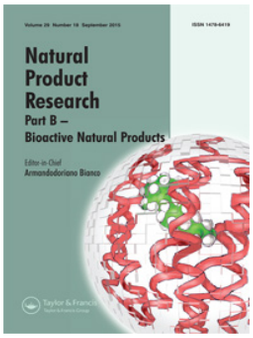

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A new antimicrobial and radical-scavenging glycoside from *Paullinia pinnata* var. *cameroonensis*

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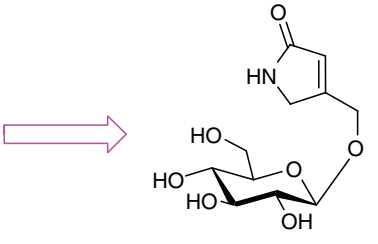

A new glycoside, pinnatoside A (**1**), together with two known compounds (**2** and **3**), were isolated from the stems of *Paullinia pinnata.* Their structures were elucidated on the basis of extensive spectroscopic analysis and chemical methods. Compound **1** showed significant antibacterial activity with a minimum inhibitory concentration (MIC) value of 1.56 μg/mL against *Escherichia coli*, and **2** displayed significant antibacterial activity with a MIC value of 1.56 μg/mL against *Enterobacter aerogenes* and *E. coli*. Equally, compound **1** exhibited the best radical-scavenging activity ($RSa_{50} = 25.07 \pm 0.49$ μg/mL).

Keywords: *Paullinia pinnata*; pinnatoside A; antimicrobial activity; radical-scavenging activity

1. Introduction

Paullinia pinnata L. (Sapindaceae), an African woody vine, has been widely used in traditional medicine in the treatment of human malaria (Chabra et al. 1991) and erectile dysfunction (Zamble et al. 2006). In the West Region of Cameroon, its leaf decoction has been used in the treatment of bacterial infections such as typhoid, syphilis, gonorrhoea, diarrhoea, and symptoms such as stomach-ache and waist pain. In East Africa, the leaves were reported to be used in the treatment of gonorrhoea, wounds and microbial infections (Annan et al. 2009). Previous phytochemical investigations showed the presence of triterpene saponins and cardiotonic

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catechol tannins (Bowden 1962; Kerharo & Adam 1974), flavone glycosides (Ehab et al. 1999), steroids and steroidal glycosides (Dongo et al. 2009), a cerebroside and a ceramide (Miemanang et al. 2006), as well as fatty acids (Chabra et al. 1991) in *P. pinnata* collected from different parts of West Africa.

However, to the best of our knowledge, there is a very limited biological investigation of the chemical constituents from the title species *P. pinnata.* As a result of our interest in this plant, we demonstrated the antityphoid and the radical-scavenging activities of the crude extracts, and obtained steroidal saponins and triterpenoid saponins (Lunga et al. 2014). In a continuation of our interest in the phytochemical and pharmacological investigation of this plant, the stems of *P. pinnata* were further investigated. As a result, a new glycoside, pinnatoside A (**1**), together with two known compounds (**2** and **3**), were isolated from the aqueous fraction of its stem methanol extract. All the compounds were evaluated for their antimicrobial activities against five bacteria, five yeasts and five dermatophytes. In this article, we report the isolation, structural elucidation, antimicrobial and radical-scavenging activities of these compounds.

2. Results and discussion

Compound **1** was isolated as a white amorphous powder. Its molecular formula was assigned as $C_{11}H_{17}NO_7$ on the basis of HR-ESI-MS peak at *m/z* 298.0899 $[M + Na]^+$ (calcd for $C_{11}H_{17}NO_7Na$, 298.0903). Its IR spectrum revealed the presence of hydroxyl (3417 cm^{-1}) and double bond (1641 cm^{-1}) functionalities. The ^1H NMR spectrum showed signals for one olefinic proton at δ_H 5.72 (1H, s, H-3), two oxygen-linked methylene protons at δ_H 4.50 (1H, d, $J = 13.2$ Hz, H-6a) and 4.37 (1H, d, $J = 13.2$ Hz, H-6b), two nitrogen-linked methylene protons at δ_H 4.22 (2H, br s, H-5) and one anomeric proton at 4.14 (1H, d, $J = 7.8$ Hz, H-1′). The ^{13}C NMR and DEPT spectra showed 11 carbon signals, 5 of which were assigned to the aglycone moiety including those ascribable to one carbonyl carbon at δ_C 165.2 (C-2) and two double bond carbons at δ_C 116.8 (C-4) and 94.2 (C-3), while the remaining were due to the sugar moiety. The correlations of H-3 with C-2, C-4 and C-5 (δ_C 61.2), of H-5 with C-2, and of H-6 with C-3, C-4 and C-5 which were observed in the HMBC spectra, led to the determination of the structure of the aglycone portion as shown (Figure 1). Acid hydrolysis liberated the D-glucose, and the β-configuration of the D-glucose was determined from the large coupling constant ($J_{1,2} > 7.0$ Hz) of the anomeric proton. The connectivity of the sugar was unambiguously established on the basis of its HMBC correlation: H-1′ with C-6 (δ_C 66.9). Based on the previous information, the structure of **1** was elucidated as shown, and named as Pinnatoside A.

