ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 16:5 (2022) 426-432

records of natural products

A New Iridoid Glucoside from the Stems of *Myoporum bontioides* (Sieb.et Zucc.) A. Gray

Tran Thi Minh [*], Nguyen Thi Viet Thanh and Vu Dinh Hoang [*]

[1]*School of Chemical Engineering, Hanoi University of Science and Technology, Hanoi 112456, Vietnam*

(Received August 30, 2021; Revised October 25, 2021; Accepted October 29, 2021)

Abstract: A phytochemical investigation of the stems of *Myoporum bontioides* (Sieb. et Zucc.) A. Gray, a semi-mangrove plant distributed along coastlines of north-eastern Vietnam and some Asian countries led to the isolation of a new iridoid glucoside (**1**), named myobontioside E, together with fourteen known compounds (**2-15**). Their structures were elucidated by means of HR ESI-MS, 1D and 2D NMR spectroscopy as well as comparison with the data reported in the literature. The cytotoxic effects on 8505C, MKN7, HT29, and T24 cell lines were assessed using SRB assay. Only iridoid **2** exhibited weak cytotoxicity against all tested cell lines with IC_{50} values ranging from 60.19 to 69.14 µM.

Keywords: *Myoporum bontioides*; Myoporaceae; iridoid glucoside; dammarane saponin; cytotoxicity.

1. Introduction

Myoporum, a genus of the family Myoporaceae, comprises approximately 32 species that occur throughout the Pacific region and Indian Ocean on Mauritius and Rodriguez islands [1]. *Myoporum bontioides* (Sieb. et Zucc.) A. Gray, a small shrubby semi-mangrove tree belonging to the family Myoporaceae, is mainly distributed along coastlines of north-eastern Vietnam, south China, Taiwan and Japan, and it has long been used to treat dermatitis, pulpitis and sciatica [2-3]. Previous chemical investigations mainly focused on the leaves of *M. bontioides* and revealed the presence of major constituents including sesquiterpenes [4-5], iridoids [6], flavonoids and phenylethanoids [7-10]. Some of these compounds have activities against bacteria [5, 10], plant pathogenic fungi [8, 11], and cancer [7]. In a previous paper, we reported the isolation and structural characterization of three flavonoids, along with the chemical composition of essential oil from the leaves of *M. bontioides* [12]. As a continuation of our investigation on this plant, we have isolated and structurally elucidated a new iridoid glucoside (**1**), together with fourteen known compounds including iridoids and iridoid glucosides (**2-7**), *O*-coumaric acid glucosides (**8-9**), aliphatic alcohol glycosides (**10-11**), a lignan glucoside (**12**), and dammarane saponins (**13-15**) from the *n*-BuOH soluble fraction of the methanol extract of *M. bontioides* stems. The cytotoxicity of iridoids and iridoid glucosides (**1-7**) against four human cancer cell lines 8505C, MKN7, HT29, and T24 was also reported.

[*] Corresponding author: E-Mail: minh.tranthi@hust.edu.vn; Phone:0084-988557877 *Fax: 0084-24-38690070*

The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products September-October 2022 EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.296.2108.2191
Available online: November 15, 2021

2. Materials and Methods

2.1. General Experimental Procedures

Optical rotations were measured with a JASCO P-2000 polarimeter (Hachioji, Japan) using MeOH as the solvent. NMR experiments were conducted on a Bruker Avance 500 MHz spectrometer with chemical shifts given in ppm (δ). HR-ESI-MS were obtained on a X500 QTOF mass spectrometer system (MA, USA). Column chromatography (CC) was performed using silica gel 60 (0.04-0.063 mm, Merck), C18 reversed-phase (RP) silica gel (150 μm, YMC), Diaion HP 20 (Mitsubishi chemical Co.), and Sephadex LH-20 (25-100 μm, Sigma-Aldrich). Thin-layer chromatography was performed using precoated silica gel 60 F254 and RP-18 F254S plates (0.25 mm, Merck), and compounds were detected by UV fluorescence at 254nm or spraying with 1% vanillin-H_2SO_4 in MeOH, followed by heating at 100 ^oC for 1-2 min.

