Chemical constituents from the rhizome of Dioscorea bulbifera L.

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Abstract

A phytochemical investigation of the rhizome of *Dioscorea bulbifera* L. led to the isolation of twenty compounds, including eight phenanthrenes (**1**–**8**), eight flavonoids (**9-16**), two diterpenes (**17**, **18**), one bibenzyl derivatives (**19**), and one acid (**20**). The structures of these compounds were elucidated on the basis of spectroscopic analysis and comparison with previous literature data. Among them, **1** and **16** were isolated from the rhizome of *Dioscorea bulbifera* L. for the first time and compounds **16** first reported from the genus *Dioscorea.* They were tested in vitro for cytotoxic activities against CT26 cell lines and Compound **1** exhibits cytotoxic activity with IC_{50} value of 83.23 μM.

Introduction

Colorectal cancer (CRC), the third most common cancer in the elders, has seriously threatened people's normal life with the symptoms of diarrhea, constipation, and anemia; There were over 140 000 cases diagnosed in the United States (US) in 2018. [1] Compared with the declining incidence of CRC in older people, it has been increasing in younger people in recent decades; Since the mid-1980s, CRC incidence have increased 2.4 percent per year for adults ages 20 to 29 and 1.0 percent per year for adults ages 30 to 39 [2].

Natural medicinal plants, the main source of new drugs and therapeutic agents, can provide a large number of small molecules that are not only uniquely diverse in structure but also have a wide range of biological activities. *Dioscorea bulbifera* L., a traditional medicinal plant in Asia, Northern Australia, America, and tropical Africa, is gaining more attention for its unique chemical compositions and a wide range of bioactivities such as anticancer, antibacterial, antiviral, anti-inflammatory, and antioxidant [3]. *D. bulbifera* is widely recognized for the treatment of cancer; The extracts of *D. bulbifera* significantly inhibited the tumor growth in colon cancer, human liver cancer and other tumor cells. It has been reported that the rhizome of *Dioscorea bulbifera* L. is rich in diterpenes, flavonoids, phenanthrene, and steroids. [4, 5]. In the present study, we reported the isolation of metabolites from *D. bulbifera*, the structural identification, and against CT26 cell lines activities evaluation of the isolates which would provide a reference for the processing and comprehensive utilization in the future.

Results And Discussion

Structural elucidation

As a result, twenty compounds were obtained (Fig. 1). Structural elucidation of the isolated compounds was done based on HR-ESI-MS, NMR analysis, and compared with the data reported in literatures.

2,2',4,4',6,6',7,7'-octahydroxy-1,1'-biphenanthrene (1)

Brown powder; HR-ESI-MS (m/z): 483.1088 $[M + H]^+$. ^{1}H-NMR (600MHz, CD_3OD), δ_H: 9.27 (1H, s, H-5,5'), 7.21 (1H, d, J = 9.2Hz, H-9,9'), 7.05 (1H, s, H-8,8'), 6.83 (1H, d, J = 9.2Hz, H-10,10'), 6.81 (1H, s, H-3,3')

^{13}C-NMR (151MHz, CD_3OD), δ_C:158.1 (C-4,4'), 154.3 (C-2,2'), 146.2 (C-6,6'), 145.1 (C-7,7'), 136.0 (C-10a,10a'), 127.8 (C-8a,8a'), 127.7 (C-9,9'), 127.0 (C-4b,4b'), 123.2 (C-10,10'), 115.6 (C-4a,4a'), 114.1 (C-5,5'), 112.4 (C-8,8'), 110.2 (C-1,1'), 102.9 (C-3,3'). The data are consistent with those reported previously [6]. Compound 1 is isolated from the rhizome of *Dioscorea bulbifera* L. for the first time.

2,7-dihydroxy-4-methoxyphenanthrene (2)

$C_{15}H_{12}O_3$, m/z 240.26; ^{1}H-NMR (600MHz, CD_3OD), δ_H: 9.33(1H, d, *J* = 9.3 Hz, H-5), 7.50 (1H, d, *J* = 8.8 Hz, H-9), 7.46 (1H, d, *J* = 8.8 Hz, H-10), 7.13 (1H, d, *J* = 2.8 Hz, H-8), 7.07 (1H, dd, *J* = 9.3, 2.8 Hz, H-6), 6.81(1H, d, *J* = 2.4 Hz, H-1), 6.75 (1H, d, *J* = 2.4 Hz, H-3), 4.08 (3H, s, 4-OCH_3).

