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Chemical constituents of the methanol extract of *Hibiscus sabdariffa* (Linn) seeds

Thanh-Tung Phan[a,b], Hoang-Khang Le[a,b], Thuy-Duong Ngo Thi[a,b], Hoang-Long Ngo[c], Kim-Thuy Tran[d], That-Quang Ton[a,b,*]

[a]*Faculty of Chemistry, University of Science, Ho Chi Minh City, Vietnam*
[b]*Vietnam National University, Ho Chi Minh City, Vietnam*
[c] *NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam.*
[d] *Binh Thuan Pharmaceutical and Medical Equipment Joint Stock Company (PhaPharco), Phan Thiet City, Vietnam*

Abstract

The flower of *Hibiscus sabdariffa* Linn (Malvaceae) is an annual herbaceous shrub, that is used in food preparation in sauces, jams, juices, jellies, syrups, flavoring, and has some medicinal uses. Most of the bioactivities of *H. sabdariffa* L. is antioxidant which has a large amount of phenolic compounds. The phytochemical investigation of the methanolic extract from the seeds led to the isolation of three compounds named 1-*O*-acetylglycerol **(1)**, 4-hydroxybenzaldehyde **(2)**, 3-*C*-(hydroxymethyl)-*β*-D-erythrofuranoside **(3)**. Their structures were elucidated by 1D-, 2D-NMR spectra as well as by comparison with those reported in the literature.

Keywords: *Hibiscus sabdariffa*, seed, 1-*O*-acetylglycerol, 4-hydroxybenzaldehyde, erythrofuranoside

Figure 1. *Hibiscus sabdariffa L.* (Malvaceae)

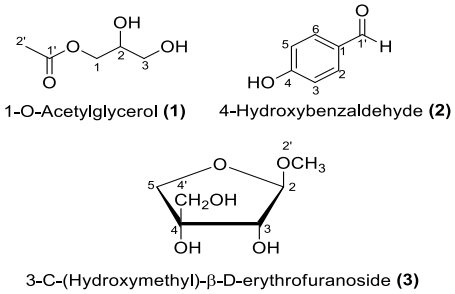

Figure 2. Chemical structures of three isolated compounds

* Corresponding author, E-mail: ttquang@hcmus.edu.vn

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1. Introduction

Hibiscus sabdariffa L. (Malvaceae), known as roselle or red sorrel **(Figure 1)** , originates in West Africa, is grown for its tops and calyxes as sour vegetables and as medicine, and has been imported to our country since the 70s of the last century. In addition to Vietnam, the tree is also widely grown in Indonesia, Thailand, India, Bangladesh and Malaysia, for the fiber in the stem and calyx (which develops together with the fruit) with a dark purple-red color to make soft drinks. [1]

Roselle seeds contained crude protein (27.78%), crude fatty oil (21.85%), carbohydrate (21.25%), crude fibre (16.44%) and ash (6.2%)[2]. Many minerals are found in seeds such as calcium, magnesium, and lysine. Seed oil is rich in unsaturated fatty acids (70%) and steroids, tocopherol have also been reported in seed oil. The main constituents of *H. sabdariffa* are organic acids, anthocyanins, polysaccharides and flavonoids[3,4]. The phytochemical investigation on methanolic extract of Roselle seeds was carried out, the result in isolation of three known compounds. Details regarding the isolation and structural elucidation of 1–3 are reported herein

2. Experimental

2.1. Material

The fresh flowers of *H. sabdariffa* L. were collected at Tuy Phong District, Binh Thuan Province, Vietnam, in January 2018. The sample was identified by Botanist Viet Hoang, Department of Ecology and Evolutionary Biology, Faculty of Biology and Biotechnology, VNUHCM–University of Science. A voucher specimen (DOC2016-HBG) has been deposited at the Department of Organic Chemistry, Faculty of Chemistry, VNUHCM-University of Science.

2.2. Extraction and isolation

Fresh flowers are divided into three parts: petals, seed coats and flower seeds. The dried seeds of *H. sabdariffa* Linn (4.3 kg) were refluxed in turn with *n*-hexane, EtOAc, and MeOH (each 30 L, 4 h x 3), to obtain the *n*-hexane (640.00 g), EtOAc (43.44 g), and MeOH (210.32 g) extracts. The MeOH extract (210.32 g) was applied to silica gel column chromatography and eluted with solvent systems of ethyl acetate : methanol (stepwise, 1 – 100% MeOH) to give 11 fractions (coded H1 – H11). The fraction H3 (12.3 g) was applied to a silica gel column, eluted with ethyl acetate : methanol (stepwise, 1 – 100% MeOH) to give 11 sub-fractions (coded H3.1 – H3.11). Sub-fraction Fr.3.1 (1329 mg) was chromatographed over a silica gel column with chloroform : methanol (stepwise, 1 – 100% MeOH) mixtures and purified by preparative TLC with chloroform : methanol (7 : 3), to afford three pure compounds: **1** (5.5 mg), **2** (5.0 mg), **3** (8.0 mg).