2.2. Plant Material

The stems of *Myoporum bontioides* (Sieb. et Zucc.) A. Gray were collected in May, 2020 from coastal areas of Thai Binh province in northern Vietnam. The plant material was identified by Prof. Tran Huy Thai, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (VAST). A voucher sample (HUST.N05) was deposited at the Department of Organic Chemistry, School of Chemical Engineering, Hanoi University of Science and Technology (HUST), Vietnam.

2.3. Extraction and Isolation

The air-dried stems (2.0 kg) of *M. bontioides* were extracted three times with MeOH (3 × 7 L) at 45 ^oC for 1 h under sonication. The three extracts were combined and evaporated under reduced pressure to obtain a residue (335 g). This crude extract was suspended in H_2O (2 L) and successively partitioned with *n*-hexane, EtOAc, and *n*-BuOH to afford *n*-hexane (27 g), EtOAc (46 g), and *n*-BuOH (120 g) residues, after removal of the solvents.

The *n*-BuOH soluble fraction (120 g) was subjected to Diaion HP20 CC, eluting with water and increasing concentration of MeOH in water (20, 40, 60, 80, 100%, v/v) to afford five fractions *Fr.1-Fr.5*. Fraction *Fr.1* (20% MeOH, 12.8 g) was chromatographed on an RP-C_{18} silica gel column, eluting with MeOH/H_2O (1:3, 1:1, v/v) to give four subfractions *Fr.1.1-Fr.1.4*. Subfraction *Fr.1.2* (0.4 g) was purified by silica gel CC with CH_2Cl_2/MeOH (9:1, v/v) and then by Sephadex LH-20 CC with MeOH to obtain compound **4** (6.8 mg). *Fr.1.3* (4.8 g) was fractionated on a silica gel CC, eluting with a CH_2Cl_2/MeOH (10:1 to 1:1, v/v) gradient to afford eight subfractions *Fr.1.3.1-Fr.1.3.8*. Subfraction *Fr.1.3.1* was purified by Sephadex LH-20 CC with MeOH and then by silica gel CC with CH_2Cl_2/acetone (5:1, v/v) to give compounds **2** (5.1 mg) and **3** (25.4 mg). *Fr.1.3.6*, *Fr.1.3.7* and *Fr.1.3.8* were purified by RP-C_{18} silica gel CC, eluting with MeOH/H_2O (1:2, v/v) to yield compounds **5** (32.8 mg), **7** (17 mg) and **8** (24.2 mg), respectively. Compound **6** (25 mg) was isolated from *Fr.1.4* (1.6 g) by RP-C18 CC, eluting with MeOH/H_2O (1:3, v/v). Fraction *Fr.2* (40% MeOH, 8.3 g) was fractionated on an RP-C_{18} silica gel CC, eluting with MeOH/H_2O (3:1, 1:1, v/v) to provide three subfractions *Fr.2.1 -Fr.2.3*. Subfraction *Fr.2.1* (0.37 g) was purified by Sephadex LH-20 CC with MeOH to give compound **9** (72 mg). *Fr.2.2* (1.1 g) was purified by Sephadex LH-20 CC with MeOH and then by RP-C_{18} silica gel CC with MeOH/H_2O (1:1, v/v) to yield compound **11** (21 mg). Compound **10** (40.5 mg) was obtained from the *Fr.2.3* by RP-C_{18} silica gel CC, eluting with acetone/H_2O (2:1, v/v). Fraction *Fr.3* (60% MeOH, 7.4 g) was subjected to silica gel CC, eluting with a CH_2Cl_2/MeOH (10:1 to 1:1, v/v) gradient to afford twelve subfractions *Fr.3.1-Fr.3.12*. Subfractions *Fr.3.5* and *Fr.3.7* were purified by RP-C_{18} silica gel CC, eluting with acetone/H_2O (1:2, v/v) to yield compounds **14** (27 mg) and **12** (17 mg). *Fr.3.10* was subjected to Sephadex LH-20 CC with MeOH, followed by RP-C_{18} silica gel CC with MeOH/H_2O (1:2, v/v) to afford compounds **1** (8 mg) and **13**

(29.7 mg). *Fr.3.11* was purified by Sephadex LH-20 CC with MeOH and then by RP-C_{18} silica gel CC with MeOH/H_2O (2:3, v/v) to yield **15** (3.5 mg).