By comparing physical and spectroscopic data with literature values, two known compounds were identified as 3-*O*-β-D-glucopyranosyloxy-4-methyl-2(5H)-furanone (**2**) (Braekman et al.

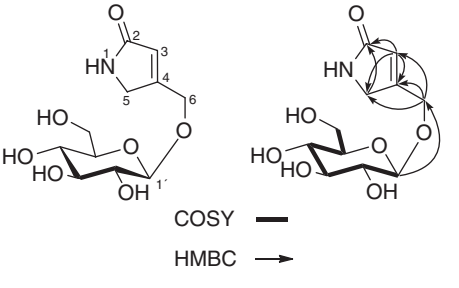
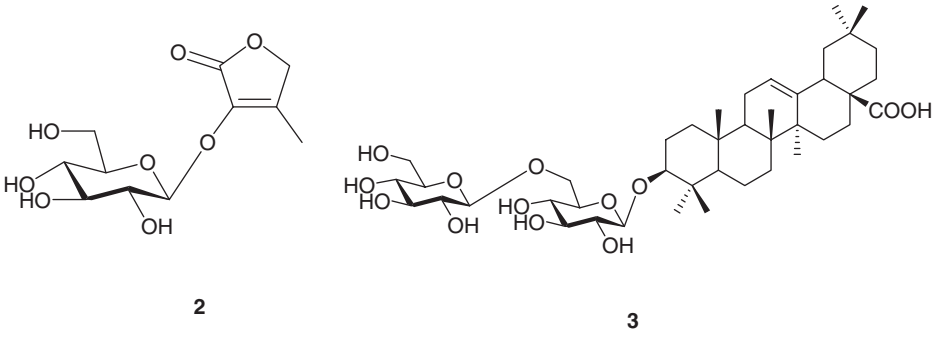


Figure 1. Structure and significant 2D correlations of compound **1**.



2 **3**

Figure 2. Structures of compounds **2** and **3**.

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1982) and 3-*O*-β-D-glucopyranosyl(1 → 6)-β-D-glucopyranosyloleanolic acid (**3**) (Ye et al. 2000) (Figure 2).

In addition, all the compounds were evaluated for their antimicrobial activities against five bacteria, five yeasts and five dermatophytes. Compound **1** was more active against bacteria [minimum inhibitory concentration (MIC) value range of 1.562–12.5 μg/mL] than yeasts (MIC value range of 12.5–100 μg/mL), and was generally inactive against dermatophytes (Table 1). The activity of **2** was comparable to that of **1** on most of the tested microorganisms. The activity of **3** was generally low on all the tested microorganisms. The minimum microbicidal concentration (MMC)/MIC ratios for the compounds were generally less than 4, suggesting a microbicidal nature of the tested compounds (Lalitagauri et al. 2004).

The result of the radical-scavenging assay showed that **1** exhibited the best activity (RSa50 = 25.07 ± 0.49), closest to that of L-ascorbic acid (5.31 ± 1.09) (Table 2). Oxidants, apart from provoking oxidative stress, can lead to serious health problems including sickle cell diseases, artherosclerosis, Parkinson's disease, heart failure, myocardial infarction, Alzheimer's disease, schizophrenia, and chronic fatigue syndrome (De Diego-Otero et al. 2009). The presence of this compound in *P. pinnata* stem could trigger its use as a natural alternative of antioxidant.

