ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. X:X (202X) XX-XX

records of natural products

Cytotoxic Diterpenoids from *Scoparia dulcis*

Lirong Wang [1], Jie Yu [2], Zonglong Nie [2] and Peipei Shan [1*]

[1]*Institute of Translational Medicine, The Affiliated Hospital of Qingdao University, College of Medicine, Qingdao University, Qingdao 266021, China*
[2]*Qingdao Central Hospital, Qingdao 266042, China*

(Received November 18, 2021; Revised January 10, 2022; Accepted January 14,, 2022)

Abstract: A new scopadulane-type diterpenoid (**1**) and one known labdane-type diterpenoid (**2**) have been isolated from the whole plants of a traditional medicinal plant, *Scoparia dulcis*. Their structures were established mainly by spectroscopic methods, and the absolute configuration of compound **1** has been determined by quantum chemical calculation of the electronic circular dichroism (ECD) spectrum and comparison with the experimental one. The cytotoxicity of **1** and **2** against two breast cancer cell lines (MCF-7 and MDA-MB231) and a cervical cancer cell line (Hela) were tested, and only compound **2** displayed inhibitory activities against these cells with IC_{50} values ranging from 8.1 to 45.2 μM, while compound **1** were inactive. In addition, compound **2** also showed *in vitro* anti-tumor activity against MCF-7, MDA-MB231, and Hela cells in a concentration-dependent manner.

Keywords: *Scoparia dulcis*, Scrophulariaceae; scopadulane diterpenoid; labdane diterpenoid; cytotoxicity.

1. Introduction

Scoparia dulcis is a herbaceous plant or small shrub originating from the tropical areas of America, and it is now widely distributed in tropical regions all over the world [1]. As the only *Scoparia* species found in China, *S. dulcis* has been mainly collected and applied in local ethno medicine by the Dai people in Yunnan Province and the Zhuang people in Guangxi Zhuang Autonomous region, and its major indications include cough, sore throat and edema [2]. Previous studies on the pharmacological effects of the solvent extracts (mainly water or ethanol) of *S. dulcis* revealed a variety of bioactivities such as anti-diabetes [3,4], anti-inflammation [5,6] and anti-oxidation [7,8]. In addition, modern phytochemical investigations on this herb have shown that it is a rich source of diverse secondary metabolites especially diterpenoids with cytotoxic [9-12], cell viability attenuating [13-15], antiviral [16,17] and antidiabetic [18,19] activities. Flavonoids with antidiabetic [19-22], NGF potentiating [23] and cytotoxic [24] properties, as well as immunomodulatory triterpenoids [25] and antiproliferative alkaloids [26], have also been reported. In a very recent review [27], Wen and co-workers have presented a comprehensive record on the phytochemistry and pharmacology of *S. dulcis*.

In our current project to search new cytotoxic agents from medicinal plants for future potential anticancer lead compounds, an intensive fractionation of the 95% ethanol extract of the whole plants of *S. dulcis* has led to the separation of one new scopadulane-type diterpenoid (1β-Hydroxydulcinol, **1**) and one known labdane-type diterpenoid (scoparinol, **2**). The present paper deals with the isolation, structure characterization, and primary anti-tumor evaluation of the two diterpenoids.

* Corresponding author: E-mail: shanpeipei@qdu.edu.cn

The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products Month-Month 2022 EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.304.2111.2266
Available online: February 15,2022

2. Materials and Methods

2.1. General Experimental Procedures

Optical rotations were recorded on a Rudolph V polarimeter (Rudolph Research Analytical, Hackettstown, USA). ECD experiments were carried out on a Chirascan photospectrometer (Applied Photophysics Ltd., Leatherhead, UK). NMR spectra in $CDCl_3$ (reference signal: δ_H 7.26, δ_C 77.16) were acquired on a Bruker Avance DRX600 spectrometer (Bruker BioSpin AG, Fallanden, Switzerland). ESIMS and HR-ESIMS analyses were conducted on Agilent 1260-6460 Triple Quad and 6545 Q-TOF MS spectrometers, respectively (Agilent Technologies Inc., Waldbronn, Germany). HPLC analyses and separations were performed on an Agilent 1260 series LC instrument (Agilent Technologies Inc., Waldbronn, Germany) equipped with an SB-C_{18} column (9.4 × 250 mm, Agilent Technologies Inc., Santa Clara, USA). Reversed phase C_{18} (RP-18) silica gel (Merck KGaA, Darmstadt, Germany), Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden), MCI gel (CHP20/P120, Mitsubishi Chemical Co. Ltd., Tokyo, Japan) and silica gel (300–400 mesh; Qingdao Marine Chemical Co. Ltd., Qingdao, China) were used for column chromatography (CC). Solvents used for CC were of at least analytical grade (Sinopharm Chemical Reagent Co. Ltd., Beijing, China) and solvents used for HPLC were of HPLC grade (Oceanpak Alexative Chemical Ltd., Goteborg, Sweden). Pre-coated silica gel GF_{254} plates (Yantai Huiyou Chemical Co. Ltd., Yantai, China) were used for thin layer chromatography (TLC) monitoring.

