 186.4 (C-9), 113.3 (C-9a), 182.1 (C-10), 136.5 (C-10a), 100.7 (C-1 ), 73.3 (C-2 ), 77.3 (C-3 ), 69.4 (C-4 ), 76.4 (C-5 ), 60.6 (C-6 ), and 21.4 (3-$CH_3$). These data matched literature reports (Gao et al., 2011), identifying the compound as **emodin-8-O-$\beta$-D-glucoside**.

**Compound 9** was obtained as a yellow powder with ESI-MS m/z: 577 [M-H]$^-$ and molecular formula $C_{27}H_{30}O_{14}$. The $^1$H-NMR (400 MHz, DMSO-$d_6$) showed $\delta$: 8.25 (1H, m, H-8), 8.20 (1H, m, H-5), 8.04 (1H, d, $J$ = 8.0 Hz, H-3), 7.95 (2H, m, H-6, 7), 7.74 (1H, d, $J$ = 8.0 Hz, H-4), 4.94 (1H, d, $J$ = 14.8 Hz, H-11a), 4.77 (1H, d, $J$ = 18.0 Hz, H-11b), 4.35 (1H, d, $J$ = 7.6 Hz, H-1 ), and 4.29 (1H, d, $J$ = 8.0 Hz, H-1 ). The $^{13}$C-NMR (100 MHz, DMSO-$d_6$) exhibited $\delta$: 158.8 (C-1), 134.0 (C-2), 133.2 (C-3), 118.7 (C-4), 134.7 (C-4a), 126.9 (C-5), 135.2 (C-6), 135.3 (C-7), 126.7 (C-8), 131.9 (C-8a), 188.6 (C-9), 115.3 (C-9a), 181.9 (C-10), 132.8 (C-10a), 64.2 (C-11), 102.6 (C-1 ), 73.5 (C-2 ), 76.8 (C-3 ), 70.0 (C-4 ), 76.1 (C-5 ), 68.3 (C-6 ), 103.3 (C-1 ), 73.6 (C-2 ), 76.9 (C-3 ), 70.1 (C-4 ), 76.6 (C-5 ), and 61.1 (C-6 ). These data were consistent with literature values (Yang et al., 2014), identifying the compound as **digiferruginol-11-O-$\beta$-gentiobioside**.

**Compound 10** was obtained as a yellow powder with molecular formula $C_{28}H_{30}O_{15}$. The $^1$H-NMR (400 MHz, DMSO-$d_6$) showed $\delta$: 8.09 (1H, d, $J$ = 8.4 Hz, H-8), 7.47 (1H, d, $J$ = 2.0 Hz, H-5), 7.40 (1H, s, H-4), 7.23 (1H, dd, $J$ = 8.0, 2.0 Hz, H-7), 5.33 (1H, d, $J$ = 7.2 Hz, H-1 ), 4.49 (1H, d, $J$ = 7.2 Hz, H-1 ), 2.15 (3H, s, -OAc), and 2.03 (3H, s, 2-$CH_3$). The $^{13}$C-NMR (100 MHz, DMSO-$d_6$) displayed $\delta$: 163.5 (C-1), 120.7 (C-2), 160.3 (C-3), 105.6 (C-4), 135.4 (C-4a), 112.6 (C-5), 161.3 (C-6), 121.6 (C-7), 129.7 (C-8), 124.1 (C-8a), 186.3 (C-9), 110.7 (C-9a), 181.7 (C-10), 132.0 (C-10a), 97.8 (C-1 ), 82.1 (C-2 ), 75.5 (C-3 ), 69.6 (C-4 ), 74.6 (C-5 ), 63.4 (C-6 ), 105.4 (C-1 ), 74.0 (C-2 ), 76.2 (C-3 ), 69.5 (C-4 ), 66.0 (C-5 ), 170.5 (C-1 ), 20.4 (C-2 ), and 8.0 (2-$CH_3$). These data were consistent with literature values (Wang et al., 1991), identifying the compound as **2-methyl-1,3,6-trihydroxy-9,10-anthraquinone-3-O-(6 -O-acetyl)-$\beta$-D-xylopyranosyl-(1$→2)− $-D-glucopyranoside**.

