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**Guetchueng, ST, Nahar, L, Ritchie, KJ, Ismail, FMD, Dempster, NM and Sarker, SD (2019) Justicalosides A and B, two new flavone glycosides from the leaves of *Ruspolia hypocrateriformis* (Vahl) Milne-Redh. (Acanthaceae). *Phytochemistry Letters*. 31. pp. 101-103. ISSN 1874-3900**

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## **Justicialosides A and B, two new flavone glycosides from the leaves of *Ruspolia hypocrateriformis* (Vahl) Milne-Redh. (Acanthaceae)**

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### ABSTRACT

Two new flavone glycosides, luteolin 7-*O*- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\beta$ -D-xylopyranoside (**1**) and chrysoeriol 7-*O*- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\beta$ -D-xylopyranoside (**2**), along with five known compounds, luteolin 7-*O*- $\beta$ -D-apiofuranosyl-(1 $\rightarrow$ 2)- $\beta$ -D-xylopyranoside (**3**), grandulosides A and B (**4** and **5**), luteolin 7-*O*-[ $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnosyl-(1 $\rightarrow$ 6)]- $\beta$ -D-glucopyranoside (**6**) and 10*H*-quindoline (**7**) were isolated from the leaves of *Ruspolia hypocrateriformis* (Acanthaceae). Their structures were elucidated by spectroscopic means, including 1D and 2D NMR, HRESIMS and by comparison with published data.

*Keywords:* *Ruspolia hypocrateriformis*; Acanthaceae; Medicinal plant; Glycosylated flavones; Luteolin; Justicialoside

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## 1. Introduction

*Ruspolia hypocrateriformis* (Vahl) Milne-Redh., synonym *Justicia hypocrateriformis* Vahl, is a small shrub with scattered growth measuring above 1 m in height and is native to Africa (Burch and Demmy, 1986). A decoction of the leaves of this plant is used as a remedy in Cameroon for the treatment of anaemia and fever associated with malaria as well as for the management of diarrhoea (Adjanohoun *et al.*, 1996; Noumi, 2015). Previous studies reported the isolation of pyrrolidine alkaloids from the roots (Roessler *et al.*, 1978; Neukomm *et al.*, 1983); the aqueous extract of the leaves was shown to possess antidiarrhoeal and antioxidant properties (Agbor *et al.*, 2014). As a part of our continuing study on the secondary metabolites of Cameroonian medicinal plants (Wansi *et al.*, 2016; Guetchueng *et al.*, 2017, 2018a, 2018b), we have undertaken the phytochemical investigation of *Ruspolia hypocrateriformis*. The present work describes the isolation and identification of two new flavone glycosides (**1** and **2**) from the leaves of this plant (Figure 1).

## 2. Results and discussion

A combination of reversed-phase solid-phase-extraction and reversed-phase preparative HPLC purification of the methanolic extract of the leaves of *R. hypocrateriformis* afforded seven compounds; two new flavone glycosides, luteolin 7-*O*- $\alpha$ -L-rhamnopynosyl-(1 $\rightarrow$ 2)- $\beta$ -D-xylopyranoside (**1**) and chrysoeriol-7-*O*- $\alpha$ -L-rhamnopynosyl-(1 $\rightarrow$ 2)- $\beta$ -D-xylopyranoside (**2**), named justicialosides A and B (Figure 1), respectively, and five known compounds, luteolin 7-*O*- $\beta$ -D-apiofuranosyl-(1 $\rightarrow$ 2)- $\beta$ -D-xylopyranoside (**3**) (Koffi *et al.*, 2013), grandulosides A and B (**4** and **5**) (Tagoutsop *et al.*, 2017), luteolin 7-*O*-[ $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnosyl-(1 $\rightarrow$ 6)]- $\beta$ -D-glucopyranoside (**6**) (Koffi *et al.*, 2013) and 10*H*-quindoline (**7**) (Subbaraju *et al.*, 2004). The structures of all compounds were elucidated by spectroscopic means, and by comparison with respective published data (for known compounds).

