SUPPLEMENTARY MATERIAL

Probing for the presence of glucosinolates in three *Drypetes* spp. (*Drypetes euryodes* (Hiern) Hutch., *Drypetes gossweileri* S. Moore, *Drypetes laciniata* Hutch.) and two *Rinorea* spp. (*Rinorea subintegrifolia* O. Ktze and *Rinorea woermanniana* (Büttner) Engl.) from Gabon

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Abstract:

Drypetes euryodes (Hiern) Hutch., *Drypetes gossweileri* S. Moore, *Drypetes laciniata* Hutch. (Putranjivaceae), *Rinorea subintegrifolia* O. Ktze, and *Rinorea woermanniana* (Büttner) Engl. (Violaceae) from Gabon were probed for the presence of glucosinolates (GLs). When present, the GLs were identified and quantified by HPLC analysis. 2-Hydroxy-2-methyl GL (**1**) was the major GL in the cork of *D. euryodes*. Moreover, 4-hydroxybenzyl GL (**2**) was the major GL in the seed of *D. gossweileri* whereas the bark contained **2** as the minor GL and benzyl GL (**3**) was the major one. In addition, 4-methoxybenzyl GL (**4**), 3-methoxybenzyl GL (**5**), and **3** were found in the root of *R. subintegrifolia*. However, no GL was detected in *D. laciniata* (leaf and stem), *D. euryodes* (leaf and stem), and *R. woermanniana* (leaf and stem-branch). Our results support the hypothesis of the existence of GLs in plants of the Putranjivaceae and Violaceae families (order Malpighiales).

Keywords: *Drypetes* spp.; *Rinorea* spp.; glucosinolates; Malpighiales

Experimental

General Experimental Procedures

HPLC-grade acetonitrile was purchased from Sigma Aldrich Chemie GmbH, (Steinheim, D). Potassium phosphate and sodium acetate were purchased from Merck (Darmstadt, Germany). Ultrapure water (pH 5.0 ± 0.2) was obtained from a Milli-Q Gradient instrument (Millipore SAS, Molsheim, F) equipped with a Millipack filter 0.22 μm (Millipore, SAS, Molsheim, F).

Plant Material

The plants were collected in February 2010 in Gabon. The samples were harvested in a forest near Andem village (Kougouleu) on the national 1 at least 30 km north of Libreville for *D. laciniata*, in a forest near Libreville (Estuaire) for *D. gossweileri*, *D. euryodes*, *R. woermanniana* and for *R. subintegrifolia* on the national 1 near Ngounié. They were identified by Mr. Raoul Niangadouma from the National Herbarium of Gabon by comparison with authenticated plants in the herbarium. The information regarding the voucher numbers are reported in Table S1.

Table S1. Studied plants from Gabon

Plant	Voucher numbers in Gabon
Drypetes euryodes	JJFE de Wilde 233
Drypetes gossweileri	AM Louis 3407
Drypetes laciniata	AM Louis 1849
Rinorea subintegrifolia	Breteler 14738
Rinorea woermanniana	Wieringa 4352

HPLC Analysis and Quantification of Desulfo-glucosinolates

Glucosinolates (GLs) were extracted as previously reported with some modifications (Barillari et al. 2005). Plant samples were reduced to a fine powder. Samples of about 500 mg were extracted for 5 min at 80°C in 2 × 5 mL EtOH-H_2O (70:30 v/v), using a U-Turrax (IKA T25) homogenizer and then centrifuged. Supernatants were combined

and each extract (1 mL) was loaded onto a mini-column filled with 0.6 mL of DEAE-Sephadex A-25 anion-exchange resin (Amersham Biosciences) conditioned with 25 mM acetate buffer (pH 5.6). After washing with 3 mL of buffer, 200 μL (0.35 U/mL) of purified sulfatase (Leoni et al. 1998) was loaded onto the mini-column which was left overnight at 30°C. The desulfo-glucosinolates (DS-GLs) were then eluted with 3 mL of ultrapure H_2O and finally injected into an HPLC. The DS-GLs were analyzed on an Agilent 1100 HPLC system equipped with an Inertsil ODS-3 column (250 × 3.0 mm, 5 μm particle size), thermostated at 30°C, and using a diode array detector. The chromatography was performed at a flow rate of 1 mL min^{-1} eluting with a gradient of H_2O (A) and acetonitrile (B) following the program: 1 min 1% B; 22 min linear gradient up to 22% B; 3 min linear gradient down to 1% B. DS-GLs were detected monitoring the absorbance at 229 nm. The amount of GL was quantified by using a calibration curve of pure DS-sinigrin solution (range from 0.14 to 1.4 mM) and the RPFs of each individual DS-GLs (Clarke 2010; De Nicola et al. 2012). Identification of the peaks was performed on the basis of retention time and UV spectra of spiked DS-GL pure standards available in our laboratory (Leoni et al. 1998).