**Compound 11** was isolated as yellow crystals with ESI-MS m/s: 271 [M+H]$^+$ and molecular formula $C_{15}H_{10}O_5$. The $^1$H-NMR (400 MHz, DMSO-$d_6$) showed $\delta$: 7.95 (1H, d, $J$ = 8.4 Hz, H-8), 7.35 (1H, dd, $J$ = 8.8, 2.4 Hz, H-7), 7.13 (1H, d, $J$ = 2.8 Hz, H-5), 7.11 (1H, s, H-4), and 2.49 (3H, s, 2-$CH_3$). The $^{13}$C-NMR (100 MHz, DMSO-$d_6$) exhibited $\delta$: 162.2 (C-1), 117.4 (C-2), 163.2 (C-3), 107.2 (C-4), 135.1 (C-4a), 112.5 (C-5), 162.3 (C-6), 121.2 (C-7), 129.3 (C-8), 124.6 (C-8a), 185.6 (C-9), 108.4 (C-9a), 181.9 (C-10), 131.7 (C-10a), and 8.1

(2-$CH_3$). These data matched literature reports (Kang et al., 2006), identifying the compound as **6-hydroxyrubiadin**.

**Compound 12** was obtained as a yellow powder with ESI-MS m/z: 241 $[M+H]^+$, 239 $[M-H]^-$ and molecular formula $C_{14}H_8O_4$. The $^1$H-NMR (400 MHz, DMSO-$d_6$) showed $\delta$: 8.31 (1H, m, H-8), 8.25 (1H, m, H-7), 7.85 (2H, m, H-6,7), 7.75 (1H, d, $J$ = 8.0 Hz, H-4), and 7.18 (1H, d, $J$ = 8.0 Hz, H-3). The $^{13}$C-NMR (100 MHz, DMSO-$d_6$) exhibited $\delta$: 150.7 (C-1), 152.7 (C-2), 120.8 (C-3), 121.2 (C-4), 123.8 (C-4a), 126.7 (C-5), 135.2 (C-6), 134.1 (C-7), 126.8 (C-8), 132.9 (C-8a), 188.8 (C-9), 116.3 (C-9a), 180.6 (C-10), and 133.6 (C-10a). These data were consistent with literature values (Itokawa et al., 1983), identifying the compound as **1,2-dihydroxyanthraquinone**.

**Compound 13** was isolated as a yellow powder with molecular formula $C_{15}H_{10}O_4$. The $^1$H-NMR (400 MHz, $CDCl_3$) showed $\delta$: 12.1 (1H, s, -OH), 12.0 (1H, s, -OH), 7.81 (1H, dd, $J$ = 8.0, 0.8 Hz, H-5), 7.66 (1H, d, $J$ = 8.0 Hz, H-6), 7.63 (1H, d, $J$ = 2.4 Hz, H-4), 7.27 (1H, dd, $J$ = 8.0, 0.8 Hz, H-7), 7.08 (1H, d, $J$ = 0.8 Hz, H-2), and 2.45 (3H, s, -$CH_3$). The $^{13}$C-NMR (100 MHz, $CDCl_3$) exhibited $\delta$: 162.4 (C-1), 124.6 (C-2), 149.4 (C-3), 121.3 (C-4), 133.2 (C-4a), 119.9 (C-5), 136.9 (C-6), 124.3 (C-7), 162.7 (C-8), 115.8 (C-8a), 192.5 (C-9), 113.7 (C-9a), 182.1 (C-10), 133.6 (C-10a), and 22.3 (3-$CH_3$). These data were consistent with literature values (Li et al., 2019), identifying the compound as **chrysophanol**.

