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CHEMICAL CONSTITUENTS OF ETHYL ACETATE EXTRACT FROM *Taxus wallichiana* ZUCC. (TAXACEAE) LEAVES COLLECTED IN HA GIANG PROVINCE

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Abstract. Investigation on the chemical constituents of ethyl acetate extract from leaves of *Taxus wallichiana* Zucc. (Taxaceae), collected in Ha Giang province, has resulted in the isolation of three metabolites for the first time: sciadopitysin (**1**), *β*-sitosterol (**2**), and isoquercitrin (**3**). Their structures were determined by spectroscopic methods.

Keywords*: Taxus wallichiana* Zucc., sciadopitysin, isoquercitrin.

1. Introduction

Taxus wallichiana Zucc. (Taxaceae), named as "cay thong do", is widely distributed in higher than 1,500 m in the mountains of Northern (such as Ha Giang, Cao Bang provinces) and Western Vietnam (such as Da Lat, Lam Dong provinces) [1, 2]. According to Vietnam traditional medicine, this plant is used for curing asthma, bronchitis, hiccough, indigestion, epilepsy, ascarid [1]. The fascinating molecule taxol [3] an important antitumor and anti-leukemic drug and its close analogs have been isolated from the plant. The genus *Taxus* was intensively investigated for the content not only of taxoids but also of other constituents, for instance, biflavones [4], flavones [5], lignans [6], diterpenoids [7]. This paper describes the isolation and structural elucidation of three metabolites from the leaves of *Taxus wallichiana* Zucc., collected in Ha Giang province.

2. Content

2.1. Materials and methods

**** Plant material***

Leaves of *Taxus wallichiana* Zucc. were collected in Bat Dai Son commune, Quan Ba distr., Ha Giang (July 2017), and identified by Prof. Dr. Do Huu Thu (Institute of Ecology, Natural Resource and Biology, VAST, Vietnam). Voucher specimens are deposited at the faculty of Chemistry, Hanoi University of Education (TW201707).

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**** General procedure***

Thin layer chromatography (TLC) was carried out on precoated Si gel GF_{254} (Merck Co., Germany) and TLC spots were viewed at 254, 302, and 366 nm and visualized by spraying with vanillin-10% H_2SO_4 solution. Column chromatography (CC) was carried out on silica gel 60 (60 - 100 μM, Merck). NMR (^1H, ^{13}C NMR, HSQC and HMBC) spectra were recorded on a Bruker Avance 500MHz instrument. The chemical shift (δ) values are given in ppm with TMS as internal standard, coupling constant *J* - by Hz. Mass spectra, including high resolution MS were recorded on an HP 5989B mass spectrometer and FT-ICR-MS (Varian 910-MS TQFTMS-7 Tesla).

**** Extraction and Isolation***

Dried powder of *T.wallichiana* leaves (2,500 g) was extracted with methanol. The methanolic extract was concentrated to give a residue (175 g) which was further partitioned into *n*-hexane, EtOAc, BuOH, and water. The ethyl acetate crude extract (13.7 g) was subjected to CC over silica gel and eluted gradient with *n*-hexane - ethyl acetate from 4:1 to 1:1, ethyl acetate - methanol from 10:1 to 0: 10. Eight fractions were successively obtained. Fraction 3 (37 mg) was chromatographed on CC using *n*-hexane - ethyl acetate as the solvent system to yield compounds **1** (4 mg), **2** (8 mg), and **3** (15 mg).

Compound 1: yellow crystals; ESI-MS positive: *m/z* $[M+H]^+$ calcd for $C_{33}H_{25}O_{10}$: 581.0 found 580.9, negative *m/z* $[M-H]^-$ calcd for $C_{33}H_{23}O_{10}$ 579.0 found 578.7; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$): Table 1.