*Myobontioside E (**1**)*: White powder. $[\alpha]_D^{25}$ = –49.3 (*c* 0.1, MeOH); HR ESI-MS: *m/z* 603.1560 [*M* + HCOOH - H]$^-$ (calcd. for $C_{25}H_{31}O_{17}$, 603.15667); ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) data are given in Table 1.

2.4. Acid hydrolysis and sugar identification

Compound **1** (2.0 mg) was refluxed with 2 N methanolic HCl (5 mL) for 2 h. After neutralization with 1 N sodium carbonate solution, the reaction mixture was extracted with $CHCl_3$ to remove the aglycone. In the aqueous layer, D-glucose was identified by the optical rotation and TLC comparison with an authentic sample [R_f 0.17, EtOAc-MeOH-H_2O (4:1:0.1, v/v), $[\alpha]_D^{25}$ +45.7].

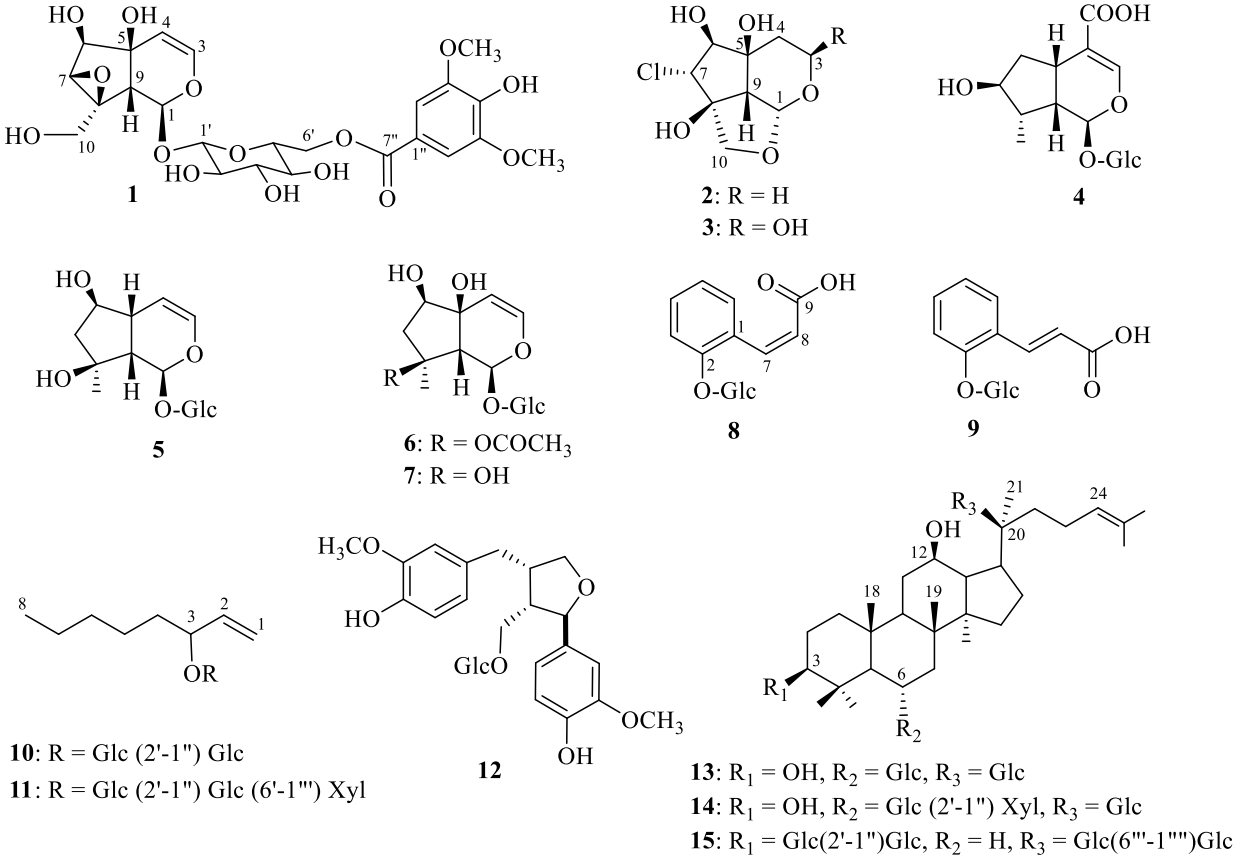

Figure 1. Chemical structures of compounds **1-15**

2.5. Cytotoxic Assay

The sulforhodamine B (SRB) method [13] was used to evaluate cytotoxic activity of seven iridoids **1-7** against four human cancer cell lines as 8505C (thyroid carcinoma), MKN7 (gastric carcinoma), HT29 (colorectal adenocarcinoma), and T24 (urinary bladder carcinoma) following the previously described protocol [14]. All tumor cell lines were supplied by Professor J. M. Pezzuto (Long-Island University, US) and Professor Jeanette Maier (Milan University, Italia). Ellipticine was used as a positive control.

3. Results and Discussion

3.1. Structure Elucidation

The *n*-BuOH soluble fraction was subjected to multiple chromatographic separations to afford fifteen compounds (**1**-**15**, Figure 1).

Myobontioside E (**1**) was isolated as a white powder. Its molecular formula was determined as $C_{24}H_{30}O_{15}$ by the HR ESI-MS at *m/z* 603.1560 $[M + FA - H]^-$ (calcd. 603.15667) and NMR spectroscopic data (Table 1). The ^{1}H NMR spectrum of **1** showed signals of two symmetrical aromatic protons at δ_H 7.35 (2H, *s*), *cis*-olefinic protons at δ_H 6.35 and 4.92 (both 1H, *d*, *J* = 6.0 Hz), an acetal proton at δ_H 5.13 (1H, *d*, *J* = 9.0 Hz), two oxygenated methylene groups at δ_H 4.60 (2H, *m*), 4.10 and 3.54 (both 1H, *d*, *J* = 13.0 Hz), an anomeric