INVESTIGATION ON CHEMICAL CONSTITUENT OF WATER FRACTION OF *PIPER BETLE* L.

KHẢO SÁT THÀNH PHẦN HOÁ HỌC PHÂN ĐOẠN NƯỚC LOÀI TRẦU KHÔNG

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ABSTRACT

From water fraction of the whole plant of *Piper betle*, three compounds including thymidine (**1**), adenosine (**2**), and 1,2-di-*O*-*β*-glucopyranosyl-4-allylbenzene **(3)** were isolated. Their chemical structures were elucidated by analyses of their NMR spectral data and comparison with those reported in the literature. Among three isolated compounds, this is the first report the isolation of 1,2-di-*O*-*β*-glucopyranosyl-4-allylbenzene from *P. betle*.

Keywords: *Piper betle, 1,2-di-O-β-glucopyranosyl-4-allylbenzene, adenosine, thymidine.*

TÓM TẮT

Từ phân đoạn nước của loài trầu không (*Piper betle*) đã được phân lập ba hợp chất gồm thymidine (**1**), adenosine (**2**), và 1,2-di-O- *β*-glucopyranosyl-4-allylbenzene **(3)**. Cấu trúc hoá học của các hợp chất này được xác định dựa trên phân tích phổ NMR, và so sánh với các số liệu phổ đã được công bố trong tài liệu tham khảo. Trong ba hợp chất phân lập được, đây là lần đầu tiên phân lập được hợp chất 1,2-di-*O*-*β*-glucopyranosyl-4-allylbenzene từ loài trầu không.

Từ khóa: *Trầu không, 1,2-di-O-β-glucopyranosyl-4-allylbenzene, adenosine, thymidine.*

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Received: 12 January 2019
Revised: 07 May 2019
Accepted: 20 February 2020

1. INTRODUCTION

Piper betle (Piperaceae family) is grown and distributed widely in the tropical countries in the southern and southeastern Asia. The leaves and rhizomes of *P. betle* have been used in traditional medicines for a long time [1]. Chemical components of *P. betle* were reported in the literature including polyphenols, carotenoids, terpenoids, alkaloids, steroids and aliphatic acids [2-6]. The methanolic extract, the essential oil, and several compounds from *P. betle* were screened and showed a lot of valuable bioactivities such as strong anti-microbial and antiseptic, antidotal, anti-inflammatory, and anti-oxidation effects [7-10]. In Vietnam, there were a few investigations focusing on chemical constituents of *P. betle* as well as its essential oil [3-4, 11]. In the contribution to clarify chemical constituent of *P. betle* growing in Vietnam, herein, we report the isolation and structural determination of three compounds from water fraction of the whole plants of *P. betle*.

2. MATERIAL AND METHODS

2.1. General experimental procedures

NMR spectra were recorded on a Jeol 600MHz FT-NMR spectrometer. Column chromatography was performed using a silica gel (Kieselgel 60, 70 - 230 mesh and 230 - 400 mesh, Merck) or RP-18 resins (150µm, YMC), thin layer chromatography (TLC) using a pre-coated silica gel 60 F_{254} (0.25mm, Merck) and RP-18 F_{254S} plates (0.25mm, Merck).

2.2. Extraction and isolation

The dried and powdered whole plants of *Piper betle* (3kg) was macerated in MeOH for three times (each 6L, 4hrs in ultrasonic bath) to give methanol extract. The methanolic residue (170g) was suspended in water (2L) and successively partitioned with *n*-hexane, dichloromethane, and ethyl acetate. The obtained water soluble fraction was poured on a diaion HP-20 column chromatography (CC), washed with water (2L) and then eluted with gradient solvent system of methanol/water (0 - 100% volume of methanol) to obtain three fractions W1 (6g), W2 (32g) and W3 (17g). The W2 fraction was subjected to a silica gel CC and eluted with gradient solvent system of dichloromethane/ methanol (0 - 100% volume of methanol) to give five fractions W2A-W2F. The W2B was repeatedly chromatographed on a silica gel column, eluting with dichloromethane/methanol/water (6/1/0.1, v/v/v) to give three smaller fractions W2B1-W2B3. Fraction W2B2 was purified on a reverse phase C18 CC using a solvent system of acetone/water (2/3, v/v) to give compound **1** (18 mg) and compound **2** (25mg). The W2D fraction was separated on a silica gel CC with acetone/dichloromethane/water (3/1/0.1, v/v/v) and further purified on a reverse phase C18 CC, eluting with methanol/water (1/1, v/v) to give compound **3** (23mg).

