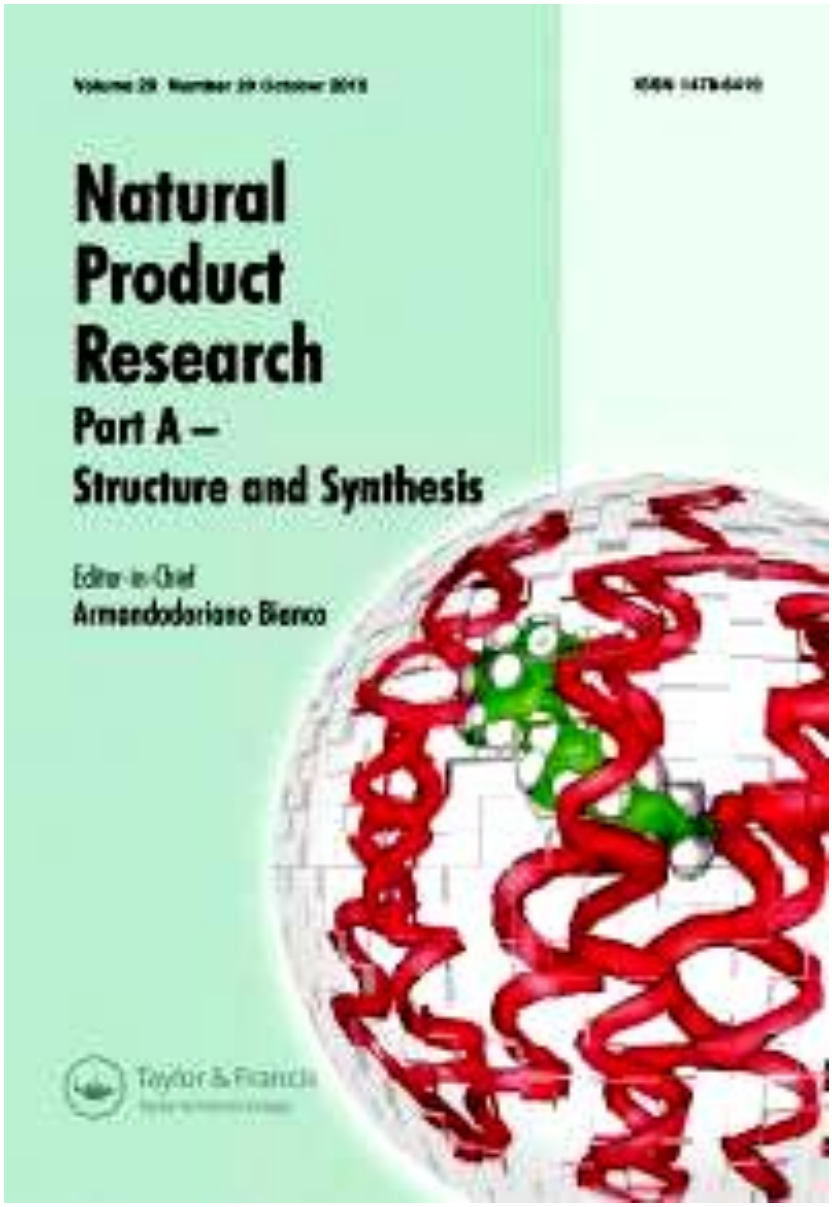

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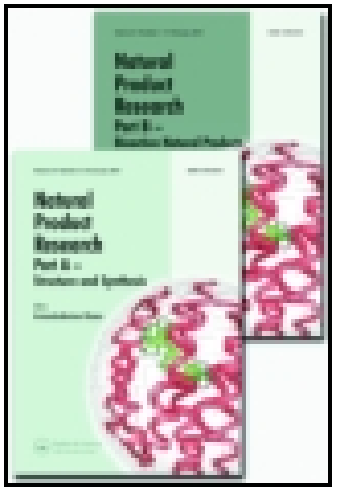

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Bioactive glycosides from the African medicinal plant Boerhavia erecta L.

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SHORT COMMUNICATION

Bioactive glycosides from the African medicinal plant *Boerhavia erecta* L.

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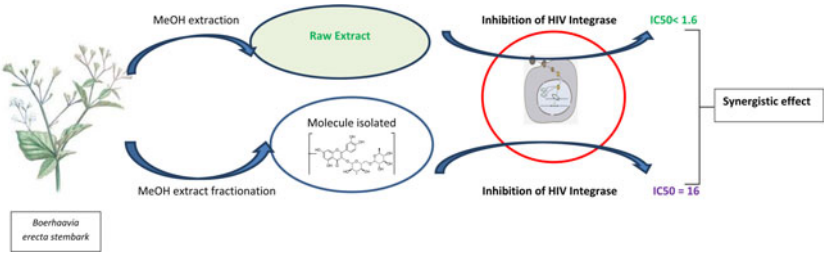

Phytochemical studies of the previously unexplored stem of *Boerhavia erecta* from Burkina Faso, resulted in the isolation of an unreported glycoside **4**, 2,3-dihydroxypropylbenzoate-3-*O*-β-[4″-methoxy] glucuronide as well as seven known glycosides (**1**–**3**, **5**–**8**). The major isolate **5** and **8** indicated a significant inhibition against HIV integrase (IC_{50} 10 and 22 μg/mL, respectively). The extracts and isolates were also tested for anti-malarial activity, but insignificant activity was observed.

