FURTHER RESULTS ON PHYTOCHEMISTRY STUDY OF *IMPATIENS CLAVIGER* HOOK. F. COLLECTED IN ME LINH, HA NOI, VIET NAM

KẾT QUẢ TIẾP THEO VỀ NGHIÊN CỨU HÓA THỰC VẬT LOÀI BÓNG NƯỚC KHỎE (*IMPATIENS CLAVIGER* HOOK. F.) THU HÁI TẠI MÊ LINH, HÀ NỘI, VIỆT NAM

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ABSTRACT

Three compounds were isolated from the butanol extract of *Impatiens claviger*'s leaves and twigs collected in Me Linh, Ha Noi. Their structures were elucidated by the mass, NMR spectroscopy and comparison with published data as two phenolic glucosides and one triterpene glycoside. One of them has been found for the first time in *Impatiens* genus.

Keywords *Impatiens claviger, phenolic glucosides, triterpene glucoside*.

TÓM TẮT

Ba hợp chất được phân lập từ cặn chiết butanol của lá và cành cây Bóng nước khỏe thu hái tại Mê Linh, Hà Nội. Cấu trúc của chúng được xác định là hai phenolic glucosid và một triterpen glucosid bằng số liệu phổ khối (MS) và phổ cộng hưởng từ hạt nhân (NMR), kết hợp so sánh với dữ liệu đã công bố. Một trong ba hợp chất lần đầu tiên được tìm thấy trong chi Bóng nước (*Impatiens*).

Từ khóa: *Bóng nước khỏe (Impatiens claviger), pheolic glucosid, triterpen glucosid.*

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1. INTRODUCTION

Balsaminaceae family contains only two genera with *Impatiens* as the main with more than 1000 species [1]. Some *Impatiens* species are known for their biological effects as antimicrobial, antioxidant, antiallergic, antipruritic, antitumoural activities,... [2,3]. Flavonoids, steroids, terpenoids, naphthoquinones, glycosides and sapogenins are the main compound classes which have been obtained from 8 studied *Impatiens* species, including *I. balsamina*, *I. textori*, *I. glandulifera*, *I. scabrida*, *I. siculifir*, *I. parviflora*, *I. bicolar*, *I. pritzellii and I. balsamina* [2, 3]. One of them, *I. balsamina* was the most studied.

Impatiens claviger Hook. f. (Vietnamese name: Bong nuoc khoe) has been found in Ba Vi, Ha Noi and Quang Nam provinces. Van Nam (China) is the originated place of this species and its leaves are used to treat sores there [4]. The first results on phytochemistry of *I. claviger* have been reported previous [5]. In continuation of the study on this plant, three compounds were isolated from *n*-butanol extract of *I. claviger*'s twigs and leaves. Their structures were determined as isotachioside (**1**), 3-{2-[β-glucopyranosyl)oxy]-4,5 (methylenedioxy)-phenyl} propanoic acid (**2**) and ginsenoside Rg1 (**3**) by the analysis of their 1D, 2D-NMR, ESI-MS spectra and compared with published data.

2. MATERIALS AND METHOD

2.1. Plant materials

Impatiens claviger's leaves and twigs were collected in Me Linh, Ha Noi, Vietnam in March 2019. A voucher specimen (VHH.ML. 3.2019.1) is deposited in Institute of Chemistry, VAST, Hanoi, Vietnam. The scientific name was identified by Dr. Vu Tien Chinh, Viet Nam National Museum of Nature, VAST, Hanoi, Vietnam.

2.2. General experimental procedures

NMR: Bruker Avance 500, Germany with TMS as internal reference (for ^{1}H) and solvent signal (for ^{13}C). ESI-MS: 5989B-MS. CC used silicagel 60G, size 0.043 - 0.063mm (Merck), TLC: precoated silica gel $G60F_{254}$ plates (Merck), spots were detected by spraying with vanillin 1% in conc. H_2SO_4 and heating at 110°C.

2.3. Extraction and isolation

The dried leaves and twigs of *I. claviger* (2.5kg) were extracted with MeOH:H_2O (95:5) (four times) at room temperature. The methanol extract was concentrated under *vacuum* and then aqueous solution was extracted with *n*-hexane, EtOAc and *n*-BuOH, successively. The solvent was evaporated in *vacuum* to afford *n*-hexane (13.0g), EtOAc (9.0g) and *n*-BuOH (26.2g) extracts.

The butanol extract (26.2g) was chromatographed on silica gel column, eluting with gradient CH_2Cl_2:MeOH to furnish 9 fractions (B1-B9). The second fraction B2 (3.7g) was rechromatographed on silica gel to yield 6 subfractions (B2.1-B2.6). Compound **1** (15mg) and **2** (18mg) were obtained by purification of subfraction B2.2 (580mg) using silica gel column, *n*- CH_2Cl_2:MeOH = 90:10. The separation of the fifth fraction B5 (2.5g) was carried out on Sephadex LH 20 with MeOH as eluate to give compound **3** (20 mg).