^{13}C-NMR (151MHz, CD_3OD), δ_C: 160.6 (C-4), 156.3 (C-2), 155.3 (C-7), 135.8 (C-8a), 134.7 (C-4b), 130.1 (C-6), 128.4 (C-9), 127.9 (C-10), 125.5 (C-10a), 117.2 (C-5), 116.0 (C-4a), 112.3 (C-8), 105.7 (C-1), 100.3 (C-3), 55.9 (4-OCH_3). The data are consistent with those reported previously [7].

2,7-dihydroxy-3,4-dimethoxyphenanthrene (3)

$C_{16}H_{14}O_4$, m/z 270.28; ^{1}H-NMR (600MHz, CD_3OD), δ_H: 9.26 (1H, d, *J* = 9.2 Hz, H-5), 7.42 (2H, d, *J* = 1.8 Hz, H-9, 10), 7.13 (1H, d, *J* = 2.8 Hz, H-8), 7.09 (1H, dd, *J* = 9.2, 2.8 Hz, H-6), 7.04 (1H, s, H-1), 3.99 (3H, s, 3-OCH_3), 3.93 (3H, s, 4-OCH_3).

^{13}C-NMR (151MHz, CD_3OD), δ_C: 155.9 (C-7), 152.6 (C-2), 150.1 (C-4), 143.2 (C-3), 134.9 (C-10a), 130.7 (C-8a), 129.0 (C-5), 127.7 (C-9), 127.2 (C-10), 124.6 (C-4b), 119.5 (C-4a), 117.5 (C-8), 112.5 (C-6), 110.1 (C-1), 61.4 (3-OCH_3), 60.3 (4-OCH_3). The data are consistent with those reported previously [8].

2,3,7-trihydroxy-4-methoxyphenanthrene (4)

$C_{15}H_{12}O_4$, m/z 256.26; ^{1}H-NMR (600MHz, CD_3OD), δ_H: 9.25 (1H, d, *J* = 9.2 Hz, H-5), 7.45 (1H, d, *J* = 8.8 Hz, H-9), 7.37 (1H, d, *J* = 8.8 Hz, H-10), 7.14 (1H, d, *J* = 2.8 Hz, H-8), 7.10 (1H, dd, *J* = 9.2, 2.8 Hz, H-6), 7.04 (1H, s, H-1), 3.87 (3H, s, 4-OCH_3).

^{13}C-NMR (151MHz, CD_3OD), δ_C: C: 155.7 (C-7), 146.4 (C-4), 145.8 (C-2), 140.6 (C-3), 135.1 (C-8a), 128.9 (C-5), 127.9 (C-10), 127.2 (C-10a), 125.3 (C-9), 124.2 (C-4b), 119.3 (C-4a), 117.2 (C-8), 112.2 (C-6), 109.6 (C-1), 59.7 (4-OCH_3). The data are consistent with those reported previously [9].

3,7-dihydroxy-2,4-dimethoxyphenanthrene (5)

$C_{16}H_{14}O_4$, m/z 270.28;^{1}H-NMR (600MHz, CD_3OD), δ_H: 9.29 (1H, d, *J* = 9.2 Hz, H-5), 7.57 (1H, d, *J* = 8.7 Hz, H-9), 7.43 (1H, d, *J* = 8.7 Hz, H-10), δ_H 7.18 (1H, s, H-1), 7.16 (1H, d, *J* = 2.7Hz, H-8), 7.11 (1H, dd, *J* = 9.2, 2.7 Hz, H-6), 4.02 (3H, s, 2-OCH_3), 3.90 (3H, s, 4-OCH_3).

^{13}C-NMR (151MHz, CD_3OD), δ_C: 156.0 (C-7), 148.9 (C-2), 145.7 (C-4), 141.1 (C-3), 135.3 (C-8a), 129.1 (C-9), 128.2 (C-10), 127.0 (C-10a), 125.5 (C-5), 124.2 (C-4a), 120.3 (C-4b), 117.3 (C-8), 112.2 (C-6), 106.1 (C-1),

69.8 (4-OCH_3), 56.4 (2-OCH_3). The data are consistent with those reported previously [8].

Hircinol (6)

$C_{15}H_{14}O_3$, m/z 242.27; ^{1}H-NMR (600MHz, CD_3OD), δ_H: 7.06 (1H, t, *J* = 7.7 Hz, H-7), 6.82 (2H, dd, *J* = 7.7, 3.7 Hz, H-6, 8), 6.55 (1H, d, *J* = 2.4 Hz, H-1), 6.50 (1H, d, *J* = 2.4 Hz, H-3), 3.94 (3H, s, 4-OCH_3), 2.65 (2H, d, *J* = 7.2 Hz, H-9), 2.61 (2H, d, *J* = 7.4 Hz, H-10).

