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# Flavonoid Glycosides from the Leaves of *Glyphaea brevis*

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## Abstract

A new flavonoid, acacetin-7-*O*- $\beta$ -D-glucopyranosyl-(1  $\rightarrow$  2)- $\beta$ -D-glucuronopyranoside (1), together with four known flavonoids, apigenin-7-*O*- $\beta$ -D-glucopyranosyl-(1  $\rightarrow$  2)- $\beta$ -D-glucuronopyranoside (2), acacetin (3), acacetin-7-*O*- $\beta$ -D-glucuronide (4) and genkwanin-5-*O*-primveroside (5) were isolated from the leaves of *Glyphaea brevis*. Their structures were elucidated by spectroscopic techniques. The isolated compounds (1 - 5) were tested for their antioxidant activity using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. The tested compounds showed slight negative antioxidant activities against DPPH radicals.

## Keywords

*Glyphaea brevis*, Malvaceae, Acacetin-7-*O*- $\beta$ -D-Glucopyranosyl-(1 $\rightarrow$ 2)- $\beta$ -D-Glucuronopyranoside

## 1. Introduction

*Glyphaea brevis* (Spreng.) Monach (Malvaceae) is a small tree distributed in a tropical region, from Guinea-bissau, Ouganda, RDC to Congo Brazzaville [1], traditionally known as fondron or koula-gboè (forest okro) in Ivory Coast. The flowers of this plant are used in revitalizing sexual impotency [2]. The leaves and the flowers are used to treat sterility [3]. The leaves are also used as a remedy for sexually, liver, eyes, pulmonary and stomach diseases, inflammations, wound healing, palpitations, abortive, anti-emetic and analgesic [1] [4]. The stem bark

of the root is used to treat diarrhea, dysentery and epilepsy [5]. Dakam *et al.* described the inhibitory effects of aqueous and hydroalcoholic extracts of the leaves of *G. brevis* on pancreatic  $\alpha$ -Amylase activity [6], while Ojelabi *et al.* (2012) showed the roles of the ethanolic extract of the leaves of *G. brevis* in the protection of the liver and as antioxidant against cadmium-mediated oxidative stress [7]. Chemical investigations of the leaves of *G. brevis* have led to the isolation of sitosterol-3-*O*- $\beta$ -D-glucopyranoside, *n*-hexacosanol, *n*-dotriacontanol, oleanolic acid, echinocystic acid and *meso*-erythritol which showed both antibacterial and antifungal activity [8]. Ekuadzi *et al.* (2014) reported the isolation of epicatechin and its dimer procyanidin B2 from the stem bark of *G. brevis* [9].

Our previous phytochemical investigation of the stem bark of the roots of *G. brevis* resulted in the identification and structural elucidation of the potent glucosidase inhibitors phenylalkyl iminosugars [10] (Gossan *et al.*, 2014). In a recent study, the results of Olugbodi *et al.* (2022) indicated that *Glyphaesia* C-enriched extract of *Glyphaea brevis* leaf enhanced the fertility by the quality of semen and improved the functional capabilities of spermatozoa [11].

In our continuous search for interesting bioactive compounds we examined the *n*-butanol extract of the leaves of *G. brevis*.

This paper describes the isolation and structural elucidation of one undescribed and four known flavonoids. Their structures were elucidated by spectroscopic techniques including MS, 1D and 2D NMR spectroscopy and by comparison with published data. All the compounds were isolated for the first time from *Glyphaea brevis*. The antioxidant activity of compounds 1 - 5, using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay was tested.

## 2. Experimental

### 2.1. General Experimental Procedures

NMR spectra were recorded in DMSO on a Bruker Avance DRX-600 spectrometer ( $^1\text{H}$  at 600 MHz and  $^{13}\text{C}$  at 200 MHz). HRESI-MS experiments were obtained using a Micromass ESI-Q-TOF micro instrument (Manchester). Optical density (OD) values in the DPPH free radical scavenging assay were read on a Fluostar omega microplate reader (BMG labtech). Silica gel 60 (63 - 200  $\mu\text{m}$ ) and LiChroprep RP-18 (40 - 63  $\mu\text{m}$ ); Merck, were used for column chromatography (CC). Glass and aluminium supported silica gel 60  $\text{F}_{254}$  (Merck) plates were used for preparative TLC. TLC spots were visualized under UV light (254 and 365 nm), followed by spraying with 50%  $\text{H}_2\text{SO}_4$  for compound detection. High Performance Flash Chromatography (HPFC) was performed with a Grace Reveleris Flash System with RP-18 Columns. Purified compounds were obtained by HPLC using a Dionex apparatus equipped with an ASI-100 autosampler, a STH 585 column, a P580 pump, a UVD 340S diode array detector and Chromeleon<sup>®</sup> software. The semipreparative HPLC was carried out using RP-18 column (Phenomenex 250  $\times$  15 mm, Luna 5  $\mu\text{m}$ ), with a binary gradient eluent  $\text{H}_2\text{O}$  with TFA (0.0025%) and  $\text{CH}_3\text{CN}$ , with a flow rate of 5 mL/min and the chromatogram was

