Chemical Constituents and Antitumor Activity of Stems and Leaves of Uncaria from Guizhou (Postprint)

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Abstract

Uncaria is rich in alkaloid constituents and abundant in resources, possessing heat-clearing and liver-calming, wind-extinguishing and fright-arresting effects. Currently, there are few reports on the chemical constituents and biological activities of Uncaria stems and leaves. To clarify the material basis of Uncaria stems and leaves and provide scientific basis for the rational development and sustainable utilization of this plant resource, this study investigated the chemical constituents of Uncaria stems and leaves and screened for antitumor activity. Silica gel column chromatography, Sephadex LH-20, and semi-preparative HPLC were employed for isolation and purification. Compound structures were identified based on physicochemical properties and spectroscopic data. The MTT assay was used to screen for in vitro antitumor activity against human leukemia cell lines K562 and HEL. Seven compounds were isolated from Uncaria stems and identified as 3,4,5-trimethoxyphenol (1), scopoletin (2), isocorynoxeine (3), corynoxeine (4), vallesiachotamine (5), rhynchophylline (6), and isorhynchophylline (7). Twelve compounds were isolated from Uncaria leaves and identified as octacosanol (8), -sitosterol (9), triacontanoic acid (10), 2-methyl-5,7-dihydroxy-chromone-7-O- -D-glucopyranoside (11), oleanolic acid (12), quercetin (13), hederagenin (14), kaempferol (15), (6R,9R)-9-hydroxymegastigman-4-en-3-one (16), ursolic acid (17), epicatechin (18), and physcion (19). The results showed that compounds 3 and 5 exhibited inhibitory effects on HEL cells with IC50 values of 17.96 g · mL-1 and 73.01 g · mL-1, respectively. Compound 5 showed inhibitory activity against K562 cells with an IC50 value of 16.45 g · mL-1. Among them, compounds 1, 8, 10, and 16 were isolated from the Uncaria plant for the first time. The chemical constituents from Uncaria stems possess certain antitumor activity.

Full Text

Chemical Constituents of Stems and Leaves from *Uncaria rhynchophylla* in Guizhou and Their Antitumor Activities

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Abstract: *Uncaria rhynchophylla* is rich in alkaloids and abundant in natural resources, with traditional functions of clearing heat, calming the liver, and extinguishing wind to relieve convulsions. However, few reports have addressed the chemical constituents and biological activities of its stems and leaves. To clarify the material basis of *U. rhynchophylla* stems and leaves and provide a scientific foundation for the rational development and sustainable utilization of this plant resource, this study investigated their chemical constituents and antitumor activities. Chromatographic techniques including silica gel column chromatography, Sephadex LH-20, and semi-preparative HPLC were employed for isolation and purification. Compound structures were identified based on physicochemical properties and spectroscopic data. The antitumor activities against human leukemia cell lines K562 and HEL were evaluated *in vitro* using the MTT assay. Seven compounds were isolated from the stems and identified as 3,4,5-trimethoxyphenol (1), scopoletin (2), isocorynoxeine (3), corynoxeine (4), vallesiachotamine (5), rhynchophylline (6), and isorhynchophylline (7). Twelve compounds were isolated from the leaves and identified as octacosanol (8), -sitosterol (9), triacontanoic acid (10), 2-methyl-5,7-dihydroxy-chromone-7-O- -D-glucopyranoside (11), oleanolic acid (12), quercetin (13), hederagenin (14), kaempferol (15), (6R,9R)-9-hydroxymegastigman-4-en-3-one (16), ursolic acid (17), epicatechin (18), and physcion (19). The antitumor activity results showed that compounds 3 and 5 inhibited HEL cells with IC values of 17.96 μg · mL ^{1} and 73.01 μg · mL ^{1}, respectively. Compound 5 also inhibited K562 cells with an IC value of 16.45 μg · mL ^{1}. Notably, compounds 1, 8, 10, and 16 were isolated from *U. rhynchophylla* for the first time. These findings demonstrate that the chemical constituents from *U. rhynchophylla* stems possess certain antitumor activities.

