

## A new adduct of iriflophene and flavonoid from *Sedum aizoon* L.

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### ABSTRACT

A new adduct of iriflophene and flavonoid, 1, 8, 10, 10b-tetrahydroxy-3-methoxy-5a-(3,4-dihydroxyphenyl)-9-(4-hydroxybenzoyl)-5a,10b-dihydro-11H-benzofuro[2,3-b]chromen-11-one (1), together with six known compounds were isolated from the EtOH extract from *Sedum aizoon* L. Their chemical structures were elucidated by analyses of spectral data. The chemotaxonomic analysis of the compounds was discussed, compounds 1–4 provide important chemotaxonomic markers for *S. aizoon*.

### 1. Subject and source

The genus *Sedum* (Crassulaceae), with about 470 species, is mainly distributed in the northern hemisphere, but it also occurs in Africa, Latin America and the southern hemisphere (Editorial Committee of Chinese Flora, 1984). There are 124 species, 1 subspecies, 14 varieties in China. (Yang et al., 2013; Wu et al., 2013. Han et al., 2020).

*Sedum aizoon* L. is a medicinal plant widely distributed in China, Japan, Korea, Mongolia, and Russia (Editorial Committee of Chinese Flora, 1984), the trivial names of *S. aizoon* in China include “tusanqi” or “jingtiansanqi”. As a traditional Chinese folk medicine, *S. aizoon* was often used for the treatment of agrypnia, traumatic bleeding, injuries and tumor (Lin et al., 2014b).

In the present study, the whole plant of *S. aizoon* was collected at Qiongdongnna Miao-Dong Autonomous Prefecture, (N 25°19'20"-27°31'40", E 107°17'20"-109°35'24"), Guizhou Province, China, in July 2019. The plant was identified as *S. aizoon* by Dr Wei Gu and the specimen voucher (QDN-2019-N-5) was deposited at The Key Laboratory of Chemistry for Natural Products of Guizhou Province and Chinese Academy of Sciences, Guiyang.

### 2. Previous work

Previous phytochemical studies of *S. aizoon* have reported the presence of flavonoids (Luo et al., 2012; Lin et al., 2014a, 2014b; Zhu et al., 2014; Xu et al., 2015; Han et al., 2017), isoflavones (Li et al., 2011), phenolic acid compounds, especially gallic acid and its derivatives (Lin et al., 2014a, 2014b; Xu et al., 2015), Terpenoids (Han et al., 2017; Xu et al., 2015) and alkaloids (Li et al., 2014; 2015; Xu et al., 2015). Recently, a series of novel adducts of iriflophene and flavonoid were isolated from *S. aizoon* (Li et al., 2017). The flavonoids and gallic acid derivatives appeared to be the major classes of compounds of *S. aizoon*.

### 3. Present study

#### 3.1. Isolation and structure elucidation

The dried powder of *S. aizoon* (10 kg) was extracted at room temperature with 80% EtOH (10L × 3 times); the extract was concentrated on a rotary evaporator at 50 °C yielding 2.33 kg of crude extract, which was suspended in H<sub>2</sub>O and extracted successively with petroleum ether (PE), ethyl acetate (EA) and n-butanol. The EA fraction was subjected to silica gel (60–80 mesh) column using PE-EA (100:1–2:1, v/v), CH<sub>3</sub>Cl, CH<sub>3</sub>Cl–MeOH (100:1–1:1, v/v), MeOH. Each fraction was evaporated to

