

# An Undescribed Triterpenoid Saponin Isolated From *Zornia diphylla*

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**Abstract:** Phytochemical investigation on the aerial parts of *Zornia diphylla* resulted in the isolation and identification of one undescribed triterpenoid saponin, zordiphyllaside I (**1**), together with 25 known compounds. Their structures were elucidated by comprehensive spectroscopic methods, including 1D and 2D NMR and MS. Notably, 24 compounds were isolated from this plant for the first time. Zordiphyllaside I showed moderate cytotoxicity against human breast carcinoma cell line (MCF-7) and human prostate cancer cell line (LNCaP), with  $IC_{50}$  values of 26.52 and 45.37  $\mu$ M, respectively.

**Keywords:** Triterpenoid saponin; *Zornia diphylla*; Fabaceae; cytotoxicity. © 2023 ACG Publications. All rights reserved.

## 1. Plant Source

In the present study, the aerial parts of *Zornia diphylla* (L.) Pers (*Z. diphylla*) were collected from Yizhou City, Guangxi Province and authenticated by Professor Peiming Yang. A voucher specimen (herbarium No.20201211) was deposited at Central Laboratory of Fengxian District Central Hospital, Shanghai, China.

## 2. Previous Studies

*Z. diphylla* (L.), known as Dingkuicao in traditional Chinese medicine belongs to the Fabaceae family and is distributed in subtropical and tropical areas, mainly in Zhejiang, Fujian, Guangxi, Yunnan and other regions of China. In traditional Chinese medicine theory, it is sweet in flavor, and has clearing heat, eliminating toxins, removing blood stasis, and relieving swelling effects [1]. Phytochemical studies on *Z. diphylla* reported the presence of flavonoid glycosides, isoflavones, phenols, and amino acids [1–3]. As part of our ongoing study on the phytochemical constituents of *Z. diphylla*, an undescribed triterpenoid saponin and 25 known compounds were isolated and identified.

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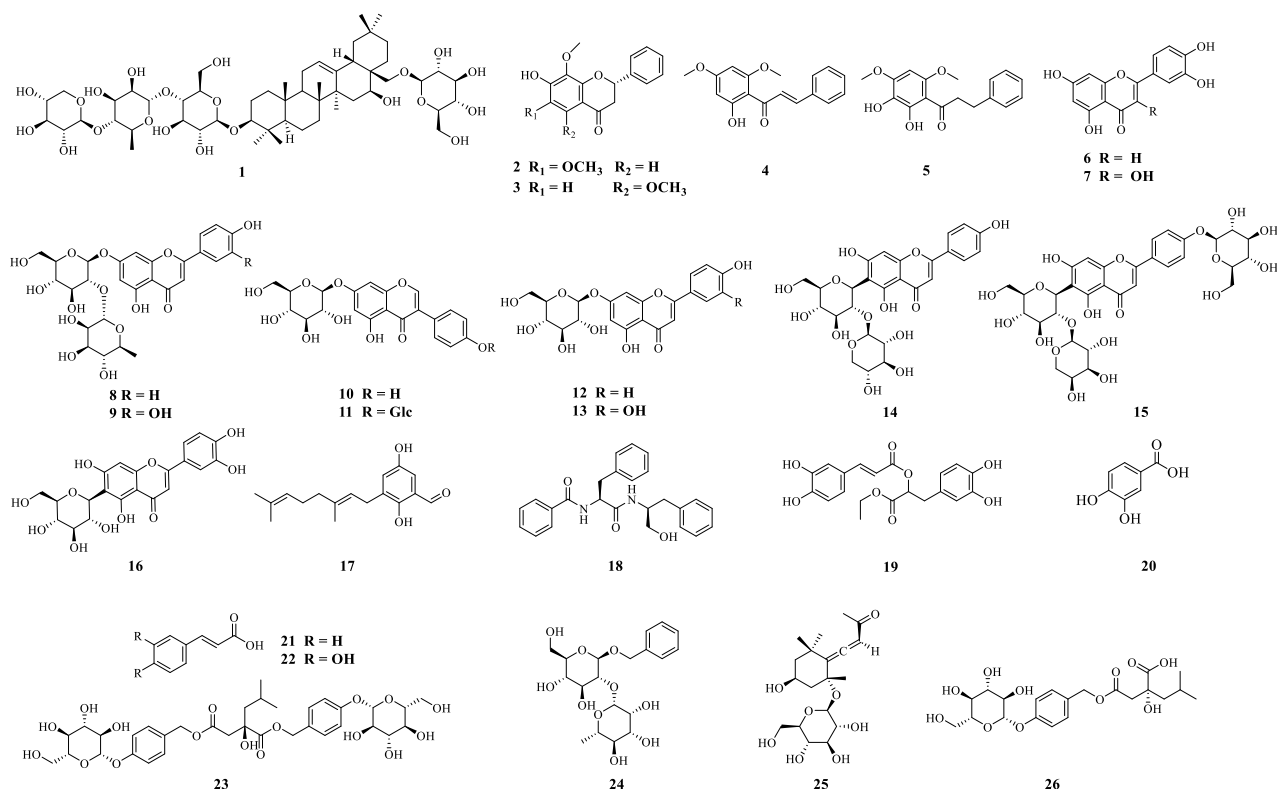
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In this study, the structure elucidation of the undescribed compound and its cytotoxic evaluation is reported.