2.2. Plant Materials

The whole plants of *Scoparia dulcis* L. were harvested in Aug. 2018 in Xishuangbanna of Yunnan province, China, and were authenticated by Prof. Wanyi Li from Yunnan Academy of Agricultural Sciences. The plant materials were compared to a previously collected local specimen (IMDY0020482). A voucher specimen is also deposited at the herbal repository room of the College of Medicine, Qingdao University (No.: SL-2018-01).

2.2. Extraction and Isolation

The dried whole plants (10 kg) of *S. dulcis* were powdered and percolated with 95% ethanol at room temperature (3 × 7 days), and the solutions were combined and evaporated under vacuum to yield the crude extract (800 g). The crude extract was partitioned between water (1.5 L) and ethyl acetate (1.0 L × 3) to give the ethyl acetate soluble part, which (210 g) was solvent-removed and then fractionated by MCI gel CC eluted with MeOH-H_2O (30%, 50%, 80%, and 95%) to obtain four elutions (A, B, C and D).

Fraction C (42 g) was subjected to a silica gel column, eluted with petroleum ether/ethyl acetate (from 10/1 to 0/1), to return eight fractions (C1–C8). Then fraction C4 (1.8 g) was separated by RP-18 CC using 80%–95% methanol/water as eluting solvent to give six subfractions (C4-1–C4-6). Fraction C4-2 was processed successively by silica gel CC (dichloromethane/methanol, from 100/1 to 50/1) and semi-preparative HPLC (80% MeCN/H_2O) to yield compound **1** (3.9 mg, t_R = 14.0 min). The fraction C6 (5.6 g) was chromatographed on a RP-18 column (MeOH/H_2O, 60%–80%) to give five subfractions (C6-1–C6-5). Subfraction C6-2 was first fractionated by Sephadex LH-20 CC (dichloromethane/methanol, 3/2) and then purified by semi-preparative HPLC (85% MeOH/H_2O) to afford compound **2** (5.4 mg, t_R = 11.6 min)

*1β-Hydroxydulcinol (**1**):* White powder; $[\alpha]^{24}_D$ −5.7 (*c* 0.33, MeOH); UV (MeOH) λ (log ε) 230 (3.93) nm; ECD (*c* 0.02, MeOH) λ (Δε) 200 (+4.4), 210 (−1.5) 230 (+9.3), 294 (+2.1); ^{1}H and ^{13}C NMR see Table 1; ESIMS *m/z* 463.2 $[M + Na]^+$, 903.4 $[2M + Na]^+$; HR-ESIMS *m/z* 441.2654 $[M + H]^+$, (calcd for $C_{27}H_{37}O_5^+$, 441.2636).

2.3. ECD Calculations

The theoretical ECD spectra of compound **1** and its enantiomer were acquired by Time-dependent Density Functional Theory (TD-DFT) method as described formerly [28].

2.4. Bioassays

2.4.1. Cell Viability Assay

The cell viability assay was performed as previously reported [29]. Briefly, MCF-7, MDA-MB231 and HeLa cells (5×10^3 cells/well) were seeded in 96-well plates. After 24 h, cells were treated with different concentrations of compound **2** or SAHA. Cell viability was measured by MTS assay, the Aqueous one Solution (Promega) was used according to the manufacturer's instructions, and the absorption at 490 nm was measured.

2.4.2. Colony Formation Assay

The colony formation assay was performed as previously reported [30]. MDA-MB231 and HeLa cells were seeded in a 6-well plate, and 24 h later, cells were treated with different concentrations of compound **2**. The culture medium was refreshed every other day. Cells were cultured for 10 days. Then the clones were fixed with 4% paraformaldehyde, stained with 0.1% crystal violet, and counted manually.

2.4.3. Cell Death Assay

MDA-MB231 and MCF-7 cells were seeded in a 6-well plate, and 24 h later, cells were treated with different concentrations of compound **2**, and 24 h later, the cell morphology changes were detected by phase-contrast microscope (Olympus, Tokyo, Japan).