Keywords: *Uncaria rhynchophylla*, chemical constituents, isolation and purification, structure identification, antitumor activity

Introduction

Uncaria rhynchophylla, belonging to the Rubiaceae family, is the dried hooked stem branch of *Uncaria rhynchophylla*, *U. macropylla*, *U. hirsuta*, *U. sinensis*, or *U. sessilifucus*. It is commonly used to treat dizziness, epilepsy, convulsions due to high fever, wind-cold with fright, infantile fright crying, and eclampsia (National Pharmacopoeia Commission, 2015). The plant is mainly produced in Jiangxi, Guangdong, Guangxi, Hunan, Yunnan, and Guizhou provinces in China (Gao et al., 2017). As one of the authentic medicinal materials in Guizhou, *U. rhynchophylla* is cultivated in over 30 counties and cities including Jianhe, Danzhai, Jinping, Rongjiang, and Kaiyang (Yang et al., 2014).

The chemical composition of *U. rhynchophylla* is diverse, encompassing alkaloids, flavonoids, triterpenoids, sterols, polyphenols, volatile oils, and glycosides (Wang & Sun, 2010; Zhang & Huang, 2020). Among these, alkaloids represent the primary active components, which can be further classified into indole and oxindole alkaloids based on structural types (Cai et al., 2019). Previous research has focused primarily on the medicinal parts of the plant, with limited reports on non-medicinal parts. To further develop and utilize these resources and avoid waste, this study conducted a preliminary investigation into the chemical constituents of the non-medicinal stems and leaves of *U. rhynchophylla*.

From the stems, we isolated seven compounds (Figure 1 [Figure 1: see original paper]) identified as 3,4,5-trimethoxyphenol (1), scopoletin (2), isocorynoxeine (3), corynoxeine (4), vallesiachotamine (5), rhynchophylline (6), and isorhynchophylline (7). From the leaves, we isolated twelve compounds identified as octacosanol (8), -sitosterol (9), triacontanoic acid (10), 2-methyl-5,7-dihydroxychromone-7-O- -D-glucopyranoside (11), oleanolic acid (12), quercetin (13), hederagenin (14), kaempferol (15), (6R,9R)-9-hydroxymegastigman-4-en-3-one (16), ursolic acid (17), epicatechin (18), and physcion (19). Compounds 1, 8, 10, and 16 were isolated from this plant for the first time. The antitumor activity results indicated that compounds 3 and 5 showed strong inhibitory effects on HEL cells, while compound 5 also demonstrated potent inhibition against K562 cells. This study enriches the understanding of the chemical basis of *U. rhynchophylla* and provides a scientific foundation for its comprehensive development and rational utilization.

Materials and Methods

1.1 Instruments and Materials

The following instruments were used: INOVA-400 MHz NMR spectrometer (Varian, USA), WIPM-500 MHz NMR spectrometer (Wuhan Institute of Mathematics and Physics, Chinese Academy of Sciences), 600 MHz superconducting NMR spectrometer (Bruker, Germany), Waters 2545 HPLC system (Waters, USA), HP-5973 mass spectrometer, HP1100-MSD LC-MS system, Sephadex LH-20

gel (40–70 m, Merck), IKA mixer, Corning cryovials, NEST cell culture dishes, Thermo ultra-low temperature freezer, Thermo cell culture incubator, NEST 96-well plates, Thermo superclean bench, Zeiss fluorescence inverted microscope, Hyclone fetal bovine serum, IKA temperature-controlled shaker, Millipore Milli-Q ultrapure water system, Grant water bath, DEME, silica gel for column chromatography (200–300 mesh and 300–400 mesh), TLC silica gel GF254 (0.20–0.25 m) (Qingdao Marine Chemical Factory), Sephadex LH-20 (MERCK), BECKMAN low-temperature centrifuge, NEST cell culture flasks, Thermo superclean bench, Fischer pipettes, NEST centrifuge tubes, NEST cell culture dishes, and Shanghai Shenbo high-pressure sterilizer.

Reagents included methanol, ethanol, and petroleum ether (Shanghai Titan Technology), Gene multi-function microplate reader, Gibico trypsin, penicillin-streptomycin, Sigma MTT, and MCI GEL CHP20P (Mitsubishi Chemical, Japan). Plant materials were provided by Changhao Jinhuang (Guizhou) Traditional Chinese Medicine Co., Ltd. from Xingren Town, Danzhai County, Guizhou Province, and identified by pharmacist He Dingxiang as stems and leaves of *Uncaria rhynchophylla* (Rubiaceae).