^{13}C-NMR (151MHz, CD_3OD), δ_C: 159.0 (C-2), 156.8 (C-4), 154.4 (C-5), 144.6 (C-10a), 141.8 (C-8a), 128.3 (C-7), 122.0 (C-4b), 120.6 (C-8), 118.2 (C-6), 115.0 (C-4a), 110.1 (C-1), 100.2 (C-3), 57.5 (4-OCH_3), 32.2 (C-10), 32.0 (C-9). The data are consistent with those reported previously [10].

7-hydroxy-2,3,4-trimethoxy-9,10-dihydrophenanthrene (7)

$C_{17}H_{18}O_4$, m/z 286.33; ^{1}H-NMR (600MHz, CD_3OD), δ_H: 8.06 (1H, d, *J* = 9.3 Hz, H-5), 6.70 (1H, s, H-1), 6.67 (2H, d, *J* = 6.9 Hz, H-6, 8), 3.85 (3H, s, 3-OCH_3), 3.86 (3H, s, 2-OCH_3), 3.70 (3H, s, 4-OCH_3), 2.68 (4H, q, *J* = 2.7Hz, H-9, 10).

^{13}C-NMR (151MHz, CD_3OD), δ_C: 157.0 (C-7), 152.7 (C-2), 152.7 (C-3), 142.6 (C-4), 140.8 (C-8a), 135.6 (C-10a), 129.5 (C-5), 125.5 (C-4b), 122.3 (C-4a), 115.3 (C-8), 114.2 (C-6), 108.8 (C-1), 61.4 (3-OCH_3), 60.8 (4-OCH_3), 56.4 (2-OCH_3), 31.3 (C-9), 31.1 (C-10). The data are consistent with those reported previously [11].

2,3,5,7-tetrahydroxy-9,10-dihydrophenanthrene (8)

$C_{14}H_{12}O_4$, m/z 244.25; ^{1}H-NMR (600MHz, CD_3OD), δ_H: 7.89 (1H, s, H-1), 6.60 (1H, s, H-4), 6.24 (1H, d, *J* = 2.4 Hz, H-6), 6.19 (1H, d, *J* = 2.4Hz, H-8), 2.60 (2H, m, H-9), 2.56 (2H, m, H-10).

^{13}C-NMR (151MHz, CD_3OD), δ_C:156.9 (C-5), 156.3 (C-7), 143.6(C-2), 143.6 (C-3), 141.9 (C-8a), 130.6 (C-10a), 126.7 (C-4a), 116.6 (C-8), 115.3 (C-4), 115.2 (C-4b), 107.6 (C-1), 102.6 (C-6), 32.2 (C-9), 30.3 (C-10). The data are consistent with those reported previously [12].

3,7-dimethoxy-3',4',5-trihydroxyflavone (9)

Yellow powder. $C_{17}H_{14}O_7$, m/z 330.07; ^{1}H-NMR(600MHz, CD_3OD), δ_H: 7.62 (1H, d, *J* = 2.2 Hz, H-2'), 7.52 (1H, dd, *J* = 8.5, 2.2 Hz, H-6'), 6.90 (1H, d, *J* = 8.5 Hz, H-5'), 6.47 (1H, d, *J* = 2.1 Hz, H-8), 6.39 (1H, d, *J* = 2.1 Hz, H-6).

^{13}C-NMR (151MHz, CD_3OD), δ_C:176.1 (C-4), 166.0 (C-7), 162.4 (C-8a), 160.3 (C-5), 155.3 (C-2), 149.5 (C-4'), 146.4 (C-3'), 141.3 (C-3), 123.1 (C-1'), 121.9 (C-6'), 116.3 (C-2'), 116.2 (C-5'), 108.3 (C-4a), 97.5 (C-6), 96.2 (C-8), 60.2 (7-OCH_3), 56.3 (3-OCH_3). The data are consistent with those reported previously [13].

3,7-dimethoxy-5,4'-dihydroxyflavone (10)

Pale yellow powder. $C_{17}H_{14}O_6$, m/z 314.08; ^{1}H-NMR(600MHz, CD_3OD), δ_H: 7.98 (2H, d, *J* = 8.9 Hz, H-2', 6'), 6.93 (2H, d, *J* = 8.9 Hz, H-3', 5'), 6.51 (1H, d, *J* = 2.1Hz, H-8), 6.41 (1H, d, *J* = 2.1 Hz, H-6).