monitored at 205, 225, 250 and 350 nm. DPPH (1,1-diphenyl-2-picrylhydrazyl radical), ascorbic acid used for the bioassay were purchased from Sigma-Aldrich, Chemical Co. (Germany).

## 2.2. Plant Material

The leaves of *Glyphaea brevis* (Spreng.) Monach (Malvaceae) were collected from Alépé, Lagunes Region, Ivory Coast, in February 2013 and identified by Prof. L. Aké Assi of FHB University. A voucher specimen (N° Aké Assi S.N-01) was deposited in the herbarium of the National Center of Floristic of FHB University of Cocody (Ivory Coast).

## 2.3. Extraction and Isolation

The air-dried leaves powder of *G. brevis* (950 g) was extracted successively with petroleum ether (10 L), and 20% aqueous methanol (14 L) for 24 h at room temperature. After concentration *under vacuum* the hydromethanolic (2 L) extract was subsequently extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 1 L) and *n*-BuOH (3 × 1 L). After removal of solvent, the *n*-BuOH extract (10 g) was subjected to flash chromatography on normal silica with CH<sub>3</sub>Cl/MeOH/H<sub>2</sub>O (90/10/0; 70/30/5; 60/40/5) to give 195 fractions of 15 mL.

The combined fractions [20 - 26] (15 mg) and [124 - 146] (75 mg) gave a white precipitate filtered in methanol and identified as pure compounds **3** (10 mg) and **5** (60 mg) respectively.

Fractions [159 - 195] (230 mg) were subjected to vacuum-liquid chromatography (VLC) in reversed-phase using a gradient of the mixture of H<sub>2</sub>O/CH<sub>3</sub>OH (80:20 to 10:90) to afford 12 sub-fractions (F<sub>1</sub> - F<sub>12</sub>). The sub-fractions [F<sub>1</sub> - F<sub>2</sub>] (18 mg) were purified by semi-preparative HPLC using a binary gradient of CH<sub>3</sub>CN-H<sub>2</sub>O from 20% to 35% CH<sub>3</sub>CN for 35 min to give compounds **2** (5 mg, t<sub>R</sub> 11.10 min). The sub-fraction F<sub>5</sub> (28 mg) was separated by semi-preparative HPLC using a gradient of CH<sub>3</sub>CN-H<sub>2</sub>O from 20% to 40% CH<sub>3</sub>CN for 32 min to afford compounds **1** (8 mg, Rt 12.9 min) and **4** (6 mg, Rt 17.5 min).

## 2.4. DPPH Free Radical Scavenging Assay

The scavenging activity of isolated compounds against DPPH was investigated by spectrophotometric methodology as described in a previous study [12]. Then, 5 µL of different concentrations of the samples (dissolved in DMSO) were added to 95 µL of a DPPH solution (158 µM, dissolved in EtOH 50%). The reaction mixture was shaken and incubated in the dark room at 37°C for 30 min. The optical density of the solution was read using a Fluostar omega microplate reader (BMG labtech) at the wavelength 515 nm. DPPH solution in EtOH was used as a control. Absorbance values were corrected for radicals decay using a blank solution. The inhibitory effect of DPPH was calculated according to the following formula:

$$\% \text{ Inhibition} = \left[ 1 - \left( A_{\text{SAMPLE}} / A_{\text{CONTROL}} \right) \right] \times 100$$

where,  $A_{\text{SAMPLE}}$  is the absorption of the blank sample (containing all reagents except the test compound) and  $A_{\text{CONTROL}}$  is the absorption of the test compound. In this assay ascorbic acid was used as a standard. The concentration providing 50% inhibition (IC<sub>50</sub>) was calculated from the plot of inhibition percentage against sample concentration by linear regression analysis. Samples were prepared at concentrations of 100, 50, 10 and 5 µg/mL. All the tests were run in triplicate. The experimental results were expressed as means ± standard deviation.