Keywords: African medicinal plant; *Boerhavia erecta*; glycosides; anti-HIV integrase activity; anti-malarial activity

1. Introduction

Burkina Faso is a country rich with medicinal plants including prospective medical sources of the *Boerhavia erecta* Linnaeus species; the aerial component of this plant is used in traditional medicine for the treatment of nervous malaria in infants, seizures in children, generalised oedema, dyspnoea, difficult delivery, haematuria, as an anti-convulsant, diuretic or spasmolytic (Hilou et al. 2004). Previous studies on the extract of the leaves of *B. erecta* revealed some activities, including anti-microbial (Perumal Samy et al. 1999) and anti-malarial effects (Hilou et al. 2004, 2006; Stintzing et al. 2004). This led to the isolation of sterols and betanin (Miralles et al., 1988; Stintzing et al. 2004). The previous investigation was driven mainly by the isolation of phenolic compounds from the leaves of the plant for anti-oxidant studies (Petrus et al. 2012). Here, we report for the first time the results of the stem bark of this plant and the bioactive constituents of polar fraction of this plant.

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2. Result and discussion

The non-polar alkanes and long chain fatty acids of the crude extract were removed by applying solid liquid (hexane, dichloromethane) back extraction. From the polar fraction, short normal phase column chromatography produced a major fraction, which was subjected to preparative HPLC >, isolating the following eight compounds (Figure 1): 3-methoxybenzoic acid 4-*O*-β-glucoside **1** (Liu et al. 2006), 3-methoxyacetophenone 4-*O*-β-glucopyranoside **2** (De Rosa et al. 1996), isorhamnetin-3-*O*-rutinoside-7-*O*-β-glucopyranoside **3** (Aquino et al. 1987), quercetin-3-*O*-rutinoside **5**, quercetin-3-*O*-β-glucopyranoside **6**, kaemferol-3-*O*-rutinoside **7** (Hamzah & Lajis 1998), and isorhamnetin-3-*O*-rutinoside **8**. Compounds **5**, **6** and **8** were previously reported from the leaves of the plant (Petrus et al. 2012), and are spectroscopically identical to that reported. Importantly, compounds **1–3** and **7** were isolated from this plant for the first time in this study.

In addition, for the previously unreported compound, we propose the structure **4** (Figure 2). The HR-ESI-MS analysis of **4** indicated a peak at *m/z* 409.1107 ($[M + Na]^+$), assigned to the molecular formula of $C_{17}H_{22}O_{10}Na$. The NMR spectral analysis suggested a molecular structure that was similar to a reported glycerol α-D-glucuronide carboxylic acid (Cai et al. 2011) and its ester **9** (Maggi et al. 2009). The ^1H and ^{13}C NMR spectral analysis indicated a singlet peak at δ 3.46 and 60.8 ppm, respectively, assigned to a methoxy group; this was in contrast to the corresponding ester OMe in **9** with values assigned in the ^1H and ^{13}C NMR spectra of δ 3.66 and 52.8 ppm, respectively (Maggi et al. 2009). This is particularly telling with the 8.0 ppm difference in the OMe in the ^{13}C NMR spectrum, with the methoxy ester more upfield compared with the methoxy substituent, as expected. gHMBC spectral analysis of **4** indicated a proton-carbon correlation between the methoxy and carbon peak at δ 83.0 (assigned to C4″ of the sugar moiety). In addition, a 3-bond correlation between H4″ and the carbonyl was evident. Thus, the molecular structure for **4** is proposed as 2,3-dihydroxypropyl-benzoate 3-*O*-β-[4″-methoxy] glucuronide. The position of the benzoic ester carbonyl peak in the ^{13}C NMR spectrum was consistent across all three derivatives (~168 ppm).

Preliminary studies on the crude extract revealed significant inhibition activity against HIV integrase in which 'fraction B' (the extract residue) showed a higher activity than the supernatant (fraction A). Further assays on the major constituents, compounds **5** and **8**, indicated moderate and less activity compared with the original extract (Table 1). The isolates were also tested against *P. falciparum* K1, which showed no significant activity (Table 1). The isolated compounds showed less inhibition relative to the fractions with the HIV integrase activity testing. This suggests a synergic mechanism playing an important role in the bioactivity. Fractions, sub-fractions and compounds **1–8** were inactive against *P. falciparum* K1 (Table 1).

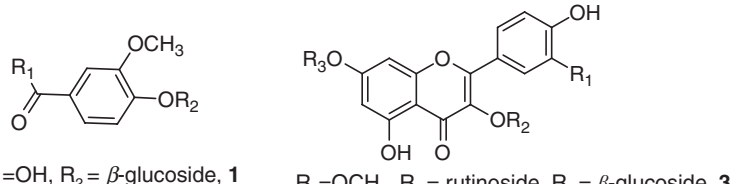

Figure 1. Molecular structure of glycosides isolated from stem of *Boerhavia erecta*. Compound **9** was isolated from *V. hookeriana* (Maggi et al. 2009).