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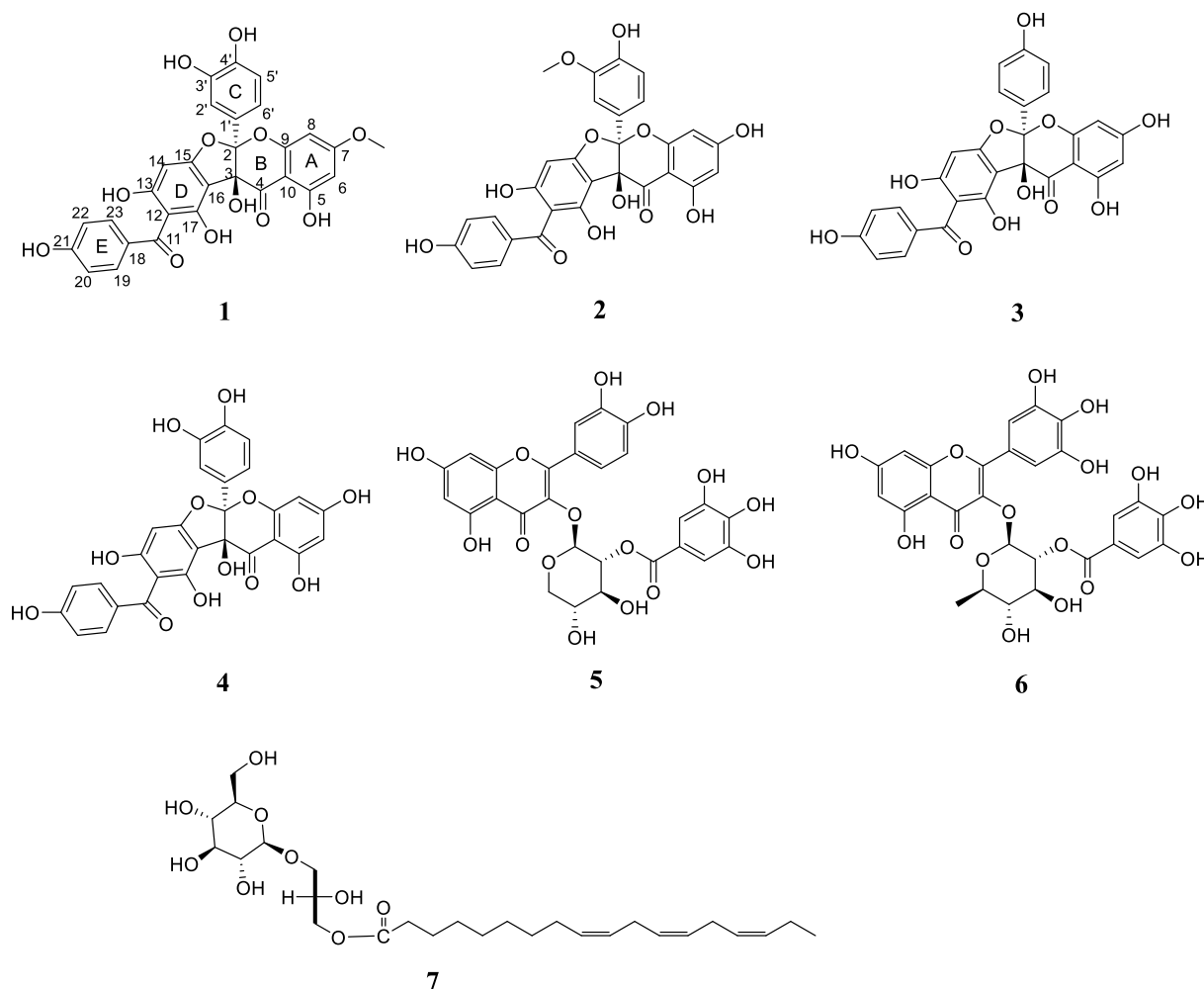
Fig. 1. Compounds 1–7 isolated from *Sedum aizoon* L.

Table 1

The  $^1\text{H-NMR}$  (150 MHz) and  $^{13}\text{C-NMR}$  (600 MHz) data for compound 1 in  $\text{C}_3\text{D}_6\text{O}$ .

