ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. X:X (2022) XX-XX

records of natural products

Chemical Constituents from the Whole Plant of *Pachysandra terminalis*

Chao Ding[#], Yuze Li[#], Ying Wu, Yu Sun, Fengrui Wang, Huawei Zhang, Yi Jiang, Dongdong Zhang[*] and Xiaomei Song[*]

School of Pharmacy, Shaanxi University of Chinese Medicine, Xianyang 712046, China

(Received May 21, 2022; Revised July 14, 2022; Accepted July 20, 2022)

Abstract: Two new compounds, butyl(*Z*)-3-((3*R*,4*R*)-5-(hydroxymethyl)-3,4-dimethyl-3,4-dihydro-2*H*-pyran-6-yl)acrylate (**1**) and (2*Z*,4*S*)-4,5-dihydroxy-3,4-dimethylpent-2-enoic acid (**2**) along with seven known ones, stigmast-5,28(29)-dien-3*β*-ol (**3**), *β*-sitosterol (**4**), carotene (**5**), fraxetin (**6**), *p*-coumaric acid (**7**), cis-*p*-hydroxycinnamic acid (**8**), ferulic acid (**9**) were obtained from the whole plant of *Pachysandra terminalis*. The structures of these compounds were elucidated by comprehensive spectroscopic methods including 1D, 2D NMR, MS, IR and ECD data analysis. Notably, compounds **3** and **5**~**8** were isolated from genus *Pachysandra* for the first time. Moreover, compounds **1**~**3** and **6**~**8** were tested their cytotoxic activities against three cancer cells, however, only compound 1 showed inhibitory effect in SW620 cells with IC_{50} value of 47.7 μM.

Keywords: *Pachysandra terminalis*; chemical composition; isolation and purification; steroids; fatty acids.

1. Introduction

Pachysandra terminalis, an evergreen plant, belongs to the genus *Pachysandra*, family Buxaceae [1-2], which is widely distributed in the South of China. It is mainly distributed in the Qinba Mountains, Shaanxi province of China [3]. The chemical constituents isolated from *P. terminalis* are mainly Pachysandra-type alkaloids, triterpenoids, volatile oils, and others [4]. Modern pharmacological studies have shown that it has antioxidant, anti-ulcer [5-7], anti-tumor [8-13], anti-bacterial [14-15], and insecticidal [16-17] activities. It was mainly used for the clinical treatment of rheumatoid arthritis and chronic bronchitis. In our continuous work to investigate more bioactive

[*] Corresponding authors: E-Mail: zhangnatprod@163.com; Phone: +86-029-38185165 (D. Zhang); songxiaom@126.com; Phone: +86-029-38185165 (X. Song)
[#]These authors contributed equally to this work

The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products Month-Month 202x EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.346.2205.2465
Available online: August 05, 2022

natural compounds from *P. terminalis*, two new compounds butyl(*Z*)-3-((3*R*,4*R*)-5-(hydroxymethyl)-3,4-dimethyl-3,4-dihydro-2*H*-pyran- 6-yl)acrylate (**1**) and (2*Z*,4*S*)-4,5-dihydroxy-3,4-dimethylpent-2-enoic acid (**2**) and seven known compounds, stigmast-5,28(29)-dien-3*β*-ol (**3**) [18], *β*-sitosterol (**4**) [19], carotene (**5**) [20], fraxetin (**6**) [21], *p*-coumaric acid (**7**) [22], *cis*-*p*-hydroxycinnamic acid (**8**) [23], ferulic acid (**9**) [24], were procured (Figure 1). In this study, we described the structure identification and cytotoxic activities of these compounds.

