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An α-glucosidase iridoid glycoside inhibitor from *Scyphiphora hydrophyllacea* (Rubiaceae)

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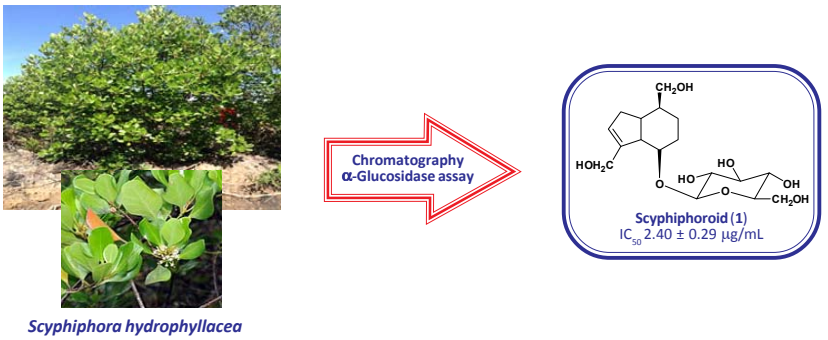
Graphical Abstract



Scyphiphora hydrophyllacea

Abstract

The stem bark of *Scyphiphora hydrophyllacea* was evaluated for a-glucosidase inhibitory activity. The lyophilized expressed juice of *S. hydrophyllacea* was found to exhibit a-glucosidase inhibition with an IC_{50} of 8.63 ± 2.16 μg/mL. The fractions of *S. hydrophyllacea* were also found to inhibit the α-glucosidase with the *n*-butanol fraction being the most active (IC_{50} 2.24 ± 0.75 μg/mL). Bioassay-guided isolation of the active constituent from the *n*-butanol fraction afforded the isolation and structure elucidation of a new iridoid glycoside named scyphiphoroid (**1**) with an IC_{50} 2.40 ± 0.29 μg/mL.

Keywords: *Scyphiphora hydrophyllacea*, α-glucosidase, iridoid glycoside, Rubiaceae, mangrove

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Introduction

Diabetes is a worldwide concern with global estimates of 422 million cases of adults living with the disease in 2014. The global prevalence has doubled since the 1980 and the majority is affected by type 2 diabetes. In 2012, 1.5 million deaths due to the disease were reported [1]. Type 2 diabetes used to occur nearly entirely among adults, but recently occurs in children as well The hyperglycemia observed in type 2 diabetes is due to the inability of the body to utilize the insulin (hormone that controls blood glucose levels) that the pancreas produces. Overtime, the uncontrolled hyperglycemia may lead to serious effects on the heart, blood vessel, eyes, kidney, and nerves [2]. One of the approaches in the management of type 2 diabetes is to control post-prandial hyperglycemia. The glucose absorption occurs in the brush border of the small intestines where the α-glucosidase hydrolyzes glucose from short chain oligosaccharides. Thus, inhibition of the α-glucosidase slows glucose absorption from the intestines [3].

Scyphiphora hydrophyllacea C.F. Gaertn. (Rubiaceae) is a mangrove plant commonly found in the coastal areas of South to Southeast Asia, Caroline Islands, Australia, and New Caledonia [4]. Phytochemical studies showed that iridoid and their glucosides, triterpenoids, flavonoids, and lignans [4–10] were commonly identified from *S. hydrophyllacea*.

An exhaustive review of literature reveals limited scientific data on the bark of *S. hydrophyllacea*. Scientific evidences for the antidiabetic claims of the bark have not been established. To address this gap, this investigation for α-glucosidase inhibitory activity of the bark extract is conducted. In this paper, we report the isolation and structure elucidation of a new iridoid glycoside, scyphiphoroid (**1**) and its α-glucosidase inhibitory activity.

Materials and methods

General considerations. NMR spectra (1D and 2D) were recorded on a Bruker AV-500 spectrometer. Column chromatography was performed on a reversed-phase MCI gel (75–150 μM bead size). TLC was performed on a pre-coated silica-gel 60 F_{254} (0.25 mm, Merck) plates and visualized in UV_{254}. The electrospray ionization – high resolution (HR-ESI) mass spectra was recorded on a SQD2, Waters Micromass spectrometer.

Plant material and extraction procedure. The stem barks of *S. hydrophyllacea* were collected from the mangrove site in Sibuyan Island, Romblon in March 2013. The samples were authenticated and identified by Dr. Maureen B. Sabit of the University of Santo Tomas Herbarium with voucher specimen no. USTH 11147.

The outermost layer of the bark was removed and the remaining layers were peeled from the hardcore. The collected peelings were pounded and squeezed to release the juice of the bark. Tannins were then removed from the bark juice extract by washing with 1% aqueous NaCl [11]. The expressed juice was lyophilized to obtain the crude bark extract (3 g).