Position	$^1\text{H-NMR}$	$^{13}\text{C-NMR}$	Position	$^1\text{H-NMR}$	$^{13}\text{C-NMR}$
11	–	196.1	6'	6.72(1H,dd, $J = 8.3, 1.9\text{Hz}$ )	119.8
4	–	193.7	2	–	118.7
7	–	170.0	20,22	6.96(2H,d, $J = 8.7\text{Hz}$ )	115.5
15	–	166.9	2'	6.84(1H,d, $J = 1.9\text{Hz}$ )	115.2
5	–	165.0	5'	6.71(1H,d, $J = 8.3\text{Hz}$ )	115.4
21	–	162.7	16	–	107.3
17	–	162.5	12	–	102.6
9	–	162.1	10	–	100.6
13	–	161.5	14	6.11(1H,s)	99.1
4'	–	147.3	6	6.14(1H,d, $J = 2.3\text{Hz}$ )	96.4
3'	–	145.2	8	6.07(1H,d, $J = 2.3\text{Hz}$ )	94.9
19,23	7.78(2H, d, $J = 8.7\text{ Hz}$ )	133.1	3	–	81.4
18	–	131.6	-OCH <sub>3</sub>	3.88(3H,s)	56.6
1'	–	125.9			

dryness to give thirteen fractions. Fraction 11 (14 g) was passed through RP-C18 column (MeOH–H<sub>2</sub>O: 20%–90%) to give ten fractions. Fraction 11–8 (222.2 mg) was separated by size exclusive Sephadex LH-20 (MeOH) and silica gel (300–400 mesh) column (CH<sub>2</sub>Cl<sub>2</sub>–MeOH) to give 1 (14.8 mg), and then by RP-HPLC (Zorbax SB-C18: 5  $\mu\text{m}$ , 250  $\times$  9.4 mm, 55% MeOH: 45% H<sub>2</sub>O,  $t_{\text{R}} = 27.5\text{ min}$ ) to give 2 (4.8 mg) and (75% MeOH–25% H<sub>2</sub>O,  $t_{\text{R}} = 14.5\text{ min}$ ) to give 3 (10.9 mg). Fraction 12 (10.3954 g) was subjected to silica gel (300–400 mesh) column to give twelve fractions. Fraction 12–11 was separated by HPLC (50% MeOH–

50% H<sub>2</sub>O,  $t_{\text{R}} = 17.2\text{ min}$ ) to give 4 (34.3 mg). Fraction 13 (49 g) was passed through RP-C18 column (MeOH–H<sub>2</sub>O: 10%–90%) to give six fractions. Fraction 13–5 (8.5501 g) was separated by silica gel (300–400 mesh) column (CH<sub>2</sub>Cl<sub>2</sub>–MeOH) to give 6 (39.6 mg), and size exclusive Sephadex LH-20 (MeOH) to give 5 (16.5 mg), then by RP-HPLC (Zorbax SB-C18: 5  $\mu\text{m}$ , 250  $\times$  9.4 mm, 40% MeOH: 60% H<sub>2</sub>O,  $t_{\text{R}} = 25.0\text{ min}$ ) to give 7 (29.5 mg).

One new adduct of iriflophenone and flavonoid (1) and six known compounds (2–7) were isolated from the ethyl acetate part of *S. aizoon*.

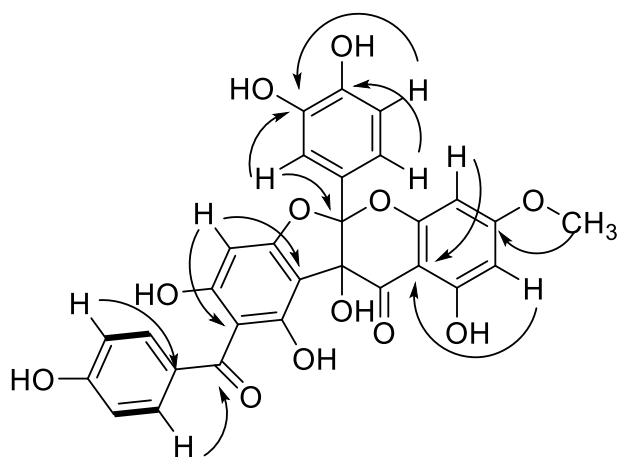


Fig. 2. Key HMBC (H→C) correlations and <sup>1</sup>H-<sup>1</sup>H COSY(H=H) observed for 1.

