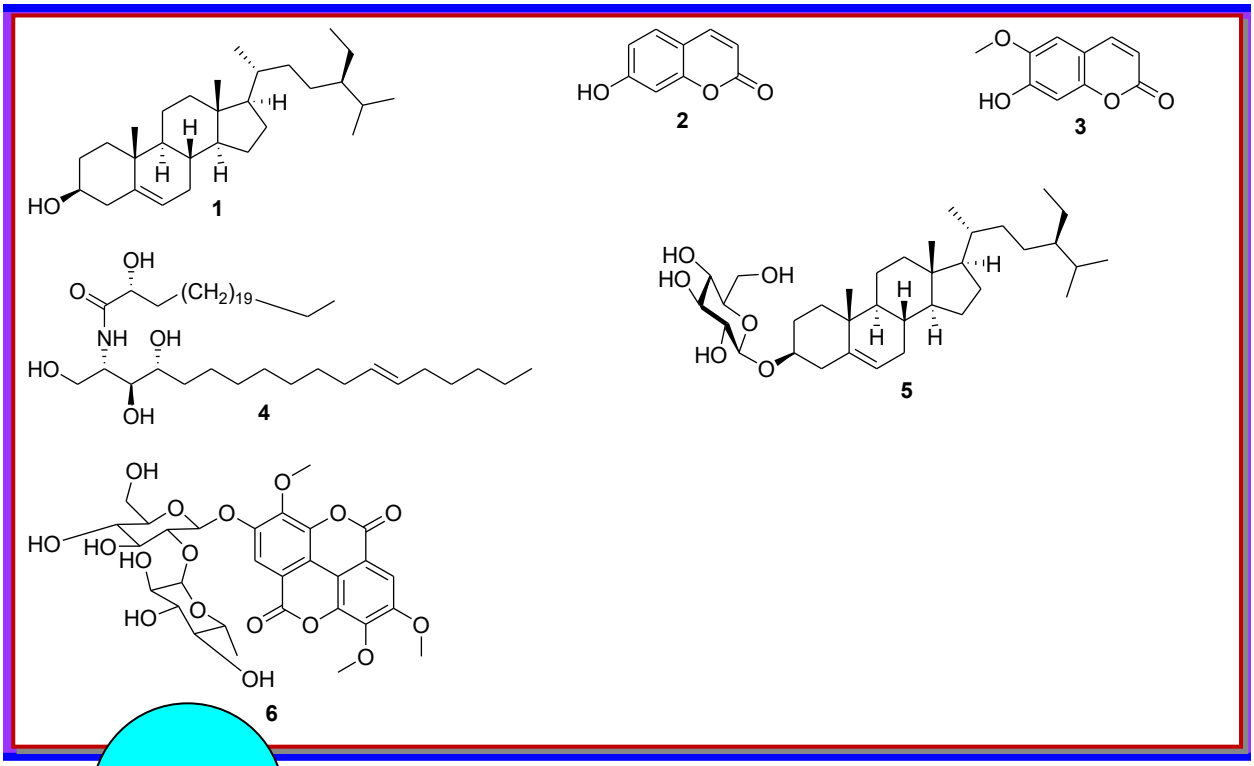

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Xanthine oxidase inhibitory activity of compounds from *Chythrantus claneianus*

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Abstract

Phytochemical investigation of the stem bark and the trunk of *Chythrantus claneianus* led to the isolation of six known compounds named *β*-sitosterol (**1**), umbelliferone (**2**), scopoletin (**3**), benjaminamide (**4**), *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (**5**) and panconoside B (**6**). All these compounds were isolated for the first time from this plant species. The chemical structures of isolates were elucidated on the basis of 1 and 2 D-NMR spectra and other spectroscopic techniques including UV–vis, FT-IR, HR-ESIMS and HR-FABMS. The isolates were tested *in vitro* for their inhibitory properties towards xanthine oxidase enzyme. Compounds **2**, **3** and **6** showed weak inhibitory activities on the enzyme with IC_{50} values ranging from 307 µM for compound **6** to 475 µM for compound **3**, while the extract and compounds **1**, **4** and **5** showed extremely weak activities with inhibition percentage less than 50%.

Introduction

Plants of the Sapindaceae family are trees or shrubs generally found in tropical or subtropical regions. In Cameroon, they are used in traditional medicine for the treatment of several ailments such as skin diseases, dysentery and rheumatism (Abdou-Shoer et al., 1993). Previous phytochemical studies on plants of this family reported the presence of various classes of secondary metabolites including flavonoids, coumarins, ellagic acids, ceramides, sterols, and saponins as major constituents (Aubreville et al., 1973, Huang et al., 2008, Li et al., 2007, Rangkadilok et al., 2005, Soh et al., 2009). Some of these compounds were shown to exhibit interesting pharmacological properties including antiplasmodial, hemolytic, antibacterial and anti-oxidant activities (Huang et al., 2008, Mesquita et al., 2005, Voutquenne et al., 2005). *Chythrantus klaineanus* is a tree or shrub of about 1-5 m high, found in Central Africa (Abdou-Shoer et al., 1993). No previous reports on the chemical constituents or biological properties of this species have been reported. As part of our ongoing search for bioactive metabolites from Cameroonian plants of the Sapindaceae family, we have investigated the MeOH extract from the mixture of the stem bark and the trunk of *C. klaineanus*.