Isolation of the iridoid glycoside. The crude bark extract of *S. hydrophyllaceae* was partitioned between H_2O and hexane. The hexane layer was separated, dried with anhy. Na_2SO_4, and concentrated under reduced pressure to yield the hexane fraction. The aqueous layer was extracted with EtOAc in the same manner to yield the EtOAc fraction. Extraction of the aqueous layer with *n*-BuOH, drying under reduced pressure, and lyophilization of the remaining aqueous layer yield the *n*-BuOH and aqueous fractions, respectively. The *n*-BuOH fraction (1.0519 g), being most active in the α-glucosidase assay, was subjected to reverse-phase column chromatography using MCI gel eluting with increasing increments of MeOH in H_2O (10:0, 500 mL), (9:1, 300 mL), (8:2, 300 mL), (7:3, 300 mL), (6:4, 300 mL), (5:5, 300 mL), (4:6, 300 mL), and (3:7, 300 mL). TLC profile of the collected fraction gave six pooled sub-fractions (Bu1 to Bu 6). Sub-fraction Bu2 (**1**, 197 mg) was ascertained to be TLC pure and was subjected to NMR analysis.

α-Glucosidase inhibitory assay. The α-glucosidase inhibition assay was done according to modified procedure [12]. A mixture of 20 µL of 0.8 U/mL yeast α-glucosidase and 120 µL of test substance or acarbose in 0.5 % dimethylsulfoxide was incubated at 37°C for 15 min. After incubation, 20 µL of 5.0 mM *p*-nitrophenyl-α-D-glucopyranoside was added then the solution was allowed to react at 37°C for 15 min. The reaction was stopped by adding 80 µL of 0.2 M Na_2CO_3. The resulting solution was read at 405 nm and percent inhibition was calculated using the formula below.

$$\textit{Percent inhibition} = \frac{\textit{Absorbance}\ \text{control} - \textit{Absorbance}\ \text{sample}}{\textit{Absorbance}\ \text{control}} \times 100$$

The mean IC_{50} of the samples using five-point concentration (0.025, 0.25, 2.50, 25, and 250 µg/mL) and their standard deviations were determined by three trials and were compared to that of acarbose. Calculation of IC_{50} values was done using four-parameter logistic equation in GraphPad Prism 6.07.

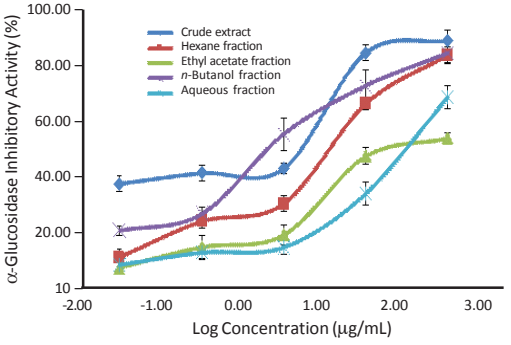

Figure 1. Concentration response curve by the *S. hydrophyllacea* crude extract and fractions for α-glucosidase inhibitory activity. Values are expressed as mean ± standard deviation (n = 9).

Table 1. α-Glucosidase IC_{50} of the *S. hydrophyllaceae* extracts

Samples	IC_{50} values (µg/mL)
Crude Extract	8.63 ± 2.16
Hexane fraction	28.33 ± 1.83
Ethyl acetate fraction	7.69 ± 2.29
n-Butanol fraction	2.24 ± 0.75
Aqueous fraction	91.39 ± 1.93
Scyphiphoroid (**1**)	2.40 ± 0.29
Acarbose*	0.64 ± 0.03

*Positive control

Results and Discussion

The concentration response of the major fractions of *S. hydrophyllaceae* on their α-glucosidase inhibitory effect was determined at a five-point increasing concentration (Fig. 1) and their IC_{50} values were compared. The α-glucosidase inhibitory activity of the major fractions of *S. hydrophyllaceae* and their IC_{50} values are shown in Table 1. The results showed that the *n*-butanol fraction was the most active α-glucosidase inhibitor with IC_{50} at 2.24 ± 0.75 μg/mL. Reverse-phased chromatography of the *n*-butanol fraction allowed the isolation of an iridoid glycoside, scyphiphoroid (**1**), with IC_{50} at 2.40 ± 0.29 μg/mL.

Results were reported as mean ± standard deviation of nine trials, except for Scyphiphoroid (**1**) ($n = 3$).