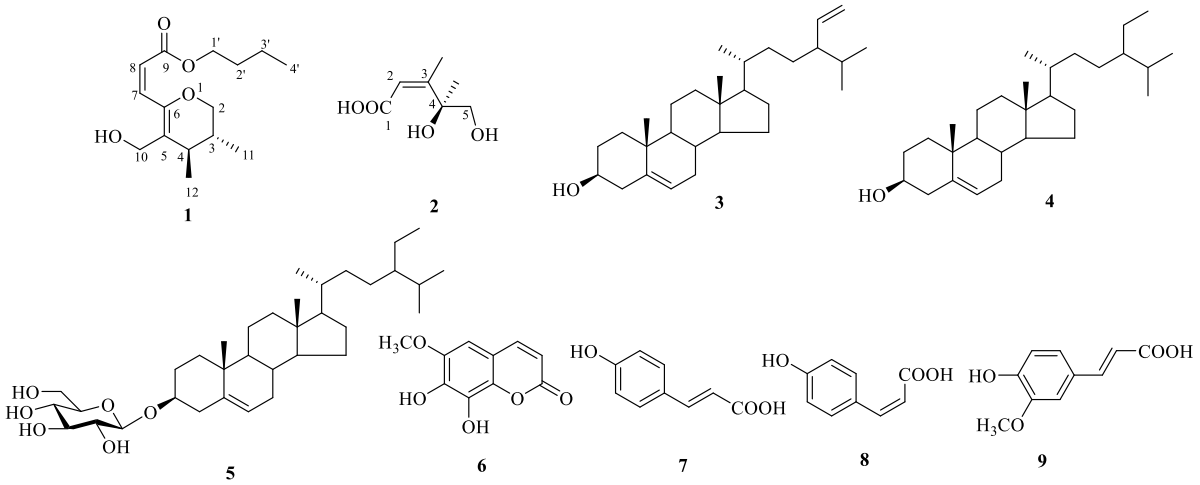

Figure 1. Structures of compounds **1–9**

2. Materials and Methods

2.1. General Experimental Procedures

The HR-ESI-MS spectra was taken on an Agilent Technologies 6550 Q-TOF and ESI-MS was performed on Waters Quattro Premier instrument. 1D and 2D NMR spectra were recorded on a Bruker-AVANCE 400 instrument with TMS as an internal standard. Semipreparative HPLC was performed on a system comprising an LC-20AP pump equipped with a SPD-20A UV detector and a Ultimate XB-C_{18} (10 mm × 250 mm, 5 μm particles). Sephadex LH-20 gel silica gel were purchased from GE Healthcare Bio-Sciences AB. Chromatographic methanol (Tianjin Comio Chemical Reagent Co., Ltd.)

2.2. Plant Material

In the present study, *Pachysandra terminalis* Sieb. et Zucc. were collected from the Baoji, Shaanxi Province, China, in 2020, and authenticated by Professor Wei Wang (School of Pharmacy, Shaanxi University of Chinese Medicine). A voucher specimen (herbarium No. 20200901) has been deposited in the Medicinal Plants Herbarium, Shaanxi University of Chinese Medicine, Xianyang, China.

2.3. Extraction and Isolation

The whole plant of *P. terminalis* (10.0 kg) was extracted with 80 % EtOH under reflux three times. After removal of EtOH solvent under reduced pressure, the extract was suspended in water and successively extracted with petroleum ether, CH_2Cl_2 and n-BuOH. The CH_2Cl_2 parts (160 g) were

chromatographed on silica gel column, eluted with gradient solvent system (CH_2Cl_2-CH_3OH, 80:1–0:1) to give thirteen fractions (Fr.1-Fr.13).

Fr. 11 (30.0 g) was subjected to Sephadex LH-20 column chromatography and eluted with CH_2Cl_2-CH_3OH (1:1) to yield Fr. 11-1~ Fr. 11-5, Fr. 11-1 (3.5 g) was purified by SP-HPLC with CH_3OH-H_2O (20:80) as mobile phase to obtained compounds **2** (t_R = 18.0 min, 16.5 mg), **9** (t_R = 25.3 min, 20.0 mg), **7** (t_R = 35.0 min, 14.0 mg) and **8** (t_R = 35.5 min, 17.0 mg). Fr. 11-3 (1.8 g) was purified by SP-HPLC with CH_3OH-H_2O (25:75) as mobile phase to obtained compounds **3** (t_R = 35.0 min, 21.6 mg) and **4** (t_R =4 0.5 min, 25.0 mg). Fr. 11-4 (3.0 g) was purified by SP-HPLC with CH_3OH-H_2O (25:75) as mobile phase to obtained compound **6** (t_R = 30.5 min, 17.0 mg). Fr. 11-5 (0.5 g) was purified by SP-HPLC with CH_3OH-H_2O (20:80) as mobile phase to obtained compounds **5** (t_R = 30.0 min, 30.6 mg) and **1** (t_R = 40.5 min, 7.0 mg) (Figure 1).

