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Research Article

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Chemical constituents of the aerial parts of *Cynanchum chinense* R. Br.

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ABSTRACT

*This study is focused on isolation and identification of bioactive ingredients of the aerial parts of the plant. The compounds were extracted and purified by D101 macroporous resins, silica gel column chromatography and Sephadex LH-20 columns. Their structures were elucidated on the basis of physico-chemical properties and NMR spectral analysis. Fifteen compounds were obtained from 95% aqueous ethanol extract of Cynanchum chinenseR. Br.and elucidated as 3-epiglochidiol(**01**), (+)-medioresinol(**02**), kaempferol-3-O-α-L-rahmnoside(**03**), catechin(**04**), 3β-hydroxyolean-12-en-29-oic acid(**05**), 6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-7-methoxy-3,4-dihydro-2-naphthaldehyde(**06**), detetrahydroconidendrin(**07**), kaempferol(**08**), 3-hydroxy-4-methoxybenzoic acid(**09**), epigallocatechin(**10**),2β,22β-dihydroxyo -lean-12-en-29-oic acid(**11**), epicatechin(**12**), p-hydrobenzoic acid(**13**). Compounds **01**, **05** and **11** were obtained from this genus, and compounds **01**, **02**, **04**, **05-07**, **10-11** and **12** were isolated from this plant for the first time.*

Key words: *Cynanchum chinense* R. Br.; Chemical Constituents

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INTRODUCTION

General methods

Optical rotations were acquired with a JASCO DIP-370 polarimeter. 1D and 2D NMR spectra were recorded on Bruker AM 400 and DRX-500 NMR spectrometers (BrukerBioSpin Group, German) with tetramethylsilane (TMS) as an internal standard. EIMS were measured on a VG Auto Spec-3000 mass spectrometer, whereas HRESIMS were measured using an API-QSTAR-TOF mass spectrometer. Column chromatography (CC) was performed with silica gel (100-200 mesh, 200-300 mesh; Qingdao Marine Chemical Factory, Qingdao, China), Diaion HP-20 (Mitsubishi Fine Chemical Industries Co., Ltd.), Sephadex LH-20 (40-70 μm; Amersham Pharmacia Biotech AB, Uppsala, Sweden), and YMC-GEL ODS-A (50 μm; YMC, Milford, MA). HPLC was performed on an Agilent 1200 liquid chromatography using Zorbax SB-C18 (5 μm, 9.4 mm × 250 mm) semi-preparative column or Zorbax SB-C18 (4.6 mm × 250 mm) analysis column with MeOH/H_2O in gradient. Preparative TLC (0.4-0.5 mm) was conducted on glass platespre-coated silica gel GF_{254} (Qingdao Marine Chemical Factory, Qingdao, China). Fractions were monitored by TLC plates, and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

Plant material

The Aerial Parts of *Cynanchum Chinense* R. Br. were collected Inner Mongolia and identified by Prof. Peifeng Xue(Inner Mongolia medical university). A voucher specimen has been deposited in the laboratory of faculty of life science and technology, Kunming University of Science and Technology.

EXPERIMENTAL SECTION

Extraction and Isolation

The air-dried and powdered the aerial parts of *CynanchumChinense* R. Br. (10 kg) were extracted with 95% EtOH at

r. t. and then concentrated under vacuum to yield an extract, which was suspended in H_2O and partitioned sequentially with EtOAc.