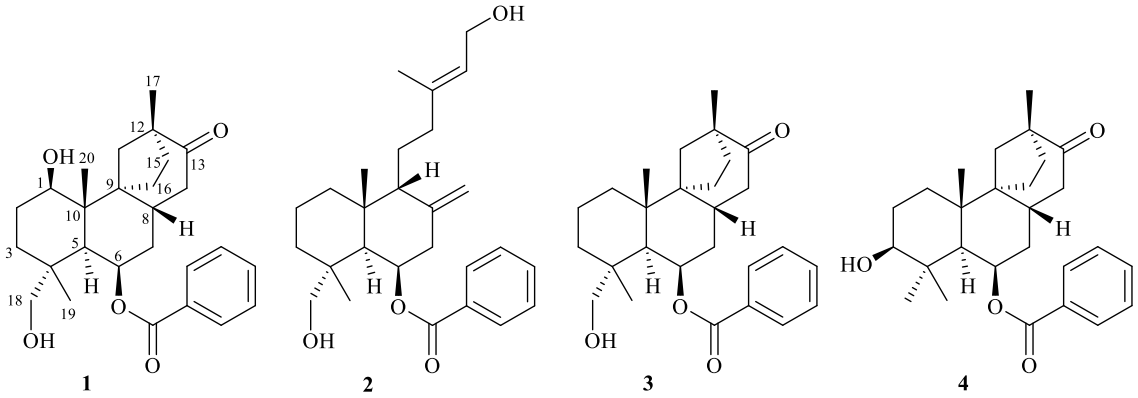

Figure 1. Chemical structures of **1** and **2** from *Scoparia dulcis* and two similar analogues.

3. Results and Discussion

Compound **1** was assigned the molecular formula $C_{27}H_{36}O_5$ by analyses of NMR and HR-ESIMS (*m/z* 441.2641, $[M+H]^+$) data, incorporating ten hydrogen deficiencies. The NMR data of (Table 1) **1** clearly showed resonances for a benzoyloxy group as evidenced by δ_H at 8.04 (dd, J = 8.3, 1.3 Hz, 2H), 7.58 (tt, J = 7.4, 1.3 Hz) and 7.47 (dd, J = 8.3, 7.4 Hz, 2H), as well as δ_C at 166.4, 133.3, 130.6, 129.7 (× 2) and 128.7 (× 2). In addition, signals for a ketone (δ_C 214.0), two oxygenated methine [δ_C 75.4, 70.4; δ_H 4.03 (dd, J = 11.3, 4.3 Hz), 5.59 (m)], an oxymethylene [δ_C 70.8; δ_H 3.60 and 3.11 (both d, J = 10.8 Hz)] and three singlet methyl [δ_C 20.6, 19.9, 14.4; δ_H 1.56, 1.07, 0.91] groups were also resolved. The benzoyl and ketone units occupied six hydrogen deficiencies, and the remaining four indicated a tetracyclic skeleton for **1**. These observations suggested that compound **1** could be a scopadulane-type diterpenoid similar to dulcinol [30] (scopadulciol [31]), which was further

corroborated by detailed examination of 2D NMR data (Figure 2) as described below. Analyses of $^{1}H–^{1}H$ COSY correlations afforded four structural fragments of CH-1/CH_2-2/CH_2-3, CH-5/CH-6/CH_2-7/CH-8/CH_2-11 and CH_2-15/CH_2-16, which were subsequently linked with other groups *via* quaternary carbons by the HMBC correlations from H_3-20 to C-1, C-5, C-9 and C-10, H_3-19 to C-3, C-4, C-5 and C-18, H-11 to C-8, C-9 and C-16, H-14 to C-13, and H_3-17 to C-12, C-13 and C-15, to confirm the scopadulane scaffold of **1** as shown (Figure 2). The benzoyloxy unit at C-6 was supported by the HMBC correlation from H-6 to C-7′ together with the chemical shift for H-6 (δ_H 5.59). Lastly, two hydroxy groups were assigned to C-1 and C-18, respectively, based on the chemical shifts for CH-1 and CH_2-18 together with the remaining elements in the molecular formula of **1**.