### 3. Present Study

The dried aerial parts of *Z. diphylla* were ground into a powder and were extracted three times with 90% ethanol under reflux. The extracts were combined and concentrated under reduced pressure. The crude extract was suspended in water and successively, was extracted with petroleum ether, ethyl acetate (EtOAc), and n-butanol (n-BuOH) to obtain the petroleum ether, EtOAc, and n-BuOH fractions. The EtOAc extract (60 g) was subjected to fractionation by silica gel column chromatography (CC), eluting with a stepwise gradient of petroleum ether in EtOAc (5%, 10%, 20%, 40%, 60%, 80%, and 100%) to yield five fractions (F1–F5). F1 (10 g) was subjected to silica gel CC, and further separated by Sephadex LH-20 eluting with chloroform (CHCl<sub>3</sub>): methanol (MeOH) (1:1, v/v) to afford compounds **17** (5.0 mg), **18** (6.0 mg), **2** (6.0 mg), **3** (50.0 mg), **4** (35.0 mg), **5** (35.0 mg), **21** (28.0 mg), **6** (40.0 mg), **7** (25.0 mg), and **19** (20.0 mg). F4 (5 g) was purified by silica gel CC eluting with dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>)-MeOH (1:1, v/v), and was then separated by reverse phase C18 CC eluting with acetonitrile (ACN): water (30: 70, v/v) to yield compounds **20** (60.0 mg) and **22** (30.0 mg).

The n-BuOH extract (80 g) was separated on a column of MCI resin eluting with a gradient of MeOH in water (10%, 25%, 50%, 75%, and 100%) to give five fractions. These fractions were separated by silica gel CC eluting with a gradient of CHCl<sub>3</sub>-MeOH (9:1 to 1:1, v/v) to give sub-fractions. These sub-fractions were isolated on a Sephadex LH-20 CC eluting with MeOH-Water (8:2, v/v), and then purified by preparative HPLC to afford compounds **8** (10.0 mg), **23** (7.0 mg), **24** (12.0 mg), **14** (8.0 mg), **15** (5.0 mg), **9** (13.0 mg), **25** (20.0 mg), **26** (16.0 mg), **16** (28.0 mg), **10** (17.0 mg), **12** (15.0 mg), **13** (6.0 mg), **1** (15.0 mg), and **11** (12.0 mg) (Figure 1).



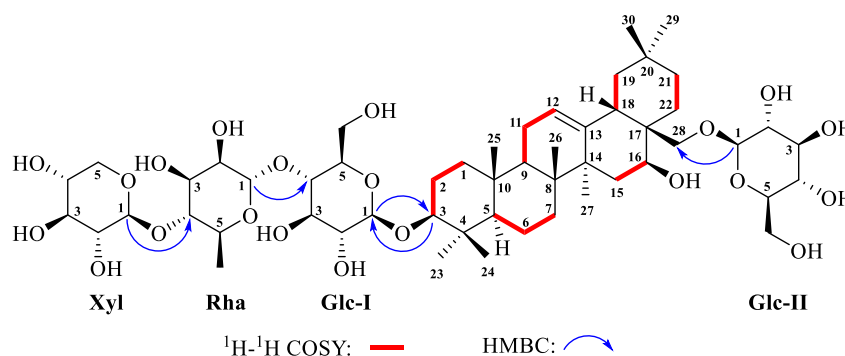
**Figure 1.** Structures of compounds **1–26**

## An undescribed triterpenoid saponin

**Table 1.**  $^1\text{H}$  (600 MHz) and  $^{13}\text{C}$  (125 MHz)-NMR data for compound **1** (pyridine-*d*<sub>5</sub>)