3. Experimental

3.1. *General experimental procedures*

Optical rotations were measured with a JASCO P-1020 digital polarimeter (Tokyo, Japan). UV spectra were obtained by using a Shimadzu UV-2401 PC spectrophotometer (Tokyo, Japan). IR spectra were recorded on a Bruker Tensor-27 infrared spectrophotometer (Madison, WI, USA) with KBr pellets. 1D and 2D NMR spectra were performed on Bruker AM-400, DRX-500 and Avance III 600 instruments (Karlsruhe, Germany) with TMS as the internal standard. ESI-MS spectra were recorded on a Bruker HTC/Esquire spectrometer (Bruker Corporation, Madison, WI, USA). HR-ESI-MS was recorded on a Waters AutoSpec Premier P776 spectrometer (Palo Alto, CA, USA). Semi-preparative HPLC was performed on an Agilent 1100 HPLC (Agilent Technologies, Santa Clara, CA, USA) with a ZORBAX SB-C_{18} (9.4 × 250 mm) column. Silica gel (100–200 and 200–300 mesh, Qingdao Marine Chemical Co., Ltd, Qingdao, P.R. China), and MCI gel (75–150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan) were used for column chromatography. Fractions were monitored by using TLC (GF254, Qingdao Marine Chemical Ltd), and by heating silica gel plates sprayed with 10% H_2SO_4 in ethanol. GC analysis was performed on an HP5890 gas chromatograph (Hewlett-Packard, Toronto, ON, Canada) equipped with a H_2 flame ionisation detector.

Table 1. MIC, MBC and MFC (μg/mL) of compounds **1**–**3** against bacteria and fungi.

Microorganism		Test substance 1	2	3	RD
Bacteria					
P. aeruginosa	MIC	6.25	3.125	100	0.048
	MBC	12.5	3.125	–	0.195
E. aerogenes	MIC	3.125	1.562	25	0.781
	MBC	3.125	3.125	25	1.562
E. coli	MIC	1.562	1.562	6.25	0.781
	MBC	1.562	3.125	6.25	0.781
S. typhi	MIC	12.5	25	100	0.195
	MBC	12.5	25	100	0.781
K. pneumoniae	MIC	3.125	6.25	6.25	0.195
	MBC	6.25	6.25	25	0.195
Yeasts					
C. albicans	MIC	25	25	25	3.125
	MFC	50	25	50	3.125
C. guilliermondii	MIC	12.5	12.5	25	6.25
	MFC	25	12.5	50	12.5
C. neoformans	MIC	12.5	6.25	25	1.562
	MFC	25	12.5	25	3.125
C. lusitaniae	MIC	25	25	25	3.125
	MFC	50	25	50	6.25
C. parapsilosis	MIC	100	50	50	3.125
	MFC	100	100	100	3.125
Dermatophytes					
T. equinum	MIC	–	–	–	6.25
	MFC	–	–	–	12.5
M. audouinii	MIC	50	50	–	6.25
	MFC	50	50	–	6.25
T. mentagrophytes	MIC	–	100	–	3.12
	MFC	–	–	–	6.25
M. gypseum	MIC	100	50	–	6.25
	MFC	100	50	–	12.5
E. floccosum	MIC	–	–	–	3.12
	MFC	–	–	–	6.25

Notes: Minimum inhibitory concentrations (MIC); minimum bactericidal concentrations (MBC); and minimum fungicidal concentrations (MFC); RD, reference drug (ciprofloxacin, nystatin and griseofulvin for bacteria, yeasts and dermatophytes respectively); –, not active at 100 μg/mL.

Table 2. Radical-scavenging activities of **1**–**3**.

Test substance	RSa_{50} (μg/mL)
1	25.07 ± 0.49^{b}
2	38.78 ± 0.93^{c}
3	na
L-Ascorbic acid	5.31 ± 1.09^{a}

Values with different superscript letters are significantly different. Waller–Dunkan ($p < 0.05$); na, not active ($RSa_{50} > 500$ μg/mL).

3.2. *Plant material*

The air-dried stems of *P. pinnata* were obtained in Dschang, West Region of Cameroon, in January 2009. The identification of plant specimens was done at the Cameroon National

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Herbarium in Yaounde where a voucher specimen was deposited under the reference number 10702/SRFCam by Mr Tadjouteu Fulbert.

3.3. *Extraction and isolation*

The air-dried stems of *P. pinnata* (2.02 kg) were powdered and extracted with MeOH (7 L × 2, 48 h each) at room temperature to give a crude extract (152.17 g) after concentration under reduced pressure. This crude extract was partitioned with petroleum ether (PE), ethyl acetate and water to obtain the PE fraction (8.08 g), EtOAc fraction (9.13 g) and aqueous residue fraction (109.89 g).

The aqueous residue fraction was mounted on a D101 macroporous resin column and eluted successively with H_2O–EtOH (10:0; 7:3; 5:5; 3:7; 0:10) to obtain five fractions denoted F1–F5, respectively. F5 (946 mg) was purified on a silica gel column, eluted with a stepwise gradient mixture of $CHCl_3$–MeOH–H_2O (8:2:0.5 → 6:4:0.5) to afford subfraction F5.1 (107 mg). The latter was further purified by HPLC, eluted with H_2O–MeOH (2:8) to afford **3** (53.0 mg). F2 (306 mg) was purified by HPLC, eluted with H_2O–MeOH (95:5) to afford **1** (101.2 mg) and **2** (49.6 mg).