The EtOAc-soluble part (100 g) was subjected to Diaion HP20 gradiently eluted with MeOH-H_2O (30%, 50%, 70%, 90% and 100%) and 50% acetone to give six fractions, Fr.1-6. Fr. 3 (7.5 g) was further subjected to silica gel (100-200 mesh) and eluted successively with gradient $CHCl_3$-MeOH (300:1, 80:1, 60:1, 40:1, 20:1, 10:1, 5:1, and 1:1) to afford six subfractions, 3.1-3.6. Fr. 3.1 (200 mg) was rechromatographedon Sephadex LH-20 with petroleum ether-$CHCl_3$-MeOH (5:5:1) followed by preparative TLC to give compounds **1** (8 mg) and **5**(7 mg). Fr. 3.4 (2 g) was fractionated by ODS CC employing a MeOH-H_2O system (gradient 40%, 60%, 80%, and 100%) as eluent to yield three fractions, 3.4.1-3.4.3. Fr.3.4.1 (700 mg) and Fr.3.4.2 (350 mg) were further chromatographed over silica gel ($CHCl_3$-MeOH 50:1) to obtained compounds **3** (17 mg), **7** (12 mg). Fr.4 (13 g) was separated by Sephadex LH-20 using $CHCl_3$-MeOH (1:1) as eluent to give five subfractions, 4.1-4.5. Fr. 4.3 (3 g) was fractionated by ODS CC using the gradient MeOH-H_2O from 40%-90% as eluent to give four fractions, 4.3.1-4.3.4. Fr. 4.3.2 (400 mg) was further chromatographed over silica gel (petroleum ether-acetone 7:1) to give compounds **8** (17 mg), **11** (9 mg). Fr. 4.3.3 (500 mg) was successively subjected to silica gel ($CHCl_3$-MeOH 50:1) and HPLC (35% MeOH) to afford compounds **2** (12 mg), **4** (10 mg), **6** (11mg). Chromatography of fraction 4.4 (2.5g) over ODS with MeOH-H_2O (40%, 60%, 80%, 100%) gave fractions 4.4.1-4.4.5. Fr. 4.4.2 (500 mg) was subjected to silica gel chromatography using eluent mixtures of petroleum ether-EtOAc (4:1) followed by preparative TLC to afford compounds **9** (16 mg), **12** (13 mg). Fr. 4.4.3 (400 mg) was successively rechromatography on Sephadex LH-20 ($CHCl_3$-MeOH 1:1) and HPLC (28% MeOH) to give compounds **10** (13 mg). Fr. 4.4.5 (600 mg) was purified on ODS CC employing 60% MeOH-H_2O as eluent followed by preparative TLC to give compounds **13** (18 mg).

Compounds^{1}H-NMR, ^{13}C-NMR spectroscopic data

KQ01[1] $C_{29}H_{48}O_2$ UV λ_{max} (MeOH); 220nm.^{1}H-NMR ($CDCl_3$, 400MHz), δ: 0.75 (3H, s), 0.79 (3H, s), 0.90 (3H, s), 0.95 (3H, s), 1.04 (3H, s), 1.67 (3H, s), 3.23 (1H, dd, *J* = 12, 4.4Hz), 3.43 (1H, dd, *J* = 12, 4.4Hz), 4.55(1H, s), 4.67 (1H, s). ^{13}C-NMR ($CDCl_3$, 100MHz), δ: 11.9 (q, C-27), 14.4 (q, C-24), 16.2 (q, C-25), 17.9 (q, C-26), 18.0 (t, C-6), 18.0 (q, C-28), 23.8 (q, C-27), 23.8 (q, C-30), 23.8 (t, C-11), 25.0 (t, C-12), 27.5 (q, C-15), 27.7(q, C-23), 29.7 (t, C-21), 34.0 (t, C-7), 34.0 (t, C-16), 35.6 (d, C-13), 37.5(t, C-2), 38.0 (s, C-4), 38.8 (t, C-22), 39.9 (s, C-10), 42.9 (s, C-8), 43.5 (s, C-17), 47.9 (d, C-19), 48.3 (d, C-18), 51.4(d, C-9), 53.1 (d, C-4), 77.3 (d, C-1), 79.0 (d, C-3), 109.4 (t, C-29), 150.8 (s, C-20).