Compound 2: white needle crystals; ESI-MS positive: *m/z* $[M-H_2O+H]^+$ calcd for $C_{29}H_{49}$: 397.0 found 397.1; ^1H NMR (500 MHz, $CDCl_3$) δ (ppm), *J* (Hz): 5.35 (t 2.5, H-6), 3.52 (m, H-3), 1.01 (s, H18), 0.92 (d 6.5, H-21), 0.87 (t 7.5, H-29), 0.83 (d 6.5, H-26), 0.81 (d 6.5, H-27), 0.68 (s, H-19); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 140.8 (C-5), 121.7 (C-6), 71.8 (C-3), 56.8 (C-14), 56.1 (C-17), 50.2 (C-9), 45.9 (C-24), 42.34 (C-4), 42.32 (C-13), 39.8 (C-12), 37.3 (C-1), 36.5 (C-20), 36.2 (C-10), 34.0 (C-22), 31.9 (C-7), 31.9 (C-8), 31.7 (C-2), 29.7 (C-25), 28.2 (C-16), 26.1 (C-23), 24.3 (C-15), 23.1 (C-28), 21.1 (C-11), 19.8 (C-18), 19.4 (C-26), 19.1 (C-27), 18.8 (C-21), 12.0 (C-19), 11.9 (C-29).

Compound 3*:* yellow powder; ESI-MS negative: *m/z* $[M-H]^-$ calcd for $C_{21}H_{19}O_{12}$: 463.0, found 462.8; ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6): Table 2.

2.2. Results and discussion

Compound **1** was obtained from ethyl acetate extract of *Taxus wallichiana* leaves. Its molecular formula was identified as $C_{33}H_{24}O_{10}$ based on its *pseudo* molecular ion peaks from ESI-MS, together with ^1H- and ^{13}C NMR data. The ^1H NMR spectrum (Table 1) shows two hydroxyl signals at δ_H 12.89 and 13.04 (each, s) shifted to the lower magnetic field due to intramolecular hydrogen bonding to the carbonyl groups and also three methoxyl groups (δ_H 3.75 3.80 3.83, each s), twelve olefinic protons which are the three pairs of *ortho* coupling protons, one pair of meta coupling protons, and four isolated protons. The ^{13}C NMR spectrum of **1** exhibited thirty-three carbons including two carbonyl signals for flavones at δ_C 181.9, 182.1, three methoxyl signals, and twenty-eight olefinic carbons which include twelve tertiary carbons and sixteen quaternary carbons. From ^1H and ^{13}C NMR spectral analysis **1** was assumed to be a

biflavonoid. The HMBC spectrum of **1** shows the crosslink peak H-2'/C-8" revealed that two flavones connected between C-3' and C-8". From the above analysis of 1D, 2D NMR and MS spectra, **1** was assumed to be sciadopitysin [8]. This compound was isolated for the first time from Vietnamese *Taxus wallichiana* Zucc. According to Gu Q. *et al*, sciadopitysin can inhibit the Aβ aggregation and reduce Aβ-induced toxicity in the primary cortical neurons, becomes a promising active component for anti-Alzheimer's disease [9].