1.2 Extraction and Separation

Dried *U. rhynchophylla* stems (15.0 kg) were pulverized and extracted three times with 90% ethanol (50 L each) by heating reflux. The extracts were combined and concentrated under reduced pressure to remove organic solvents, yielding a crude extract. The extract was dissolved in 5% hydrochloric acid, filtered, and the filtrate pH was adjusted to 9 with saturated sodium hydroxide solution. The solution was then extracted four times with chloroform. The chloroform layers were combined and evaporated under reduced pressure to obtain 200.0 g of chloroform fraction. This fraction (200.0 g) was dissolved in an appropriate solvent, mixed with 350.0 g of silica gel (60–80 mesh) as a sample, and subjected to column chromatography over 2.0 kg of silica gel (300–400 mesh) with a gradient elution of chloroform–methanol (100:1 → 0:1). Fractions of approximately 800 mL were collected and concentrated under reduced pressure. The concentrated fractions were analyzed by TLC under UV light and after staining with 5% phosphomolybdic acid. Similar fractions were combined, yielding nine major fractions (Fr.1–Fr.9).

Fraction Fr.2 (5.0 g) was separated by MCI column chromatography using a methanol–water gradient (20:80 → 100:0) to give six subfractions. Subfraction Fr.2-1 (505.2 mg) was further purified by repeated silica gel column chromatography with petroleum ether–ethyl acetate (10:1 → 1:1) to afford compounds 1 (6.0 mg) and 2 (7.0 mg). Subfraction Fr.2-6 (240.3 mg) was recrystallized to yield compound 3 (5.0 mg). Subfraction Fr.2-5 (1.3 g) was subjected to repeated silica gel column chromatography and semi-preparative HPLC to obtain compounds 4 (3.2 mg) and 5 (6.0 mg). Fraction Fr.3 (4.0 g) was purified by Sephadex LH-20 gel column (methanol–water 1:1) and silica gel gradient elution (petroleum ether–ethyl acetate 8:1 → 2:1), followed by semi-preparative HPLC

to yield compounds 6 (15.3 mg) and 7 (4.0 mg).

Dried *U. rhynchophylla* leaves (7.5 kg) were pulverized and extracted three times with 90% ethanol (25 L each) by reflux. The combined extract was concentrated under reduced pressure to remove organic solvents. The resulting total extract was suspended in water and successively extracted three times each with petroleum ether, ethyl acetate, and *n*-butanol. The solvents were recovered under reduced pressure to obtain petroleum ether (5.0 g), ethyl acetate (291.0 g), and *n*-butanol (330.0 g) fractions. The ethyl acetate fraction (291.0 g) was dissolved in an appropriate organic solvent and mixed with 500.0 g of silica gel (60–80 mesh) as a sample. It was then subjected to column chromatography over 3 kg of silica gel (300–400 mesh) with gradient elution of chloroform–methanol (100:1 → 0:1). Fractions of approximately 800 mL were collected, concentrated under reduced pressure, and analyzed by TLC. Similar components were combined, yielding eleven fractions (Fr.1–Fr.11).

Fraction Fr.3 (3.0 g) was separated by MCI column chromatography (methanol–water 20:80 → 100:0) to give six subfractions. Subfraction Fr.3-1 (820.1 mg) was purified by silica gel column chromatography (petroleum ether–ethyl acetate 20:1 → 5:1) to afford compounds 8 (20.0 mg) and 9 (15.2 mg). Fraction Fr.4 (6.0 g) was separated by silica gel gradient elution (petroleum ether–ethyl acetate 100:1 → 10:1) into seven subfractions (Fr.4-1–Fr.4-7). Subfraction Fr.4-5 (1.5 g) was purified by silica gel column chromatography (petroleum ether–ethyl acetate 10:1) to yield compounds 10 (8.0 mg) and 11 (5.0 mg). Subfraction Fr.4-7 (602.4 mg) was recrystallized from methanol to obtain compound 12 (6.1 mg). Fraction Fr.6 (5.0 g) was separated by silica gel gradient elution (petroleum ether–ethyl acetate 50:1 → 10:1) into nine subfractions (Fr.6-1–Fr.6-9). Subfraction Fr.6-4 (1.6 g) was purified by Sephadex LH-20 gel column (methanol–chloroform 1:1) to afford compound 13 (6.3 mg). Subfraction Fr.6-9 (803.2 mg) was purified by Sephadex LH-20 gel column (methanol–chloroform 1:1) and semi-preparative HPLC to yield compounds 14 (6.3 mg), 15 (6.2 mg), and 16 (3.0 mg). Fraction Fr.7 (6.0 g) was separated by MCI column chromatography (methanol–water 30:70 → 100:0), followed by repeated silica gel gradient elution (petroleum ether–ethyl acetate 20:1 → 0:1), Sephadex LH-20 gel column (50% methanol), and semi-preparative HPLC to obtain compounds 17 (25.1 mg), 18 (20.3 mg), and 19 (8.2 mg).