Table 1. NMR data for compound **1** and the most similar known compounds **3** and **4** in $CDCl_3$

	1		**3**	**4**	
	δ_C	δ_H	δ_H	δ_C	δ_H
1	75.4	4.03, dd (11.3, 4.3)		32.4	
2	29.5	α 1.66, m		27.5	
		β 1.86, m			
3	35.3	α 1.74, m		79.2	3.20, dd (8.9, 6.2)
		β 1.19, dt (13.0, 3.3)			
4	38.5			39.8	
5	43.4	1.71, d (2.3)	1.79, d (2)	50.4	1.20, d (2.2)
6	70.4	5.59, m	5.63, td (3, 2)	70.3	5.74, q (2.6)
7	35.7	β 1.76, m	1.81, ddd (15, 5, 3)	35.9	
		α 1.65, m	1.76, ddd (15, 12, 3)		
8	37.4	2.46, tt (11.1, 4.8)		35.8	2.47, m
9	53.5			53.1	
10	44.4			39.2	
11	48.8	a 2.89, d (12.6)	1.83, d (12)	45.6	
		b 1.60, d (12.6)	1.54, d (12)		
12	52.0			52.5	
13	214.0			213.5	
14	42.9	a 2.21, dd (16.0, 6.2)	2.23, dd (16, 12)	42.7	2.27, dd (16, 6.6)
		b 2.02, dd (16.0, 12.1)	2.01, dd (16, 6)		1.98, dd (16, 12)
15	36.8	1.75, m (2H)		36.8	
16	23.4	a 2.28, m		23.9	2.10, br t (9.0)
		b 1.78, m			1.72, m
17	19.9	1.07, s	1.09, s	19.9	1.10, s
18	70.8	a 3.60, d (10.8)	3.59, d (11)	28.5	1.11, s
		b 3.11, d (10.8)	3.13, d (11)		
19	20.6	0.91, s	0.93, s	16.8	0.97, s
20	14.4	1.56, s	1.53, s	21.4	1.51, s
1′	130.6			130.8	
2′/6′	129.7	8.04, dd (8.3, 1.3)	8.02, br d (7.5)	129.8	8.05, d (7.6)
3′/5′	128.7	7.47, dd (8.3, 7.4)	7.57, br t (7.5)	128.8	7.47, t (7.6)
4′	133.3	7.58, tt (7.4, 1.3)	7.45, brt (7.5)	133.3	7.59, t (7.6)
7′	166.4			166.3	

The relative configuration of **1** was established by analysis of NOESY data (Figure 2) and proton-proton couplings. In the NOESY spectrum, H_3-19 showed correlations with both H_3-20 and H-2 at δ_H 1.86, suggesting that ring A adopted a chair conformation and this H-2, Me-19 and Me-20 were 1,3-diaxially bonded. Similarly, the correlations of H_3-20 with H-8 and H-2′(6′) also indicated the

chair conformation for ring B and the mutual 1,3-diaxial relationship for H-8, Me-20, and the benzoyloxy unit. The aforementioned protons and groups were thus co-planar and were assigned to be β-orientated as in dulcinol [31] (scopadulciol [32]). Then the coupling pattern of H-1/H-2 (J = 11.3 Hz, 1,2-diaxial relationship) and the NOESY correlation of H-1/H-5 implied that H-1 and H-5 were α-axially located and 1-OH was thus β-directed. Finally, the NOESY correlations of H-8/H-11 (δ_H 1.60), H-1/H-16 (δ_H 2.28) and H-7α/H-16 (δ_H 1.78) suggested that CH_2-11 was in β-equatorial position and CH_2-16 was in α-axial position in ring B, which established the relative configuration of the bridge ring as shown. The absolute configuration of **1** was determined to be (1*R*,4*R*,5*S*,6*R*,8*S*,9*S*,10*S*,12*S*) by comparing its experimental ECD curve with the calculated one (Figure 3), with a very good match. Moreover, its ECD curve was also consistent with that of a very recently reported analogue, scopadulcic acid D [13]. On reviewing the literature, two compounds with >95% similarity to **1**, namely dulcinol (**3**) [31] (scopadulciol [32]) and *iso*-dulcinol (**4**) [33], were previously described from the same species. Compound **1** was identified as the 1β-hydroxy derivative of dulcinol (**3**) with extra signals for an oxymethine unit and loss of signals for a methylene group in the NMR spectra. Meanwhile, compared with *iso*-dulcinol (**4**), the location of the hydroxy group in ring A of **1** shifted to C-1 and the 18-OH was lost in **1**.

In addition to the new molecule **1**, another known diterpenoid co-metabolite (**2**) of the labdane type was also isolated and structurally characterized as scoparinol [31], based on the comparison of spectroscopic data with those in the literature.

