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

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Abstract

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 (IC₅₀ 10 and 22 μ g/mL, respectively). The extracts and isolates were also tested for anti-malarial activity, but insignificant activity was observed.

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

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Abstract

Phytochemical studies of the previously unexplored stem of *Boerhavia erecta* from Burkina Faso-Africa, 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 (IC₅₀ 10 and 22 μ g/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 which the aerial component of the plant is used in traditional medicine for the treatment of nervous malaria in infants, seizures in children, generalized edema, dyspnea, difficult delivery, hematuria, 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; Hilou et al., 2006; Stinzing et al., 2004). This led to the isolation of sterols and betanin (Stinzing et al., 2004; Miralles et al., 1988). 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.

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 eight compounds (Figure 1): 3-methoxybenzoic acid 4-*O*- β -glucoside **1** (Liu et al., 2006), 3-methoxyacetophenone 4-*O*- β -glucopyranoside **2** (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 et al., 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.

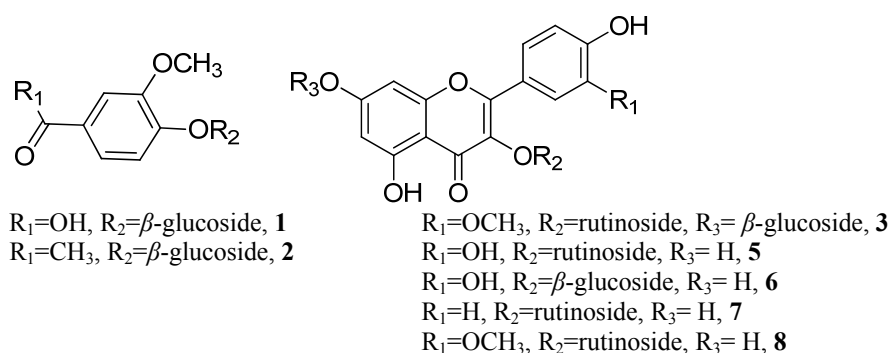


Figure 1. Molecular structure of glycosides isolated from stem of *Boerhavia erecta*. Compound **9** was isolated from *V. hookeriana* (Maggi et. al, 2009).

Additionally, for the previously unreported compound, we propose the structure **4** (Figure 2). The HRESI 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 which 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 δ 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 δ 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 to 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). Additionally, 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).

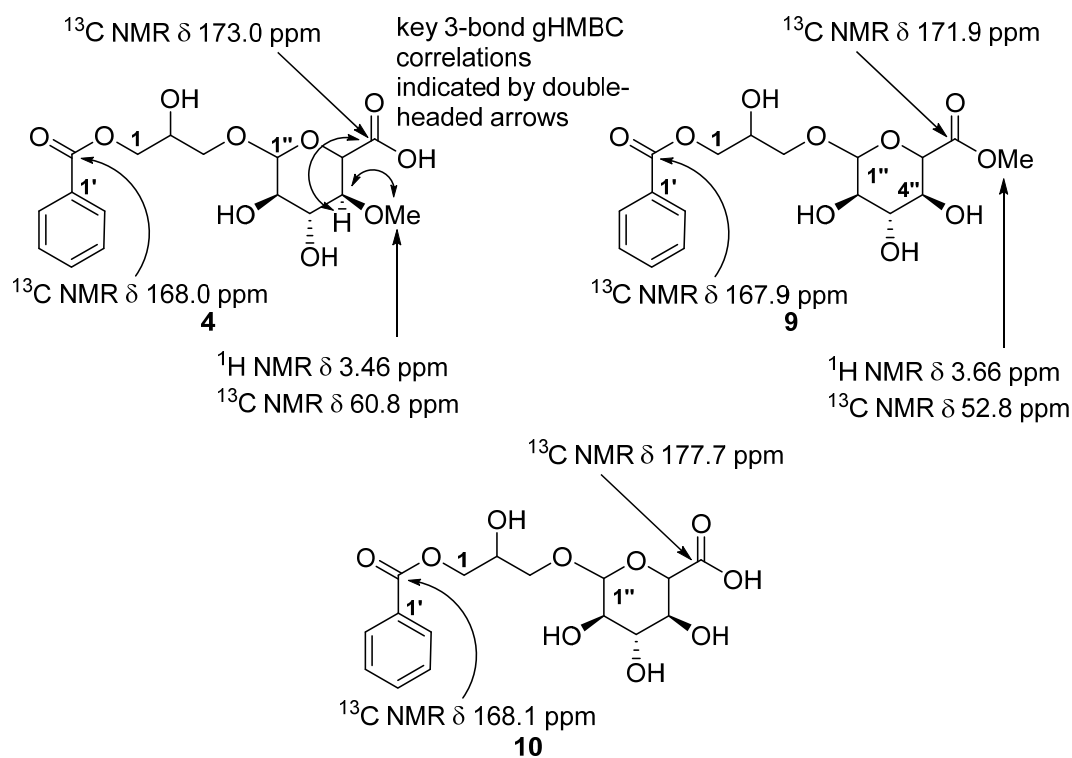


Figure 2: Proposed structure of **4** and the known α -D-glucuronide carboxylates **9** and **10**. Key NMR assignments are indicated (single-headed arrows) while key HMBC correlations (double-headed arrows) are also shown. For a larger set of correlations for **4**, see SI.

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 to 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).

Table 1. Anti-HIV integrase and anti-plasmodial activities (IC₅₀ in µg/mL) of the extracts and pure compounds isolated from *B. erecta*

Entry	Anti-HIV	integrase	Anti-malarial activity	
	activity		Activity	IC ₅₀ (µg/mL)
	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	-

Note: 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. IC₅₀ of positive control: Dihydroartemisinin = 2.20 nM, Mefloquine = 0.0310 µM.

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 IC₅₀ 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 (IC₅₀ 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 (IC₅₀ 15 µg/mL). Quercetin possessed anti-integrase activity with IC₅₀ of 4 µg/mL (Raghavan et al., 1995).

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

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