^{13}C-NMR (151MHz, CD_3OD), δ_C: 176.1 (C-4), 165.0 (C-7), 162.5 (C-8a), 161.3 (C-4'), 160.2 (C-5), 155.5 (C-2), 141.3 (C-3), 131.1 (C-2',6'), 122.7 (C-1'), 116.4 (C-3',5'), 108.6 (C-4a), 97.2 (C-6), 96.1 (C-8), 60.2 (7-OCH_3), 56.4 (3-OCH_3). The data are consistent with those reported previously [9].

Kaempferol (11)

Yellow powder. $C_{15}H_{10}O_6$, m/z 286.05; ^{1}H-NMR (600MHz, CD_3OD), δ_H: 8.10 (2H, d, *J* = 8.9 Hz, H-2', 6'), 6.92 (2H, d, *J* = 8.9 Hz, H-3', 5'), 6.41 (1H, d, *J* = 2.1 Hz, H-8), 6.19 (1H, d, *J* = 2.1 Hz, H-6).

^{13}C NMR (151 MHz, MeOD), δ_C: 177.3 (C-4), 165.5 (C-7), 162.5 (C-5), 160.5 (C-4'), 158.2 (C-9), 148.0 (C-2), 137.1 (C-3), 130.6 (C-2',6'), 123.7(C-1'), 116.3 (C-3',5'), 104.5 (C-10), 99.2 (C-6), 94.4 (C-8).The data are consistent with those reported previously [14].

Myricetin (12)

Yellow acicular crystal. $C_{15}H_{10}O_8$, m/z 318.04; ^{1}H-NMR (600MHz, CD_3OD), δ_H: 7.36 (2H, s, H-2', 6'), 6.38 (1H, d, *J* = 2.1 Hz, H-8), 6.19 (1H, d, *J* = 2.1Hz, H-6). The data are consistent with those reported previously [12].

Quercetin (13)

Yellow acicular crystal. $C_{15}H_{10}O_7$, m/z 318.04; ^{1}H-NMR (600MHz, CD_3OD), δ_H: 7.74 (1H, d, *J* = 2.2 Hz, H-2'), 7.64 (1H, dd, *J* = 8.5, 2.2 Hz, H-6'), 6.89 (1H, d, *J* = 8.5 Hz, H-5'), 6.39 (1H, d, *J* = 2.1 Hz, H-8), 6.19 (1H, d, *J* = 2.1 Hz, H-6).

^{13}C NMR (151 MHz, MeOD), δ_C:177.3 (C-4), 165.5 (C-7), 162.5 (C-5), 158.2 (C-9), 148.7 (C-2), 147.9 (C-4'), 146.2 (C-3'), 137.2 (C-3), 124.1 (C-1'), 121.6 (C-6'), 116.2 (C-5'), 115.9 (C-2'), 104.5 (C-10), 99.2 (C-6), 94.4 (C-8).The data are consistent with those reported previously [14].

Hyperoside (14)

Yellow acicular crystal. $C_{21}H_{20}O_{12}$, m/z 464.10; ^{1}H-NMR(600MHz, CD_3OD), δ_H: 7.72 (1H, d, *J* = 2.2 Hz, H-2'), 7.60 (1H, dd, *J* = 8.5, 2.2 Hz, H-6'), 6.88 (1H, d, *J* = 8.5 Hz, H-5'), 6.41 (1H, d, *J* = 1.9 Hz, H-8), 6.22 (1H, d, *J* = 2.1 Hz, H-6), 5.27 (1H, d, *J* = 7.7 Hz, Glu-1")3.72 (1H, dd, *J* = 11.9, 2.4 Hz, Glu-6"), 3.58 (1H, dd, *J* = 11.9, 5.4 Hz, Glu-6"), 3.49 (1H, dd, *J* = 9.1, 7.6 Hz, Glu-2"), 3.43(1H, t, *J* = 9.0Hz, Glu-3"), 3.36 (1H, m, Glu-4"), 3.23 (1H, ddd, *J* = 9.7, 5.4, 2.4 Hz, Glu-5")

^{13}C-NMR (151MHz, CD_3OD), δ_C: 179.5 (C-4), 166.0 (C-7), 163.0 (C-5), 159.0 (C-2), 158.4 (C-8a), 149.8 (C-4'), 145.9 (C-3'), 135.6 (C-3), 123.1 (C-6'), 123.0 (C-1'), 117.5 (C-5'), 116.0 (C-2'), 105.6(C-4a), 104.2 (C-1"), 99.9 (C-6), 94.7 (C-8), 78.4 (C-5"), 78.1 (C-3"), 75.7 (C-2"), 71.2 (C-4"), 62.5 (C-6"). The data are consistent with those reported previously [14].