### 3. Results and Discussion

The n-BuOH fraction extract of the leaves of *G. brevis* was subjected to a series of chromatographic separations to yield five flavonoids **1** - **5** (Figure 1). Their structures were elucidated based on the combination of various NMR (<sup>1</sup>H and <sup>13</sup>C NMR, COSY, HSQC, HMBC, and NOESY) spectroscopic and mass spectrometric (HR-EI-MS) techniques.

The known compounds **2**-**5** were readily identified from their spectral data and by comparison with reported data in the literature, as apigenin 7-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucuronopyranoside (**2**) [13], acacetin (**3**), acacetin-7-*O*-β-D-glucuronide (**4**) [14] and genkwain-5-*O*-primeveroside (**5**) [15].

Compound **1** was obtained as an amorphous yellow powder. The ESIMS spectra of **1** revealed [M + Na]<sup>+</sup> (positive ion mode) ion peak at *m/z* 645, suggesting the molecular formula C<sub>28</sub>H<sub>30</sub>O<sub>16</sub>.

The <sup>1</sup>H and <sup>13</sup>C-NMR spectra of **1** were closely similar to that of **2**, except for the presence of OCH<sub>3</sub>-4' in the flavone moiety of **1** (Table 1). Thus, the <sup>1</sup>H NMR spectrum of **1** showed an isolated proton at δ 6.97 (1H, s), a methoxyl group at δ 3.88, a *meta*-coupled protons at δ 6.52 and 6.92 (each 1H, d, *J* = 2.3 Hz) assigned to H-6 and H-8, respectively, and an *ortho*-coupled AA'BB'-type protons at δ 8.07 and 7.15 (each 2H, d, *J* = 8.7 Hz) assignable to H-2', 6' and H-3', 5' respectively. The above spectral evidence and comparison with literature data readily allowed to recognize a typical acacetin framework in **1** [14]. The spectrum further displayed signals at δ<sub>H</sub> 5.40 (1H, d, *J* = 7.2 Hz, H''-1), 3.58 (1H, m, H''-2), 3.57 (1H, m, H''-3), 3.49 (1H, t, *J* = 9.4 Hz, H''-4), 4.11 (1H, d, *J* = 9.4 Hz, H''-5). These proton shifts in correlation with downfield signals at δ<sub>C</sub> 98.0, 82.2, 74.9,

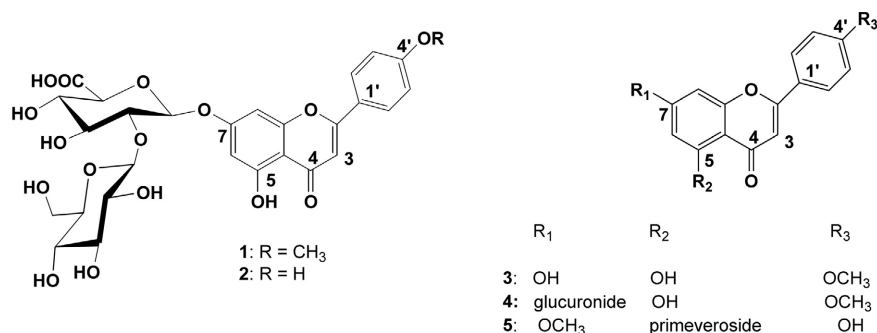


Figure 1. Structures of compounds **1** - **5**.

**Table 1.** NMR (ppm) data for **1** and **2** (in DMSO- $d_6$ ,  $^1H$ : 600 MHz,  $^{13}C$ : 150 MHz)  $J$ (Hz).

N°	<b>1</b>		<b>2</b>	
	$\delta_C$	$\delta_H$	$\delta_C$	$\delta_H$
2	163.9		164.3	
3	103.8	6.97 s	103.1	6.85 s
4	182.1		182.0	
5	161.1		161.0	
6	99.5	6.52 d (2.3)	99.6	6.50 d (2.3)
7	162.5		162.7	
8	95.0	6.92 d (2.3)	95.0	6.95 d (2.3)
9	156.9		156.8	
10	105.5		105.4	
1'	122.7		121.0	
2', 6'	128.4	8.07 d (8.7)	128.6	7.98 d (8.7)
3', 5'	114.7	7.15 d (8.7)	116.0	6.98 d (8.7)
4'	162.5		161.4	
OCH <sub>3</sub>	55.6	3.88 s		
glucuronic acid				
1''	98.0	5.40 d (7.2)	98.1	5.28 d (7.2)
2''	82.2	3.58 m	82.5	3.51 m
3''	74.9	3.57 m	75.4	3.52 m
4''	70.8	3.49 t (9.4)	71.3	3.32 t (9.4)
5''	75.0	4.11 d (9.4)	74.0	3.88 d (9.4)
6''	170.0		171.0	
glucose				
1'''	104.6	4.49 d (7.8)	104.8	4.48 d (7.8)
2'''	74.6	3.00 td (7.8, 3.4)	74.7	3.00 td (7.8, 3.4)
3'''	76.2	3.20 td (8.8, 3.4)	76.2	3.20 td (8.8, 3.4)
4'''	69.6	3.15 m	69.6	3.12 m
5'''	76.9	3.16 m	76.9	3.14 m
6'''	60.5	3.49 m	60.5	3.45 m