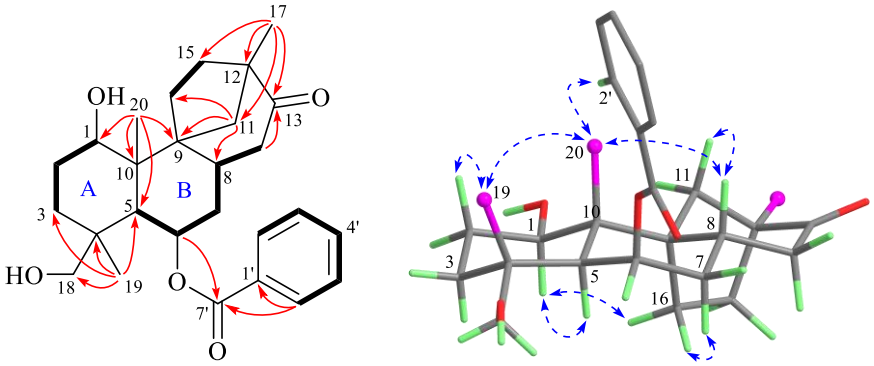

Figure 2. ^{1}H–^{1}H COSY (bond lines), selected HMBC (arrows) and NOESY (double-headed dashed arrows) correlations for compound **1** (For clarity, all methyl groups were simplified to pink balls).

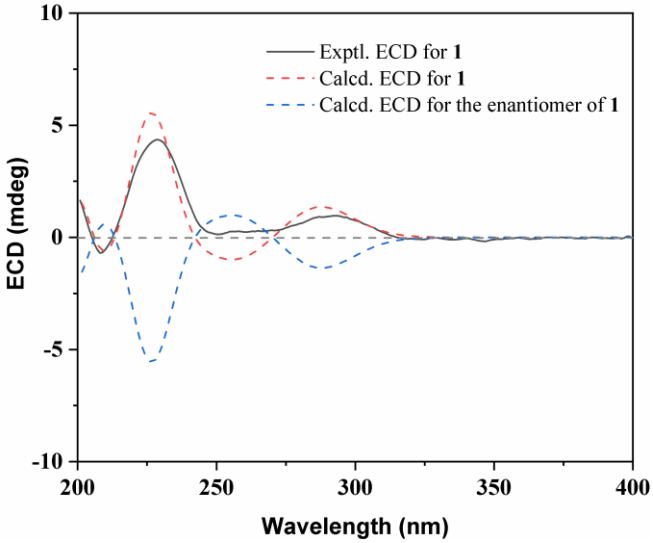

Figure 3. Experimental and calculated ECD spectra for compound **1**

Table 2. Cytotoxicity of **1** and **2** against three female cancer cells (IC_{50} in μM)