2.4. Spectroscopic Data

Butyl(Z)-3-((3R,4R)-5-(hydroxymethyl)-3,4-dimethyl-3,4-dihydro-2H-pyran-6-yl)acrylate ***(1):*** A reddish brown oily solid, $[\alpha]_D^{20}$-11.2 (*c* 0.05, CH_3OH); IR ν_{max} (KBr) (cm^{-1}): 3305, 2950, 2834, 1735 and 1452 cm^{-1}; ^{1}H-NMR (400 MHz, CH_3OH) and ^{13}C-NMR (100 MHz, CH_3OH) spectral data, see Table 1; HR-ESI-MS: *m/z* 269.1748 $[M+H]^+$ (calcd. for $C_{15}H_{25}O_4$, 269.1753).

(2Z, 4S)-4,5-dihydroxy-3,4-dimethylpent-2-enoic acid ***(2):*** A yellow oily solid, $[\alpha]_D^{20}$ -2.8 (*c* 0.05, CH_3OH); IR ν_{max} (KBr) (cm^{-1}): 3342, 2938, 2883, 1738, 1430 and 1032; ^{1}H-NMR (400 MHz, CH_3OH) and ^{13}C-NMR (100 MHz, CH_3OH) spectral data, see Table 2; HR-ESI-MS: *m/z* 161.0814 $[M+H]^+$ (calcd. for $C_7H_{13}O_4$, 161.0814).

3. Results and Discussion

3.1. Structure Elucidation

Compound **1** was isolated as a reddish brown oily solid. The molecular formula $C_{15}H_{25}O_4$ was supported by the positive HR-ESI-MS molecular ion peak at *m/z* 269.1748 $[M+H]^+$ (calculated 269.1753 $[M+H]^+$). The IR spectrum displayed for hydroxy (3305 cm^{-1}), carbonyl (1735 cm^{-1}) and double bonds (1452 cm^{-1}). The ^{1}H NMR data of **1** (Table 1) exhibited three methyl signals at δ_H 0.98 (3H, t, CH_3-4′), 1.00 (3H, d, *J* = 7.8 Hz, CH_3-12), 1.06 (3H, d, *J* = 7.8 Hz, CH_3-11), three hypoxia-methylene signals at δ_H 3.58, 3.88 (2H, d, *J* = 2.5 Hz, H-10), 3.33 and 3.99 (2H, t, *J* = 8.2 Hz, H-2), 4.29 (2H, t, *J* = 6.6 Hz, H-1′) and a double bond signal at δ_H 7.62 (1H, d, *J* = 10.1 Hz, H-7) and 7.72 (1H, d, *J* = 10.1 Hz, H-8). The ^{13}C NMR data of **1** (Table 1) displayed 15 carbon signals, three of which belongs to the methyl groups at (δ_C 11.8, 14.1, 15.9), three of which were determined as methylene groups at (δ_C 64.5, 66.7, 74.4), four of which were confirmed as two double bond signals at (δ_C 106, 133.6, 129.9, 132.4) and carbonyl carbon signal at δ_C 169.3. In addition, five of which were determined as methylene groups at (δ_C 20.3, 31.7, 64.5, 66.7, 74.4) in the DEPT-135 spectrum. The 2D NMR data analysis confirmed the conclusion above.

The ^{1}H-^{1}H COSY correlations (Figure 2) from H-12/H-3, H-3/H-4 and H-4/H-11, accompanied with the HMBC correlations (Figure 2) of H-12/C-2, C-3 and C-4, H-11/C-3, C-4 and C-5, H-10/C-4, C-5 and C-6, the six-membered ring is an alkene ether structure based on C-6 (δ_C 133.6) and C-2 (δ_C 74.4), C-5 (δ_C 106.8) is connected with hydroxymethyl. In the HMBC spectrum (Figure 2), the

