Journal of Science and Technology ***54*** *(4A) (2016) 221-226*

CHEMICAL CONSTITUENTS FROM THE SEEDS OF *ANNONA RETICULATA* L. IN VIETNAM

Nguyen Huy Hung

Faculty of Chemistry, Vinh University, 182, Le Duan street, Vinh City, Nghe An province

Email: *huyhung2602@gmail.com*

Received: 15 August 2016; Accepted for publication: 7 October 2016

ABSTRACT

In the present study, the chemical investigation of the seeds of *A. reticulata* L. (Annonaceae) in Vietnam has resulted in the identification of four known compounds, including two triterpenoids (rotundic acid (**1**), pedunculoside (**2**)), and two phenolic compounds (eleutheroside B (**3**), sinapaldehyde glucoside (**4**). Their chemical structures were elucidated on the basis of spectroscopic analysis, including homonuclear and heteronuclear correlation NMR (^{1}H-NMR, ^{13}C-NMR, COSY, HSQC, and HMBC) experiments.

Keywords: *Annona reticulata*, Annonaceae, triterpenoids, phenolic.

1. INTRODUCTION

The Annonagenus (Annonaceae) consists of about 119 species, most of which are shrubs and trees widely distributed in the tropical and subtropical regions, including the Southeast Asia countries. In Indian folk medicine, various species of Annona have been used as vermifuges, anti inflammatory agents, in wound healing, as antimalarial agents and in the treatment of diarrhoea and dysentery [1]. Seed, leaf, stem and roots of *Annona reticulata* L. are insecticidal, antihelmenthic, suppurant and are used against inflammatory tumors. Leaf decoction is used for nervous shock, indigestion and abdominal pain [2] and leaf paste is applied externally for boils [3]. The phytochemical studies revealed that the plant contains acetogenins, alkaloids, diterpenoids, triterpenoids, phytosterols, phenolic compounds and flavonoids [4]. In the present study, we describe isolation and structural characterization of four compounds, including two triterpenoids, rotundic acid (**1**), pedunculoside (**2**), and two phenlic compounds eleutheroside B (**3**), sinapaldehyde glucoside (**4**). Their chemical structures were established on the basis of physical, chemical and spectroscopic methods UV, IR, 1D (^{1}H- and ^{13}C) NMR and 2D (COSY, HSQC and HMBC) NMR spectrum, mass spectrometry (MS) and by comparison with literature data.

2. MATERIALS AND METHODS

2.1. General procedures

Melting points were determined using Yanagimoto MP-S3 apparatus. The UV spectra were obtained on a Hitachi UV-3210 spectrophotometer, IR spectra (KBr) were obtained on a Shimadzu FTIR-8501 spectrophotometer. The electrospray ionization- mass spectra (ESI-MS) were determined using an Agilent 1200 LC-MSD Trap spectrometer. Silica gel $60F_{254}$ was used in thin layer chromatography analysis. ^{1}H and ^{13}C NMR, COSY, HMBC, HSQC, DEPT spectra recorded on a Bruker Avance-500 spectrometer using tetramethylsilane (TMS) as internal standard. Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70–230 mesh and 230–400 mesh, E. Merck, Darmstadt, Germany). The compounds were visualized by spraying with 10 % (v/v) H_2SO_4 followed by heating at 110 ^{o}C for 10 min.

2.2. Plant materials

The seeds of *Annona reticulata* L. (Annonaceae) were collected during October 2010 in Tien Giang, Vietnam. The plant materials were identified and authenticated by Dr. Tran Huy Thai, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology. A voucher specimen (Viet-TSWu-20101015) was deposited at the Herbarium of the Vinh University.