KQ02[2] $C_{20}H_{22}O_6$ UV λ_{max} (MeOH); 229nm, 279nm.^{1}H-NMR(400MHz, $CDCl_3$) δ: 6.89-6.9l(2H, m, H-2′, 5′), 6.82(1H, dd, J=8.1, 1.8Hz, H-6′), 6.58(2H, s, H-2, 6), 5.60(1H, s, OH-4or4′), 5.50(1H, s, OH-4′or 4), 4.75 (1H, d, J=4.5Hz, H-7′), 4.72 (1H, d, J=4.5Hz, H-7), 4.23-4.30 (2H, m, H-9, 9′), 3.90, 3.88 (9H, s, H-10, 10′, 11), 3.88-3.90 (2H, m, H-9, 9′), 3.08-3.1 (2H, m, H-8, 8′).^{13}C-NMR (100MHz, $CDCl_3$) δ: 147.1 (C-3, 5), 146.6, 145.2 (C-3′, 4′), 134.2 (C-4), 132. 8(C-1′), 132.1 (C-1), 118.9 (C-6′), 114.2 (C-5′), 108.5 (C-2′), 102.6 (C-2, 6), 86.1, 85.8 (C-7, 7′), 71.8, 71.6 (C-9, 9′), 56.3, 55.9 (C-10, 10′, 11), 54.4, 54.1 (C-8, 8′).

KQ03[3] **$C_{21}H_{20}O_{10}$**UV λ_{max} (MeOH); 224nm, 255nm, 265nm, 330nm, 366nm. ^{1}H-NMR (400MHz, DMSO-d_6) δ: 12.5 (1H, brs, 5-OH), 10.1 (1H, brs, 4′-OH), 9.56 (1H, brs, 3-OH), 8.08 (2H, d, J = 8.5 Hz, H-2′, 6′), 6.92 (2H, d, J =8.5Hz, H-3′, 5′), 6.81 (1H, d, J = 2.1 Hz, H-8), 6.41(1H, d, J = 2.1 Hz, H-6), 5.53(1H, d, J=1.0Hz, Rha-H-1), 1.12(3H, d, J = 6.0 Hz, Rha-H-6).^{13}C-NMR(100MHz, DMSO-d_6) δ: 176.1 (C-4), 161.4 (C-7), 160.4 (C-5), 159.4 (C-7), 155.8 (C-9), 147.5 (C-2), 1356.1 (C-3), 129.7 (C-2′, 6′), 121.6 (C-1′), 115.5 (C-3′, 5′), 104.7 (C-10), 98.9(C-6), 98.4 (Rha-C-1), 94.4(C-8), 71.6(Rha-C-4), 70.3 (Rha-C-3), 70.1(Rha-C-2), 69.9(Rha-C-5), 18.0(Rha-C-6).

KQ04[4] **$C_{16}H_{16}O_6$**UV λ_{max} (MeOH); 227nm, 279nm. ^{1}H-NMR (DMSO-d_6, 400MHz), δ: 2.49 (1H, dd, *J* = 20, 8 Hz, H-4β), 2.66 (1H, dd, *J* = 12, 8 Hz, H-4α), 3.80 (1H, dd, *J* = 12, 0.8 Hz, H-3), 4.46 (1H, d, *J* = 12 Hz, H-2), 5.67 (1H, s, H-6), 5.88 (1H, s, H-8), 6.60 (2H, d, *J* = 12 Hz, H-2′ , 4′), 6.68 (1H, s, H-4′). ^{13}C-NMR (DMSO-d_6, 100MHz), δ: 27.9 (t, C-4), 66.3(d, C-3), 81.0 (d, C-2), 93.8 (d, C-8), 95.1 (d, C-6), 99.1 (d, C-10), 114.5 (d, C-2′), 115.1 (d, C-5′), 118.5 (d, C-6′), 130.6 (s, C-1′), 144.9 (s, C-4′), 144.9(s, C-3′), 155.4 (s, C-5), 156.2(s, C-7) , 156.5 (s, C-9).