No.	$\delta_{\text{C}}$	$\delta_{\text{H}}$ ( $\delta$ in ppm, <i>J</i> in Hz)
1	38.8 (d)	1.41 (overlap), 0.88 (overlap)
2	26.6 (d)	2.23 dd (14.0, 4.0), 1.82 (overlap)
3	88.8 (t)	3.39, dd (11.8, 4.5)
4	39.5 (q)	
5	55.8 (t)	0.80, d (12.0)
6	18.6 (d)	1.58 (overlap), 1.41(overlap)
7	32.9 (d)	1.56 (overlap), 0.89 (overlap)
8	40.3 (q)	
9	47.2 (t)	1.59 (overlap)
10	36.8 (q)	
11	23.9 (d)	1.94 (overlap), 1.85 (overlap)
12	123.1 (t)	5.34, t (3.0)
13	143.6 (q)	
14	43.7 (q)	
15	36.5 (d)	2.17 t (12.6), 1.74 dd (13.3, 4.7)
16	65.3 (t)	4.62 (overlap)
17	41.4 (q)	
18	44.0 (d)	2.67, dd (14.0, 4.8)
19	46.8 (d)	1.87 (overlap), 1.18 (overlap)
20	31.0 (q)	
21	34.1 (d)	1.58 (overlap), 1.36 (overlap)
22	25.1 (d)	2.68 d (4.8), 2.05 td (14.2, 4.2)
23	28.2 (s)	1.34, s
24	17.0 (s)	1.03, s
25	15.8 (s)	0.86, s
26	17.1 (s)	1.18, s
27	27.3 (s)	1.37, s
28	74.5 (d)	4.13 (overlap), 4.29 (overlap)
29	33.5 (s)	0.89 s
30	24.5 (s)	1.05 s
Glc-I 1	106.8 (t)	4.94, d (7.7)
2	75.8 (t)	4.06 (overlap)
3	78.8 (t)	4.25 (overlap)
4	79.1 (t)	4.03 (overlap)
5	78.3 (t)	4.05 (overlap)
6	63.0 (d)	4.59 (overlap), 4.42 (overlap)
Rha 1	101.6 (t)	6.44, br. s
2	72.6 (t)	4.83 (overlap)
3	71.8 (t)	4.24 (overlap)
4	85.2 (t)	4.42 (overlap)
5	68.2 (t)	4.76 (overlap)
6	19.1 (s)	1.90, d (6.1)
Xyl 1	107.3 (t)	5.18, d (7.4)
2	77.9 (t)	4.08 (overlap)
3	78.6 (t)	4.11 (overlap)
4	71.0 (t)	4.21 (overlap)
5	67.5 (d)	3.57, t (10.8) 4.29 (overlap)
Glc-II 1	103.2 (t)	4.84, d (7.4)
2	76.1 (t)	4.22 (overlap)
3	79.6 (t)	4.24 (overlap)
4	71.8 (t)	4.21 (overlap)
5	78.2 (t)	3.84, dt (8.5, 3.8)
6	62.7 (d)	4.59 (overlap), 4.42 (overlap)