2.3. Extraction and Isolation

The air-dried and powdered seeds of *A. reticulata* L. (3.0 kg) were soaked with methanol (5 L × 3) at room temperature (7 days), and the combined extracts were concentrated under reduced pressure to give deep brown syrup (184.0 g). The crude extract was suspended into water (1 L) and partitioned with n-hexane (1 L × 3), ethyl acetate (1 L × 5), and n-butanol (1 L × 3 times), successively to afford hexane (10.0 g), ethyl acetate (94.5 g), butanol (12.0 g), and water solubles fractions, respectively.

The ethyl acetate fraction was applied to silica gel column chromatography with *n*-hexane and acetone gradients (50:1 to 2:1) to afford eight fractions. Fraction 3 (4.7 g) was subjected to silica gel column chromatography eluted with *n*-hexane : ethyl acetate (8:1 ; 6 :1 and 4 :1) to afford eleutheroside B (**3**), sinapaldehyde glucoside (**4**). Fraction 5 (3.4 g) was subjected to silica gel column chromatography eluted with dichloromethane : methanol (10 :1 and 7 :1) to afford rotundic acid (**1**), pedunculoside (**2**).

Rotundic acid (**1**): white needles; mp 272-273^{0}C; UVλ_{max}^{EtOH} (nm): 195, 275, 219; IRν_{max}^{KBr}cm^{-1}: 3566, 3627, 3649 (OH), 1700 (C=O), 1615 (C=C); ^{1}H-NMR (500 MHz, CD_3OD) δ (ppm): 5.31 (1H, *t*, *J* = 3.0 Hz, H-12), 3.63 (1H, *dd*, *J* = 11.5, 4.5 Hz, H-3), 3.55 (1H, *d*, *J* = 11,0 Hz, H-23), 3.31 (1H, *d*, *J* = 11,0 Hz, H-23), 2.59 (1H, *dt*, *J* = 11.5, 4.5 Hz, H-16), 2.52 (1H, *br s*, H-18), 2.0 (2H, *m*, H-11), 1.83 (1H, *m*, H-15), 1.78 (1H, *m*, H-9), 1.76 (4H, *m*, H-21,22), 1.67 (1H, *m*, H-7), 1.64 (1H, *m*, H-1), 1.53 (1H, *dt*, *J* = 11.5, 4.5 Hz, H-16), 1.46 (2H, *m*, H-6), 1.39 (1H, *m*, H-20), 1.36 (3H, *s*, H-27), 1.30 (1H,*m*, H-7), 1.21 (3H, *s*, H-29), 1.18 (1H, *m*, H-5), 1.03 (1H, *m*, H-1), 1,0 (3H, *s*, H-25), 0.95 (3H, *d*, *J* = 7,0 Hz, H-30), 0.82 (3H, *s*, H-26), 0.74 (3H, *s*, H-24); ^{13}C-NMR (125 MHz, CD_3OD) δ (ppm): 182.3 (C-28), 140.0 (C-13), 129.5 (C-12), 74.2(C-3), 73.6 (C-19), 67.6 (C-23), 55.1 (C-18), 48.9 (C-5), 49.1 (C-17), 48.5 (C-9), 43.3 (C-4), 43.1 (C-20), 42.7 (C-14), 41.0 (C-8), 39.5 (C-1), 39.0 (C-22), 37.9 (C-10), 33.7 (C-7), 29.6 (C-15), 27.4 (C-2), 27.3 (C-21), 27.1 (C-29), 26.6 (16), 24.9 (C-27), 24.7 (C-11), 19.2 (C-6), 17.5 (C-26), 16.6 (C-30), 16.3 (C-25), 12.7 (C-24); ESI-MS *m/z* 489 $[M+H]^{+}$.