*2.2.1. 1-O-acetylglycerol (**1**)*

White amorphous solid. ^{1}H-NMR (DMSO-d_6, 500 MHz): δ_H 4.03 (1H, *dd*, 11.2; 4.1, H-1a), 3.88 (1H, *dd*, 11.2; 6.7, H-1b), 3.61-3.64 (1H, *m*, H-2), 3.30-3.35 (2H, *m*, H-3), 2.00 (3H, *s*, H-2'). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ_C 170.5 (C-1'), 69.3 (C-2), 62.7 (C-3), 65.7 (C-1), 20.8 (C-2').

*1.1.2. 4-hydroxybenzaldehyde (**2**)*

Yellow amorphous solid. ^{1}H-NMR (DMSO-d_6, 500 MHz): δ_H 9.78 (1H, *s*, H-1'), 7.75 (1H, *d*, 8.5, H-3, 5), 6.92 (1H, *d*, 8.5, H-2, 6). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ_C 130.3 (C-1), 132.1 (C-2, 6), 115.9 (C-3, 5), 163.4 (C-4), 191.0 (C-1').

*1.1.3. 3-C-(hydroxymethyl)-β-D- erythrofuranoside (**3**)*

Yellow amorphous solid. ^{1}H-NMR (DMSO-d_6, 500 MHz): δ_H 4.69 (1H, *d*, 3.5, H-2), 3.73 (1H, *s*, H-3), 3.84 (1H, *d*, 9.0, H-5a), 3.59 (1H, *d*, 9.0, H-5b), 3.24 (3H, *s*, H-2') ,3.30 (2H, *dd*, 12.0, 5.5, H-4'). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ_C 109.8 (C-2), 75.7 (C-3), 78.7 (C-4), 73.2 (C-5), 54.9 (C-2'), 62.9 (C-4').

3. Results and discussion

The dried seeds of *H. sabdariffa* Linn were extracted in turn with *n*-hexane, EtOAc and MeOH, to yield n-hexane-, EtOAc-, MeOH-soluble fractions. The detailed chromatographic fractionation of the MeOH-soluble fractions *H. sabdariffa* led to the isolation of three compounds: 1-*O*-acetylglycerol (**1**), 4-hydroxybenzaldehyde (**2**), 3-*C*-(hydroxymethyl)-*β*-D-erythrofuranoside (**3**) **(Figure 2)**.

Compound **1** was isolated as a white amorphous solid. The ^{1}H-NMR spectrum of **1** revealed two double of doublet signals at δ_H 4.03 (1H, *dd*, 11.2; 4.1, H-1a), 3.88 (1H, *dd*, 11.2; 6.7, H-1b) of two protons incommensurate methylene. Additionally, in the higher magnetic field, has two proton signals of oxygenated methine group from 3.35 to 3.65 ppm and one methyl proton signal at δ_H 2.00 (3H, *s*, H-2') **(Table 2)**. The ^{13}C-NMR spectrum showed 5 carbon signals comprising a carbonyl carbon at δ_C 170.5 (C-1'), two oxymethylene carbons at δ_C 62.7 (C-3), 65.7 (C-1), an oxymethine carbon at δ_C 69.3 (C-2) and a methyl carbon at δ_C 20.8 (C-2') **(Table 1)**. The HMBC correlations showed a cross-peak of methyl to carbonyl carbon C-1' and the remaining HMBC correlations confirmed the structure. The good compatibility between its NMR data and those reported in the literature [5] confirmed the structure of **1** to be 1-*O*-acetylglycerol (**1**) **(Figure 3)**.

Compound **2** was isolated as a yellow amorphous solid. ^{1}H-NMR spectrum of **2** showed three signals of five protons. At the low magnetic field has an aldehyde proton signal at δ_H 9.78 (1H, *s*, H-1'), moreover, two ortho-coupling doublet proton signals at δ_H 7.75 (2H, *d*, 8.5, H-2,6), δ_H 6.92 (2H, *d*, 8.5, H-3,5) of a 1,4-disubstituted benzene ring **(Table 2)**. ^{13}C-NMR spectrum show 4 signals of six aromatic carbons with a signal at δ_C 163.4, (C-4) is a carbon signal attached directly to the oxygen atom and one carboxyl carbon δ_C 191.0 (C-1') at the low magnetic zone **(Table 1)**. The good compatibility between its NMR data and those reported in the literature [6] confirmed the structure of 2 to be 4-hydroxybenzaldehyde.