**Table 2**  
Antioxidant activity of compounds (n = 3, ±SD).

Compounds	IC <sub>50</sub> (μmol•L <sup>-1</sup> )
Vc	1.020 ± 0.029
1	0.245 ± 0.018
4	0.744 ± 0.013
5	0.415 ± 0.023
6	0.365 ± 0.016

The structures of isolated compounds were elucidated by extensive spectroscopic analysis, including NMR, optical rotation experiments and HR-ESI-MS, and by comparison with the literature data. The six known compounds were identified as 1,3,8,10,10b-pentahydroxy-5a-(4-hydroxy-3-methoxyphenyl)-9-(4-hydroxybenzoyl)-5a,10b-dihydro-11H-benzofuro[2,3-b]chromen-11-one (2) (Li et al., 2017), 1, 3, 8, 10,10b-pentahydroxy-9-(4-hydroxybenzoyl)-5a-(4-hydroxyphenyl)-5a,10b-dihydro-11H-benzofuro[2,3-b]chromen-11-one (3) (Li et al., 2017), 5a-(3,4-dihydroxyphenyl)-1, 3, 8, 10, 10b-pentahydroxy-9-(4-hydroxybenzoyl)-5a,10b-dihydro-11H-benzofurochromen-11-one (4) (Li et al., 2017) (see Fig. 1), quercetin-3-O-α-arabinopyranoside-2''-gallate (5) (Ouyang et al., 2015), myricetin-3-O-α-rhamnopyranoside-2''-gallate (6) (Zhou et al., 2017), monoacylmonogalactosylglycerol (7) (Tuntiwachuttikul et al., 2004). Compounds 5 and 7 were isolated from genus *Sedum* for the first time.

Compound 1 was obtained as a yellow amorphous solid. The HR-ESI-MS data showed quasi-molecular ion peak at m/z 559.0872 [M-H]<sup>-</sup> (calcd for m/z 559.0871), corresponding to a molecular formula of C<sub>29</sub>H<sub>19</sub>O<sub>12</sub>. A broad IR absorption band centered at 3342 cm<sup>-1</sup> confirmed the presence of hydroxyl groups while a band with a shoulder at 1446 cm<sup>-1</sup> showed the presence of conjugated carbonyl functionalities. Both of 1D (Table 1) and 2D-NMR data of compound 1 were in good agreement with 5a-(3,4-dihydroxyphenyl)-1,3,8,10,10b-pentahydroxy-9-(4-hydroxybenzoyl)-5a,10b-dihydro-11H-benzofurochromen-11-one<sup>[4]</sup>, except for the <sup>1</sup>H-NMR spectrum for compound 1 displayed a singlet proton at δ<sub>H</sub> 3.88 (3H, s, -OCH<sub>3</sub>) corresponding to the characteristic of methoxyl. Combining with HMBC spectra, it certificated that the methoxyl connected to C-7 (Fig. 2).

The absolute configuration of compound 1 was determined by comparison of the calculated electronic circular dichroism (ECD) with experimental ECD. It proves that the measured ECD spectra and the calculated ECD spectra basically tallies (Figure S14). The absolute configurations of compounds 2–4 (Figure S15–S17) were identical to that of 1, as evidenced by similar curves in the ECD spectra, which was confirmed by comparison with Lawsonia biflavone A (Li et al., 2014).

Thus, the structure of compound 1 was assigned as 2S, 3S-1, 8, 10, 10b-tetrahydroxy-3-methoxy-5a-(3,4-dihydroxyphenyl)-9-(4-

hydroxybenzoyl)-5a,10b-dihydro-11H-benzofuro[2,3-b]chromen-11-one, an iriflophene unit was connected with isorhamnetin via a furan ring.