Scyphiphoroid (**1**) (Fig. 2) was obtained as a brown, amorphous solid. The molecular formula was established to be $C_{16}H_{26}O_9$ by HR-ESI-MS ($[M + Na]^+$ at *m/z* 385.1576, calculated *m/z* 385.1590) and is supported by ^1H and ^{13}C NMR (Table 2). The molecular formula has four index of hydrogen deficiency attributed to three rings and one double bond. The ^1H-NMR showed the presence of an acetal proton (δ 5.10, d, $J = 7.0$ Hz, H-1), an olefinic proton (δ 5.74, br s, H-7), and three oxygenated methylenes (δ 3.60, m, H_2-3, δ 4.09, d, $J = 8.5$ Hz, H-10a, and δ 4.20, d, $J = 8.5$ Hz, H-10b; δ 3.48–3.50, m, H-11a and δ 3.70–3.73, m, H-11b). The ^{13}C NMR showed an acetal carbon (δ 95.6, C-1), olefinic carbons (δ 125.6, C-7 and δ 144.4, C-8) and three oxygenated methylene carbons

Table 2. NMR data of Scyphiphorin (**1**)

Position	Scyphiphorin (**1**)	
	^{13}C NMR 125 MHz, DMSO-d6	*^1H NMR, 500 MHz, DMSO-d6*
1	95.7	5.10 (d $J = 7.0$ Hz)
2	–	–
3	69.6	3.60 (m)
4	38.3	2.74–2.78 (m)
5	35.2	3.04–3.12 (m)
6	48.6	3.20–3.26 (m)
7	125.6	5.74 (s)
8	144.4	–
9	45.9	2.61–2.64 (m)
10	59.5	4.09 (d $J = 8.5$ Hz) 4.20 (d $J = 8.5$ Hz)
11	60.9	3.48–3.50 (m) 3.70–3.73 (m)
1’	98.5	4.60 (d $J = 7.0$ Hz)
2’	73.4	3.03–3.07 (m)
3’	76.7	3.22–3.25 (m)
4’	71.3	3.51–3.55 (m)
5’	77.3	3.16–3.20 (m)
6’	63.9	3.44–3.48 (m) 3.65–3.68 (m)

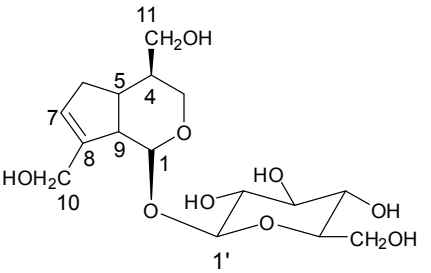

Figure 2. Structure of Scyphiphoroid (**1**)

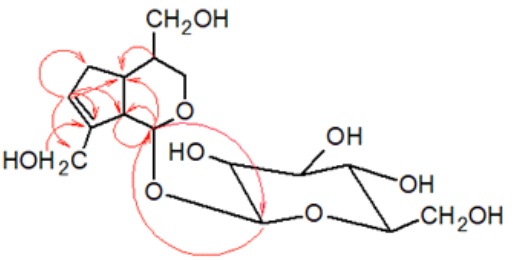

Figure 3. Selected HMBC correlations of Scyphiphoroid (**1**)

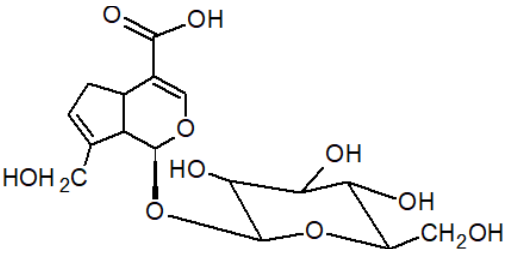

Figure 4. Structure of geniposidic acid

(δ 69.6, C-3; δ 59.5, C-10; and, δ 60.9, C-11). Additionally, ^{1}H and ^{13}C signals corresponding to a β-D-glucopyranosyl moiety was also observed with the anomeric proton at d 4.60 (δ, *J* = 7.0 Hz, H-1'). To further support the structure, important HMBC correlations (Fig. 3) were observed between H-11 methylenes to δ 69.6 (C-3), δ 38.3 (C-4), αvδ δ 35.2 (C-5); H-10 methylenes to δ 125.6 (C-7) and δ 144.4 (C-8); H-7 olefinic proton to δ 59.6 (C-10); H-1 methine to δ 98.5 (C-1'); H-1' anomeric proton to δ 95.7 (C-1) (Fig. 3). An NOE correlation was also observed between the methine protons at H-1 and H-4. Hence, the relative structure of **1** was deduced as shown in Fig. 2.

The indicated ^{1}H and ^{13}C chemical shifts (Table 1) are typical of an iridoid glucoside which are commonly isolated in previous studies of *S. hydrophyllaceae*. The structure of **1** is also similar to geniposidic acid [6]. The main differences are indicated by the presence of a double bond in C-3 and C-4 and carboxylic acid in C-11 in geniposidic acid. Although the solvents used to measure the ^{13}C NMR data for **1** (DMSO-d_6) and genisipodic acid (CD_3OD) [6] are different, these differences are evident in the observed ^{13}C NMR signals, i.e., δ 69.6 (C-3), δ 38.3 (C-4), αvδ δ 60.9 (C-11) in 1; δ 152.9 (C-3), δ 113.4 (C-4), and δ 171.9 (C-11) in geniposidic acid.