1.3 Antitumor Activity Assay

The antiproliferative effects of compounds from *U. rhynchophylla* stems and leaves against human leukemia cell lines K562 and HEL were determined by the MTT method. Logarithmic-phase cells were suspended in RPMI1640 medium containing 10% fetal bovine serum and seeded in 96-well plates at 8,000 cells per well. The plates were incubated overnight at 5% CO to allow complete adaptation. Test compounds were dissolved in DMSO and prepared as five concentration gradients in culture medium. Positive controls (paclitaxel, adriamycin, vincristine, cisplatin, and 5-fluorouracil) were prepared at equivalent

concentrations. The negative control consisted of culture medium with 0.25% DMSO. Each group had five replicate wells. After 48 h of treatment, the supernatant was removed by centrifugation, and MTT solution (5 mg • mL [1]) in culture medium was added. Following incubation at 37 °C in 5% CO for 4 h, the supernatant was discarded, DMSO was added, and the plates were shaken at 37 °C in the dark until formazan crystals were completely dissolved. Optical density was measured at 490 nm using a microplate reader. Cell viability (%) = (experimental OD - blank control OD) / (control OD - blank control OD) × 100%, and inhibition rate (%) = 100% - cell viability (%). The half-maximal inhibitory concentration (IC) was calculated using the formula: IC = log [1][Xm - i(p - 5)], where i represents the logarithm of (maximum dose/adjacent dose) and Xm represents the logarithm of maximum concentration.

Results

2.1 Structural Identification

Compound 1: White amorphous powder. ESI-MS m/z: 207 [M + Na] . [1]H-NMR (500 MHz, CDCl) H: 6.09 (2H, s, H-2, 6), 3.81 (6H, s, 3, 5-OCH), 3.78 (3H, s, 4-OCH). [13]C-NMR (125 MHz, CDCl) C: 153.8 (C-3, 5), 152.3 (C-1), 131.9 (C-4), 92.9 (C-2, 6), 61.1 (4-OCH), 56.0 (3, 5-OCH). These data were consistent with literature values (DE Oliveira et al., 2014), identifying compound 1 as 3,4,5-trimethoxyphenol.

Compound 2: White powder. ESI-MS m/z: 215 [M + Na] . [1]H-NMR (500 MHz, CDCl) H: 7.60 (1H, d, J = 9.5 Hz, H-4), 6.92 (1H, s, H-5), 6.85 (1H, s, H-8), 6.27 (1H, d, J = 9.5 Hz, H-3), 3.96 (3H, s, 6-OCH). [13]C-NMR (125 MHz, CDCl) C: 161.5 (C-2), 149.7 (C-6, 9), 144.0 (C-7), 143.3 (C-4), 113.4 (C-5), 111.5 (C-3), 107.5 (C-10), 103.2 (C-8), 56.4 (6-OCH). These data matched literature values (Ma et al., 2009), identifying compound 2 as scopoletin.

Compound 3: White powder, positive reaction with Dragendorff' s reagent. ESI-MS m/z: 383 [M + H] . [1]H-NMR (400 MHz, CDCl) H: 8.44 (1H, s, 1-NH), 7.47 (1H, d, J = 7.3 Hz, H-9), 7.28 (1H, s, H-17), 7.17 (1H, m, H-11), 7.05 (1H, t, J = 7.1 Hz, H-10), 6.89 (1H, d, J = 7.7 Hz, H-12), 5.52 (1H, m, H-19), 4.94 (2H, m, H-18), 3.70 (3H, s, OCH), 3.58 (3H, s, COOCH), 3.31 (1H, m, H-3), 3.21 (1H, d, J = 10.9 Hz, H-15), 2.07-1.97 (2H, m, H-6a,6), 1.08 (1H, d, J = 11.4 Hz, H-14a). [13]C-NMR (125 MHz, CDCl) C: 184.3 (C-2), 170.8 (C-22), 162.0 (C-17), 142.5 (C-19), 142.1 (C-13), 136.4 (C-8), 130.0 (C-11), 127.7 (C-9), 124.9 (C-10), 117.9 (C-18), 114.5 (C-16), 111.8 (C-12), 74.6 (C-3), 63.8 (C-21), 61.2 (C-OCH), 59.3 (C-7), 56.5 (C-5), 53.5 (C-COOCH), 45.0 (C-20), 40.1 (C-15), 38.0 (C-6), 32.1 (C-14). These data were consistent with literature values (Kim et al., 2011), identifying compound 3 as isocorynoxeine.