KQ05[5]**$C_{30}H_{48}O_3$**UV λ_{max} (MeOH); 230nm.^{1}H-NMR (DMSO-d_6, 400MHz), δ: 0.84 (3H, s), 0.89 (3H, s), 0.91 (3H, s), 0.94 (3H, s), 1.10 (3H, s), 1.22 (3H, s), 1.46 (3H, s), 2.50 (1H, t), 3.35 (1H, dd, *J* = 11.5, 5 Hz), 5.19 (1H, t).^{13}C-NMR (DMSO-d_6, 100MHz), δ: 15.3 q, C-25), 16.1 (q, C-24), 16.6 (q, C-26), 19.2 (t, C-6), 19.2 (q, C-30), 19.2 (t, C-11), 25.8 (q, C-27), 25.8 (t, C-15), 25.8 (t, C-16), 27.9 (t, C-2), 28.2 (q, C-23), 28.2 (q, C-28), 28.2 (t, C-21), 32.1 (t, C-7), 32.1 (s, C-17), 36.5 (t, C-22), 38.4 (s, C-10), 38.4 (t, C-1), 38.4 (s, C-4), 41.2 (s, C-8), 41.2 (t, C-19), 41.2 (s, C-14), 41.6 (s, C-30), 45.5 (d, C-18), 47.1 (d, C-9), 54.7 (d, C-5), 76.8 (d, C-3), 122.3(d, C-11), 143.9 (s, C-13), 179.7 (s, C-29).

KQ06[6] $\mathbf{C_{20}H_{20}O_6}$ UV λ_{max} (MeOH); 256nm, 362nm. ^{1}H-NMR (DMSO-d_6, 400MHz) *δ*: 7.4 9(1H, s, H-1),3.01 (1H, m, H-3),4.25 (1H, s, H-4), 6.65 (1H, s, H-5), 7.1 (1H, s, H-8) 6.61 (1H, J=1.8, H-2′), 6.54 (1H, J=8.2, H-5′), 6.15 (1H, J=8.2, J=1.8, H-6′), 9.45 (1H, s, H-2α), 3.32 (1H, m, H-3α), 2.90 (1H, m, H-3α), 3.65 (3H, s, 7-OCH_3), 3.80 (3H, s, 3′- OCH_3).^{13}C-NMR (DMSO-d_6, 100MHz) *δ*: 41.9 (C-3), 42.1 (C-4), 48.6 (C-5-OCH_3), 55.6 (C-7-OCH_3), 55.7 (C-3′-OCH_3), 60.6 (C-5α), 111.5 (C-2′), 113.0 (C-5), 115.2 (C-5′), 117.4 (C-8), 119.2 (C-6′), 122.7 (C-9), 132.8 (C-10), 134.4 (C-2), 136.0 (C-1′), 144.8 (C-4′), 146.6 (C-7), 147.1 (C-1), 147.3 (C-3′), 149.7 (C-6), 192.5 (C-2α).

KQ07[7] $\mathbf{C_{20}H_{16}O_6}$ UV λ_{max} (MeOH); 223nm, 265nm, 367nm. ^{1}H-NMR (DMSO-d_6, 400MHz) δ: 10.10 (6-OH), 9.28 (4′-OH), 8.30 (1H, s, H-1), 7.62 (1H, s, H-8), 7.18 (1H, s, H-5), 6.99 (1H, d, J=1.8HZ, H-2′), 6.95 (1H, d, t, =8.4HZ, H-5′), 6.85 (1H, dd, J=1.8, 8.4HZ, H-6′), 5.38 (1H, d, J=14.4HZ, Ha-3α), 5.27 (1H, d, J=14.4 HZ, Hb-3α), 3.92 (3H, s, OCH_3-3′), 3.80 (3H, s, OCH_3-7).^{13}C-NMR (DMSO-d_6, 100MHz) δ: 171.2 (C-2a), 150.2 (C-6), 149.5 (C-7), 147.9 (C-3′), 146.4 (C-4′), 137.5 (C-3), 131.6 (C-10), 131.2 (C-4), 129.1 (C-9), 126.8 (C-1′), 123.3 (C-1), 121.9 (C-6′), 119.9 (C-2), 115.8 (C-5′), 113.3 (C-2′), 108.5 (C-8), 107.4 (C-5), 69.4 (C-3α), 55.8 (OCH_3-3′), 55.6 (OCH_3-7).