correlation between the proton signal at H-7 (δ_H 7.62) with the carbon signal at C-6 (δ_C 133.6), suggested that two double bonds are connected through C-6 and C-7. In the ^{1}H-^{1}H COSY spectrum (Figure 2), there is a correlation between H-7 and H-8, accompanied with the HMBC correlations (Figure 2) of H-8/C-9 and C-7, description of the double bond and the C-9 (δ_C 169.3) carbonyl group related. Finally, H-4′/H-3′, H-2′ and H-1′ was found in the ^{1}H-^{1}H COSY correlation spectrum, and the HMBC spectrum (Figure 2) shows that H-1′/C-9, C-2′ and C-3′, indicates that the carbonyl group (C-9) is linked to the n-butanol group, which is the n-butanol ester, demonstrated the 2D structure of **1** as butyl-3-(5-(hydroxymethyl)-3,4-dimethyl-3,4-dihydro-2*H*-pyran-6-yl) acrylate. In the NOESY spectrum of **1** (Figure 2), correlations between H-11/H-3 and H-12/H-4 deduced the *β*-configuration of H-3 and CH_3-11 and *α*-configuration of CH_3-12 and H-4. Coupling constants of $J_{7,8}$ = 10.1 Hz confirmed the *Z* configuration of $\Delta^{7,8}$. To further determine the absolute configuration of **1**, the ECD curves (Figure 3) were simulated of **1** [(3*R*, 4*R*)-**1** and (3*S*, 4*S*)-**1**]. The experimental and calculated ECD curves of (3*R*, 4*R*)-**1** matched well [25]. Thus, the structure of **1** was assigned as butyl(*Z*)-3-((3*R*,4*R*)-5-(hydroxymethyl)- 3,4-dimethyl-3,4-dihydro-2*H*-pyran-6-yl) acrylate (Figure 1).

Table 1. ^{1}H-NMR (400 MHz, in CD_3OD) and ^{13}C-NMR (100 MHz, in CD_3OD) spectral data of compound **1**

Position	δ_C	δ_H	Position	δ_C	δ_H
2	74.4, CH_2	2a, 3.33, t, (8.2) 2b, 3.99, t, (8.2)	10	64.5, CH_2	3.88, d, (2.5)
3	52.0, CH	1.46, m	11	11.8, CH_3	1.06, d, (7.8)
4	40.4, CH	2.11, m	12	15.9, CH_3	1.00, d, (7.8)
5	106.8, C	—	1′	66.7, CH_2	4.29, t, (6.6)
6	133.6, C	—	2′	31.7, CH_2	1.72, m
7	132.4, CH	7.62, d, (10.1)	3′	20.3, CH_2	1.44, m
8	129.9, CH	7.72, d, (10.1)	4′	14.1, CH_3	0.98, t, (7.5)
9	169.3, C	—			