Compound 4: White powder, positive reaction with Dragendorff' s reagent. ESI-MS m/z: 383 [M + H] . [1]H-NMR (400 MHz, CDCl) H: 8.20 (1H, s, 1-NH),

7.27 (1H, s, H-17), 7.23 (1H, d, J = 2.8 Hz, H-9), 7.04 (1H, t, J = 7.6 Hz, H-10), 6.87 (1H, d, J = 7.7 Hz, H-12), 5.51 (1H, m, H-19), 4.99–4.85 (2H, m, H-18), 3.73 (3H, s, OCH), 3.62 (3H, s, COOCH), 3.39 (1H, t, J = 6.8 Hz, H-3), 3.28 (1H, dd, J = 10.8, 4.0 Hz, H-15), 3.00 (1H, m, H-20), 2.07–1.98 (2H, m, H-6a, 6b), 1.90 (1H, m, H-14b), 1.24 (1H, t, J = 7.2 Hz, H-14a). ^{13}C-NMR (150 MHz, CDCl) C: 181.2 (C-2), 159.7 (C-17), 140.8 (C-19), 139.5 (C-13), 133.7 (C-8), 127.9 (C-11), 123.3 (C-9), 122.6 (C-10), 115.4 (C-18), 111.6 (C-16), 109.3 (C-12), 75.0 (C-3), 58.7 (C-OCH), 56.6 (C-7), 54.8 (C-5), 51.2 (C-COOCH), 42.6 (C-20), 34.7 (C-6), 28.8 (C-14). These data matched literature values (Chen et al., 2009), identifying compound 4 as corynoxeine.

Compound 5: Brown powder, positive reaction with Dragendorff' s reagent. ESI-MS m/z: 373 [M+Na] . ^{1}H-NMR (400 MHz, CDCl) H: 9.38 (1H, s, H-21), 8.02 (1H, s, H-1), 7.68 (1H, s, H-17), 7.49 (1H, d, J = 7.7 Hz, H-10), 7.31 (1H, d, J = 7.9 Hz, H-11), 7.20–7.09 (2H, m, H-9, 12), 6.68 (1H, q, J = 7.3 Hz, H-19), 4.48 (1H, d, J = 11.5 Hz, H-3), 4.02 (1H, d, J = 4.9 Hz, H-15), 3.65 (3H, s, OCH), 2.93 (1H, m, H-6), 2.82 (1H, d, H-6), 2.17 (1H, d, H-14), 2.10 (3H, d, J = 7.5 Hz, H-18), 1.93 (1H, m, H-14). ^{13}C-NMR (100 MHz, CDCl) C: 195.9 (C-21), 168.3 (C-228), 152.9 (C-19), 147.4 (C-17), 146.4 (C-16), 136.2 (C-13), 132.4 (C-2), 126.8 (C-8), 122.1 (C-9), 119.8 (C-12), 118.1 (C-10), 111.0 (C-10), 108.4 (C-7), 94.1 (C-20), 51.1 (C-5), 50.7 (OCH), 49.3 (C-3), 34.1 (C-14), 28.3 (C-15), 22.0 (C-6), 15.1 (q, C-18). These data were consistent with literature values (Tian et al., 2014), identifying compound 5 as vallesiachotamine.