Pedunculoside (**2**): white needles; mp 213 – 214^{0}C; UV λ_{max}^{MeOH} nm: 219, 199, 275; IRν_{max}^{KBr} cm^{-1}: 3390 (OH), 1702 (C=O), 1612 (C=C); ^{1}H-NMR (500 MHz, CD_3OD) δ (ppm):

5.35 (1H, *br s*, H-1'), 5.33 (1H, *br s*, H-12), 3.81 (1H, *dd*, J = 12.0, 2.0 Hz, H-6'), 3.70 (1H, *dd*, *J* = 12.0, 4.5 Hz, H-6'), 3.63 (1H, *dd*, *J* = 11.5, 4.5 Hz, H-3), 3.55 (1H, *d*, *J* = 10.5 Hz, H-23), 3.42 (1H, *m*, H-5'), 3.38 (1H, *m*, H-4'), 3.35 (1H, *m*, H-2'), 3.34 (1H, *m*, H-3'), 3.31 (1H, *d*, *J* = 10.5 Hz, H-23), 2.62 (1H, *dt*, *J* = 11.5, 4.5 Hz, H-16), 2.53 (1H, br s, H-18), 2.0 (2H, *m*, H-11), 1.86 (1H, *m*, H-15), 1.82 (1H, *m*, H-22), 1.77 (1H, *m*, H-21), 1.74 (1H, *m*, H-9), 1.67 (2H, *m*, H-1,7), 1.65 (2H, *m*, H-16,22), 1.64 (2H, *m*, H-2), 1.46 (2H, *m*, H-6), 1.39 (1H, *m*, H-20), 1.35 (3H, *s*, H-27), 1.30 (1H, *m*, H-7), 1.28 (1H, *m*, H-21), 1.22 (3H, *s*, H-29), 1.18 (1H, *m*, H-5), 1.02 (1H, *m*, H-1), 1.0 (3H, *s*, H-25), 1.0 (1H, *m*, H-15), 0.95 (3H, *d*, *J* = 7.0 Hz, H-30), 0.8 (3H, *s*, H-26), 0.73 (3H, *s*, H-24); ^{13}C-NMR (125 MHz, CD_3OD) δ (ppm): 177.9 (C-28); 140.2 (C-13), 129.4 (C-12), 95.8 (C-1'), 80.2 (C-5'), 79.9 (C-3'), 75.0 (C-2'), 74.2 (C-3) 73.7 (C-19), 72.2 (C-4'), 67.6 (C-23), 62.5 (C-6'), 55.0 (C-18), 49.5 (C-17), 49.0 (C-5), 48.5 (C-9), 43.3 (C-4), 42.9 (C-20), 42.7 (C-14), 41.2 (C-8), 39.6 (C-1), 38.3 (C-22), 37.9 (C-10), 33.7 (C-7), 29.7 (C-15), 27.4 (C-2), 27.2 (C-21), 27.1 (C-29), 26.5 (C-16), 24.7 (C-27), 24.7 (C-11), 19.3 (C-16), 17.7 (C-26), 16.6 (C-30), 16.3 (C-25), 12.7 (C-24); HR-ESI-MS *m/z*: 673.3923 $[M+Na]^+$.

Eleutheroside B (**3**): White amorphous; mp 191-192^{0}C; UV (MeOH) λ_{max} (nm): 220, 264; IRν_{max}^{KBr} cm^{-1}: 3560, 3380, 1650, 1510, 1465; ESI-MS *m/z* 395 $[M+Na]^+$. ^{1}H and ^{13}C-NMR spectra data in Table 1.

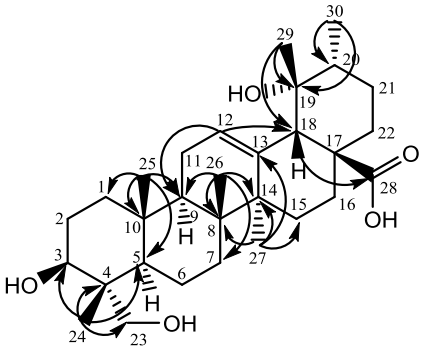
Sinapaldehyde glucoside (**4**): white powder; UV λ_{max} (nm): 205, 238, 315; IR ν_{max}^{KBr}: 3420, 1683, 1506; HR-ESI-MS: *m/z* 371.1282 $[M+H]^+$. ^{1}H and ^{13}C-NMR spectra data in Table 1.