Thymidine (**1**): White amorphous powder; ESI-MS *m/z*: 243 $[M+H]^+$; ^{1}H-NMR (600 MHz, DMSO-d_6) δ_H ppm: 1.86 (3H, s, H-7), 2.21 (2H, m, H-2′), 3.70 (1H, dd, J = 3.0, 12.0 Hz, H-5′), 3.77 (1H, dd, J = 3.6, 12.0 Hz, H-5′), 3.87 (1H, m, H-4′), 4.37 (1H, m, H-3′), 6.26 (1H, t, J = 6.6 Hz, H-1′), 7.79 (1H, s, H-6); ^{13}C-NMR (150 MHz, DMSO-d_6) δ_C ppm: 12.4 (C-7), 41.2 (C-2′),

62.8 (C-5′), 72.2 (C-3′), 86.2 (C-1′), 88.8 (C-4′), 111.5 (C-5), 138.2 (C-6), 152.4 (C-2), 166.4 (C-4).

Adenosine (**2**): White amorphous powder; ESI-MS *m/z*: 268 $[M+H]^+$ ^{1}H-NMR (600 MHz, DMSO-d_6) δ_H ppm: 3.55 (1H, m, H-5′), 3.67 (1H, m, H-5′), 3.96 (1H, m, H-4′), 4.12 (1H, dd, *J* = 5.5, 11.0 Hz, H-3′), 4.61 (1H, dd, *J* = 6.2, 11.0 Hz, H-2′), 5.87 (1H, d, *J* = 6.2 Hz, H-1′), 8,13 (1H, s, H-2), 8.35 (1H, s, H-8); ^{13}C-NMR (150 MHz, DMSO-d_6) δ_C ppm: 62.2 (C-5′), 71.2 (C-3′), 73.9 (C-2′), 86.4 (C-4′), 88.4 (C-1′), 120.0 (C-5), 140.5 (C-8), 149.7 (C-4), 153.0 (C-2), 156.2 (C-6).

1,2-di-*O*-*β*-glucopyranosyl-4-allylbenzene (3): Pale yellow amorphous powder; ESI-MS *m/z*: 497 $[M+Na]^+$ ^{1}H-NMR and ^{13}C-NMR (See Table 1).