KQ8[8] $\mathbf{C_{15}H_{10}O_6}$UV λ_{max} (MeOH); 220nm, 265nm, 367nm. ^{1}H-NMR(DMSO-d_6, 400MHz)*δ*: 12.48 (1H, s, 5-OH), 8.03 (2H, d, J=8.8 Hz, H-2′, 6′), 6.93 (2H, d, J=8.8 Hz, H-3′, 5′), 6.90 (1H, s, H-3), 6.42 (1H, s, H-8), 6.17 (1H, d, H-6).^{13}C-NMR(DMSO-d_6, 100MHz) δ: 175.91 (C-4), 164.0 (C-2), 164.0 (C-7), 160.7 (C-4′), 159.2 (C-9), 156.2 (C-5), 129.5 (C-2′, 6′), 121.7 (C-1′), 115.5 (C-3′, 5′), 104.43 (C-10), 103.3 (C-3), 98.3 (C-6), 93.5 (C-8).

KQ9[9] $\mathbf{C_8H_8O_4}$ UV λ_{max} (MeOH); 214nm, 255nm, 290nm. ^{1}H-NMR (DMSO-d_6, 400MHz) δ:2.49 (3H, s, -CH_3), 6.83 (1H, s, J=8.5HZ, -OH), 7.41-7.49 (2H, m, H-2, 6), 12.5 (1H, s, -COOH).^{13}C-NMR(DMSO-d_6, 100MHz) δ:55.5 (s, -OCH_3), 112.7 (s, C-2), 115.0 (s, C-6), 121.7 (s, C- 1), 123.5 (s, C-5).

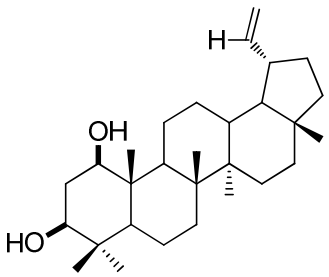
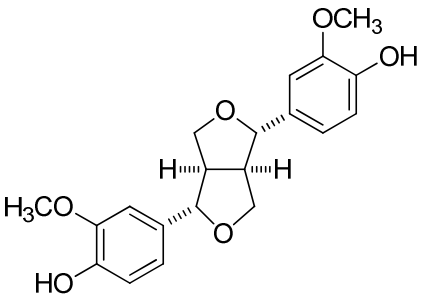
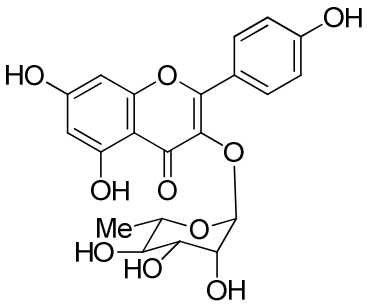
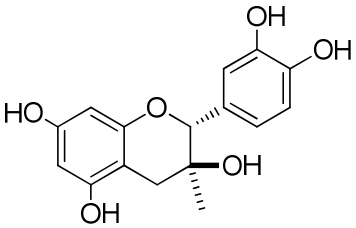
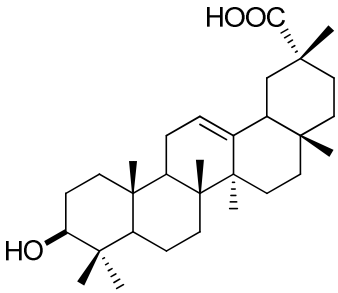
KQ10[10] $\mathbf{C_{16}H_{16}O_7}$ UV λ_{max} (MeOH); 230nm. ^{1}H-NMR (DMSO-d_6, 400MHz), δ: 2.63 (1H, d, *J* = 12 Hz, H-4β), 2.68 (1H, dd, *J* = 12 , 4 Hz, H-4α), 4.13 (1H, d, *J* = 4 Hz, H-3), 4.65 (1H, d, *J* = 4 Hz, H-2), 5.88 (1H, d, *J* = 2 Hz, H-6), 6.3 (1H, d, *J* = 2 Hz, H-8), 6.44 (2H, s, H-2′, 6′).^{13}C-NMR (DMSO-d_6, 100MHz), δ: 28.2 (t, C-4), 65.0 (d, C-3), 78.1 (d, C-2), 94.2 (d, C-8), 95.0 (d, C-6), 98.6 (d, C-10), 106.0 (d, C-2′, 6′), 130.0 (s, C-1′), 132.1 (s, C-4′), 145.4 (s, C-3′, 5′), 155.8 (s, C-5), 156.2 (s, C-7) , 156.5 (s, C-9).