3. RESULTS AND DISCUSSION

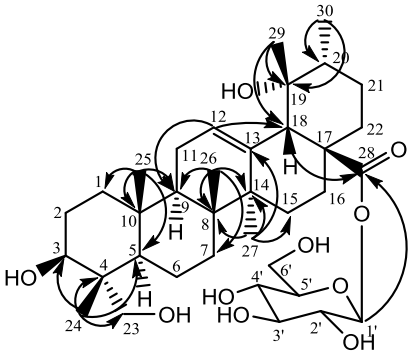
Compound 1 was isolated as white needles and its mass spectral data suggested the molecular formula as $C_{30}H_{48}O_5$. The IR spectrum showed absorption peaks in the region (3649 - 3566) cm^{-1-} indicating the presence of hydroxyl groups (-OH). The absorption band at 1720 cm^{-1} indicated the presence of (C=O) stretching. The absorption band at 1615 cm^{-1} indicated the presence of (C=C) stretching. The ^{1}H-NMR spectrum showed resonance signals of olefinic proton at δ_H 5.31 (1H, *t*, *J* = 3.0 Hz, H-12), a proton of oxymethine group at δ_H 3,63 (1H, *dd*, *J* = 11.5, 4.5 Hz, H-3) and two methylene protons of $-CH_2OH$ group at δ_H 3.55 (1H, *d*, *J* = 11.0, H_a-23), 3.31 (1H, *d*, *J* =11.0, H_b-23), presence of five methyl signals appeared as singlets signals at 1.36 (H-27), 1.21 (H-29), 1.0 (H-25), 0.82 (H-26), 0.74 (H-24) and presence of a methyl signal appeared as doublet at 0,95 (3H, *d*, *J* = 7.0 Hz, H-30). The ^{13}C-NMR spectrum showed the presence of thirty carbon signals including a resonance signal of carbonyl carbon at δ_C 178.9, two olefinic carbons at δ_C 126.8, 138.6 and three oxigenated at δ_C 73.6 (C-19), 74.2 (C-3), 67,6 (C-23). The HMBC spectrum showed ^{3}*J*-HMBC correlations between H-23 to C-24, H-24 to C-3, H-24 to C-5 and H-23 to C-3; ^{2}*J*-HMBC correlations between H-23 and H-24 to C-4; ^{3}*J*-HMBC correlations between H-18 to C-12 and C-28. A careful comparison of the obtained NMR data of **1** with the literature [5], allowed us to identify **1** as a rotundic acid.

Compound 2 was isolated as white needles and its mass spectral data suggested the molecular formula as $C_{36}H_{58}O_{10}$. Its IR spectrum showed absorption peaks in the region 3390 cm^{-1-} indicating the presence of hydroxyl groups (-OH). The absorption bands at 1720 cm^{-1} indicated the presence of (C=O) stretching. The absorption band at 1615 cm^{-1} indicated the presence of (C=C) stretching. A careful comparison of the MS, IR, NMR data obtained for **2** with the **1** displayed that **2** was ester of **1** with one β-glucosyl unit. The carbon signals of the sugar moiety at δ_C 95.8 (C-1'), 80.2 (C-5'), 79.9 (C-3'), 75.0 (C-2'), 72.2 (C-4') and 62.5 (C-6') were well consistent with those of glucose. The location of the *β*-glucosyl unit was determined

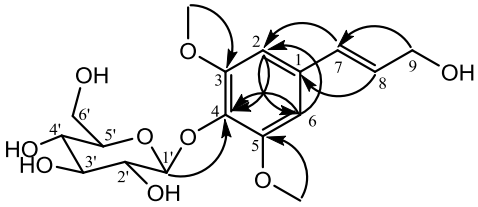
by ^{3}J-HMBC correlations between H-1' to C-28. A careful comparison of the NMR data obtained for **2** with the literature [6] indicated that **2** was pedunculoside.