	MCF-7	MDA-MB231	Hela
SAHA	14.2±5.64	6.91±2.22	5.21±0.54
1	>50	>50	>50
2	45.2±8.21	26.4±5.79	8.10±1.82

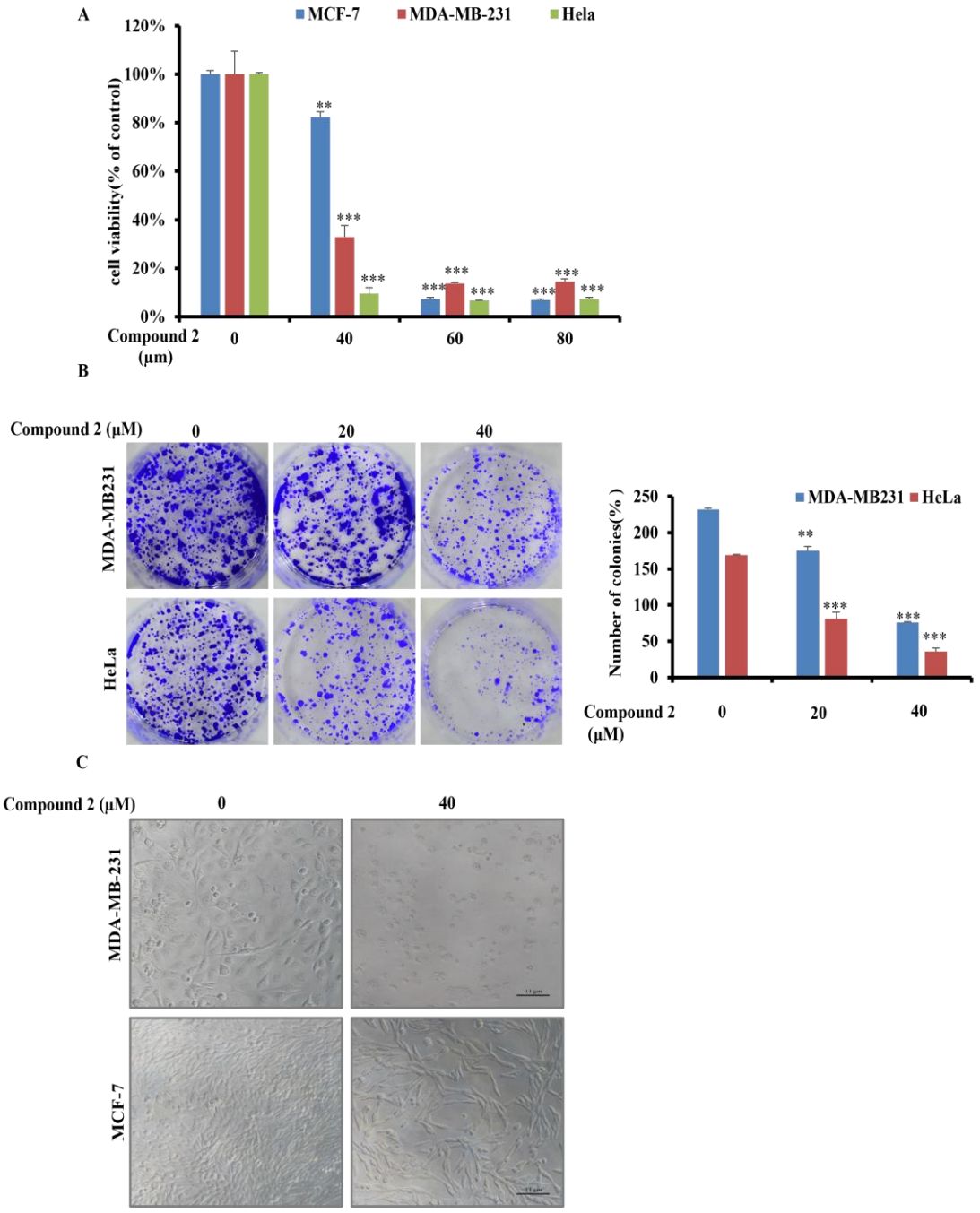

Figure 4. Compound **2** exerts promising anti-breast cancer activity *in vitro*.

(**A**) MCF-7, MDA-MB231 and HeLa cells were treated with compound **2**, and after 48 h, the MTT assay was performed. The bars indicate the mean ± SD. (**B**) MDA-MB231 and HeLa cells were seeded on 6-well plates. After 12 h, cells were treated with indicated concentrations of compound **2**. On day 10, the number of colonies was counted in experiments repeated three times. Results represent the average of three replications. (**C**) MDA-MB231 and MCF-7 cells were treated with the indicated concentrations of compound **2** for 24 h. The cell morphologies were detected by interference light microscopy.

Compounds **1** and **2** were assessed for their cytotoxicities toward the breast cancer cells (MCF-7 and MDA-MB231), as well as the cervical cancer cells (Hela). As shown in Table 2, compound **2** exhibited different degrees of inhibitory activity against the three cell lines with IC_{50} values of 45.2±8.21, 26.4±5.79 and 8.10±1.82 μM, respectively, and suberoylanilide hydroxamic acid (SAHA) was used as a positive control. To further investigate the anticancer activities of compound **2**, the cell viability of the three cancer cells under treatment of compound **2** was measured by using MTS assay. As shown in Figure 4A, compound **2** significantly inhibited the growth of the tested cancer cells in a dose-dependent manner. Since colony formation is close to the pathological process of tumor cells *in vivo*, we then performed colony formation assay. As shown in Figure 4B, compound **2** significantly inhibited the cell colony formation in a dose-dependent manner. Furthermore, cell morphology detected by phase-contrast microscope proved that compound **2** induced obvious death of cancer cells (Figure 4C). These results demonstrated that compound **2** exerts promising anti-breast cancer activity *in vitro*.

Acknowledgments

We thank the National Natural Science Foundation of China (No. 91849209, 81803016, 81703360), the Natural Science Foundation of Shandong Province (No. ZR2019HB012), the Qingdao science and technology plan fund (No. RZ1900002629), the Qingdao Applied Basic Research Project (No. 19-6-2-50-cg) and the Postdoctoral Science Foundation of China (No. 2019M650157) for funding supports.

Supporting Information

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

ORCID
Lirong Wang: 0000-0001-7154-2016
Jie Yu: 0000-0001-8321-8862
Zonglong Nie: 0000-0002-4439-5054
Peipei Shan: 0000-0002-0553-4728
Lirong