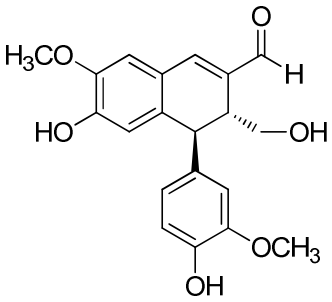
KQ11[11] $\mathbf{C_{30}H_{48}O_4}$ ^{1}H-NMR (DMSO-d_6, 400MHz) δ: 0.91 (3H, s), 0.87 (3H, s), 1.00 (3H, s), 1.09 (3H, s), 1.22 (3H, s), 1.27 (3H, s), 1.44 (3H, s), 1.58 (3H, s), 2.50 (2H, t, *J* = 12Hz), 2.99 (2H, s), 3.50 (1H, brs), 3.31 (1H, brs, *J* = 12, 4.4Hz), 5.15(1H, s), 5.18 (1H, s). ^{13}C-NMR (DMSO, 100MHz) δ:15.3 (q, C-25), 16.1 (q, C-26), 16.1 (t, C-6), 20.4 (t, C-16), 20.4 (q, C-24), 20.4 (q, C-30), 24.6 (t, C-11), 24.6 (q, C-27), 26.0 (t, C-2), 28.2 (t, C-15), 28.2 (q, C-23), 36.5 (t, C-7), 36.5 (t, C-1), 37.9 (s, C-10), 37.9 (s, C-8), 38.4 (t, C-19), 39.3 (s, C-4), 40.1 (s, C-17), 41.9 (t, C-2), 41.9 (s, C-14), 47.1 (s, C-20), 47.1 (d, C-18), 47.1 (d, C-9), 54.7 (d, C-5), 73.0 (d, C-3), 76.8 (d, C-22), 122.3 (d, C-12), 143.4 (s, C-13), 179.0 (s, C-29).

KQ12[12] $\mathbf{C_{16}H_{16}O_6}$ UV λ_{max} (MeOH); 228nm, 279nm. ^{1}H-NMR (DMSO-d_6, 400MHz) δ: 4.72 (1H, s, H-2), 3.99 (1H, d, J=3.3 Hz, H-3), 2.68 (1H, dd, J=4.5, 16.35 HZ, H4-α), 2.45 (1H, dd, J=3.6, 16.35HZ, H-4-β), 5.70 (1H, d, J=2.1 HZ, H-6), 5.88 (1H, d, J=2.1 Hz, H-8), 6.64 (2H, s, H-5′, 6′), 6.88 (1H, s, H-2′). ^{13}C-NMR (DMSO-d_6, 100MHz), δ: 28.2 (t, C-4), 64.9 (d, C-3), 78.1 (d, C-2), 94.1 (d, C-8), 95.1 (d, C-6), 98.5 (d, C-10), 114.8 (d, C-2′), 114.9 (d, C-5′), 118.0 (d, C-6′), 130.6 (s, C-1′), 144.5 (s, C-4′), 144.5 (s, C-3′), 155.8 (s, C-5), 155.3 (s, C-7), 156.6 (s, C-9).

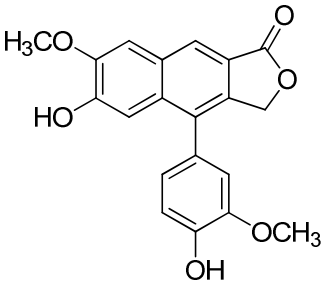
KQ13[13] $\mathbf{C_7H_6O_3}$ UV λ_{max} (MeOH); 253nm.^{1}H-NMR (DMSO-d_6, 400MHz) δ: 7.78 (2H, d, J=9.4 Hz, H-2, H-6), 6.81 (2H, d, J=9.4 Hz, H-3, H-5). ^{13}C-NMR(DMSO-d_6, 100MHz) δ: 167.3 (C=O), 161.7 (C-1) , 131.6 (C-5), 131.7 (C-3), 121.5 (C-4), 115.2(C-2), 115.2(C-6).