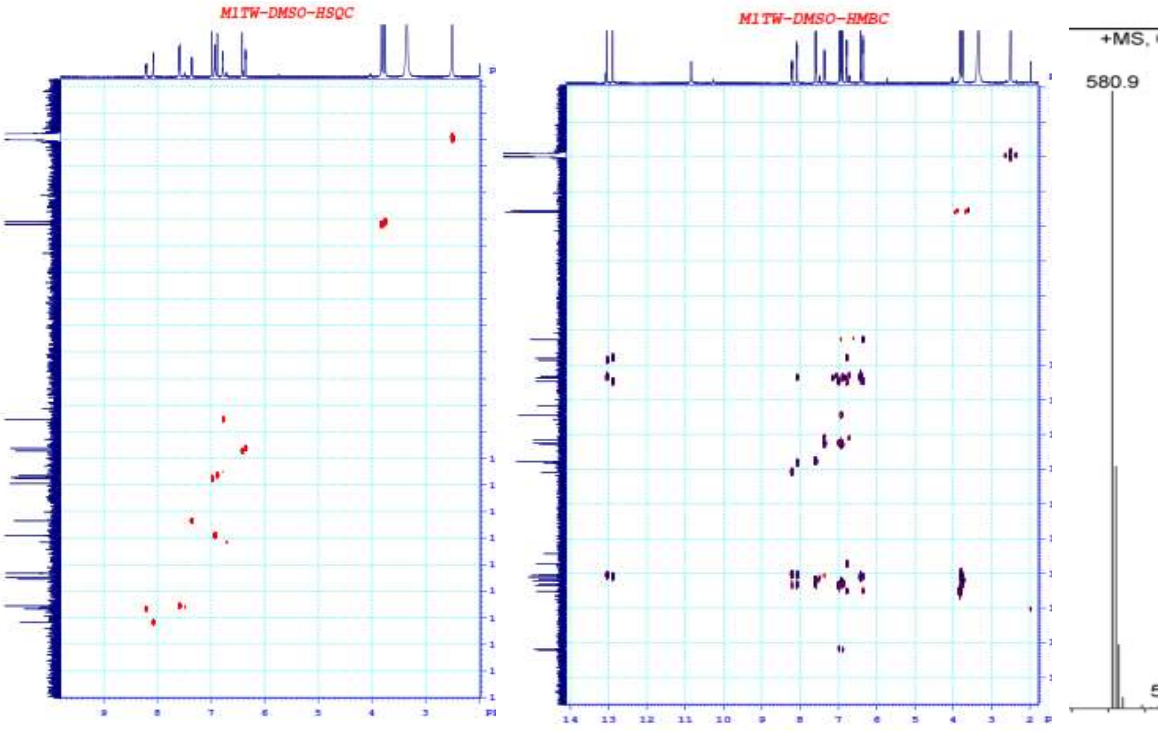
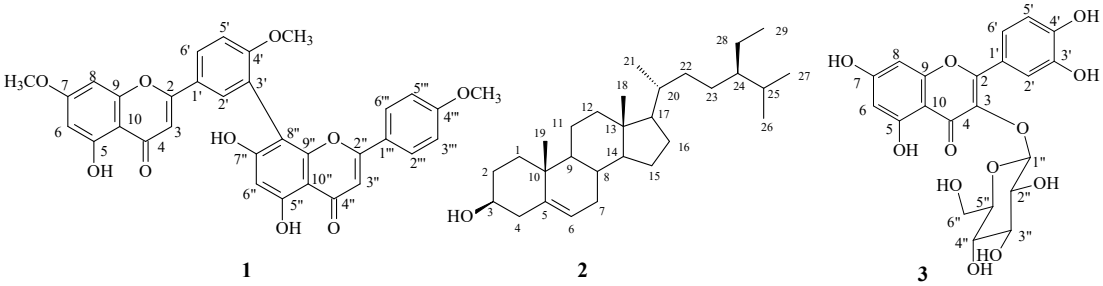


Figure 1. HSQC, HMBC and +MS spectra of compound 1



1 **2** **3**

Figure 2. Structures of compounds 1, 2 and 3

Table 1. ^{1}H NMR and ^{13}C NMR data of compounds 1 (δ ppm, J Hz)

No	^{1}H NMR	^{13}C NMR	No	^{1}H NMR	^{13}C NMR	No	^{1}H NMR	^{13}C NMR
2	-	163,1	5’	7.36 d 9.0	111.8	3’’’	6,92 d 9,0	114,5
3	6.98 s	103,7	6’	8.22 dd 9.0 2.5	128.3	4’’’	-	165,2
4	-	181.9	2”	-	163.6	5’’’	6.92 d 9.0	114.5
5	-	160.6	3”	6.88 s	103.2	6’’’	7.59 d 9.0	127.8
6	6.35 d 2.0	98.1	4”		182.1	7-OCH_3	3.75 s	55.5
7	-	162.2	5”	-	161.1	4’-OCH_3	3.80 s	55.9
8	6.78 m	92.7	6”	6.42 s	98.6	4’’’-OCH_3	3.83 s	56.0
9	-	157.3	7”	-	161.8	HO-5	13.04 s	-
10	-	103.6	8”	-	103.8	HO-5”	12.89 s	-
1’	-	122.8	9”	-	154.3	HO-7”	10.85 s	-
2’	8.08 d 2.5	130.9	10”	-	104.8			
3’	-	122.4	1’’’	-	121.6			
4’	-	160.6	2’’’	7.59 d 9.0	127.8			