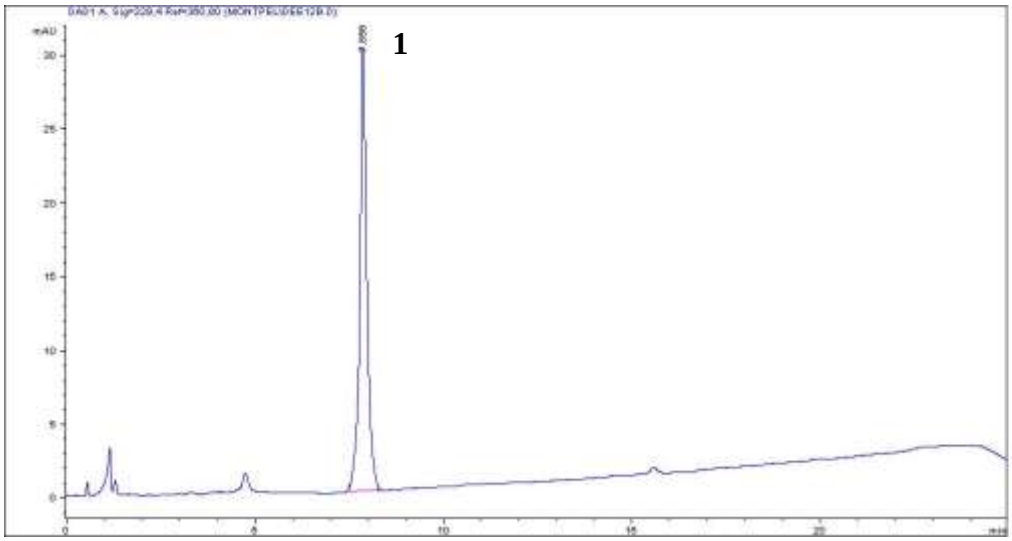

Figure S1. HPLC chromatogram of DS-GLs in *Drypetes euryodes* cork ethanolic extract. **1:** desulfo 2-hydroxy-2-methylbutyl GL (t_R 7.9 min).

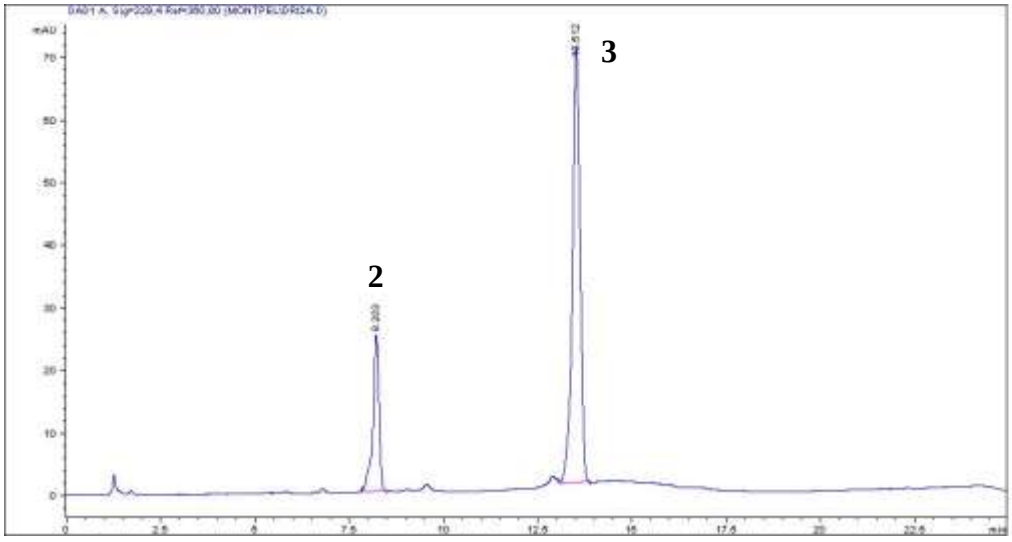

Figure S2. HPLC chromatogram of DS-GLs in *Drypetes gossweileri* bark ethanolic extract. **2:** desulfo 4-hydroxybenzyl GL (t_R 8.2 min), **3:** desulfo benzyl GL (t_R 13.5 min).