proton at δ_H 4.78 (1H, *d*, *J* = 8.0 Hz), along with other sugar proton signals at δ_H 3.30 - 3.64, two symmetrical methoxy groups at δ_H 3.92 (6H, *s*), and one non-deshielded methine proton δ_H 2.55 (1H, *d*, *J* = 9.0 Hz). The ^{13}C NMR spectrum exhibited signal for a carboxyl carbon at δ_C 167.9, four carbon signals for a symmetrical aromatic ring, a carbon signal for two methoxy groups at δ_C 57.0, nine carbon signals for C_9-type iridoid aglycone, and six carbon signals for a glucosyl moiety, which included an anomeric carbon signal at δ_C 99.8. The ^{1}H and ^{13}C NMR signals of **1** (Table S1) were in good agreement with those of myobontioside B, a known iridoid glucoside isolated from the same species of Japan [6], except that the signals of the ferulic acid in myobontioside B replaced by signals of a syringic acid. The syringyl moiety was linked by an ester bond to the hydroxyl group of C-6′ (δ_C 64.4, δ_H 4.60), which was confirmed by the HMBC correlation from H_2-6′ to C-7″ (δ_C 167.9). Noted that the HMBC spectrum also showed the correlations from two methoxy groups (δ_H 3.92) to C-3″, 5″ (δ_C 149.0), from H-2″, 6″ (δ_H 7.35) to the carboxyl carbon C-7″ (δ_C 167.9). In addition, the NOESY spectrum of **1** exhibited correlation between δ_H 3.92 (OCH_3) and δ_H 7.35 (H-2″, 6″), indicating the syringyl moiety. The relative configuration of **1** was confirmed according to structure of myobontioside B and based on *J* values and NOESY spectroscopic analysis. The NOE correlations of H-6 (δ_H 3.81) with H-7 (δ_H 3.45), and also of H-6 with H-1 (δ_H 5.13) indicated that H-1, H-6 and H-7 were on the same side and designated as α-oriented (Figure 2). The small coupling constant ($J_{6,7}$ = 1.0 Hz) between H-6 and H-7 also supported this conformation. The large coupling constant ($J_{1,9}$ = 9.0 Hz) between H-1 and H-9 suggested that they were present in *trans*-diaxial relationship and positioning H-9 in *β*-orientation [6, 15]. Based on this evidence, the compound **1** was determined to be a new compound as macfadyenoside-6′-syringic acid ester.