Isotachioside (ICB2, **1**): (-)-HR-ESIMS: *m/z* = 337.0694 $[M+Cl]^-$ (calc. 337.0696 for $C_{13}H_{18}O_8Cl^-$). ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 6.22 dd, 1H, *J* = 3.0 & 9.0 (H-5); 6.89 d, 1H, *J* = 9.0 (H-6); 6.39 d, 1H, *J* = 3.0 (H-3); 4.66 d, 1H, *J* = 7.0 (H-1′); 3.1 – 3.6 m, 6H (H-2′-H-6′)

3-{2-[β-glucopyranosyl)oxy]-4,5 (methylenedioxy)-phenyl}propanoic acid (ICB3, **2**): (+)-ESIMS: *m/z* = 373 $[M+H]^+$. ^{1}H NMR (500MHz, DMSO-d_6): δ_H 6.77 s, 1H (H-3); 6.70s, 1H (H-6); 2.71m, 2H (H_2-7); 2.14m, 2H (H_2-8), 5.90s, 2H (-OCH_2O-); 4.52 d, 1H, *J* = 7.5 (H-1′); 3.1 – 3.6m, 6H (H-2′-H-6′)

Ginsenoside Rg1 (ICB4, **3**): (-)-HR-ESI-MS: *m/z* = 985.4992 $[M-H]^-$ (calc. 985.5014 for $C_{49}H_{77}O_{20}^-$). ^{1}H NMR (500 MHz, CD_3OD): δ_H 3.39m, 1H (H-3); 4.12m, 1H (H-6); 3.69m, 1H (H-12); 5.13m, 1H (H-24), 4.63 d, 1H, *J* = 7.5 (H-1′); 4.37 d, 1H, *J* = 7.5 (H-1″).

^{13}C NMR (125MHz) of compounds **1**-**3**: Table 1

3. RESULTS AND DISCUSSION

From *n*-butanol extract of the twigs and leaves of *I. claviger* three compounds were isolated by column chromatography method. Their structures were elucidated by NMR spectroscopic analysis in comparison with those data published in the literature, including isotachioside (**1**),

Table 1. ^{13}C-NMR data (125 MHz) of compounds 1-3

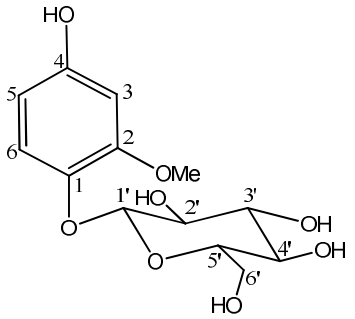
C	δ_C			3 (CD_3OD)		
	1 (DMSO-d_6)	2 (DMSO-d_6)	C	δ_C	C	δ_C
1	139.4 C	125.8 C	**1**	40.5 CH_2	**21**	22.8 CH_3
2	149.9 C	150.0 C	**2**	27.6 CH_2	**22**	36.6 CH_2
3	100.9 CH	99.7 CH	**3**	79.1 CH	**23**	24.2 CH_2
4	152.7 C	145.1 C	**4**	40.4 C	**24**	125.9 CH
5	106.0 CH	141.7 C	**5**	61.8 CH	**25**	132.3 C
6	117.4 CH	108.7 CH	**6**	80.9 CH	**26**	25.8 CH_3
7	-	26.9 CH_2	**7**	45.3 CH_2	**27**	17.9 CH_3
8	-	39.5 CH_2	**8**	41.9 C	**28**	31.4 CH_3
9	-	176.8 C	**9**	49.9 CH	**29**	16.1 CH_3
1′	101.5 CH	103.6 CH	**10**	40.2 C	**30**	17.1 CH_3
2′	73.3 CH	73.4 CH	**11**	31.5 CH_2	**1′**	105.6 CH
3′	76.9 CH	77.1 CH	**12**	71.7 CH	**2′**	75.5 CH
4′	69.8 CH	69.9 CH	**13**	50.6 CH	**3′**	78.3 CH
5′	76.9 CH	76.5 CH	**14**	52.4 C	**4′**	71.2 CH
6′	60.8 CH_2	60.9 CH_2	**15**	31.0 CH_2	**5′**	77.9 CH
-OMe	55.6 CH_3	-	**16**	27.2 CH_2	**6′**	62.9 CH_2
-OCH_2O-		100.6 CH_2	**17**	53.1 CH	**1″**	98.2, CH
			18	17.8 CH_3	**2″**	74.9, CH
			19	17.9 CH_3	**3″**	79.1, CH
			20	84.9 C	**4″**	71.0, CH
					5″	78.0, CH
					6″	62.3, CH_2

3-{2-[β-glucopyranosyl)oxy]-4,5 (methylenedioxy)-phenyl}propanoic acid (**2**), and ginsenoside Rg1 (**3**).