70.8, 75.0 and 170.0 in the  $^{13}C$ -NMR, HSQC and HMBC spectra, demonstrated the presence of an *O*- $\beta$ -D-glucuronic acid moiety [13]. The glucose part of **1** was identified by the presence of six carbons signals at  $\delta_C$  104.6, 74.6, 76.2, 69.6, 76.9, 60.5 (**Table 1**) in the  $^{13}C$  spectrum and one anomeric proton doublet at 4.49 (1H, d,

**Table 2.** DPPH radical scavenging activities of compound **1 - 5**.

Compounds	IC <sub>50</sub> ± S.D. (µg/mL)
<b>1</b>	<sup>a</sup> ND
<b>2</b>	<sup>a</sup> ND
<b>3</b>	150.2 ± 0.8
<b>4</b>	<sup>a</sup> ND
<b>5</b>	<sup>a</sup> ND
<b>Ascorbic acid<sup>b</sup></b>	30.5 ± 0.5

<sup>a</sup>Not determined: IC<sub>50</sub> value > 200 µg/mL. <sup>b</sup>Used as a positive control.

$J = 7.8$  Hz, H<sup>'''</sup>-1) in the <sup>1</sup>H NMR spectrum. The coupling constant of H<sup>'''</sup>-1 ( $J = 7.8$  Hz) indicated the  $\beta$ -D configuration of the terminal  $\beta$ -glucopyranose unit. The location of the glucuronic acid unit at C-7 of acacetin moiety was confirmed by the correlation between H<sup>''</sup>-1 and C-7 in the HMBC spectrum. The attachment of the glucosyl unit at C<sup>''</sup>-2 of acacetin glucuronide moiety was confirmed by the upfield shift of C<sup>''</sup>-2 as well as by the cross-peak between C<sup>''</sup>-2 and H<sup>'''</sup>-1 in the HMBC spectrum. Accordingly, **1** was characterized as acacetin-7-*O*- $\beta$ -D-glucopyranosyl-(1  $\rightarrow$  2)- $\beta$ -D-glucuronopyranoside.

All isolated compounds **1 - 5** were tested for their antioxidant activities in the DPPH radical scavenging assay and showed no activity for **1**, **2**, **4** and **5** (**Table 2**). These results confirmed those of previous studies, which showed weak or negative effects against free radical scavenging activities in compounds **2**, **3**, **4** and **5** possessing acacetin and apigenin nucleus [13] [16] [17] [18]. This very weak activity may be attributed to the smaller number of phenolic groups or the blocked 4-hydroxy group in the B-ring [19]. Furthermore, the presence of the sugar groups on acacetin (**1** and **4**) and apigenin (**2** and **5**) could also be attributed to the inactivity. Very frequently, the antioxidant activities of natural phenolic compounds, such as flavonoid glycosides depend on the number or sequence of sugars [19]. Shimoda *et al.* (2011) showed the decrease of DPPH free-radical scavenging activity of daidzein in its glucosylation form  $\beta$ -glucosides and  $\beta$ -primeveroside [20].

#### 4. Conclusion

The phytochemical investigation of the leaves of *Glyphaea brevis* resulted in the identification of a new flavonoid acacetin-7-*O*- $\beta$ -D-glucopyranosyl-(1  $\rightarrow$  2)-glucuronopyranoside (**1**) together with apigenin-7-*O*- $\beta$ -D-glucopyranosyl-(1  $\rightarrow$  2)-glucuronopyranoside (**2**) acacetin (**3**), acacetin-7-*O*-glucuronide (**4**) and genkwanin 5-*O*-primveroside (**5**). All the compounds were isolated for the first time from *Glyphaea brevis*. The antioxidant activity of compound **1** using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay showed no antioxidant activity against DPPH radicals.

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## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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