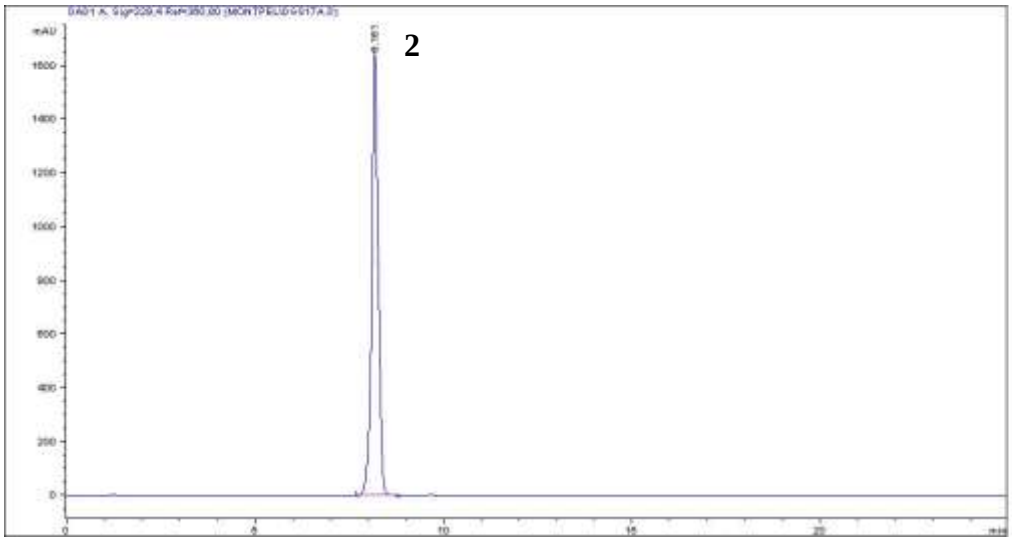

Figure S3. HPLC chromatogram of DS-GLs in *Drypetes gossweileri* seed ethanolic extract. **2:** desulfo 4-hydroxybenzyl GL (t_R 8.2 min).

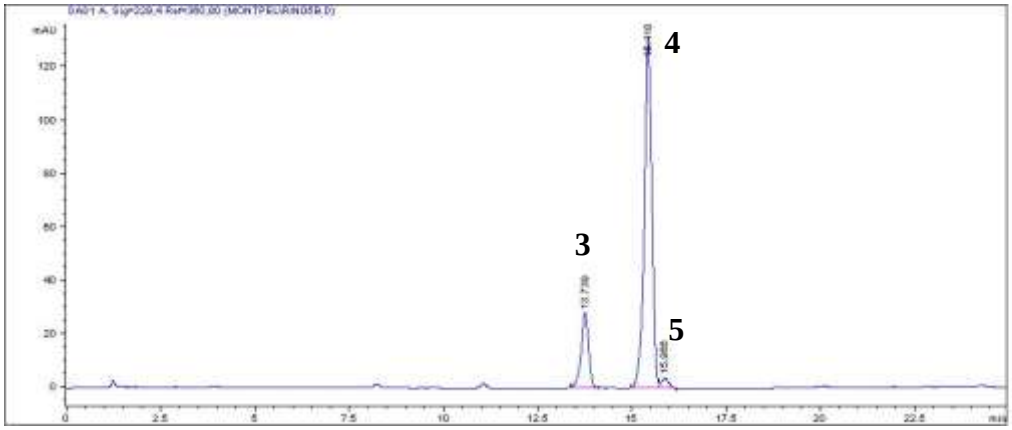

Figure S4. HPLC chromatogram of DS-GLs in *Rinorea subintegrifolia* root ethanolic extract. **3:** desulfo benzyl GL (t_R 13.7 min), **4:** desulfo 4-methoxybenzyl GL (t_R 15.4 min), **5:** desulfo 3-methoxybenzyl GL (t_R 15.9 min).

Table S2. Distribution of glucosinolates in *Drypetes euryodes*, *Drypetes gossweileri*, *Dryptetes laciniata*, *Rinorea subintegrifolia*, and *Rinorea woermanniana*.

	Glucosinolates[a] (μmol/g dry weight)					
Plant	**1**	**2**	**3**	**4**	**5**	**Total**
D. euryodes						
Leaf	ND [b]	ND	ND	ND	ND	ND
Stem	ND	ND	ND	ND	ND	ND
Cork	3.19 ± 0.49[c]	ND	ND	ND	ND	3.19 ± 0.49
D. gossweileri						
Bark	ND	2.30 ± 0.12	16.11 ± 0.56	ND	ND	18.41 ± 0.68
Seed	ND	171.41 ± 8.93	ND	ND	ND	171.41 ± 8.93
D. laciniata						
Leaf	ND	ND	ND	ND	ND	ND
Stem	ND	ND	ND	ND	ND	ND
R. subintegrifolia						
Root	ND	ND	2.56 ± 0.52	5.99 ± 1.06	0.20 ± 0.03	8.75 ± 1.61
R. woermanniana						
Leaf	ND	ND	ND	ND	ND	ND
Stem and branch	ND	ND	ND	ND	ND	ND

[a] Glucosinolates: **1:** 2-hydroxy-2-methylbutyl GL, **2:** 4-hydroxybenzyl GL, **3:** benzyl GL, **4:** 4-methoxybenzyl GL, **5:** 3-methoxybenzyl GL.
[b] "ND" = not detected. [c] Values represent mean ± standard error, n = 3.

References

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