References

[1] Flora of China Editorial Committee of Chinese Academy of Sciences(1979). Flora of China, Beijing. Science Press. **67**(**2**), 85.
[2] W.T. Wan, Y.J. Song, L.J Xu and P.G. Xiao (2015). An overview on modern research and application potency of *Scoparia dulcis*, *Zhongcaoyao*. (in Chinese) **46**(**16**), 2492-2498.
[3] M. Latha and L. Pari (2003). Modulatory effect of *Scoparia dulcis* in oxidative stress-induced lipid peroxidation in streptozotocin diabetic rats, *J. Med. Food.* **6**, 379-386.
[4] M. Latha and L. Pari (2004). Effect of an aqueous extract of *Scoparia dulcis* on blood glucose, plasma insulin and some polyol pathway enzymes in experimental rat diabetes, *Brazilian J. Med. Biol. Res.* **37**, 577-586.
[5] S.M.D.F. Freire, J.A.D.S. Emim, A.J. Lapa, C. Souccar and L.M.B. Torres (1993). Analgesic and anti-inflammatory properties of *Scoparia dulcis* L. extracts and glutinol in rodents, *Phytother. Res.* **7**, 408-414.
[6] J.C. Tsai, W.H. Peng, T.H. Chiu, S.C. Lai and C.Y. Lee (2011). Anti-inflammatory effects of *Scoparia dulcis* L. and betulinic acid, *Am. J. Chin. Med.* **39**, 943-956.
[7] L. Pari and M. Latha (2004). Protective role of *Scoparia dulcis* plant extract on brain antioxidant status and lipid peroxidation in STZ diabetic male wsitar rats, *BMC. Complem. Alternat. Med.* **4**, 1-8.
[8] A.Y. Coulibaly, M. Kiendrebeogo, P.G. Kehoe, P.A.E.D. Sombie, C.E. Lamien, J.F. Millogo and O.G. Nacoulma (2011). Antioxidant and anti-inflammatory effects of *Scoparia dulcis* L, *J. Med. Food.* **14**, 1576-1582.
[9] M. Nur-e-Alam, S. Ahmed, M. Yousaf, S. Khan, R. Mothana and A. Al-Rehaily (2020). Isolation and characterization of cytotoxic and anti-inflammatory constituents from *Scoparia dulcis* L, *Journal of Chemical Research*. **44**(**7-8**), 381-387.

[10] K. Hayashi, T. Hayashi and N. Morita (1992). Cytotoxic and antitumor activity of scopadulcic acid from *Scoparia dulcis* L, *Phytotherap. Res*. **6**(**1**), 6-9.

[11] M. Ahsan, S.K.N. Islam, A.I. Gray and W. H. Stimson (2003). Cytotoxic diterpenes from *Scoparia dulcis*, *J. Nat. Prod*. **66**, 958-61.

[12] M.G. Phan, T.S. Phan, K. Matsunami and H. Otsuka (2006). Chemical and biological evaluation on scopadulane-type diterpenoids from *Scoparia dulcis* of Vietnamese origin, *Chem. Pharm Bull.* **54**, 546-549.

[13] L. Dong, W. Sun, Z. Huang, C. Zhang, C. Liu, X. Zhang and S. Zhang (2021). Two new scopadulane diterpenoids from *Scoparia dulcis* attenuated palmitate-induced viability in MIN6 cells, *Fitoterapia* **155**, 105051.

[14] C.Y. Zhang, L.Z. Chen, Y.Y. Li, N. Wei, L. Zhang, L. Dong, Y. Zhang and X.P. Zhang (2020). Scoparic acid E: a new labdane diterpenoid on attenuating palmitate induced viability in MIN6 cells from *Scoparia dulcis*, *Rec. Nat. Prod*. **14**(**5**), 383-386.

[15] W.Y. Sun, Y. Zhang, Y.Y. Li, L.Z. Chen, L. Zhang, C.Y. Zhang and X.P. Zhang (2020). Two new labdane diterpenoids from Scoparia dulcis to attenuate palmitate-induced viability in MIN6 cells, *Phytochemistry Letters*. **38**, 25-27.

[16] K. Hayashi, T. Hayashi (1996). Scopadulciol is an inhibitor of herpes simplex virus type 1 and a potentiator of acyclovir, *Antivir. Chem. Chemother.* **7**, 79-85.

[17] K. Hayashi , S. Niwayama , T. Hayashi, R. Nago, H. Ochiai and N. Morita (1988). *In vitro* and *in vivo* antiviral activity of scopadulcic acid B from *Scoparia dulcis*, Scrophulariaceae, against herpes simplex virus type 1, *Antivir. Res*. **9**, 345-354.

[18] M. Latha , L. Pari , K.M. Ramkumar , P. Rajaguru, T. Suresh, T. Dhanabal, S. Sitasawad and R. Bhonde (2009). Antidiabetic effects of scoparic acid D isolated from *Scoparia dulcis* in rats with streptozotocin-induced diabetes, *Nat. Prod. Res.* **23**, 1528-1540.

[19] Q. Liu, Q.M. Yang, H.J. Hu, L. Yang, Y.P. Yang, G.X. Chou and Z.T. Wang (2014). Bioactive Diterpenoids and Flavonoids from the Aerial Parts of *Scoparia dulcis*, *J. Nat. Prod*.. **77**(**7**), 1594-1600.