A review of literature revealed a range of compounds isolated from medicinal plants having α-glucosidase inhibitory activity. The natural products with α-glucosidase inhibitory activity contain within the chemical framework includes terpene, alkaloid, quinine, flavonoid, phenol, phenylpropanoid and sterol structures. Most of the compounds with sugar moieties work by competitive and reversible inhibition of the α-glucosidase [12, 13]. Scyphiphoroid (**1**) may possibly exert its α-glucosidase inhibitory activity through the same mechanism. As with other α-glucosidase inhibitors, the presence of the alcohol functional groups in the iridoid ring may relate to the α-glucosidase inhibitory activity of **1**. This is the first report of an iridoid glycoside isolated from the stem bark of *S. hydrophyllacea* having an α-glucosidase inhibitory and may explain in part evidence for the antidiabetic claim of this stem bark.

Conclusion

This is the first report on the α-glucosidase inhibitory activity of the crude and semi-crude extracts of the stem bark of *S. hydrophyllacea*. Further purification of the *n*-butanol extract afforded a new iridoid glucoside, scyphiphoroid (**1**), with an α-glucosidase IC_{50} of 2.40 μg/mL.

Acknowledgments

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References

[1] *WHO Mortality Database*. (World Health Organization, **2018**). www.who.int/healthinfo/mortality_data/en/ (2018 October 11)
[2] Dabelea D. The accelerating epidemic of childhood diabetes. *Lancet* **2009**; 373(9680), 1999–2000.
[3] Bremner GM & Stevens CW. *Pharmacology* (4th Ed.). (The Netherlands: Elsevier, **2013**).
[4] Zeng YB, Mei WL, Wang H, Li XN, & Dai HF. Scyphiphin D, a new iridoid glucoside dimer from *Scyphiphora hydrophyllacea*. *Journal of Asian Natural Products Research* **2010**; 12(11), 1010–1014.
[5] Feng CL, Gong MF, Zeng YB, Dai HF, & Mei WL. Scyphiphin C, a new iridoid from *Scyphiphora hydrophyllacea*. *Molecules* **2010**; 15(4), 2473 2477.
[6] Tao SH, Wu J, Qi SH, Zhang S, Li MY, & Li QX. Scyphiphorins A and B, two new iridoid glycosides from the stem bark of a Chinese mangrove *Scyphiphora hydrophyllacea*. *Helvetica Chimica Acta* **2007**; 90(9), 1718–1722.
[7] Zeng YB, Wen LM, You XZ, Ling Z, Hong K, & Dai HF. Two new epimeric pairs of iridoid from mangrove plant *Scyphiphora hydrophyllacea*. *Chinese Chemical Letters* **2007**; 18(12), 1509 1511.
[8] Zeng YB, Mei WL, Zhao YX, & Dai HF. Two new noriridoids from *Scyphiphora hydrophyllacea*. *Zeitschrift fur Naturforschung B* **2008**; 63, 108–110.
[9] Li YJ, Tang HX, Li J, Xie L, Cao WQ, Liu YP, & Fu YH. Chemical constituents from stems and leaves of mangrove plant, *Scyphiphora hydrophyllacea*. *Chinese Traditional and Herbal Drugs* **2019**; 50(23), 5677–5682.
[10] Samarakoon SR, Ediriweera MK, Wijayabandara L, Fernando N, Tharmarajah L, Tennekoon KH, Piyathilaka P, & Adhikari A. Isolation of cytotoxic triterpenes from the mangrove plant, *Scyphiphora hydrophyllacea* C.F. Gaertn (Rubiaceae). *Tropical Journal of Pharmaceutical Research* **2018**; 17(3), 475–481.
[11] Wall ME, Wani MC, Brown DM, Fullas F, Olwald JB, Josephson FF, Thorton NM, Pezzuto JM, Beecher CW, Farnsworth NR, Cordell GA, & Kinghorn AD. Effect of tannins on screening of plant extracts for enzyme inhibitory activity and techniques for their removal. *Phytomedicine* **1996**; 3(3), 281–285.
[12] Feng J, Yang XW, & Wang RF. Bio-assay guided isolation and identification of a-glucosidase inhibitors from the leaves of *Aquilaria sinensis*. *Phytochemistry* **2011**; 72, 242–47.
[13] Yin Z, Zhang W, Feng F, Zhang Y, & Kang W. a-Glucosidase inhibitors isolated from medicinal plants. *Food Science and Human Wellness* **2014**; 3, 136 174.