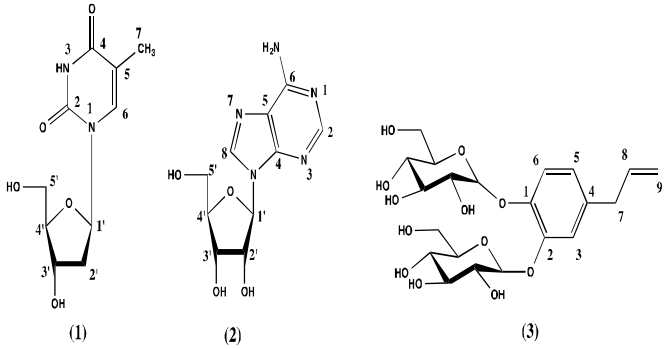

Figure 1. Chemical structures of **1-3** from Piper betle

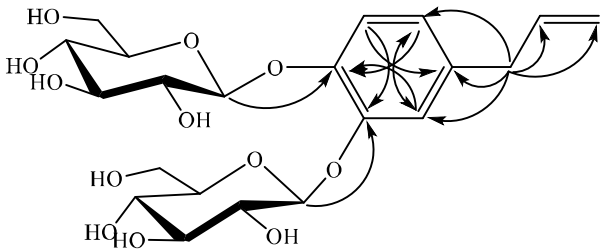

Figure 2. Important HMBC correlations (H → C) of compound **3**

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white amorphous powder. The ^{1}H-NMR spectrum of **1** exhibited proton signals at δ_H 7.79 (1H, s), 6.26 (1H, t, *J* = 6.6 Hz), 4.37 (1H, m), 3.87 (1H, m), 3.77 (1H, dd, *J* = 3.0, 12.0 Hz), 3.70 (1H, dd, *J* = 3.6, 12.0 Hz), 2.21 (2H, m), and methyl group at 1.86 (3H, s). The ^{13}C-NMR spectrum of **1** contained signals of ten carbons at δ_C 166.4, 152.4, 138.2, 111.5, 88.8, 86.2, 72.2, 62.8, 41.2 and 12.4 ppm. By DEPT spectrum, the signals at δ_C 62.8 and 41.2 were determined to be CH_2 groups. The chemical structure of **1** was then clearly elucidated by analyses of 2D-NMR spectra such as HSQC and HMBC. Based on the correlations between protons and carbons showed in HSQC spectra, assignments of carbon and proton were obtained including one olefin group at δ_H 7.79/ δ_C 138.2, one anomeric group at δ_H 6.26/ δ_C 86.2, two oxymethin groups at δ_H 4.37/ δ_C 72.2 and δ_H 3.87/ δ_C 88.8, one oxymethylene group at δ_H 3.77 and 3.70/ δ_C 62.8, one methylene group at δ_H 2.21/ δ_C 41.2, and one methyl group at δ_H 1.86/ δ_C 12.4. Three remaining carbon signals at δ_C 166.4, 152.4, and 111.5 did not observe correlations with proton in the HSQC spectrum which were assigned for nonprotonated carbons. An anomeric carbon signal at δ_C 86.2 and HMBC correlation between anomeric proton (δ_H 6.26) and carbon C-2 (δ_C 152.4), C-6 (δ_C 138.2) suggested for the N-glycoside linkage. The HMBC correlation between methyl protons (δ_H 1.86) and C-6 (δ_C 138.2)/ C-5 (δ_C 111.5)/ C-4 (δ_C 166.4) indicated the assignment of C-4/C-5/C-6 and a double bond at C-5/C-6. In addition, the chemical shift of C-4 (δ_C 166.4) allowed to determine carbonyl group of C-4. Also, chemical shift of C-2 at δ_C 152.4 ppm can be predicted other carbonyl group at C-2 and the linkage between C-2 and C-4 via a nitrogen atom. Five carbon signals of monosaccharide suggested the presence of a pentose. In addition, signals of methylene group C-2′ (δ_H 2.21/ δ_C 41.2) suggested for the presence of a 2-deoxypentose. The NMR data of **1** was compared and

Table 1. NMR spectral data for compound **3** and reference compound

No.	$^{\#}\delta_C$	$^{a,b}\delta_C$	$^{a,c}\delta_H$ (mult., *J* in Hz)	No.	$^{\#}\delta_C$	$^{a,b}\delta_C$	$^{a,c}\delta_H$ (mult., *J* in Hz)	No.	$^{\#}\delta_C$	$^{a,b}\delta_C$	$^{a,c}\delta_H$ (mult., *J* in Hz)
1	146.8	147.5	-	1-*O*-Glc				2-*O*-Glc			
2	148.5	149.2	-	1′	103.3	104.1	4.80 (d, 7.8)	1″	103.5	104.3	4.82 (d, 7.8)
3	120.0	120.9	7.08 (d, 1.8)	2′	74.7	75.1	3.48 (m)	2″	74.7	75.1	3.48 (m)
4	137.3	137.4		3′	77.3	78.2	3.46 (m)	3″	77.3	78.2	3.46 (m)
5	124.7	124.7	6.83 (dd, 1.8; 8.4)	4′	70.9	71.3	3.39 (m)	4″	70.9	71.3	3.39 (m)
6	120.0	120.9	7.15 (d, 8.4)	5′	77.8	77.7	3.32 (m)	5″	77.8	77.7	3.32 (m)
7	40.3	40.6	3.29 (m)	6′	62.0	62.4	3.83 (m) 3.70 (m)	6″	62.0	62.4	3.83 (m) 3.70 (m)
8	138.6	138.8	5.93 (m)								
9	116.3	116.0	5.03 (m)								

Measured in [a)] CD_3OD, [b] 150MHz, [c] 600MHz, [#)] reported data in CD_3OD [12]

observed in good agreement with those reported of thymidine in the literature [13]. Consequently, compound **1** was determined to be thymidine.

Compound **2** was isolated as a white amorphous powder. The ^{1}H-NMR spectrum of **2** exhibited the signals at δ_H 8.13 (s), 8.35 (s), 5.87 (d, *J* = 6.2 Hz), 4.61 (dd, *J* = 6.2, 11.0 Hz), 4.12 (dd, *J* = 5.5, 11.0 Hz), 3.96 (m), 3.67 (m), and 3.55 (m). The ^{13}C-NMR spectrum of **2** showed ten carbons at δ_C 156.2, 153.0, 149.7, 140.5, 120.0, 88.4, 86.4, 73.9, 71.2, 62.2. By DEPT spectrum, these signals were separated into one CH_2group atδ_C 62.2, three non-protonated carbons at δ_C 156.2, 149.7, 120.0; and remaining six CH groups. Among them, five carbon signals at δ_C 88.4, 73.9, 71.2, 86.4, 62.2 suggested for the presence of a ribose moiety. The chemical shift of C-1′ (δ_C 88.4), and HMBC correlation from H-1′ (δ_H 5.87) to C-4 (δ_C 149.7), C-8 (δ_C 140.5) suggested the presence of a N-glycoside linkage. In the aglycone moiety, there were only two signals of sp^2 methine groups at δ_H 8.13/ δ_C 153.0 and δ_H 8.35/ δ_C 140.5 which were suggested for the structure of adenine alkaloid. The ^{13}C-NMR spectral data of **2** was also in good consistence with that reported data of adenosine in the literature [14]. The NMR assignment of **2** was confirmed again by HMBC spectrum. Example, HMBC correlations between anomeric proton H-1′ (δ_H 5.87) and C-4 (δ_C149.7), C-8 (δ_C140.5) indicated the assignment of chemical shift values of C-4 and C-8, respectively. HMBC correlations between H-8 (δ_H 8.35) and C-4 (δ_C 149.7)/ C-5 (δ_C 120.0) were demonstrated for assignment of C-5. And HMBC correlations between H-2 (δ_H 8.13) and C-4 (δ_C 149.7)/ C-6 (δ_C 156.2) were supported for assignment of C-6. Similarly, the signals of monosaccharide moiety were assigned by 2D-spectra and spin-spin coupling constant observing in ^{1}H-NMR spectrum. Oxymethylene group was deduced by the signal at δ_H 3.67, 3.55/ δ_C 62.2. HMBC correlations from H-5′ (δ_H 3.67, 3.55) to C-4′ (δ_C 86.4), C-3′ (δ_C 71.2) consisted to the data of C-3′ and C-4′. Then, the remaining oxymethin signal at δ_C 73.9 was assigned to C-2′. Adenosine was one of nucleoside. This compound was known as a main component of *Cordyceps sinensis*, played an important role in circulatory system, controlling immune system [15].