Both compounds **1** and **2** were isolated as yellow amorphous powders. The molecular formula C<sub>26</sub>H<sub>28</sub>O<sub>14</sub> of **1** was determined from its HR ESI-MS spectrum obtained in +ve ion mode, where the sodiated ion peak was observed at *m/z* 565.1559, calculated for [C<sub>26</sub>H<sub>28</sub>O<sub>14</sub>+H]<sup>+</sup>, 565.1552, consistent with thirteen DBE. The <sup>1</sup>H NMR (Table 1) spectrum of **1** displayed 17 signals, which could be attributed to a glycosylated molecule bearing two

sugar moieties. The aglycone part of the compound was identified as luteolin by the presence of an ABX system resonating at  $\delta_{\text{H}}$  7.25 (d,  $J = 8.3$  Hz), 7.50 (dd,  $J = 2.2, 8.3$  Hz) and 7.88 (d,  $J = 2.2$  Hz), characteristic of a 3,4-dihydroxylated B ring; an AX system appearing as two doublet signals at  $\delta_{\text{H}}$  6.94 and 6.96 ( $J = 2.1$  Hz), typical of a *meta*-dihydroxylated A ring, and one singlet at  $\delta_{\text{H}}$  6.91 assignable to the H-3 proton of the luteolin C ring. The sugar moieties were identified by the presence of two anomeric proton signals at  $\delta_{\text{H}}$  5.62 (d,  $J = 7.5$  Hz) and 6.38 (d,  $J = 1.6$  Hz) and a doublet methyl at  $\delta_{\text{H}}$  1.78 ( $J = 6.1$  Hz) showing a cross peak correlation in the HSQC spectrum with the carbon signal resonating at  $\delta_{\text{C}}$  18.6 suggesting a rhamnose as one of the sugars. Complete assignment of the protons and carbons (Table 1) of the sugar units in **1** was achieved by analysis of its 2D NMR spectra including COSY, HSQC, HMBC and TOCSY spectra. The sugars were identified as  $\beta$ -D-xylopyranose and  $\alpha$ -L-rhamnose with their anomeric protons resonating at  $\delta_{\text{H}}$  5.62 (d,  $J = 7.5$  Hz) and 6.38 (d,  $J = 1.6$  Hz), respectively, by comparison of their spectroscopic data with those reported in the literature (Beier and Mundy, 1980; Agrawal, 1992). In the HMBC spectrum (Figure 2), a correlation could be observed between the proton H-1'' ( $\delta_{\text{H}}$  5.62) of the xylose and the carbon C-7 ( $\delta_{\text{C}}$  163.2) of the aglycone, confirming its direct attachment to the luteolin skeleton. Other key correlations observed between the proton H-1''' ( $\delta_{\text{H}}$  6.38) of the rhamnose and the carbon C-2'' of the xylose ( $\delta_{\text{C}}$  77.4) and between the proton H-2'' ( $\delta_{\text{H}}$  4.47) of the xylose and the carbon C-1''' ( $\delta_{\text{C}}$  102.3) of the rhamnose confirmed attachment of the rhamnosyl unit on the carbon C-2'' of the xylose, making it a disaccharyl moiety linked to luteolin skeleton. The  $^{13}\text{C}$  NMR data (Table 1) for all the 26 carbon atoms required for this luteolin glycoside could be fully obtained from HSQC and HMBC spectra. Thus, compound **1** was identified as luteolin 7-*O*- $\alpha$ -L-rhamnopynosyl-(1 $\rightarrow$ 2)- $\beta$ -D-xylopyranoside, a new flavone glycoside, and was named justicialoside A.

The molecular formula  $\text{C}_{27}\text{H}_{30}\text{O}_{14}$  of **2** was determined from its HR ESI-MS spectrum in +ve ion mode by the presence of the peak at  $m/z$  579.1713 calculated for  $[\text{C}_{27}\text{H}_{30}\text{O}_{14}+\text{H}]^+$ , 579.1708, consistent with thirteen DBE. Its 1D and 2D NMR spectra, similar to those of **1**, also suggested a luteolin glycoside skeleton. The only difference was the presence of an additional signal in the  $^1\text{H}$  NMR (Table 1) spectrum of **2** of a singlet attributable to a methoxyl group at  $\delta_{\text{H}}$  3.99 (3H). This was confirmed by the  $^1\text{H}$ - $^{13}\text{C}$  direct correlation observed in its HSQC spectrum between this signal and the carbon resonating at  $\delta_{\text{C}}$  55.4. In the HMBC spectrum, a correlation was observed between the signal of the methoxy proton

and a carbon resonating at  $\delta_C$  147.7 confirmed its position on the C-3' carbon of the luteolin skeleton (Figure 2). Thus, all the  $^1H$  and  $^{13}C$  NMR data (Table 1) of **2** led to its identification as chrysoeriol 7-*O*- $\alpha$ -L-rhamnopynosyl-(1 $\rightarrow$ 2)- $\beta$ -D-xylopyranoside, a new flavone glycoside, and was given the trivial name justicialoside B.