(+) Catechin (15)

Dark red powder. $C_{15}H_{14}O_6$, m/z 290.08; ^{1}H-NMR (600MHz, CD_3OD), δ_H: 6.85 (1H, d, *J* = 2.0 Hz, H-2'), 6.77 (1H, d, *J* = 8.1 Hz, H-5'), 6.73 (1H, dd, *J* = 8.2, 2.0 Hz, H-6'), 5.94 (1H, d, *J* = 2.3 Hz, H-8), 5.87 (1H, d, *J* = 2.3 Hz, H-6), 4.58 (1H, d, *J* = 7.5 Hz, H-2), 3.99 (1H, td, *J* = 7.9, 5.5 Hz, H-3), 2.86 (1H, dd, *J* = 16.1, 5.5 Hz, H-4*α*), 2.52 (1H, dd, *J* = 16.1, 8.2 Hz, H-4*β*).

^{13}C-NMR (151MHz, CD_3OD), δ_C: 157.8 (C-8a), 157.5 (C-7), 156.9 (C-5), 146.2 (C-4'), 146.2 (C-3'), 132.2 (C-1'), 120.0(C-2'), 116.0(C-5'), 115.2 (C-6'), 100.8 (C-4a), 96.2 (C-6), 95.4 (C-8), 82.8 (C-2), 68.8 (C-3), 28.5 (C-4). The data are consistent with those reported previously [14].

Quercetin-3-O -β- **D-glucopyranoside-(3'→O-3''')-quercetin-3''-O** -β- **D-galactopyranoside (16)**

^{1}H-NMR (600MHz, CD_3OD), δ_H: 7.84 (1H, d, *J* = 2.2 Hz, H-2'''), 7.71 (1H, d, *J* = 2.1 Hz, H-2'), 7.58 (1H, d, *J* = 8.4 Hz, H-6', 6'''), 6.86 (1H, dd, *J* = 8.4, 3.2 Hz, H-5', 5'''), 6.38 (1H, dd, *J* = 4.1, 2.1 Hz, H-8, 8''), 6.19 (1H, d, *J* = 2.1 Hz, H-6, 6''), 5.25 (1H, d, *J* = 7.6 Hz, H-Glu-1''''), 5.16 (1H, d, *J* = 7.8 Hz, H-Gal-1''''), 3.87 (1H, d, *J* = 3.4 Hz, H-Gal-4''''), 3.82 (1H, dd, *J* = 9.7, 7.8 Hz, H-Gal-2''''), 3.71 (1H, dd, *J* = 11.9, 2.3 Hz, H-Glu-6''''), 3.64 (1H, dd, *J* = 11.2, 6.0 Hz, H-Gal-6''''), 3.57 (3H, td, *J* = 11.6, 5.8 Hz, H-Glu-1'''', Gal-3'''', 5''''), 3.48 (2H, m, H-Glu-2'''', 5''''), 3.43 (1H, t, *J* = 9.0 Hz, Gal-6''''), 3.35 (1H, t, *J* = 9.3 Hz, H-Glu-4''''), 3.23 (1H, dq, *J* = 7.3, 2.7 Hz, H-Glu-3'''').

^{13}C-NMR (151MHz, CD_3OD), δ_C: 179.5 (C-4''), 179.4 (C-4), 166.2 (C-7''), 166.1 (C-7), 163.0 (C-5, 5''), 158.9(C-2), 158.7 (C-2''), 158.4 (C-9, 9'') 149.93 (C-4'''), 149.84 (C-4'), 145.8 (C-3'''), 145.7(C-4'), 135.7 (C-3''), 135.6 (C-3), 123.1 (C-6'''), 123.06 (C-6'), 122.9 (C-1'''), 122.8 (C-1'), 117.7 (C-2'''), 117.5 (C-2'), 116.0 (C-5'''), 115.9 (C-5'), 105.6 (C-10''), 105.5 (C-10), 105.4(C-Gal-1''''), 104.31 (C-Glu-1''''), 99.9 (C-6, 6''), 94.7 (C-8, 8''), 78.3 (C-5''''), 78.1 (C-3''''), 77.1 (C-5''''), 75.7 (C-2''''), 75.0 (C-3''''), 73.1 (C-2''''), 71.2 (C-4''''), 70.0 (C-4''''), 62.5 (C-6''''), 61.9 (C-6''''). The data are consistent with those reported previously [15].