Table 1. ^{1}H (at 500 MHz) and ^{13}C (at 125 MHz) NMR data for compound **1** in CD_3OD

Position	δ_C	δ_H, *J* in Hz	**Position**	δ_C	δ_H, *J* in Hz
1	95.5 (CH)	5.13 (1H, *d*, *J* = 9.0)	**Glc**-1′	99.8 (CH)	4.78 (1H, *d*, *J* = 8.0)
2	-	-	2′	74.7 (CH)	3.30 (1H, *dd*, *J* = 8.0, 9.0)
3	142.6 (CH)	6.35 (1H, *d*, *J* = 6.0)	3′	77.5 (CH)	3.45 (1H, *m*)
4	108.0 (CH)	4.92 (1H, *d*, *J* = 6.0)	4′	71.8 (CH)	3.45 (1H, *m*)
5	74.4 (C)	-	5′	76.0 (CH)	3.64 (1H, *m*)
6	78.7 (CH)	3.81 (1H, *d*, *J* = 1.0)	6′	64.4 (CH_2)	4.60 (2H, *m*)
7	63.2 (CH)	3.45 (1H, *m*)	**Acyl**-1″	121.3 (C)	-
8	66.5 (C)	-	2″, 6″	108.4 (CH)	7.35 (2H, *s*)
9	50.9 (CH)	2.55 (1H, *d*, *J* = 9.0)	3″, 5″	149.0 (C)	-
10	61.5 (CH_2)	4.10 (1H, *d*, *J* = 13.0)	4″	142.6 (C)	-
		3.54 (1H, *d*, *J* = 13.0)	7″	167.9 (C)	-
			3″,5″-OCH_3	57.0 (CH_3)	3.92 (6H, *s*)

The structures of fourteen isolates (**2**-**15**) were identified as myopochlorin (**2**) [6], 3-hydroxymyopochlorin (**3**) [16], 8-*epi*-loganic acid (**4**) [17], ajugol (**5**) [18], 8-*O*-acetylharpagide (**6**) [19], harpagide (**7**) [20], *cis*-melilotoside (**8**) and *trans*-melilotoside (**9**) [21-22], octane-1-en-3-ol-3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranoside (**10**) [23], ebracteatoside B (**11**) [24], lariciresinol-9-

O-*β*-D-glucopyranoside (**12**) [25], ginsenoside Rg1 (**13**) [26], notoginsenoside-R1 (**14**) [27], and ginsenoside Rb1 (**15**) [28] by detailed analysis of their 1D- and 2D-NMR spectroscopic data in comparison with those reported in the literature. This is the first report on the isolation of aliphatic alcohol glycosides (**10-11)** and dammarane saponins (**13-15)** from this genus [3].

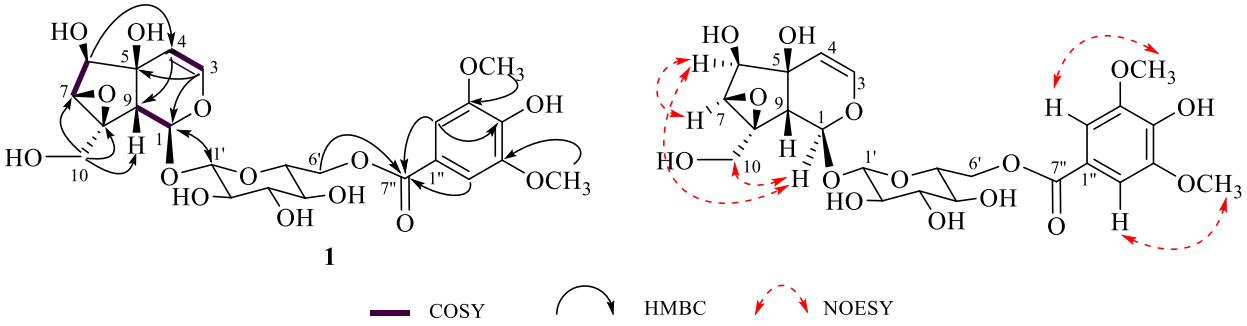

Figure 2. ^{1}H-^{1}H COSY and the selected HMBC, NOESY correlations of compound **1**

3.2. Cytotoxicity Activity

Iridoids and iridoid glucosides **1-7** were evaluated *in vitro* for their cytotoxicity against the 8505C, MKN7, HT29, and T24 human cancer cell lines using SRB method with ellipticine as the positive control [13-14]. As the obtained results, only myopochlorin (**2**) exhibited weak cytotoxicity against all the tested cancer cell lines with the IC_{50} values of 60.19 ± 1.75, 64.25 ± 1.41, 67.65 ± 1.32, and 69.14 ± 1.37 μM, respectively. The other compounds were inactive in this test (IC_{50} > 100 μM).