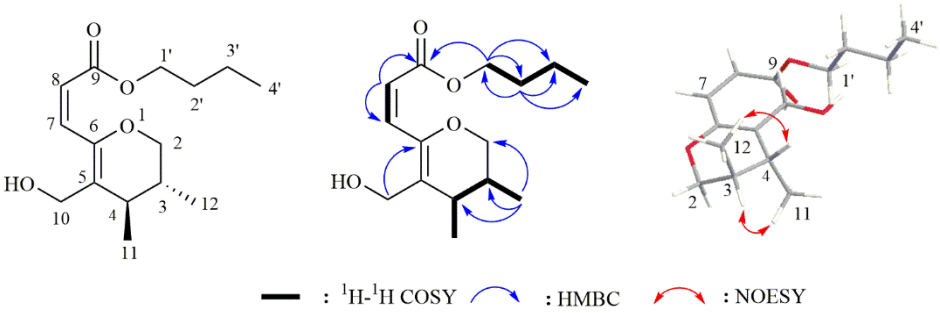

Figure 2. Key ^{1}H - ^{1}H COSY, HMBC and NOESY correlations of compound **1**

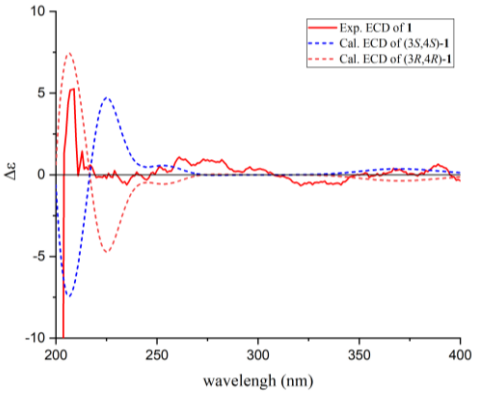

Figure 3. Experimental and calculated ECD spectra of **1**

Compound **2** was isolated as a yellow oily solid. The molecular formula $C_7H_{13}O_4$ was supported by the positive HR-ESI-MS molecular ion peak at *m/z* 161.0814 $[M+H]^+$ (calculated 161.0814 $[M+H]^+$). The IR spectrum displayed for hydroxy (3342 cm^{-1}), carbonyl (1738 cm^{-1}) and double bonds (1430 cm^{-1}). The ^1H NMR data of **2** (Tab. 2) exhibited two methyl signals at δ_H 1.38 (3H, s, CH_3-4), 2.07 (3H, d, J = 1.2 Hz, CH_3-3), one hypoxia-methylene signals at δ_H 3.70 (2H, m, H-5) and a trisubstituted double bond at δ_H 5.83 (1H, d, J = 1.4 Hz, H-2). The ^{13}C NMR data of **2** (Table 2) displayed 7 carbon signals, two of which belongs to the methyl groups at (δ_C 11.8, 18.2), one of which were determined as methylene groups at δ_C 63.9, two of which were confirmed as double bond signals at (δ_C 116.7, 172.0) and carbonyl carbon signal at δ_C 173.6. In addition, one of which were determined as methylene groups at δ_C 63.9 in the DEPT-135 spectrum. The 2D NMR data analysis confirmed the conclusion above. The HMBC correlations (Figure 4) correlations from CH_3-3/C-2, C-3 and C-4, prove the correlation between double bond and methyl group, CH_3-4/C-3, C-4 and C-5, H-5/C-3, C-4 and CH_3-4, H-2/CH_3-3, C-4 and C-1 disclosed the 2D structure of **2** as 4,5-dihydroxy-3,4-dimethylpent- 2-enoic acid. In the NOESY spectrum of **2**, correlations between H-2 and H-3(CH_3-3), indicated the *Z* configuration of $\Delta^{2,3}$. To further determine the absolute configuration of **2**, the ECD curves (Figure 5) were simulated of **2** [(4*S*)-**2** and (4*R*)-**2**]. The experimental and calculated ECD curves of (4*S*)-**2** matched well [26]. Therefore, the structure of **2** was assigned as (2*Z*,4*S*)-4,5- dihydroxy-3,4-dimethylpent-2-enoic acid (Figure 1).

Table 2. ^1H-NMR (400 MHz, in CD_3OD) and ^{13}C-NMR (100 MHz, in CD_3OD) spectral data of compound **2**

Position	δ_C	δ_H
1	173.6, C	—
2	116.7, CH	5.83, d, (1.4)
3	172.0, C	—
4	90.7, C	—
5	63.9, CH_2	3.70, m
CH_3-3	11.8, CH_3	2.07, d, (1.2)
CH_3-4	18.2, CH_3	1.38, s

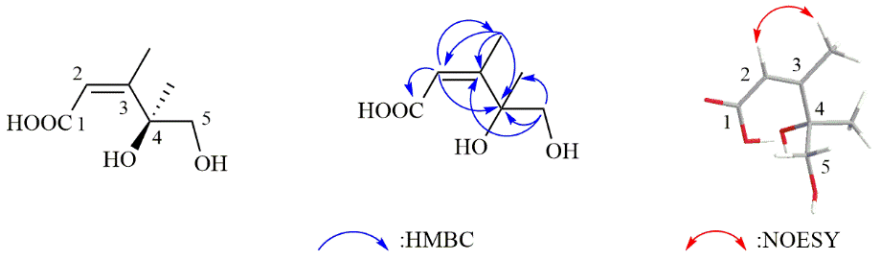

Figure 4. Key HMBC and NOESY correlations of compound **2**

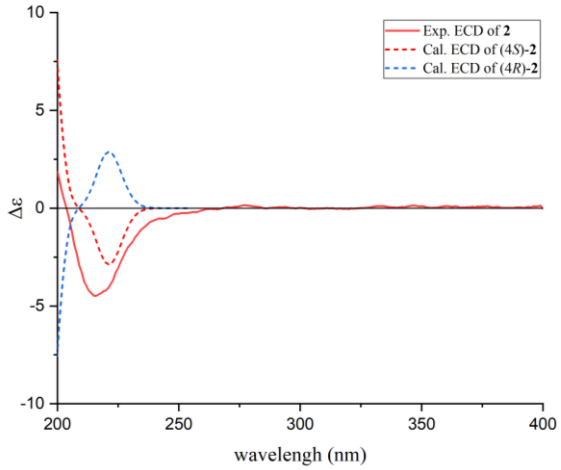

Figure 5. Experimental and calculated ECD spectra of **2**

3.2. Cytotoxicity Assay

The cytotoxic activities assay toward three human tumor cell (A549, HCT116 and SW620) lines were measured by the MTT method as we reported previously [4] for compounds **1**~**3** and **6**~**8**, using cisplatin as positive control. The experimental results (Tab. 3) showed that these compounds showed weak cytotoxicity in the human cancer cell lines.