8-epidiosbulbin E acetate (17)

White powder.$C_{21}H_{24}O_7$, m/z 388.15; ^{1}H-NMR (600MHz, CD_3OD), δ_H: 7.65 (1H, m, H-16), 7.50 (1H, t, J = 1.8 Hz, H-15), 6.56 (1H, dd, *J* = 1.8, 0.9 Hz, H-14), 5.56 (1H, dd, *J* = 12.7, 3.5 Hz, H-12), 5.09 (1H, q, *J* = 2.7 Hz, H-6), 4.88 (1H, t, *J* = 5.2 Hz, H-2), 2.70 (1H, dt, *J* = 15.0, 2.6 Hz, H-7a), 2.51 (1H, m, H-3a, 4, 10), 2.17 (3H, m, H-1a, 8, 11a), 2.04 (1H, ddd, *J* = 15.0, 6.2, 2.5 Hz, H-7b), 2.05 (1H, ddd, *J* = 15.0, 6.2, 2.5 Hz, H-5), 1.92(1H, m, H-11b), 1.90 (3H, s, 21-CH_3),1.88(1H, m, H-3b), 1.59 (1H, ddd, *J* = 15.0, 6.2, 2.5 Hz, H-1b), 1.19 (3H, s, 19-CH_3).

^{13}C-NMR (151MHz, CD_3OD), δ_C: 179.2 (C-20), 174.6 (C-18), 172.3 (C-17), 144.9 (C-15), 141.5 (C-16), 126.7 (C-13), 109.5 (C-14), 79.1 (C-12), 72.0 (C-2), 71.9 (C-6), 46.8 (C-8), 43.9 (C-4), 41.9 (C-5), 40.3 (C-9), 39.3 (C-10), 35.6 (C-3), 32.8 (C-11), 28.9 (C-1), 27.5 (C-7), 21.7 (C-21), 21.0 (C-19). The data are consistent with those reported previously [16].

8-epidiosbulbin E (18)

White powder. $C_{19}H_{22}O_6$, m/z 346.14. ^{1}H-NMR (600MHz, $CDCl_3$),^{1}H NMR (600 MHz, Chloroform-*d*). δ_H: 7.57 (t, *J* = 1.2 Hz, 1H, H-16), 7.40 (t, *J* = 1.8 Hz, 1H, H-15), 6.94 (dd, *J* = 1.9, 0.9 Hz, 1H, H-14), 5.30 (dd, *J* = 11.0, 5.4 Hz, 1H, H-12), 4.88 (t, *J* = 5.1 Hz, 1H, H-2), 4.71 (d, *J* = 5.7 Hz, 1H, H-6), 2.76 (d, *J* = 5.4 Hz, 1H, H-7a), 2.64–2.60 (m, 1H, H-10), 2.60–2.55 (m, 1H, H-4), 2.18–2.12 (m, 2H, H-3a, 8), 2.11–2.06 (m, 2H, H-11,1a), 2.01(1H, d, *J* = 15.0, 6.2, 2.5 Hz, H-5),1.98 (ddd, *J* = 12.0, 5.5, 1.1 Hz, 1H, H-7b), 1.93 (d, *J* = 11.9 Hz, 1H, H-11b), 1.80 (d, *J* = 11.7 Hz, 1H, H-3b), 1.66–1.59 (m, 1H, H-1b), 1.25 (s, 3H, 19-CH_3). The data are consistent with those reported previously [17].

Batatasin Ⅲ (19)

White powder. $C_{15}H_{16}O_3$, m/z 244.29.^{1}H-NMR (600MHz, CD_3OD), δ_H:7.06 (1H, t, *J* = 7.8 Hz, H-5'), 6.65 (1H, dt, *J* = 7.6, 1.2 Hz, H-6'), 6.62 (2H, t, J = 2.0 Hz, H-4', 2'), 6.24 (1H, t, J = 1.8 Hz, H-6), 6.22 (1H, t, J = 1.8 Hz, H-2), 6.18 (1H, t, J = 1.8 Hz, H-4), 3.70 (3H, s, 5-OCH_3), 2.79 (2H, m, a), 2.76 (2H, m, a').