Acknowledgments

This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 104.01-2018.36.

Supporting Information

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

ORCID

Tran Thi Minh: 0000-0003-0784-4217
Nguyen Thi Viet Thanh: 0000-0001-8857-0224
Vu Dinh Hoang: 0000-0002-2112-6732

References

[1] G. S. Richmond and E. L. Ghisalberti (1995). Cultural, food, medicinal uses and potential applications of *Myoporum* species (Myoporaceae), *Econ. Botan.* **49**, 276–285.
[2] H. H. Pham (1999). An Illustrated Flora of Vietnam. Hanoi Young Publishing House (in Vietnamese), Hanoi, Vietnam. **3**, p. 11.
[3] A. Musa (2021). Phytochemistry, pharmacological potency, and potential toxicity of *Myoporum* spp. *Rec. Nat. Prod.* **15**, 148-168.
[4] Q. Wang, C. Ma and J. Zhai (2000). Furanoeudesmane-B, a new eudesmane sesquiterpenoid from *Myoporum bontioides*, *Acta Cryst.* **C56**, e569.

[5] L. M. Dong, L. L. Huang, H. Dai, Q. L. Xu, J. K. Ouyang, X. C. Jia, W. X. Gu and J. W. Tan (2018). Anti-MRSA sesquiterpenes from the semi-mangrove plant *Myoporum bontioides* A. Gray, *Mar. Drugs* **16**, 438-445.

[6] M. Kanemoto, K. Matsunami, H. Otsuka, T. Shinzato, C. Ishigaki and Y. Takeda (2008). Chlorine-containing iridoid and iridoid glucoside and other glucosides from leaves of *Myoporum bontioides*, *Phytochemistry* **69**, 2517-2522.

[7] J. R. Weng, L. Y. Bai, W. Y. Lin, C. F. Chiu, Y. C. Chen, S. W. Chao and C. H. Feng (2017). A flavone constituent from *Myoporum bontioides* induces M-Phase cell cycle arrest of MCF-7 breast cancer cells, *Molecules* **22**, 472-484.

[8] L. L. Huang, J. W. Li, C. L. Ni, W. X. Gu and C. Y. Li (2011). Isolation, crystal structure and inhibitory activity against *Magnaporthe Grisea* of (2*R*, 3*R*)-3,5,7-trihydroxyflavanone 3-acetate from *Myoporum Bontioides* A. Gray, *Chin. J. Struct. Chem.* **30**, 1298-1304.

[9] T. Iwashina and G. Kokubugata (2010). Flavonoids in the leaves and flowers of *Myoporum bontioides* native to northernmost region in the Myoporaceae, *Bull. Natl. Mus. Nat. Sci. Ser. B.* **36**, 117-125.

[10] H. Ye, H. Dai, L. Wu, Y. Guo and W. Gu (2014). Chemical constituents from leaves of *Myoporum bontioides* and their bacteriostatic activities, *J. Trop. Subtrop. Bot.* **22**, 307-313.

[11] D. Yecheng, Y. Zhen, Y. Yanzhen and B. Xiulian (2008). Inhibitory activity against plant *pathogenic fungi* of extracts from *Myoporum bontioides* A. Gray and identification of active ingredients, *Pest Manag. Sci.* **64**, 203-207.

[12] T. T. Minh, T. T. Ngoan, N. T. M. Thuong, H. K. Toan, N. X. Truong, T. T. Huong, G. T. P. Ly, D. T. T. Ai, N. D. Khanh (2020). Chemical compositions and bioefficacy against *Spodoptera litura* of essential oil and ethyl acetate fraction from *Myoporum bontioides* leaves, *Vietnam J. Chem.* **58**, 57-62.

[13] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney and M. R. Boyd (1990). New colorimetric cytotoxic assay for anticancer-drug screening, *J. Natl. Cancer Inst.* **82**, 1107-1112.