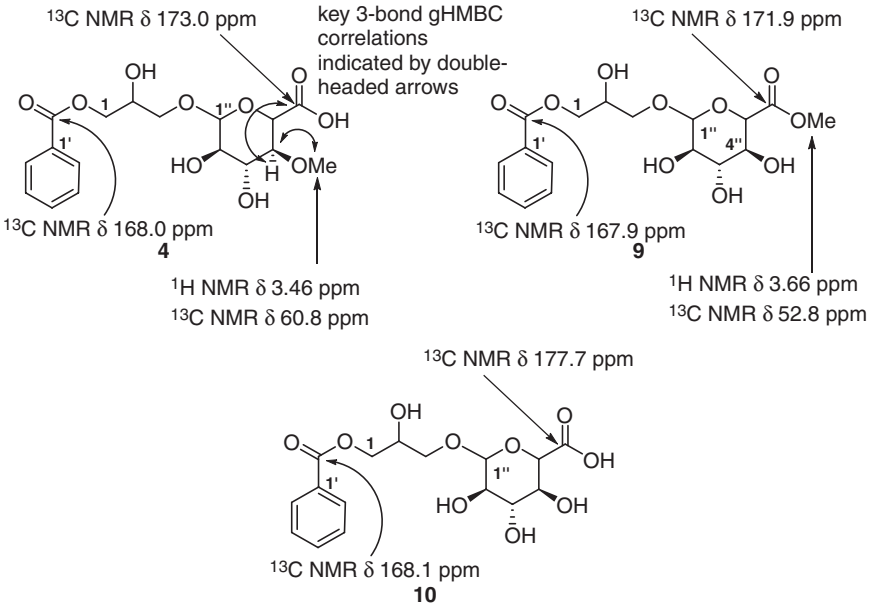

Figure 2. Proposed structure of **4** and the known α-D-glucuronide carboxylates **9** and **10**. Key NMR assignments are indicated (single-headed arrows) while key HMBC correlations (double-headed arrows) are also shown. For a larger set of correlations for **4**, see Supporting Information.

Quercetin has been reported to inhibit HIV-infected cells (Tang et al. 1994), which were later found to have diverse modes of action including anti-protease (PR), anti-integrase (IN) and anti-transcriptase (RT) activities. Quercetin and rutin revealed inhibition against HIV-1 protease with IC_{50} values of 34 and 28 μg/mL, respectively, although methoxylation or glucosylation at 3,7,4′ reduced its activity (Xu et al. 2000). Quercetin was a weak HIV-1 RT inhibitor (IC_{50} 150–200 μg/mL) possibly due to structural planarity, which weakens its intercalating properties (Tan et al. 1991); however, glucosylation at 3′ increased the activity (IC_{50} 15 μg/mL). Quercetin possessed anti-integrase activity with IC_{50} of 4 μg/mL; Raghavan et al. 1995).

Table 1. Anti-HIV integrase and anti-plasmodial activities (IC_{50} in μg/mL) of the extracts and pure compounds isolated from *B. erecta*.

Entry	Anti-HIV integrase activity IC_{50} (μg/mL)	Anti-malarial activity	
		Activity	IC_{50} (μg/mL)
Crude extract	<1.6	na	na
Fraction A	16	inactive	–
Fraction B	<0.4	inactive	–
Fraction B1	<10	na	na
Fraction B2	<46	na	na
Fraction B4	>50	na	na
5	<10	inactive	–
8	<22	inactive	–

Notes: na, data not available. No activity. Compounds **1**–**4**, **6** and **7** were inactive against *P. falciparum* K1 and were not tested against HIV integrase. Anti-malarial testing, final conc. of samples: 10 μg/mL, negative control: 0.1% DMSO. IC_{50} of positive control: Dihydroartemisinine = 2.20 nM, Mefloquine = 0.0310 μM.

3. Conclusions

A previously unreported derivative of the glycerol α-D-glucuronide class of compounds (**4**) was isolated from the stem of *B. erecta*, which was proposed as 2,3-dihydroxypropyl-benzoate 3-*O*-β-[4″-methoxy] glucuronide. Moderate anti-HIV integrase activity was shown by the major constituents (**5** and **8**). The anti-malarial testing against *P. falciparum* K1 indicated none of the purified compounds possessed anti-plasmodial activity. However, the major compounds **5** and **8** were previously reported to have anti-malarial activity against *P. falciparum* (FCR3, cycloguanil-resistant from Gambia) (Murakami et al. 2001). Therefore, these compounds show significant differences in their *P. falciparum* inhibitory activity between different strains.

Supplementary material

Experimental details relating to this article are available online; ^{1}H, ^{13}C, COSY, gTOCSY, HSQC, HMBC, HR-ESI-MS and MS/MS spectra of compounds **4**.

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