[20] J.E. Beh, L.T. Khoo, J. Latip, M.P. Abdullah, N.B.M. Alitheen, Z. Adam, A. Ismail and M. Hamid (2013). SDF7, a group of Scoparia dulcis Linn. derived flavonoid compounds, stimulates glucose uptake and regulates adipocytokines in 3T3-F442a adipocytes, *J. Ethnopharmacol.* **150**(**1**), 339-352.

[21] V.J. Sharma and U.D. Shah(2010). Antihyperglycemic activity of flavonoids from methanolic extract of aerial parts of Scoparia dulcis in streptozotocin induced diabetic rats, *Int. J. Chem. Tech Res*. **2**(**1**), 214-218.

[22] M. Kawasaki, T. Hayashi, M. Arisawa, N. Morita and L.H. Berganza(1988). 8-Hydroxytricetin 7-glucuronide, a b-glucuronidase inhibitor from Scoparia dulcis, *Phytochemistry* **27**(**11**), 3709-11.

[23] Y.S. Li, X.G. Chen, M. Satake, Y. Oshima and Y. Ohizumi (2004). Acetylated Flavonoid Glycosides Potentiating NGF Action from *Scoparia dulcis*, *J. Nat. Prod*. **67**(**4**), 725-727.

[24] T. Hayashi, K. Uchida, K. Hayashi, S. Niwayama and N. Morita (1988). A cytotoxic flavone from *Scoparia dulcis* L. *Chem. Pharm. Bull.* **36**, 4849-4851.

[25] K.R. Sharma1, A. Adhikari, A. Jabeen, , N. Dastagir, S.K. Kalauni, M.I. Choudhary and Y.R. Pokharel (2015). Immunomodulatory Studies on Triterpenoids from S*coparia dulcis* Linn, *Biochem. Pharmacol.* **4**, 4. Doi:10.4172/2167-0501.1000182

[26] W.H. Wu, T.Y. Chen, R.W. Lu, S.T. Chen and C.C. Chang (2012). Benzoxazinoids from *Scoparia dulcis* (sweet broomweed) with antiproliferative activity against the DU-145 human prostate cancer cell line, *Phytochemistry* **83**, 110-115.

[27] Z.K. Jiang, J.H. Sung, X.Y. Wang, Y.Y. Zhang, Y.M. Wang, H.F. Zhou and L. Wen (2021). A review on the phytochemistry and pharmacology of the herb *Scoparia dulcis* L. for the potentialtreatment of metabolic syndrome, *RSC Adv.*. **11**(**50**), 31235-31259.

[28] J.H. Yu, Z.P. Yu, Y.Y. Wang, J. Bao, K.K. Zhu, T. Yuan and H. Zhang (2019). Triterpenoids and triterpenoid saponins from *Dipsacus asper* and their cytotoxic and antibacterial activities, *Phytochemistry* **162**, 241–249.

[29] Q.X. Ma, Q.Q. Xu, J.J. Zhao, W.W. Zhang, Q. Wang, J. Fang, Z.M. Lu, J. Liu and L.N. Ma (2021). Coupling HDAC4 with transcriptional factor MEF2D abrogates SPRY4-mediated suppression of ERK activation and elicits hepatocellular carcinoma drug resistance, *Cancer Lett.* **520**, 243-254.

[30] P.P. Shan, F.F. Yang, H.Z. Qi, Y.J. Hu, S.J. Zhu, Z.Q. Sun, Z. Zhang, C.X. Wang, C.X. Hou, J. Yu, L.R. Wang, Z.X. Zhou, P.F. Li, H. Zhang and K. Wang (2021). Alteration of MDM2 by the small molecule YF438 exerts antitumor effects in triple-negative breast cancer, *Cancer Res.* **81**, 4027-4040.

[31] M. Ahmed and J. Jakupovic (1990). Diterpenoids from *Scoparia dulcis*, *Phytochemistry* **29**, 3035-3037.

[32] T. Hayashi, S. Asano, M. Mizutani, N. Takeguchi, T. Kojima, K. Okamura and N. Morita (1991). Scopadulciol, an Inhibitor of Gastric H^+,K^+-ATPhase from *Scoparia dulcis*, and its structure-activity relation-ships, *J. Nat. Prod*. **54**, 802-809.

[33] M. Ahsan, S.K.N. Islam, A.I. Gray and W.H. Stimson (2003). Cytotoxic Diterpenes from *Scoparia dulcis*, *J. Nat. Prod*. **66**, 958-961.