Table 3 Cytotoxic activities of compounds **1**~**3** and **6**~**8** on A549, HCT116 and SW620 cancer cell lines. (IC_{50}, μM) [a]

Compounds	A549	HCT116	SW620
Cisplatin	32.1 ± 1.3	43.5 ± 3.3	32.5 ± 3.4
1	>100	>100	47.7 ± 2.5
2	>100	>100	>100
3	>100	>100	>100
6	>100	>100	>100
7	>100	>100	>100
8	>100	>100	>100

[a] IC_{50} values are means from three independent experiments (average ± SD) in which each compound concentration was tested in three replicate wells; Cisplatin as positive control.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (82104368), Shaanxi Provincial Science and Technology Department Project (2021JQ-744), Shaanxi University of

Chinese Medicine Postgraduate Innovation Program (2021CX06) and Shaanxi University of Chinese Medicine Science and Technology Innovation Team Project (2019-YL12).

Supporting Information

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

ORCID
Chao Ding: 0000-0001-9165-3063
Yuze Li: 0000-0001-7571-3214
Ying Wu: 0000-0002-0645-1254
Yu Sun: 0000-0001-5465-7989
Fengrui Wang: 0000-0002-0688-0315
Huawei Zhang: 0000-0003-4970-3818
Yi Jiang: 0000-0003-1200-1441
Dongdong Zhang: 0000-0003-0956-1261
Xiaomei Song: 0000-0003-1906-1578

References

[1] Y. Y. Zhang, Y. Z. Li, S. Q. Huang, Y. W. Cui, H. W. Zhang, W. L. Huang, C. Deng, W. Wang and X. M. Song (2020). Two new pregnane alkaloids from *Pachysandra terminalis Sieb.* et Zucc, *Nat. Prod. Res.* **35**, 1-7.

[2] L. Zhang, H. Y. Cheng, J. T. Wang, N. Zhang and G. Zhang (2015). Pharmacognosical studies on *Pachysandra terminalis* Sieb. et Zucc, *Jilin J. Chin. Med.* **35**, 1071-1073.

[3] W. Q. Qiu (2012). Compendium of commonly used chinese herbs with colourful illustrations of the original plants, M. Beijing. Ancient Chinese Medical Books Publishing House. 210.

[4] Y. Sun, C. Ding, F. R. Wang, Y. Y. Zhang, W. L. Huang, H. W. Zhang, Y. Z. Li, D. D. Zhang and X. M. Song (2021). Pregnane alkaloids with BRD4 inhibitory and cytotoxic activities from *Pachysandra terminalis*, *Phytochem. Lett.* **45**, 63-67.

[5] M. H. Qiu, R. L. Nie and Z. R. Li (1994). Chemical structure and activity screening of Pachysandra-type alkaloids, *Plant. Diversity.* **16**, 296-300.

[6] M. M. Hagiwara, K. Watanabe, H. Watanabe, M. Shimizu and T. Kikuchi (1984). Effects on gastric acid secretion of a steroidal alkaloid, epipachysamine-A, extracted from *Pachysandra terminalis* sieb. et zucc, *J. Pharm. Dyn.* **7**, 263-267.

[7] H. Watanabe, K. Watanabe, M. Shimadzu, T. Kikuchi and Z. Liu (1986). Anti-ulcer effect of steroidal alkaloids extracted from *Pachysandra terminalis*, *Planta. Med.* **2**, 56-58.

[8] M. H. Qiu, N. Nakamura, B. S. Min and M. Hattori (2005). Two new pregnanone derivatives with strong cytotoxic activity from *Pachysandra axillaris*, *Chem. Biodivers.* **2**, 866-871.

[9] S. Funayama, T. Noshita, K. Shinoda, N. Haga, S. Nozoe, M. Hayashi and K. Komiyama (2000). Cytotoxic alkaloids of *Pachysandra terminalis*, *Biol. Pharm. Bull.* **23**, 262-264.

[10] S. N. Ma (2013). Anti-metastatic effect of ionone alkaloids from *Pachysandra terminalis* and its mechanism, *Tianjin Med. Univ*.

[11] M. N. Jin, S. N. Ma, H. Y. Zhai, N, Qin, H. Q. Duan and D. X. Kong (2015). A new megastigmane alkaloid from *Pachysandra terminalis* with antitumor metastasis effect, *Chem. Nat. Compd*. **51**, 311-315.