Compound 6: White powder, positive reaction with Dragendorff' s reagent. ESI-MS m/z: 393 [M + Na] . ^{1}H-NMR (400 MHz, CDCl) H: 8.23 (1H, s, NH-1), 7.46 (1H, s, H-9), 7.18 (1H, td, J = 7.7, 1.2 Hz, H-11), 7.04 (1H, t, J = 6.1 Hz, H-10), 6.87 (1H, d, J = 7.7 Hz, H-5a), 3.35 (1H, dd, J = 10.8, 3.6 Hz, H-12b), 3.30 (1H, dd, J = 8.4, 1.5 Hz, H-5b), 0.83 (1H, t, J = 7.1 Hz, H-18). ^{13}C-NMR (100 MHz, CDCl) C: 134.0 (C-8), 109.2 (C-12), 72.4 (C-3), 58.2 (C-21), 56.8 (C-7), 35.5 (C-6). These data matched literature values (Chou et al., 2009), identifying compound 6 as isorhynchophylline.

Compound 7: White powder, positive reaction with Dragendorff' s reagent. ESI-MS m/z: 385 [M + H] . ^{1}H-NMR (400 MHz, CDCl) H: 8.46 (1H, s, NH-1), 7.27 (1H, s, H-9), 7.17 (1H, d, J = 7.7 Hz, H-11), 7.04 (1H, t, J = 7.5 Hz, H-10), 6.90 (1H, d, J = 7.4 Hz, H-12), 3.72 (3H, s, OCH), 3.62 (3H, s, COOCH), 2.44 (1H, dd, J = 17.1, 8.4 Hz, H-5a), 2.02 (1H, dd, J = 12.6, 7.1 Hz, H-6a), 1.66 (1H, t, J = 10.4 Hz, H-21a), 0.82 (3H, t, J = 7.2 Hz, H-18). ^{13}C-NMR (100 MHz, CDCl) C: 181.5 (C-2), 159.8 (C-17), 140.9 (C-13), 133.8 (C-8), 127.7 (C-11), 123.1 (C-10), 109.3 (C-12), 58.2 (C-21), 56.0 (C-7), 55.0 (C-5), 37.8 (C-15), 34.8 (C-6), 31.9 (C-14), 24.2 (C-19), 11.3 (C-18). These data were consistent with literature values (Sun et al., 1995), identifying compound 7 as rhynchophylline.

Compound 8: White granular crystals. ^{1}H-NMR (600 MHz, CDCl) H: 3.64 (2H, dd, J = 11.2, 6.4 Hz, CH OH), 1.56 (2H, m, CH CH OH), 1.27 (50H, m), 0.88 (3H, t, J = 7.0 Hz, CH). ^{13}C-NMR (150 MHz, CDCl) C: 63.1 (C-1), 32.8 (C-2), 31.9 (C-26), 29.7 (C-3, 25), 25.7 (C-3), 22.7 (C-27), 14.1 (C-28). These

data matched literature values (Jiang and Xu, 2011), identifying compound 8 as octacosanol.

Compound 9: Colorless crystals. ESI-MS: m/z 415 [M + H] . ^{1}H-NMR (500 MHz, CDCl) H: 5.40 (1H, d, J = 2.1 Hz, H-6), 3.57 (1H, m, H-3), 1.06 (3H, s, H-19), 0.97 (3H, d, J = 6.2 Hz, H-21), 0.73 (3H, s, H-18). ^{13}C-NMR (125 MHz, CDCl) C: 140.8 (C-5), 121.7 (C-6), 71.8 (C-3), 56.1 (C-14), 56.1 (C-17), 50.1 (C-9), 42.3 (C-4, 13), 39.8 (C-12), 37.3 (C-1), 36.5 (C-10), 36.1 (C-20), 34.0 (C-22), 32.0 (C-7), 31.7 (C-8), 29.7 (C-2), 29.2 (C-25), 28.2 (C-16), 26.1 (C-23), 24.3 (C-15), 23.1 (C-28), 21.1 (C-11), 19.8 (C-19), 19.4 (C-27), 19.0 (C-26), 18.8 (C-21), 12.0 (C-18, 29). These data were consistent with literature values (Wang, 2014), identifying compound 9 as -sitosterol.

Compound 10: White amorphous crystals. ^{1}H-NMR (500 MHz, CDCl) H: 2.39 (2H, t, J = 7.3 Hz, 29-CH), 1.65 (2H, m, J = 4.8 Hz, 28-CH), 1.30 (52H, s, 2–27-CH), 0.93 (3H, t, J = 6.3 Hz, -CH). ^{13}C-NMR (125 MHz, CDCl) C: 33.8 (C-2), 31.6 (C-3), 29.7 (C-4-27), 24.7 (C-28), 22.7 (C-29), 14.1 (C-30). These data matched literature values (Zhang and Rao, 2016), identifying compound 10 as triacontanoic acid.