**Compound 14** was obtained as a light yellow amorphous powder with ESI-MS m/z: 257 $[M+H]^+$ and molecular formula $C_{14}H_8O_5$. The $^1$H-NMR (400 MHz, $CD_3OD$) showed $\delta$: 8.12 (1H, d, $J$ = 8.8 Hz, H-8), 7.50 (1H, d, $J$ = 2.8 Hz, H-5), 7.16 (1H, dd, $J$ = 8.8, 2.4 Hz, H-7), 7.15 (1H, overlapped d, $J$ = 2.4 Hz, H-4), and 6.53 (1H, d, $J$ = 2.0 Hz, H-2). The $^{13}$C-NMR (100 MHz, $CD_3OD$) exhibited $\delta$: 166.5 (C-1), 109.3 (C-2), 166.4 (C-3), 108.9 (C-4), 137.0 (C-4a), 113.8 (C-5), 164.7 (C-6), 122.2 (C-7), 130.5 (C-8), 126.8 (C-8a), 187.2 (C-9), 110.7 (C-9a), 183.9 (C-10), and 132.8 (C-10a). These data were consistent with literature values (El-Gamal et al., 1995), identifying the compound as **6-hydroxyxanthopurpurin**.

**Compound 15** was isolated as a yellow powder with ESI-MS m/z: 239 $[M-H]^-$ and molecular formula $C_{14}H_8O_4$. The $^1$H-NMR (400 MHz, DMSO-$d_6$) showed $\delta$: 8.15 (2H, dd, $J$ = 6.8, 2.8 Hz, H-5, H-8), 7.88 (2H, m, H-6, 7), 7.10 (1H, d, $J$ = 2.4 Hz, H-4), and 6.57 (1H, d, $J$ = 2.4 Hz, H-2). The $^{13}$C-NMR (100 MHz, DMSO-$d_6$) exhibited $\delta$: 164.8 (C-1), 107.7 (C-2), 165.5 (C-3), 108.4 (C-4), 135.0 (C-4a), 126.9 (C-5), 134.7 (C-6), 134.5 (C-7), 126.4 (C-8), 132.9 (C-8a), 185.9 (C-9), 109.4 (C-9a), 181.9 (C-10), and 132.8 (C-10a). These data were consistent with literature values (Gui et al., 2017), identifying the compound as **1,3-dihydroxyanthraquinone**.

## 4. Discussion and Conclusion

*Rubia oncotricha* is used medicinally for its roots and rhizomes, traditionally valued for cooling blood, stopping bleeding, and relieving cough and phlegm. However, few studies have investigated its chemical constituents. The genus *Rubia* is rich in anthraquinones, particularly polyhydroxyanthraquinones with significant antioxidant properties and therapeutic potential against cancer and other diseases. Based on chemotaxonomic relationships, we hypothesized that *R. oncotricha* would also contain abundant anthraquinones. Previous work by Wang et al. (2018) on methanol extracts primarily yielded low-polarity anthraquinone aglycones. To discover additional anthraquinone constituents and explore their bioactivities, we employed multiple separation techniques to isolate fifteen anthraquinones, including both glycosides and aglycones. Three compounds were obtained from the genus *Rubia* for the first time, and ten from this species.

Literature surveys revealed that several isolated compounds possess notable pharmacological properties. Compound **8** significantly reduces 3-hydroxy-3-methylglutaryl-CoA reductase activity for lipid-lowering effects and exhibits antitumor activity. Compound **11** inhibits lipopolysaccharide-induced inflammation, suggesting potential as an anti-inflammatory candidate. Compound **13** demonstrates anticancer, neuroprotective, and lipid-regulating activities. Compounds **1** and **2** were isolated in relatively large quantities and may serve as marker compounds for quality control of *R. oncotricha* from different sources and batches. Our previous HPLC fingerprinting studies on *R. oncotricha* identified compounds **1** and **2** as characteristic constituents, warranting future quantitative analysis for comprehensive quality evaluation.

In summary, this study aligns with reports of anthraquinone richness in *Rubia* species, expands the chemical knowledge of *R. oncotricha*, and provides a scientific basis for its future development and utilization.

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