[12] H. Y. Zhai, C. Zhao, N. Zhang, M. N. Jin, S. A. Tang, N. Qin, D. X. Kong and H. Q. Duan (2012). Alkaloids from *Pachysandra terminalis* inhibit breast cancer invasion and have potential for development as antimetastasis therapeutic agents, *J. Nat. Prod.* **75**, 1305-1311.

[13] M. H. Qiu and D. Z. Li (2002). Preliminary chemical taxonomy of family *Buxaceae*, *Chin. J. Appl. Envir. Biol.* **8**, 387-391.

[14] H. Zhao and X. Y. Xue (2014). A novel kind of pregnane alkaloid extracted from *Pachysandra terminalis* as a potential nonresistance-inducing antibiotic agent against methicillin-resistant staphylococcus aureus, *Professional Committee of Chemotherapy Pharmacology, Chin. Pharmacol. Soc.* 120-130.

[15] H. Zhao, X. Y. Wang, M. K. Li, Z. Hou, Y. Zhou, Z. Chen, J. R. Meng, X. X. Luo, H. F. Tang and X. Y. Xue (2015). A novel pregnane-type alkaloid from *Pachysandra terminalis* inhibits methicillin-resistant staphylococcus aureus in vitro and in vivo, *Phytother. Res.* **29**, 373-380.

[16] J. Du and M. Y. Zhou (2015). Progress in the treatment of human African Trypanosomiasis, *Pro. Mod. Biomed.* **15**, 1154-1159.

[17] D. Flittner, M. Kaiser, P. Mäser, N. P. Lopes and T. J. Schmidt (2021). The alkaloid-enriched fraction of *Pachysandra terminalis* (Buxaceae) shows prominent activity against *trypanosoma brucei rhodesiense*, *Molecules* **26**, 591-591.

[18] Y. Sun, B. L. Hou, Y. Yang and Y. H. Pei (2006). Studies on the chemical constituents of *Ulva lactuca* L, *J. Shenyang Pharm. Univ.* 148-150.

[19] Z. L. Ji, D. S. Ma and F. P. Miao (2014). Chemical constituents from *Trichoderma longibrachiatum*, an endophytic fungus derived from marine green alga *Codiunm fragile*, *J. Shenyang Univ. (Nat. Sci.)* **26**, 277-280+319.

[20] J. X. Zeng, B. B. Xu, Y. Bi, J. Wang, G. Ren, H. L. Wang, L. Zhang and H. Zou (2017). Chemical constitutes from *Plantaginis Semen* (II), *Chin. J. Exper. Tradit. Med. Form.* **23**, 81-84.

[21] O. Emi, O. Yoshiko, Y. Mikio and S. Motoyoshi (1996). Pharmacologically active components of a Peruvian medicinal plant, fluanarpo (*Jatropha cilliata*), *Chem. Pharm. Bull.* **44**, 333-336.

[22] J. Q. Zhao, J. Hao, Y. R. Ma, J. T. Deng, H. Q. Huang, X. Z. Yang and K. J. Pang (2021). Chemical constituents from *Hypericum attenuatum*, *Chin. Tradit. Pat. Med.* **43**, 664-669.

[23] B. S. Min, U. Youn and K. Bae (2008). Cytotoxic compounds from the stem bark *of Magnolia obovate*, *Nat. Prod. Sci.* **14**, 90-94

[24] Y. H. Fei, Z. Chen, X. R. Li, Q. M. Xu and S. L. Yang (2014). Chemical constituents from seeds of *Helianthus annuus*, *Chin. Tradit. Herb. Drug.* **45**, 631-634.

[25] D. D. Zhang, D. Q. Ruan, J. Y. Li, Z. Q. Chen, W. L. Zhou, F. J. Guo, K. X. Chen, Y. M. Li and R. Wang (2020). Four undescribed sulfur-containing indole alkaloids with nitric oxide inhibitory activities from *Isatis tinctoria* L. roots, *Phytochemistry* **174**, 112337.

[26] D. D. Zhang, Y. Sun, Z. Q. Chen, Q. Jia, W. L. Zhou, K. X. Chen, Y. M. Li and R. Wang (2020). Bisindole alkaloids with nitric oxide inhibitory activities from an alcohol extract of the *Isatis indigotica* roots, *Fitoterapia* **146**, 104654.