Zordiphyllaside I (**1**), obtained as a white amorphous powder, was found to have a molecular formula of  $C_{53}H_{88}O_{21}$  as deduced from its  $[M + H]^+$  ion at  $m/z$  1061.5892 in the positive HR-ESI-MS (calculated mass, 1061.5896; the mass error is 0.37 ppm). The  $^{13}C$  NMR spectrum of **1** displayed 53 carbon signals, of which 30 were assigned to the aglycone and 23 to sugar units (Table 1). Detailed analysis of the NMR data ( $^1H$ -NMR,  $^{13}C$ -NMR, HSQC,  $^1H$ - $^1H$  COSY, and HMBC) revealed seven  $sp^3$  carbons at  $\delta_C$  28.2, 17.0, 15.8, 17.1, 27.3, 33.5, and 24.5 in correlation with seven methyl singlets at  $\delta_H$  1.34, 1.03, 0.86, 1.18, 1.37, 0.89, and 1.05, and an  $sp^2$  carbon at  $\delta_C$  123.1 correlated with an olefinic proton at  $\delta_H$  5.34 indicating the presence of an olean-12-ene type aglycone [4]. A series of ROESY correlations between H-3, H-23, H-5, H-9, H-27, H-16, and H-29 confirmed the  $\alpha$  orientation for these protons. The series of ROESY cross-peaks between H-24, H-25, H-26, H-28, H-18, and H-30 demonstrated their  $\beta$  orientation. Hence, the aglycone was established as olean-12-ene- $3\beta$ ,  $16\beta$ , 28-triol. Compound **1** had four sugar residues indicated by four anomeric protons at  $\delta_H$  4.94 (1H, d,  $J = 7.7$  Hz), 6.44 (1H, brs), 5.18 (1H, d,  $J = 7.4$  Hz), and 4.84 (1H, d,  $J = 7.4$  Hz), and four protonated carbons at  $\delta_C$  106.8, 101.6, 107.3, and 103.2 observed in the HSQC spectrum. These were identified from their NMR spectroscopic data as  $\beta$ -glucose,  $\alpha$ -rhamnose, and  $\beta$ -xylose. Absolute configurations of the sugar moieties were assigned as D for glucose and xylose, and L for rhamnose by HPLC analysis with authentic sugar standards after acid hydrolysis [5]. The correlations observed in the HMBC spectrum between Glc-I H-1 ( $\delta_H$  4.94) with aglycone C-3 ( $\delta_C$  88.8), aglycone H-3 ( $\delta_H$  3.39) with Glc-I C-1 ( $\delta_C$  106.8), Rha H-1 ( $\delta_H$  6.44) with Glc-I C-4 ( $\delta_C$  79.1), and Xyl H-1 ( $\delta_H$  5.18) with Rha C-4 ( $\delta_C$  85.2) established that the xylopyranosyl residue was linked to C-4 of the rhamnopyranosyl residue, the rhamnopyranosyl residue was linked to C-4 of the glucopyranosyl residue, and that the trisaccharide chain was attached to position C-3 of the aglycone (Figure 2). In addition, the anomeric proton of Glc-II ( $\delta_H$  4.84) showed long range correlation to  $\delta_C$  74.5 (C-28) suggesting the connection of another glucose residue to C-28. Complete assignment of the protons and carbons of **1** was achieved with a combination of  $^1H$ ,  $^{13}C$ , COSY, DEPT, HSQC, HMBC, and ROESY experiments. The structure of **1** was established as  $3\beta$ -[ $\beta$ -D-xylopyranosyl-(1 $\rightarrow$ 4)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 4)-] $\beta$ -D-glucopyranosyl-28 $\beta$ - $\beta$ -D-glucopyranosylolean-12-ene-16 $\beta$ -ol, named zordiphyllaside I.



**Figure 2.** Key  $^1H$ - $^1H$  COSY and HMBC correlations of **1**.

The known compounds were identified as 7-hydroxy-6,8-dimethoxyflavanone (**2**) [6], 7-hydroxy-5,8-dimethoxyflavanone (**3**) [7], flavokawain B (**4**) [8], 2',3'-dihydroxy-4',6'-dimethoxychalcone (**5**) [8], luteolin (**6**) [9], quercetin (**7**) [9], rhoifolin (**8**) [10], lonicerin (**9**) [10], genistein-7-O- $\beta$ -D-glucopyranoside (**10**) [11], genistein-7-4'-O- $\beta$ -diglucoside (**11**) [12], apigenin 7-O-glucoside (**12**) [13], luteoloside (**13**) [13], 2''-xyloxyisovitexin (**14**) [14], vaccarin (**15**) [15], isoorientin (**16**) [16], 3-geranyl-2,5-dihydroxy-benzaldehyde (**17**) [17], aurantiamide (**18**) [18], ethyl rosmarinat (**19**) [19], protocatechuic acid (**20**) [20], cinnamic acid (**21**) [20], *trans*-caffeic acid (**22**) [20], militarine (**23**) [21], benzyl O- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\beta$ -D-glucopyranoside (**24**) [22], citroside A (**25**) [23], and gymnoside II (**26**) [21], respectively, by comparison of their spectroscopic data with published data. Except for compounds **7** and **12**, the remaining 24 of these compounds were isolated from *Z. diphylla* for the first time. Cytotoxic activities toward human breast carcinoma cell line, MCF-7, and human prostate cancer cell line, LNCaP, were measured by MTT assay methods.

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Compound **1** displayed moderate cytotoxicity to MCF-7 and LNCaP cells with IC<sub>50</sub> values of 26.52 ± 2.30 μM and 45.37 ± 3.28 μM, respectively. Paclitaxel was used as the positive control for MCF-7 cells with IC<sub>50</sub> = 7.90 ± 0.25 μM, and doxorubicin was used as the positive control for LNCaP cells with IC<sub>50</sub> = 12.86 ± 0.89 μM.

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## Supporting Information

Supporting Information accompanies this paper on <http://www.acgpubs.org/journal/records-of-natural-products>

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