^{13}C-NMR (151MHz, CD_3OD), δ_C: 162.2 (C-5), 159.3 (C-3), 158.3 (C-3'), 145.4(C-1), 144.6 (C-1'), 130.2 (C-5'), 120.8 (C-6'), 116.3 (C-2'), 113.7 (C-4'), 109.0 (C-4), 106.5 (C-6), 99.9 (C-2), 55.5 (5-OCH_3), 39.1 (C-a), 38.8 (C-a'). The data are consistent with those reported previously [14].

Protocatechuic acid (20)

White powder. $C_7H_6O_4$, m/z 154.03; ^{1}H-NMR (600MHz, CD_3OD), δ_H: 7.43(2H, m, H-5, 6), 6.79 (1H, d, *J* = 2.1Hz, H-2)

^{13}C-NMR (150MHz, CD_3OD), δ_C: 170.3 (C-COOH), 151.6 (C-4), 146.1 (C-3), 124.0 (C-1), 123.2 (C-6), 117.8 (C-5), 115.8 (C-2). The data are consistent with those reported previously [18].

In the present study, twenty compounds, including eight phenanthrenes (**1**–**8**), eight flavonoids (**9–16**), two diterpenes (**17**, **18**), one bibenzyl derivatives (**19**), and one acid (**20**) were isolated from the rhizome of *Dioscorea bulbifera*. Among them, compound **1** is first reported from the *Dioscorea bulbifera* and compound **16** first reported from the genus *Dioscorea.*

Cytotoxic activity against CT26 cell lines

Compound **1** and compound **16** against CT26 cell lines were evaluated at different concentrations (2.5, 5, 10, 20, 40, 80μg/ml) to calculate IC_{50} values. The results were shown in Table 1.

Table 1
Cytotoxic activity of the isolates of *Dioscorea bulbifera* against CT26 cell lines

Compound	IC_{50} (μM)
1	83.23
16	>100

Conclusion

The rhizome of *D. bulbifera* was reported rich in diosbulbin, phenanthrenes, and flavonoids. During our study, compounds **1** is first reported from the *Dioscorea bulbifera* showed week cytotoxic against CT26 cell lines and compounds **16** first reported from the genus *Dioscorea.* Besides this, 18 known compounds based on NMR-guided method were isolated from the rhizome of *Dioscorea bulbifera*. This study enriches the phytochemical study of *Dioscorea bulbifera* and provides new sights to this plant on its chemical profile.

Material And Methods

Instruments

The NMR spectra were measured in methanol-d_4, on a Bruker ARX-400 or AV600 instrument with TMS as an internal standard. ESI-MS spectra were recorded on Waters Quattro micro API LC/MS/MS spectrometer (Waters, USA). HPLC was performed on JAI LC9103 Recycling preparative HPLC (Japan Analytical Industries, Japan) equipped with JAIGEL-ODS-AP-P column and JAIGEL-GS310 column using a JAI refractive index detector and a JAI UV-3702 detector with MultiChro 2000 workstation.

Plant Materials

The rhizome of *Dioscorea bulbifera* L. was collected from Bozhou, China, in July 2018. The plant herbarium specimen was collected, authenticated and deposited in Shenyang Pharmaceutical University.

Extraction And Isolation

PE, EtOAc and n-BuOH extracts of the rhizome of *Dioscorea bulbifera* L. (9 kg) were obtained using the method described previously [19]. the EtOAc extract and the 30% EtOH, 70% EtOH, and 95% EtOH fractions of n-butanol extract were then separated. These extracts were subjected to repeated separations over Sephadex LH-20 and silica gel, and were further purified by Recycling preparative HPLC to obtain compounds **1** (5.9 mg), **2** (4.0 mg), **3** (2.6 mg), **4** (7.6 mg), **5** (4.3 mg), **6** (4.0 mg), **7** (3.7 mg), **8** (6.9 mg), **9**

(9.7 mg), **10** (6.9 mg), **11** (6.7 mg), **12** (8.4 mg), **13** (7.9 mg), **14** (8.9 mg), **15** (90.5 mg), **16** (41.8 mg), **17** (500.0 mg), **18** (57.0 mg), **19** (3.2 mg), **20** (15.6 mg).

Cytotoxicity Against Ct26 Cell Lines

Cell viability was determined by MTT assay. Cells (CT26) were cultured in DMEM medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) and antibiotics antimycotics (PSF; 100 units/mL penicillin G sodium, 100 µg/mL streptomycin, and 250 ng/mL amphotericin B). The cells were incubated at 37°C and 5% CO_2 in a humidified atmosphere. Cells were seeded in 96-well plates and incubated for 12 h. After the indicated treatments, cells were incubated with 0.5 mg/mL MTT for 4 h. The crystal was dissolved with dimethyl sulfoxide. All compounds were solved in DMSO (final concentration of 0.1%[v/v]), stored at -20°C and diluted to desired concentration (2.5, 5, 10, 20, 40, 80µg/ml) in culture medium immediately prior to each experiment. Absorbance was measured at 490 nm.