Compound **2** was obtained from ethyl acetate extract of *Taxus wallichiana* leaves. Its molecular formula was identified as $C_{29}H_{50}O$ based on its *pseudo* molecular ion peak $[M-H_2O+H]^+$ from ESI-MS, together with ^{1}H- and ^{13}C NMR data. The ^{1}H NMR spectrum of **2** shows the presence of two methyl singlets at δ_H 0.68, and 1.01 ppm; three methyl doublets that appeared at δ_H 0.81, 0.83 and 0.92 ppm; and a methyl triplet at δ_H 0.87 ppm. The ^{1}H NMR spectra also showed the presence of one olefinic proton at δ_H 5.35 ppm suggesting them belonged to a double bond, >C=CH-. The proton that appeared as a multiplet at δ 3.52 ppm was assigned for a methine proton bonded to carbinol carbon (C**H**OH). From the above analysis, we suggested that compound **2** should belong to the group of sterols. The ^{13}C NMR spectrum of compound **2** shows the presence of six methyl (δ_C 11.9, 12.0, 18.8, 19.1, 19.4, 19.8), eleven methylene, nine methine, and three quaternary carbons. Among them, two carbon signals at δ_C 140.8 and 121.7 were assigned for a double bond, >C=CH-. The carbon signal at δ_C 71.8 was assigned for cyclic carbinol carbon of a sterol (C-3). The above spectral data supported the presence of sterol skeleton having hydroxyl group at C-3 position with one double bond at

C-5/C-6 with six methyl groups. Thus, compound **2** was assigned as the known *β*-sitosterol [10]. This compound is very popular in many plants.

The molecular formula of compound **3** was found to be $C_{21}H_{20}O_{12}$ by FT-ICR-MS. Analysis of its ^{1}H NMR spectra revealed that it has five aromatic protons at δ_H 7.58, 7.57, 6.84 6.40 and 6.20 ppm; seven *O*-glycoside protons from δ_H 3.09 to 5.45 ppm, suggesting that **3** should be an *O*-glycoside flavonoid (Table 2). The ^{13}C NMR spectrum has resonances of 21 carbons (Table 2) including one carbonyl signal for flavones at δ_C 177.4, fourteen olefinic carbons which include five tertiary carbons and nine quaternary carbons, six aliphatic carbons of monosaccharide. The HMBC spectrum of **3** shows the crosslink peak between C-3 and H-1", revealed that monosaccharide moiety should connect to flavone by C-3/C-1" bond. Compound **3** has very similar spectral data with those of isoquercitrin [11]. Therefore, compound **3** was determined as isoquercitrin. This compound was isolated for the first time from Vietnamese *Taxus wallichiana* Zucc. According to Chen Q. *et al*, isoquercitrin can inhibit the progression of pancreatic cancer *in vivo* and *in vitro* by regulating opioid receptors and the mitogen-activated protein kinase signaling pathway [12].

Table 2. ^{1}H NMR and ^{13}C NMR data of compounds 3 (δ ppm, J Hz)

No	^{1}H NMR	^{13}C NMR	No	^{1}H NMR	^{13}C NMR	No	^{1}H NMR	^{13}C NMR
2	-	156.3	10	-	103.9	1"	5.45 d 7.5	100.9
3	-	133.3	1'	-	121.1	2"	3.26 m	74.1
4	-	177.4	2'	7.58 d 2.0	116.2	3"	3.23 m	76.5
5	-	161.2	3'	-	144.7	4"	3.09 m	69.9
6	6.20 d 2.0	98.6	4'	-	148.4	5"	3.09 m	77.5
7	-	164.1	5'	6.84 d 9.0	116.2	6"	3.58 d 9.0 3.32 brd s	60.9
8	6.40 d 2.0	93.4	6'	7.57 m	121.5	HO-5	12.63 s	-
9	-	156.13						

3. Conclusions

Chemical composition of ethyl acetate extract of the leaves of *Taxus wallichiana* Zucc. collected in Ha Giang province, has been investigated. Three metabolites, sciadopitysin (**1**), *β*-sitosterol (**2**), and isoquercitrin (**3**) were isolated for the first time and structurally elucidated by MS, 1D, and 2D NMR spectroscopies.

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