### 3. Materials and methods

#### 3.1. General experimental procedures

Analytical HPLC was performed on a Dionex UPLC 3000 (ThermoScientific, UK) coupled with a photo-diode-array (PDA) detector (ThermoScientific). Extracts and fractions were analyzed on a Phenomenex C<sub>18</sub> column (150 × 4.6 mm, 5  $\mu$ m, Phenomenex, USA). An Agilent 1200 Infinity series preparative HPLC system coupled with a PDA detector (Agilent, UK) was used to isolate compounds; a Hichrom ACE C<sub>18</sub> preparative column (150 × 21.2 mm, 5  $\mu$ m) was used. The column temperature was set at 25°C. The chromatogram was monitored at variable UV–vis wavelengths (205, 320 and 366 nm). NMR spectroscopic analyses were performed in CD<sub>3</sub>OD or Pyr-d<sub>5</sub> solution on a Bruker AMX600 NMR spectrometer (600 MHz for  $^1H$  and 150 MHz for  $^{13}C$ ). HR ESI-MS analyses were performed on a Xevo G2-S ASAP or LTQ Orbitrap XL1 spectrophotometer or on an on an Agilent 6200 Series Accurate-Mass Time-of-Flight (TOF) LC/MS.

#### 3.2. Plant material

The leaves of *Ruspolia hypocrateriformis* were collected in Melen, Yaoundé, Centre Region, Cameroon, in June 2015, and identified at the Cameroon National Herbarium, where the voucher specimen 37822/SFR was deposited.

#### 3.3. Extraction and isolation of compounds

The air-dried and powdered leaves (450.5 g) were successively extracted with *n*-hexane, DCM and MeOH to obtain 13.6, 10.2 and 41.8 g of dark green, brown green and dark brown extracts, respectively, using a Soxhlet extractor. A portion of the dried MeOH extract (2 g) was suspended in 10 mL of 10% MeOH-water and loaded on to a Strata C-18-E cartridge (Phenomenex, USA) (20 g), previously washed with MeOH (50 mL), followed by

equilibration with water (100 mL). The cartridge was eluted with MeOH-water mixture of decreasing polarity to obtain four fractions: 20, 50, 80 and 100% MeOH in water (200 mL each) (F1-F4, respectively). All fractions were concentrated to dryness using a combination of rotary evaporator and freeze-dryer and stored at 4°C until further use. Fraction F2 (428.6 mg) was purified by preparative HPLC using an ACE prep-column (150×21.2 mm, Hichrom Ltd, UK), flow rate 10 mL/min, mobile phase gradient of water (A) and methanol (B) both containing 0.1% TFA: 30-75% B, 0-20 min, monitored at wavelengths 320 and 366 nm to yield compounds **1** (3.3 mg), **3** (5.1 mg), **5** (0.9 mg), **6** (4.4 mg) and **7** (1.6 mg) having retention times 17.3, 15.5, 18.9, 10.2 and 17.8 min, respectively. F3 (361.8 mg) was subjected to preparative RP-HPLC as above using a different mobile phase gradient of water (A) and methanol (B) both containing 0.1% TFA: 45 - 50 % B, 0-10 min and 50 - 60 % B from 10 - 30 min monitored at wavelengths 205 nm to yield compounds **2** (2.6 mg), **4** (2.9 mg), and more of **3** (3.1 mg) and **5** (1.2 mg) having the retention 17.5, 19.2, 14.7 and 24.8 min, respectively.

### 3.3.1. *Justicialoside A (1)*

Yellow powder (3.3 mg);  $[\alpha]_D^{25} = -38.5$  (c 0.001, MeOH). HR ESI-MS  $m/z$  565.1559,  $[M+H]^+$  (calculated for  $C_{26}H_{28}O_{14}H^+$ , 565.1552). See Table 1 for  $^1H$  NMR (600 MHz, Pyr- $d_5$ ) and  $^{13}C$  NMR (150 MHz, Pyr- $d_5$ ) data.

### 3.3.2. *Justicialoside B (2)*

Yellow powder (2.6 mg);  $[\alpha]_D^{25} = -29.3$  (c 0.003, MeOH). HR ESI-MS  $m/z$  579.1713  $[M+H]^+$  (calculated for  $C_{27}H_{30}O_{14}H^+$ , 579.1708). See Table 1 for  $^1H$  NMR (600 MHz,  $CD_3OD$ ) and  $^{13}C$  NMR (150 MHz,  $CD_3OD$ ) data.

## 4. Conclusion

Two new luteolin glycosides (**1** and **2**), together with five known phytochemicals, were isolated from the leaves of *Ruspolia hypocrateriformis* (Vahl) Milne-Redh. (synonym *Justicia hypocrateriformis*).

## Acknowledgments

Part of mass spectrometry data were acquired at the EPSRC UK National Mass Spectrometry Service (NMSS) at Swansea University. We thank the Commonwealth Commission for the award of a PhD scholarship to STG.