Statistical analysis

The data of bioactivity assays are represented as the means ± SD for three independent experiments. GraphPad Prism 8 statistical soft was used to perform data analysis.

Declarations

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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Figures

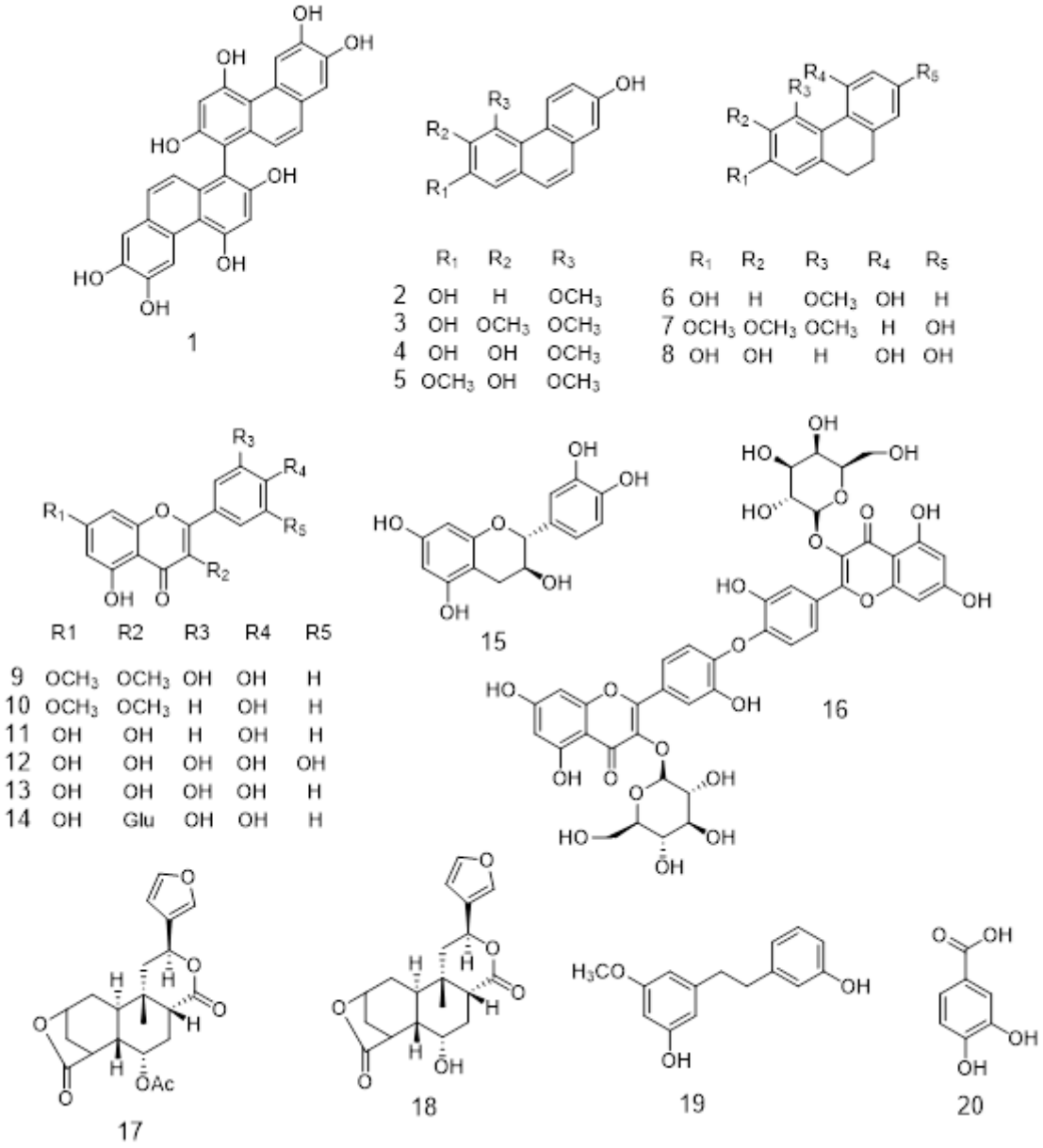

Figure 1

Chemical structures of compounds 1–20 isolated from the rhizome of *D. bulbifera.*

Supplementary Files

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