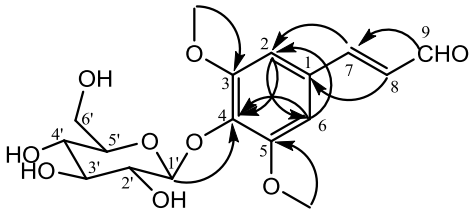
Rotundic acid (**1**)



Pedunculoside (**2**)



Eleutheroside B (**3**)



Sinapaldehyde glucoside (**4**)

Figure 1. Significant HMBC of compounds **1**-**4.**

Compound 3 was isolated as white amorphous powder and its mass spectral data suggested the molecular formula as $C_{17}H_{24}O_9$. The ^{1}H-NMR spectrum of **3** indicated the presence of a glucose moiety at δ_H 3.03 to 3.59, an anomeric proton at δ_H 4.9 (1H, *d, J* = 7.5 Hz, H-1'), and phenylpropanoid skeleton at δ_H 6.33 (1H, *dt, J* = 16.0, 5.0 Hz, H-8), 6.46 (1H, *d, J* = 16.0 Hz, H-7), and 4.1 (2H, *br s*, H-9). The coupling constant of *J* = 16.0 Hz was attributable to one pair of *trans* protons which is the hallmark of cinnamic acid derivatives, *m*-substituted aromatic ring system signals were observed at δ_H 6.72 (2H, *s*, H-2 and H-6), and two O-methyl groups were revealed at 3.76 (6H, *s*, 3- and 5-OCH_3). In the ^{13}C NMR data, an anomeric carbon signal at δ_C 102.6 (C- 1') and the peaks derived from a glucose moiety between δ_C 60.9 (C-6') and 77.2 (C-5') were confirmed. The signal at δ_C 56.4 (3,5-OCH_3) indicated two O-methyl carbons. On the basis of the ^{1}H and ^{13}C NMR data, compound **3** was assumed as eleutheroside B (syringin) and belongs to the phenylpropanoid glucosides [7].

Compound 4 was isolated as a white powder. The molecular formula of **4** was established as $C_{17}H_{22}O_9$. Compounds **3** and **4** have similar structural signals. Comparison of the ^{1}H-NMR, ^{13}C-NMR, HMBC spectra between **3** and **4** displayed that the CH_2OH (C-9) group was oxidized to aldehyde (δ_H 9.66 (1H, *d, J* = 7.5 Hz) and δ_C 196.0) and these were also supported of determining the molecular formula. Thus, the structure of **4** was assigned as sinapaldehyde glucoside that was consistent to the reported literature values of [8].

Table 1. ^{1}H and ^{13}C-NMR spectra data of compounds **3** (*DMSO-d_6*) and **4** (*CD_3OD*).

	Compound **3**			Compound **4**		
No	DEPT	δ_C	δ_H	DEPT	δ_C	δ_H
1	C	132.7		C	132.0	
2	CH	104.5	6.72 (1H, *s*)	CH	107.8	7.06 (1H, *s*)
3	C	152.7		C	155.1	
4	C	133.9		C	138.8	
5	C	152.7		C	155.1	
6	CH	104.5	6.72 (1H, *s*)	CH	107.8	7.06 (1H, *s*)
7	CH	128.5	6.46 (1H, *d*, 16.0 Hz)	CH	154.7	7.63 (1H, *d*, *J* =16.0 Hz)
8	CH	130.2	6.33 (1H, *d*, 16.0 Hz)	CH	129.2	6.73 (1H, *dd*, *J* = 16.0, 8.0 Hz)
9	CH_2	61.5	4.10 (2H, *br s*)	CHO	196.0	9.66 (1H, *d*, *J* = 7.5 Hz)
	OCH_3	56.4	3.76 (6H, *s)*	OCH_3	57.1	3.92 (6H, *s)*
1’	CH	102.6	4.90 (1H, *d*, 7.5 Hz)	CH	104.7	5.06 (1H, *d*, *J* = 7.5 Hz)
2’	CH	74.2	3.20 (1H, *m*)	CH	77.9	3.45 (1H, *m*)
3’	CH	76.6	3.19 (1H, *m*)	CH	75.7	3.52 (1H, *m*)
4’	CH	70.0	3.14 (1H, *m*)	CH	71.3	3.44 (1H, *m*)
5’	CH	77.2	3.03 (1H, *m*)	CH	78.5	3.26 (1H, *m*)
6’	CH_2	60.9	3.59 (1H, *m*) 3.42 (1H, *m*)	CH_2	62.5	3.80 (1H, *dd*, *J* = 12.0, 2.0 Hz) 3.68 (1H, *dd*, *J* = 12.0, 5.5 Hz)