## Appendix A. Supplementary data

NMR and MS spectra of compounds **1** and **2**.

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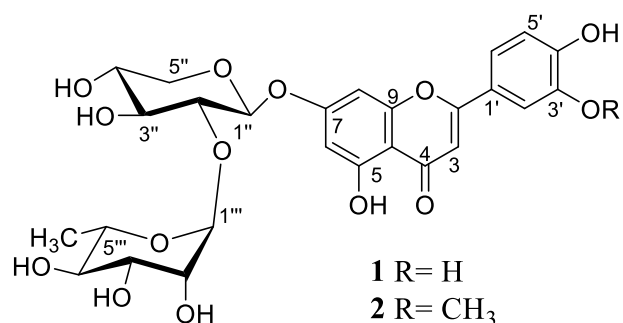
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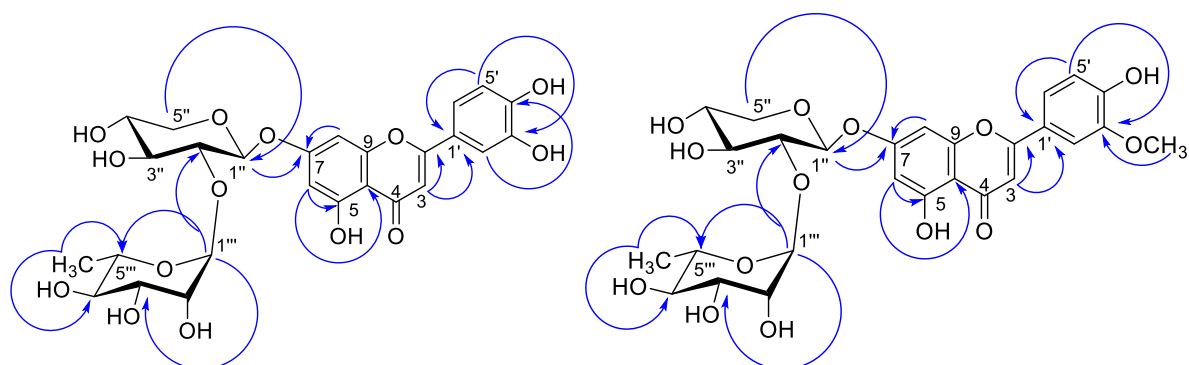
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**Figure 1.** Structures of justicialosides A (**1**) and B (**2**)



**Figure 2.** Key HMBC correlations compounds **1** and **2**



**Table 1.**  $^1\text{H}$  NMR (600 MHz) and  $^{13}\text{C}$  NMR (150 MHz) data of compounds **1** and **2**

Position	<b>1<sup>a</sup></b>		<b>2<sup>b</sup></b>	
	$\delta_{\text{H}}$ m ( <i>J</i> in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ m ( <i>J</i> in Hz)	$\delta_{\text{C}}$
2	-	165.1	-	165.0
3	6.91 s	104.0	6.73 s	103.2
4	-	182.5	-	182.1
5	-	162.4	-	160.9
6	6.94 d (2.1)	99.9	6.46 d (2.1)	99.5
7	-	163.2	-	162.5
8	6.96 d (2.1)	95.0	6.79 d (2.1)	94.5
9	-	157.4	-	156.8
10	-	106.5	-	105.1
1'	-	122.3	-	121.5
2'	7.88 d (2.2)	114.1	7.55 d (1.9)	109.5
3'	-	147.6	-	147.7
4'	-	151.0	-	150.2
5'	7.25 d (8.3)	116.5	6.97 d (8.4)	115.3
6'	7.50 dd (2.2, 8.3)	119.4	7.58 dd (1.9, 8.4)	120.5
1''	5.62 d (7.5)	100.2	5.21 d (7.3)	99.0
2''	4.47 dd (7.6, 9.0)	77.4	3.69 m	77.6
3''	4.28 m	78.6	3.61 m	70.7
4''	4.75 m	70.5	3.60 m	77.2
5''	3.80 m	67.0	3.49 m	65.6
	4.31 m		3.98 m	
1'''	6.38 d (1.6)	102.3	5.28 d (1.5)	101.2
2'''	4.80 m	72.1	3.96 m	70.7
3'''	4.54 dd (3.3, 9.3)	72.4	3.61 m	69.7
4'''	4.32 m	74.0	3.41 t (9.6)	72.6
5'''	4.75 m	69.9	3.89 m	68.6
6'''	1.78 d (6.1)	18.6	1.32 d (6.2)	16.9
OCH <sub>3</sub>	-	-	3.99 s	55.4

a- ran in Pyr-d<sub>5</sub>; b- ran in CD<sub>3</sub>OD