Compound 11: White needle crystals. ESI-MS m/z: 353 [M - H] . ^{1}H-NMR (500 MHz, DMSO-d) H: 12.86 (1H, s, 5-OH), 6.68 (1H, d, J = 1.4 Hz, H-8), 6.45 (1H, d, J = 1.4 Hz, H-6), 6.29 (1H, s, H-3), 5.11 (1H, d, J = 5.1 Hz, H-1), 2.42 (3H, s, CH). ^{13}C-NMR (125 MHz, DMSO-d) C: 182.5 (C-4), 168.9 (C-9), 163.3 (C-7), 161.6 (C-5), 157.9 (C-2), 108.8 (C-3), 105.5 (C-10), 99.9 (C-6), 94.9 (C-8), 77.6 (C-3), 76.8 (C-5), 73.5 (C-2), 70.0 (C-4), 61.0 (C-6). These data were consistent with literature values (Guo and Zeng, 2010), identifying compound 11 as 2-methyl-5,7-dihydroxy-chromone-7-O- -D-glucopyranoside.

Compound 12: White powder. ESI-MS m/z: 455 [M - H] . ^{1}H-NMR (600 MHz, CDCl) H: 5.30 (1H, t, J = 3.5 Hz, H-12), 3.24 (1H, dd, J = 11.3, 4.2 Hz, H-3), 2.84 (1H, dd, J = 13.8, 4.2 Hz, H-18), 1.15 (3H, s), 1.01 (3H, s), 0.95 (3H, s), 0.93 (3H, s), 0.92 (3H, s), 0.79 (3H, s), 0.77 (3H, s). ^{13}C-NMR (150 MHz, CDCl) C: 183.2 (C-28), 143.6 (C-13), 122.6 (C-12), 79.1 (C-3), 55.2 (C-5), 47.6 (C-9), 46.5 (C-17), 45.9 (C-19), 41.6 (C-14), 41.0 (C-18), 39.3 (C-8), 38.8 (C-4), 38.4 (C-1), 37.1 (C-10), 33.8 (C-21), 33.1 (C-7), 32.6 (C-22), 32.4 (C-29), 30.7 (C-20), 28.1 (C-23), 27.7 (C-15), 27.2 (C-2), 26.0 (C-27), 23.6 (C-11), 23.4 (C-30), 22.9 (C-16), 18.3 (C-6), 17.1 (C-26), 15.6 (C-24), 15.3 (C-25). These data matched literature values (Liu and Ge, 2010), identifying compound 12 as oleanolic acid.

Compound 13: Yellow powder. ESI-MS m/z: 301 [M - H] . ^{1}H-NMR (600 MHz, MeOD) H: 7.75 (1H, d, J = 2.1 Hz, H-2), 7.65 (1H, dd, J = 8.5, 2.2 Hz, H-6), 6.90 (1H, d, J = 8.5 Hz, H-5), 6.41 (1H, d, J = 2.1 Hz, H-8), 6.19 (1H, d, J = 2.1 Hz, H-6). ^{13}C-NMR (150 MHz, MeOD) C: 175.9 (C-4), 164.2 (C-7), 161.1 (C-5), 156.8 (C-9), 147.4 (C-2), 146.6 (C-4), 144.8 (C-2), 135.8 (C-3), 122.7 (C-1), 120.27 (C-6), 114.82 (C-5), 114.59 (C-2), 103.11 (C-10), 97.83 (C-6), 93.01 (C-8). These data were consistent with literature values (Xu,

2003), identifying compound 13 as quercetin.

Compound 14: White powder. ESI-MS m/z: 471 [M - H] . ^{1}H-NMR (600 MHz, DMSO-d) H: 4.17 (1H, d, J = 4.9 Hz, H-3), 3.07 (1H, dd, J = 10.3, 3.4 Hz, H-3), 2.74 (1H, dd, J = 13.8, 4.1 Hz, H-18), 1.10 (3H, s, Me), 0.72 (3H, s, Me), 0.53 (3H, s, Me). ^{13}C-NMR (150 MHz, DMSO-d)

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