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**

*D. euryodes* 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).

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1. Introduction

*Drynepet gossweileri* S. Moore (Putranjivaceae) is a tree used in Africa for diverse therapeutic applications such as the treatment of headache, intercostal pain, kidney pain, and bronchopneumonia. It has also been used as vermifuge, aphrodisiac, and against gonorrhoea (Bouquet 1969). The stem bark extract was found to have antifungal properties (Ngouana et al. 2011) and to display some toxicity towards mice (Tessier & Paris 1978). However, no toxic effect was noticed in albinos Wistar rats (Ngouana et al. 2011). Furthermore, a methanolic extract of *D. gossweileri* stem bark was found to have cytotoxic and DNA-damaging activities (Ngouela et al. 2003). The ethanolic and crude aqueous extracts of *D. gossweileri* bark were also active against bacteria responsible for urinary tract infections (Ijah & Oyebanji 2003). Dichloromethane and ethyl acetate extracts of *D. gossweileri* have shown effective insecticide activities against *Sitophilus zeamais* (Motsch.) and *Rhyzopertha dominica* (F.) (Aba Toumnonou et al. 2013). The crude stem bark extract of *D. gossweileri* has shown anti-microbial and phytotoxic properties against *Lemna minor* L. (Schmelzer & Gurib-Fakim 2008). Phytochemical screenings of stem bark extract and fractions indicated the presence of alkaoids, phenols, flavonoids, saponins, anthocyanins, anthraquinones, sterols, cardiac glycosides, tannins, phlobatannins, and essential oils (Ijah & Oyebanji 2003; Ngouana et al. 2011; Aba Toumnonou et al. 2013). The essential oil of *D. gossweleiri* bark (origin Gabon) did not show good antioxidant or antiradical activities but has shown bacteriostatic and bactericidal activities (Agnaniet et al. 2003; Voundi et al. 2015). This essential oil contained benzyl isothiocyanate (ITC) (56.5, 93.9 and 86.7% in plants from Gabon, Central African Republic and Cameroon, respectively) and benzyl cyanide (42.3, 5.7 and 12.6%, respectively) (Eyele Mvé-Mba et al. 1997; Voundi et al. 2015). Those constituents are indicative of the existence of benzyl glucosinolate (GL) (3) in the plant (Figure 1). Another study confirmed the presence of the above major constituents in *D. gossweileri* bark essential oil, together with benzyl alcohol, several benzyl esters, and terpenes (Agnaniet et al. 2003). In addition, non-volatiles have been isolated from the stem bark of *D. gossweleiri* (Dupont et al. 1997). In other respects, several other secondary metabolites were isolated from the stem bark of *D. gossweleiri* (Ngouela et al. 2003; Ata et al. 2011).

The whole stems of *Drypetes laciniata* Hutch. (Putranjivaceae) were shown to contain several friedelane-type ketones, olean-12-en-28-oic acid derivatives, a mixture of sterols, and chikusetsusaponin IVa methyl ester (Fannang et al. 2011).

In African traditional medicine, *Rinorea subintegrifolia* O. Ktze (Violaceae) is used as a fragrant agent during ancestral cults, as expectorant, against eye diseases and to treat heart disease, fever, headache, rheumatism, stomach ache, constipation, œdema, and malaria (Agnaniet et al. 2003; Tokuoka 2008; Lekana-Douki et al. 2011). However, the methanolic extract of *R. subintegrifolia* was not active *in vitro* against *Plasmodium falciparum* Welch.
(Lekana-Douki et al. 2011). The essential oil obtained from roots of Gabonese *R. subintegri folia* contained benzyl- and *p*-methoxybenzyl cyanides, benzyl- and *p*-methoxybenzyl ITCs, benzyl- and *p*-methoxybenzyl alcohols (Agnaniet et al. 2003). This investigation led to think that the plant would contain *3* and *p*-methoxybenzyl GL (4) (Figure 1). Furthermore, the essential oil of *R. subintegri folia* did not have good antioxidant or antiradical activities (Agnaniet et al. 2003).

No previous phytochemical study was reported in the literature for *Drypetes euryodes* (Hiern) Hutch. (Putranjivaceae) or *Rinorea woermanniana* (Büttner) Engl. (Violaceae).

GLs are sulphur-containing secondary metabolites present in all species of the order Brassicales (Montaut et al. 2016) and in some families of the order Malpighiales (Eyele Mvé-Mba et al. 1997; Agnaniet et al. 2003; Voundi et al. 2015). GL degradation products – mainly ITCs, nitriles, thiocyanates and oxazolidinethiones – are known to be responsible for various biological activities (Mithen et al. 2000; Brader et al. 2006). The aim of this work was to probe for the presence of GLs in *D. euryodes*, *D. gossweileri*, *D. laciniata*, *R. subintegri folia* and *R. woermanniana* growing wild in Gabon. GLs were extracted, analysed as desulfo-glucosinolates (DS-GLs) and quantified by HPLC.