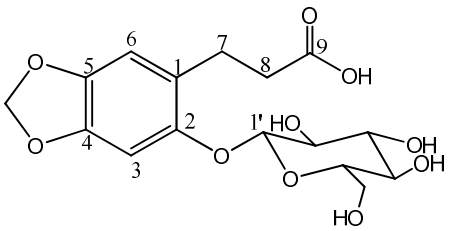
Compound **1** was isolated as an white powder. It's negative mode HR-ESIMS showed pseudo-molecular ion peak at *m/z* = 337.0694 $[M+Cl]^-$, calc. 337.0696 for $C_{13}H_{18}O_8Cl^-$. 1D-NMR spectra indicated three protons of trisubstituted aromatic ring, including two doublets at δ_H 6.89 d, *J* = 9.0 (H-6); 6.39 d, *J* = 3.0 (H-3) and one double doublet at δ_H 6.22 dd, *J* = 3.0 & 9.0 (H-5) as well as six carbons in the range of 100 - 153ppm. Besides, the signals at δ_H 3.70 s, δ_C 55.6 proved the presence of a methoxy group in the molecular **1**. A glucopyranosyl unit was confirmed by the signals of an anomeric methin at δ_H 4.66 d, *J* = 7.0, δ_C 101.5 together with four oxygenated methin (δ_H 3.18 – 3.42; δ_C 72.4 – 76.9) and one oxy-methylene at δ_H 3.28 - 3.65; δ_C 60.8. These spectroscopic data are total identical with those of methoxyhydroquinone-l-O-β-D-glucopyranoside named isotachioside, which was isolated previously from *Berchemia racemosa* [6].

Compound **2** was obtained as light yellow powder. Its NMR spectra showed the signals of 1,2,4,5-substituted aromatic ring, revealing two singlets at δ_H 6.70 (H-6) and 6.77 (H-3); six carbons at δ_C 100.0 - 151.3; a methylenedioxy group at δ_H 5.90 s, 2H; δ_C 100.6. In addition, the presence of a propanoyloxy group was showed by the signals of two methylene groups at δ_H 2.14, 2.71 (each m, 2H, H_2-7, H_2-8) and a carboxy group at δ_C 176.8. The anomeric methine at δ_H 4.52 d, *J* = 7.5, δ_C 103.6 proved a β-D-glucopyranose unit in **2**. The correlations between C-2 and H-1'; C-1, C-9 and H-7, H-8 in the HMBC confirmed the connection positions of propanoyloxy and β-D-glucopyranose groups in the structure of compound **2**. The above mentioned evidence and compared with published data led to conclude that **2** is 3-{2-[β-glucopyranosyl)oxy]-4,5 (methylenedioxy)-phenyl} propanoic acid. This phenol derivative was isolated as new compound from *Tetracentron sinense* in 2006 [7].

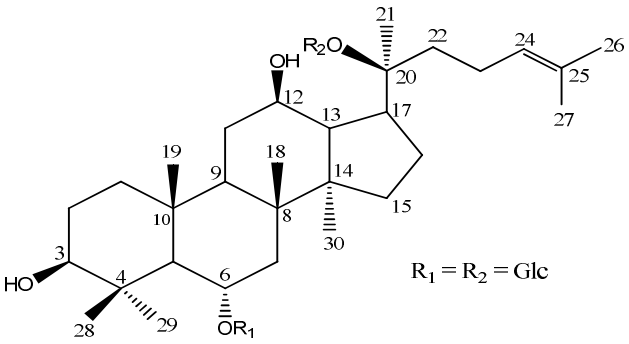
Compound **3**, an white powder, showed signals of a triterpene glycoside in its NMR spectra. The aglycone part was revealed by typical signals of hydroxy damarane, containing a double bond >CH=C< at δ_H 5.13 m (H-24), δ_C 125.9 (C-24), 132.3 (C-25), 8 singlet methyls and three oxy-methin groups at δ_H 3.38m, 3.69m, 4.12m, δ_C: 79.1, 71.7, 80.9, respectively. In addition, the presence of two pyranoglucosyl units was demonstrated by signals of two anomeric methines at δ_H 4.37 d, *J* = 7.5, δ_C 105.6; δ_H 4.63 d, *J* = 7.5, δ_C 98.2. The connection of two glucose groups were proved by HMBC correlations between C-6 (80.9ppm) and anomer proton at δ_H 4.37; C-20 (84.9ppm) and δ_H 4.63 and. Finally, the structure of **3** was established as a dammarane glycosides, named Ginsenoside Rg1 by comparison with those of it in published article [8]. Ginsenoside Rg1 is one of nine protopanaxatriol glycosides isolated from crude root saponins of *Panax notoginseng* [8].



1: Isotachioside



2: 3-{2-[-glucopyranosyl)oxy]-4,5-(methylenedioxy)-phenyl}propanoic acid



3: Ginsenoside Rg1

Figure 1. The structure of compounds 1-3

4. CONCLUSION

The further results on phytochemistry study of the leaves and twigs of *Impatiens claviger* led to the isolation of isotachioside, 3-{2-[β-glucopyranosyl)oxy]-4,5 (methylenedioxy)-phenyl}propanoic acid and ginsenoside Rg1 from butanol extract.

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THÔNG TIN TÁC GIẢ

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