Compound **3** was isolated as a pale yellow amorphous powder. The ^{1}H-NMR spectrum of **3** exhibited three aromatic protons of ABX spin coupled system at δ_H 7.08 (1H, d, *J* = 1.8 Hz), 6.83 (1H, dd, *J* = 1.8; 8.4 Hz), and 7.15 (1H, d, *J* = 8.4 Hz), indicating the presence of a 1,3,4-three substituted phenyl group. The vinyl (CH_2=CH) group were recognized by three olefinic proton signals at δ_H 5.93 (1H, m) and 5.03 (2H, m). Two anomeric protons signals were showed at δ_H 4.80 and 4.82 (each 1H, d, *J* = 7.8 Hz) suggested the presence of two monosaccharide units. Analysis of ^{13}C-NMR spectrum of **3** revealed twenty-one carbons. Among them, twelve carbons at δ_C 104.1/ 104.3, 75.1/ 75.1, 78.2/ 78.2, 71.3/ 71.3, 77.7/ 77.7, and 62.4/ 62.4 characterised for two glucopyranosyl groups. At the same time, the spin-spin coupling constant of anomeric proton (*J* = 7.8 Hz) indicated the β-glucopyranosyl linkages. Next, HMBC correlations between H-7 (δ_H 3.29) and C-3 (δ_C 120.9)/ C-4 (δ_C 137.4)/ C-5 (δ_C 124.7)/ C-8 (δ_C 138.8)/ C-9 (δ_C 116.0) indicated the location of propenyl group at C-4. In addition, HMBC correlations between H-3 (δ_H 7.08)/ H-5 (δ_H 6.83) and C-1 (δ_C 147.5) and downfield chemical shift of C-1 (δ_C 147.5) indicated an oxygenated carbon C-1. Similarly, HMBC correlations between proton H-6 (δ_H 7.15) and C-4 (δ_C 137.4)/ C-2 (δ_C 149.2), in combination with downfield chemical shift of C-2 (δ_C 149.2) suggested another oxygenated sp^2 carbon C-2. Finally, HMBC correlations between anomeric protons H-1′ (δ_H 4.80) and C-1 (δ_C 147.5), H-1″ (δ_H 4.82) and C-2 (δ_C 149.2) were confirmed the positions of two glucopyranosyl groups at C-1 and C-2. Consequently, compound **3** was determined to be 1,2-di-*O*-*β*-glucopyranosyl-4-allylbenzene. The ^{13}C-NMR data of **3** was well consisted with those reported data of 1,2-di-*O*-*β*-D-glucopyranosyl-4-allylbenzene in the literature (Table 1). This compound was isolated previously from *Alpinia officinarum*, a species belonging Zingiberaceae family and was expected causing anti-oxidation activity of *Alpinia officinarum* [12]. However, until now, this is the first report on the isolation of 1,2-di-*O*-*β*-glucopyranosyl-4-allylbenzene from *Piper betle*.

4. CONCLUSIONS

Three compounds including thymidine, adenosine, and 1,2-di-*O*-*β*-glucopyranosyl-4-allylbenzene were isolated from water soluble fraction of the whole plants of *P. betle*. Their NMR spectral data were well consisted with those reported in the literature. To the best of our knowledge, this is the first report on the isolation of 1,2-di-*O*-*β*-glucopyranosyl-4-allylbenzene from *P. betle*.

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THÔNG TIN TÁC GIẢ

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Khoa Công nghệ Hóa, Trường Đại học Công nghiệp Hà Nội