3.4. *Pinnatoside A (1)*

White amorphous powder; $[\alpha]_D^{23}$ – 38.0 ($c = 0.35$, MeOH); UV (MeOH) λ_{max}(log ε) 211 (4.21); IR (KBr) ν_{max} 3417, 2921, 1641, 1414, 1375, 1287, 1076 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6): δ_H 5.72 (1H, s, H-2), 4.22 (2H, br s, H-5), 4.50 (1H, d, $J = 13.1$ Hz, H-6a), 4.37 (1H, d, $J = 13.1$ Hz, H-6b), 4.14 (1H, d, $J = 7.8$ Hz, H-1′), 3.67 (1H, dd, $J = 11.7, 5.0$ Hz, H-6′a), 3.47 (1H, m, H-6′b), 3.14 (1H, m, H-3′), 3.09 (2H, m, H-5′ and 4′), 2.97 (1H, m, H-2′); ^{13}C NMR (150 MHz, DMSO-d_6): δ_C 165.2 (s, C-2), 116.8 (s, C-4), 102.7 (d, C-1′), 94.2 (d, C-3), 77.1 (d, C-3′), 76.7 (d, C-5′), 73.3 (d, C-2′), 69.9 (d, C-4′), 66.9 (t, C-6), 61.2 (t, C-5), 60.9 (t, C-6′); ESI-MS *m/z*: 276 $[M + H]^+$; HR-ESI-MS *m/z*: 298.0899 $[M + Na]^+$ (calcd for $C_{11}H_{17}NO_7Na$, 298.0903).

3.5. *Acidic hydrolysis of 1 and GC analysis*

Acid hydrolysis and sugar analysis were carried out as described in the previously published papers (Qin et al. 2012, 2013). The absolute configuration of the sugar residues was determined to be D-glucose (t_R 10.67 min).

3.6. *Antimicrobial assay*

The microorganisms used in this study were obtained from the American Type Culture Collection (ATCC), 'Ecole Nationale Vétérinaire d'Alfort' (E), 'centre Pasteur' ofYaounde-Cameroon and 'Institut Pasteur' of Paris-France (IP). They included five bacteria strains: *Salmonella typhi* ATCC 6539, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 11296, *Escherichia coli* ATCC10536, *Enterobacter aerogenes* ATCC 13048; five yeasts: *Candida albicans* ATCC 2091, *Candida guilliermondii*, *Cryptococcus neoformans* IP 90526, *Candida lusitaniae* ATCC 200950 and *Candida parapsilosis* ATCC 22019 and five dermatophytes: *Trichophyton equinum* E1424, *Microsporum audouinii* E1421, *Trichophyton mentagrophytes* E1425, *Microsporum gypseum* E1420 and *Epidermophyton floccosum*.

The MIC and MMC of the isolated compounds were determined by the broth microdilution method in 96-well micro-titre plates as described in the literature elsewhere (Tchakam et al. 2012). The compounds were evaluated at a final concentration range of 100–0.78 μg/mL.

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Ciprofloxacin for bacteria, nystatin for yeast and griseofulvin for dermatophytes were used as positives controls.

3.7. *Radical-scavenging assay*

The radical-scavenging activities of the compounds were evaluated spectrophotometrically using the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical as formally described (Lunga et al. 2014). Experiments were carried out in triplicates and the percentages of DPPH reduction (%RSa) by test samples were compared with that of L-ascorbic acid, and were converted to RSa_{50}, which is the amount of sample necessary to decrease by 50% the total free radical DPPH (Yassa et al. 2008). The data were subjected to one-way analysis of variance, and differences between samples at $P \leq 0.05$ were determined by Waller–Duncan test and the results were expressed as mean ± standard deviation of three replicates.

4. Conclusion

One new glycoside, pinnatoside A (**1**), together with two known compounds (**2** and **3**), were isolated from the stems of *P. pinnata*. The antimicrobial and radical-scavenging activities for these compounds were also evaluated. Compound **1** showed significant antibacterial activity with a MIC value of 1.56 μg/mL against *E. coli*, and the best radical-scavenging activity. Compound **2** displayed significant antibacterial activity with a MIC value of 1.56 μg/mL against *E. aerogenes* and *E. coli*.

Supplementary material

Supplementary material relating to this article is available online, alongside Figures S1–S6.

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