### 2. Results and discussion

The extraction of various plant parts of *D. euryodes*, *D. gossweileri*, *D. laciniata*, *R. subintegri folia* and *R. woermanniana*, the HPLC analysis and quantification of DS-GLs (Barillari et al. 2005; Clarke 2010; De Nicola et al. 2012) were performed as described in the supplementary material section (Figures S1–S4 and Tables S1–S2). Identification of the peaks was performed on the basis of retention times and UV spectra of spiked DS-GLs pure standards available in our laboratory (Leoni et al. 1998).

No GL was detected in the leaf and stem of *D. laciniata* or in the leaf and stem-branch of *R. woermanniana*. This could be paralleled to the fact that these species are odourless (Raponda-Walker & Sillans, 1995). Additionally, no GL was detected in the leaf and stem of *D. euryodes* whereas 2-hydroxy-2-methylbutyl GL (also called glucocleomin, 1) was identified for the first time as the only GL in the cork of this species (Figure 1).

#### 2.1. GLs in *D. gossweileri*

The results of our investigations showed that 4-hydroxybenzyl- (also called glucosinalbin, 12.5%, 2) and benzyl GL (also called glucotropaeolin, 87.5%, 3) are present in the bark of *D. gossweileri*. Furthermore, 2 was the only GL found in the seeds of *D. gossweileri*. The unusual high quantity of 2 (171.14 μmol/g dry weight) (Table S2) in this plant is indicative that *D. gossweileri* represents a sound novel source of this GL. Our investigations confirmed the presence of 3 in the cork of *D. gossweileri*, as surmised by the detection of benzyl ITC in the essential oil obtained from the plant bark (Eyele Mvé-Mba et al. 1997; Voundi et al. 2015). However, we were able to identify 2 as a minor GL in the bark, whereas no previous study mentioned the presence of 4-hydroxybenzyl ITC in *D. gossweileri* essential oil. This can be explained by the fact that this ITC, resulting from the degradation of 2, is unstable in aqueous media, producing 4-hydroxybenzyl alcohol under release of a thiocyanate ion (Borek & Morra 2005). Interestingly, the smell of the bark of *D. gossweileri* was reported to be very similar to the smell of *Pentadiplandra brazzeana* Baill. root (family Pentadiplandraceae, order Brassicales).
and described as a particular mixture of horseradish and methyl salicylate (Bouquet 1969). This similarity could be attributed partly to the presence of 3, which is prone to undergo degradation into benzyl ITC in both plants.

2.2. GLs in R. subintegrifolia

The HPLC profile of R. subintegrifolia root revealed the presence of three GLs, the major being 4-methoxybenzyl GL (also called glucoaubrietin, 68.5%, 4) followed by 3 (29.2%), and 3-methoxybenzyl GL (also called glucolimnanthin, 2.3%, 5). Our investigations confirmed the presence of 3 and 4 in the root of R. subintegrifolia hypothesized from the detection of benzyl- and 4-methoxybenzyl ITCs in the essential oil of the root by one of us (Agnaniet et al. 2003). We also have been able to identify 5 as a minor GL in the root whereas no previous study ever mentioned the presence of 3-methoxybenzyl ITC in R. subintegrifolia root essential oil. Our GL profile based on DS-GL analysis does not fit previous results regarding the composition of the essential oil from the root. In fact, the essential oil was constituted of benzyl ITC (1.4–29%), benzyl cyanide (64–87.7%), 4-methoxybenzyl ITC (0.6–0.8%), and 4-methoxybenzyl alcohol (0.4–0.5%) (Agnaniet et al. 2003). This apparent discrepancy can be explained by the high instability of arylaliphatic ITCs under hydrodistillation conditions (De Nicola et al. 2013). Genetic and environmental factors may also account for the observed differences. Interestingly, a similar GL profile was observed for the root of R. subintegrifolia and for the root of P. brazzeana (De Nicola et al. 2012).

Arylaliphatic GLs, biosynthesized from Tyr and Phe, were found in D. gossweileri and R. subintegrifolia whereas the aliphatic GL 1 was identified in D. euryodes. The close GL profiles of D. gossweileri and R. integrifolia would indicate a close relationship between these two genera. This is supported by a phylogenetic analysis of the order Malpighiales which showed that Putranjivaceae and Violaceae are grouped in the same clade (Tokuoka & Tobe 2006).

3. Conclusions

The probing of the presence of GLs in plants of the order Malpighiales (D. euryodes, D. gossweileri, D. laciniata, R. subintegrifolia and R. woermanniana) growing wild in Gabon enabled the identification and quantification of 5 known GLs. More species in the Violaceae and Putranjivaceae families and other families of the order Malpighiales should be screened in the future for the presence of GLs, to delineate a better overview of the distribution and diversity of GLs in these plant families.

Supplementary material

Experimental details relating to this paper are available online, alongside Tables S1–S2 and Figures S1–S4.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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