Name, formula and structure of compounds

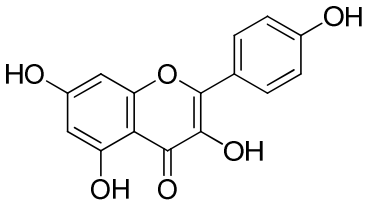
Name	Formula	Structure
3-epiglochidiol(**01**)	$C_{29}H_{48}O_2$	
(+)-medioresinol(**02**)	$C_{20}H_{22}O_6$	
kaempferol-7-O-α-L- rahmnoside(**03**)	$C_{21}H_{20}O_{10}$	
Catechin(**04**)	$C_{16}H_{16}O_6$	
3β-hydroxyolean-12-en-29-oic acid(**05**)	$C_{30}H_{48}O_3$	
6-hydroxy-4-(4-hydroxy-3-methoxyphenyl) -3-hydroxymethyl-7-methoxy-3,4-dihydro -2-naphthaldehyde(**06**)	$C_{20}H_{20}O_6$	



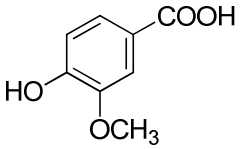
detetrahydroconidendrin(**07**) $C_{20}H_{16}O_6$



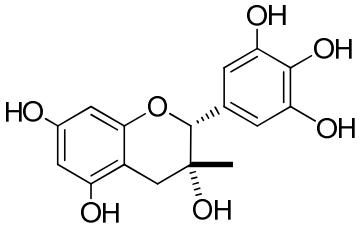
apigenin(**08**) $C_{15}H_{10}O_6$



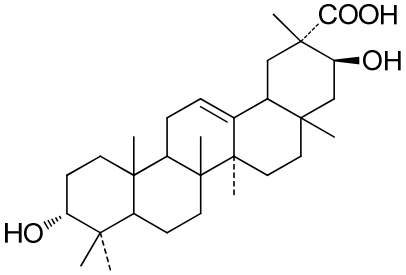
3-hydroxy-4-methoxybenzoic acid(**09**) $C_8H_8O_4$



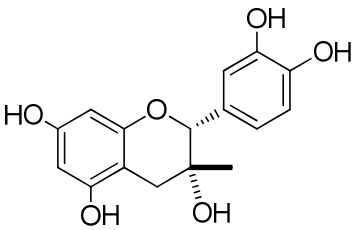
epigallocatechin(**10**) $C_{16}H_{16}H_7$



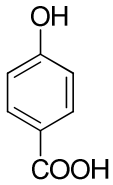
2β,22β-dihydroxyolean-1
-en-29-oic acid(**11**) $C_{30}H_{48}O_4$



epicatechin(**12**) $C_{16}H_{16}O_6$



p-hydrobenzoic acid(**13**) $C_7H_6O_3$



CONCLUSION

Cynanchumchinense R. Br. is a perennially indigenous herb in many frigid regions including north China. As a traditional Chinese medicine, it has been used in China for fever, detoxication, analgesia and the milk treatment for excrescence, etc. Now Protease, Flavonoid glycosides, Diterpeneesters and Organic acids have been isolated from *Cynanchumchinense* R.Br. *Cynanchumchinense* R. Br. can affect cardiac muscle, immunity, anti-convulsant and kill virus in clinical efficacy. The compounds were extracted and purified by D101 macroporousresins, silica gel column chromatography and Sephadex LH-20 columns. Their structures were elucidated on the basis of physic chemical properties and NMR spectral analysis. This study is focused on isolation and identification of bioactive ingredients of the aerial parts of the plant. Fifteen compounds were obtained from 95% aqueous ethanol extract of *Cynanchumchinense* R.Br. Compounds **01**, **05** and **11** were obtained from this genus, and compounds **01**, **02**, **04**, **05-07**, **10-11** and **12** were isolated from this plant for the first time.

This research summarized systemic scientific data, for established the foundation of the pharmacognosy, quality control, quality standard, isolation and identification of chemical components, pharmacological activity of *Cynanchumchinense* R. Br., which is definitely helpful for further research on *Cynanchunchinens*e R. Br.

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