[14] T. T. Minh, N. H. Thu, H. K. Toan, T. T. Quang, N. T. V. Thanh, T. T. Huong and T. V. Sung (2022). Three new phenolic sulfates from *Acrostichum aureum* collected from coastal area of Thai Binh province, Vietnam and their cytotoxic activity, *Rec. Nat. Prod.* **16**, 66-73.

[15] E. Davini, C. Iavarone and C. Trogolo (1983). Effect of an 8-hydroxymethyl substituent on the base-catalyzed ring opening of 7,8-epoxyiridoid glucosides, *J. Org. Chem.* **48**, 2854-2857.

[16] C. Lémus, R. Grougnet, E. N. Ellong, W. Tian, M. L. Bornet, S. Adenet, K. Rochefort, S. Michel and H. Dufat (2015). Phytochemical study of *Capraria biflora* L. Aerial parts (Scrophulariaceae) from Martinique island (French West Indies), *Phytochem. Lett.* **13**, 194–199.

[17] Z. Dogan, K. Ishiuchi, T. Makino and I. Saracoglu (2019). New acylated iridoid glucosides from *Scutellaria glaphyrostachys* Rech.f. and chemotaxonomic importance for the genus *Scutellaria*, *Phytochem. Lett.* **32**, 157-161.

[18] S. Damtoft, S. R. Jensen and B. J. Nielsen (1981). ^{13}C and ^{1}H NMR spectroscopy as a tool in the configurational analysis of iridoid glucosides, *Phytochemistry*, **20**, 2717-2732.

[19] Z. Min, M. Mizuo, S. Wang, M. Iinuma and T. Tanaka (1990). Two new neo-clerodane diterpenes in *Ajuga decumbens*, *Chem. Pharm. Bull.* **38**, 3167-3168.

[20] Y. J. Yu, J. C. Do, K. Y. Jung, S. Y. Kwon and K. H. Son (1998). Studies on the constituents of the Herbs of *Ajuga multiflora*, *Kor. J. Pharmacogn.* **29**, 75-78.

[21] J. Monton and F. Van der Kooy (2014). Identification of *cis*- and *trans*-melilotoside within an *Artemisia annua* tea infusion, *Eur. J. Med. Plants* **4**, 52-63.

[22] P. M. Giang, V. M. Trang, D. T. V. Huong, S. Kawakami and H. Otsuka (2019). Thymol derivatives from *Eupatorium fortunei*, *Rec. Nat. Prod.* **13**, 434-439.

[23] N. Li, X. Li, B. L. Hou and D. L. Meng (2008). New disaccharoside from *Camptosorus sibiricus* Rupr. *Nat. Prod. Res.* **22**, 1379–1383.

[24] T. Kanchanapoom, R. Kasai, C. Picheansoonthon and K. Yamasaki (2001). Megastigmane, aliphatic alcohol and benzoxazinoid glycosides from *Acanthus ebracteatus*, *Phytochemistry* **58**, 811–817.

[25] H. K. Cho, W. S. Suh, K. H. Kim, S. Y. Kim and K. R. Lee (2014). Phytochemical constituents of *Salsola komarovii* and their effects on NGF induction, *Nat. Prod. Sci.* **20**, 95-101.

[26] D. S. Kim, Y. J. Chang, U. Zedk, P. Zhao, Y. Q. Liu and C. R. Yang (1995). Dammarane saponins from *Panax ginseng*, *Phytochemistry* **40**, 1493-1497.

[27] J. Zhou, M. Wu, S. Taniyasu, H. Besso, O. Tanaka, Y. Saruwatari, and T. Fuwa (1981). Dammarane saponins of Sanchi Ginseng, roots of *Panax notoginseng* (Burk.) F. H. Chen (Araliaceae): Structures of new saponins Notoginsenoides-R1 and –R2, and identification of Ginsenoides-Rg_2 and –Rh_1, *Chem. Pharm. Bull*. **29**, 2844-2850.

[28] J. G. Cho, M. K. Lee, J. W. Lee, H. J. Park, D. Y. Lee, Y. H. Lee, D. C. Yangand N. I. Baek (2010). Physicochemical characterization and NMR assignments of ginsenosides Rb1, Rb2, Rc, and Rd isolated from *Panax ginseng*, *J. Ginseng Res.* **34**, 113-121.