4. CONCLUSION

Four compounds were isolated and characterized from the seeds of *A. reticulata* L. including two triterpenoids, rotundic acid (**1**), pedunculoside (**2**), and two phenlic compounds eleutheroside B (**3**), sinapaldehyde glucoside (**4**).

REFERENCES

1. Pham Hoang Ho – Cay co Viet Nam, NXB Tre, 1999, tr. 244.
2. Weniger B., Rouzier M., Daguilh R., Henrys D., and Henrys J. H. - Anton R., Traditional medicine in the Central Plateau of Haiti, J. Ethnopharmacol. **17** (1) (1986) 13-30.
3. Girach R. D., Aminuddin Siddiqui P. A., and Khan S. A. - Traditional plant remedies among the Kondh of District Dhenkanal (Orissa), Int. J. pharmacogn. **32** (3) (1994) 274-283.

4. Jamkhande P. G. and Wattamwar A. S. - *Annona reticulata* Linn. (Bullock's heart): Plant profile, phytochemistry and pharmacological properties,. J. Tradit. Complement. Med. **5** (3) (2015) 144-152.
5. Nakatani M., Miyazaki Y., Iwashita T., Naoki H., and Hase T. - Triterpenes from *Ilex rotunda* fruits, Phytochemistry. **28** (5) (1989) 1479-1482.
6. Ding P., Qiu J. Y., Ying G., and Dai L. - Chemical Constituents of *Millettia speciosa*, Chin. Herb. Med. **6** (4) (2014) 332-334.
7. Yang E. J., Kim S. I., Ku H. Y., Lee D. S., Lee J. W., Kim Y. S., and Song K. S. - Syringin from stem bark of *Fraxinus rhynchophylla* protects Aβ (25–35)-induced toxicity in neuronal cells, Arch. Pharm. Res. **33** (4) (2010) 531-538.
8. Zheng L. P., He Z. G., Wu Z. J., and Zhang C. - Chemical constituents from *Dendropanax dentiger*, Chem. Nat. Compd. **48** (5) (2012) 883-885.

TÓM TẮT

THÀNH PHẦN HÓA HỌC CỦA HẠT BÌNH BÁT (*ANNONA RETICULATA* L.) Ở VIỆT NAM

Nguyễn Huy Hùng

Khoa Hóa học, Trường Đại học Vinh, 182, Lê Duẩn, Thành phố Vinh, Tỉnh Nghệ An

Email: *huyhung2602@gmail.com*

Từ hạt bình bát (*A. reticulata* L.) đã phân lập được bốn hợp chất gồm hai hợp chất triterpenoid gồm rotundic acid (**1**), pedunculoside (**2**); hai hợp chất phenolic gồm eleutheroside B (**3**), sinapaldehyde glucoside (**4**). Cấu trúc của các hợp chất này được xác định dựa trên sự kết hợp nhiều phương pháp phổ, bao gồm UV, IR, MS, NMR (^{1}H-NMR, ^{13}C-NMR, COSY, HSQC và HMBC).

Từ khóa: Annona reticulata, Annonaceae, triterpenoids, phenolic.