Table 1: The ^{13}C-NMR (DMSO-d_6, 125 MHz) data of compounds 1-3

N^{o}	1	2	3
1	65.7	130.3	
2	69.3	132.1	109.8
3	62.7	115.9	75.7
4		163.4	78.7
5		115.9	73.2
6		132.1	
1'	170.5	191.0	
2'	20.8		54.9
4'			62.9

Table 2: The ^{1}H-NMR (DMSO-d_6, 500 MHz) data of compounds 1-3

Nº	1	2	3
1a	4.03 (1H, *dd*, 11.2; 4.1)		
1b	3.88 (1H, *dd*, 11.2; 6.7)		
2	3.61-3.64 (1H, *m*)	7.75 (1H, *d*, 8.5)	4.69 (1H, *d*, 3.5)
3	3.30-3.35 (2H, *m*)	6.92 (1H, *d*, 8.5)	3.73 (1H, *s*)
4			
5a		6.92 (1H, d, 8.5)	3.84 (1H, *d*, 9.0)
5b			3.59 (1H, *d*, 9.0)
6		7.75 (1H, *d*, 8.5)	
1'		9.78 (1H, *s*)	
2'	2.00 (3H, *s*)		3.24 (3H, *s*)
4'			3.30 (2H, *dd*, 12.0, 5.5)

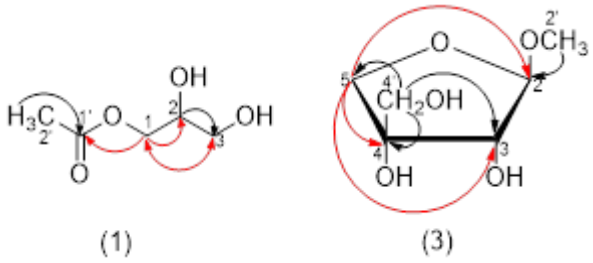

Figure 3. Keys HMBC of compounds 1 and 3

Compound **3** was isolated as a yellow amorphous solid. ^{1}H-NMR spectrum of **3** indicated an anomeric proton signal at δ_H 4.69 (1H, *d*, 3.5, H-1) with a coupling constant is 3.5 Hz showed that a signal of a furan ring. Additionally, the high magnetic field has an oxymethine proton signal at δ_H 3.73 (1H, s, H-3), three signals of two oxymethylene protons at δ_H 3.84 (1H, *d*, 9.0, H-5a), δ_H 3.59 (1H, *d*, 9.0, H-4b), 3.30 (2H, *dd*, 12.0; 5.5, H-4')] and a methoxy proton signal at δ_H 3.24 (3H, *s*, H-2') **(Table 2)**. ^{13}C-NMR spectrum showed an anomeric carbon signal δ_C 109.8, C-2 and 4 carbon signals of oxygenated methine, methylene group δ_C 62.9, (C-4'), 73.2, (C-5), 75.7 (C-3) và 78.7 (C-4) and methoxy carbon signal at δ_C 54.9 (C-2') **(Table 1)**. The methoxy group was assigned at C-2 by the HMBC correlation of the methoxy protons with carbons C-2. The remaining HMBC correlations confirm the furan ring structure **(Figure 3)**. The good compatibility of its NMR data with those reported in the literature [7] suggested that **3** was methyl 3-*C*-(hydroxymethyl)-*β*-D-erythrofuranoside.

4. Conclusions

From the dried seeds of *H. sabdariffa* Linn collected at Binh Thuan province, three compounds were isolated, including 1-*O*-acetylglycerol (**1**), 4-hydroxybenzaldehyde (**2**), 3-*C*-(hydroxymethyl)-*β*-

D-erythrofuranoside (**3**). Although these compounds were already reported in other plants, but this is the first time they are known from this species.

Declaration of Competing Interest

The authors declare no competing interests.

Author contributions

Thanh-Tung Phan, Hoang-Khang Le interpreted NMR and MS data and searched the bibliography.

Thuy-Duong Ngo-Thi, Hoang-Long Ngo, Kim-Thuy Tran, Quang Ton-That contributed to conducting experiments and acquiring MS and NMR data and gave the final correction for the manuscript.

Corresponding author: Quang Ton-That, Faculty of Chemistry, University of Science, Vietnam National University, Ho Chi Minh City, Vietnam, 227 Nguyen Van Cu Street, ward 4, district 5, Ho Chi Minh City e-mail: ttquang@hcmus.edu.vn.

All authors have read and agreed to the published version of the manuscript.

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