2S, 3S-1, 8, 10, 10b-tetrahydroxy-3-methoxy-5a-(3, 4-dihydroxyphenyl)-9-(4-hydroxybenzoyl)-5a,10b-dihydro-11H-benzofuro[2,3-b]chromen-11-one (1): yellowish amorphous solid; [α]<sub>D</sub> 19.42 (c 0.11, MeOH); UV (MeOH) λ<sub>max</sub> (log ε): 197.20 nm (4.68); <sup>1</sup>H and <sup>13</sup>C-NMR (C<sub>3</sub>D<sub>6</sub>O): Table 1; HR-ESI-MS: m/z 559.0872 [M - H]<sup>-</sup> (calcd for [C<sub>29</sub>H<sub>19</sub>O<sub>12</sub>]<sup>-</sup> 559.0871).

### 3.2. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The antioxidant activity of compounds was determined by the 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) assay (Shahidi et al., 2015; Grajeda-Iglesias et al., 2016), with some modifications. Briefly, 160 μL of each sample (0.1563–5 μg/mL) was mixed with 40 μL DPPH solutions (0.15 mmol/L) and incubated in the dark at room temperature for 30 min. The absorbance of the mixture was then measured at 517 nm. Vitamin C was used as a positive control. The result was showed in Table 2, compounds 1, 4, 5 and 6 exhibited significant antioxidant activity with the IC<sub>50</sub> values of 0.245, 0.744, 0.415 and 0.365 μM, respectively.

## 4. Chemotaxonomic significance

In the study, four dimer of adducts of iriflophene and flavonoids were isolated from *S. aizoon* (1–4). Compound 1 is new, whereas compounds 2–4 was previously reported on *S. aizoon* (Li et al., 2017). So far, this type of compound has not been reported on other species of Crassulaceae. Therefore, it can be inferred that compounds 1–4 could be used as the chemotaxonomic makers of *S. aizoon*. Compound 5 was first isolated from *Lasiobema japonica* Maxim (Iwagawa et al., 1990) and compound 7 was first isolated from *Citrus hystrix* DC. Cat. (Murakami et al., 1995). In this study, compounds 5 and 7 were isolated from the family Crassulaceae for the first time. To date the distribution of these compounds within Crassulaceae is unknown. Previous phytochemical studies of genus *Sedum* members have reported a series of Flavonoids and their glycosides. Compound 6, also called gallomyricitrin, was first isolated from *S. selskianum* Regel et Maack (Zapesochayna et al., 1975) and have also been obtained from *S. sediforme* Pau (Winekenstädte et al., 2015). The present study revealed the close chemotaxonomic relationships between *S. aizoon* and the above species to a certain extent. Previously, compound 6 was isolated from the family Leguminosae (Nicollier and Thompson, 1989; Moharram et al., 2006; Hsieh et al., 2010; Walaa et al., 2016; Falcowski et al., 2019), Myricaceae (Sun et al., 1991; Yoshikawa et al., 1998; Masuda et al., 2010; Kuo et al., 2004), Polygonaceae (Nugroho et al., 2014; Kong et al., 2014; Lin and Chou, 2000), Dilleniaceae (Rodrigues et al., 2008), and Euphorbiaceae (Lee et al., 2017). These findings provide new evidence for the chemical characteristics of the Crassulaceae, and the gallomyricitrin might serve as a chemotaxonomic marker for family Crassulaceae.

In conclusion, The chemotaxonomic analysis indicate that compounds 1–4 provide important chemotaxonomic markers for *S. aizoon*. Compounds 6 could be consider as the characteristic components of Crassulaceae. The study provides new chemotaxonomic markers for *S. aizoon* and extends the chemical constituents of the family Crassulaceae.

### Authors statement

The corresponding author is responsible for ensuring that the descriptions are accurate and agreed by all authors.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bse.2020.104119>.

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