Xanthine oxidase (XO, EC 1.1.3.22) is a complex metalloflavoprotein which catalyzes the oxidation of hypoxanthine and xanthine into uric acid, plays a vital

role in disorders such as hyperuricemia and gout. XO inhibitors may be useful for treatment of these diseases. For the treatment of hyperuricemia and gout allopurinol, oxypurinol, and febuxostat have been used widely (Higgins et al., 2011; Nguyen et al., 2005).

Materials and Methods

General: Isolation of compounds was done with normal phase column chromatography using silica gel (70-230 mesh, Merck, Germany). Melting points were obtained on a Gallenkamp melting point apparatus. Optical rotations were measured with a JASCO DIP-360 polarimeter. The structures of compounds were elucidated using spectral methods UV–vis, fourier transform infrared spectroscopy, 1D and 2D NMR, HR-ESIMS and HR-FABMS. Ultraviolet spectra were recorded on a Hitachi UV 3200 spectrophotometer. A JASCO 320-A spectrophotometer was used for scanning IR spectroscopy using KBr pellets. 1D and 2D NMR spectra were run on a Bruker spectrometer operating at 75, 100, 125, 150, 300, 400, 500, and 600 MHz, where chemical shifts (δ) were expressed in ppm with reference to the solvent signals. Electron impact mass spectra were obtained on a Varian MAT 311A mass spectrometer. Fast atom bombardment mass spectra were measured on a JEOL-HX 110 mass spectrometer. Column chromatography was performed on silica gel 230-400 mesh (Merck). Fractions were monitored by thin layer chromatography using precoated aluminum-backed silica gel 60 F 254 sheets. Spots were visualized under ultraviolet light (254 and 365 nm) or using ceric sulfate reagent.

Plant material: The stem bark and the trunk of *C. klaineanus* were collected in April 2008 from Mount Kala in the Centre Region of the Republic of Cameroon. The plant was identified by Mr. Nana Victor, botanist at the National Herbarium of Cameroon, where a voucher specimen (no. HNC 1489) has been deposited.

Extraction and isolation: 2.5 kg of air-dried and powdered mixture of the stem bark and the trunk of *C. klaineanus* were extracted with MeOH (5 L) at room temperature for 48 hours and filtered. The extract was concentrated to dryness under vacuum to give 40 g of a dark brownish crude extract. The extract was extracted selectively with EtOAc. The EtOAc-soluble fraction was evaporated to dryness under vacuum to give a dry residue (13 g) which was then subjected to medium pressure liquid chromatography over silica gel 230-400 mesh (Merck) eluted successively with *n*-hexane, mixtures of *n*-hexane/EtOAc, EtOAc, and EtOAc/MeOH of increasing polarities. Subfractions (119), of 500 mL each, were collected and combined according to their thin layer chromatography profiles on pre-coated silica gel 60 F_{254} plates developed with *n*-hexane/EtOAc mixtures to yield 4 fractions (F1-4). Fraction F1 was subjected to column chromatography over silica gel (Merck, 70-230 mesh) eluted with an *n*-hexane/EtOAc mixture (9.5: 0.5 to 7: 3). This resulted in the isolation of **1** (21 mg) and **2** (63 mg). Fraction F2 was also subjected to successive column chromatography over silica gel (Merck, 70-230 mesh) eluted with a mixture of *n*-hexane and EtOAc (6: 4) to give **3** (33 mg) and **4** (151 mg). Fraction F3 was eluted with an *n*-hexane/EtOAc mixture of increasing polarity (5: 5 to 1: 9) to yield **5** (230 mg). Fraction F4 was subjected to column chromatography over silica gel (Merck, 70-230 mesh) with a CH_2Cl_2/MeOH mixture (9.5: 0.5 to 8: 2) to yield **6** (320 mg).

*Acid hydrolysis of compound **6**:* A solution of 10 mg of compound **6** in 10 mL MeOH/H_2O (1 : 1) mixed with 5 mL 2N HCl was refluxed at 100°C for 3 hours. After evaporation of the organic solvent under vacuum and threefold extraction of the aqueous phase with EtOAc, the aglycone was identified as 3,3′,4′-tri-*O*-methylellagic acid using NMR techniques. The neutralized aqueous portion resulted in the detection of glucose and rhamnose by comparison with standard sugar samples on thin layer chromatography plates using *n*-BuOH/Me_2CO/H_2O (4 : 5 : 1) as solvents and using $(NH_4)_6Mo_7O_{24}Ce(SO_4)_2H_2SO_4$ reagent for visualization.

In vitro xanthine oxidase inhibitory activity assay: Xanthine (X-0626) and xanthine oxidase (EC 1.1.3.22) (from butter milk) were obtained from Sigma Aldrich, (Japan). The xanthine oxidase inhibitory activity of compounds was determined by measuring the rate of hydroxylation of the substrate (xanthine) with the formation of uric acid, which is a colorless product of the reaction which shows absorption at 295 nm (Lee et al., 1998). Briefly, the reaction mixture containing 10 μL of 1 mmol/L of isolates was dissolved in dimethyl sulfoxide, 150 μL of phosphate buffer (0.05 mmol/L, pH 7.4), 0.003 units of xanthine oxidase dissolved in buffer (20 μL), and 20 μL of 0.1 mmol/L xanthine as substrate for enzyme. After addition of xanthine oxidase, the mixture was incubated for 10 min at room temperature and pre-read in the ultraviolet region at 295 nm. The substrate was added to reaction mixture, and subsequent continuous reading for 15 min at an interval of 1 min was observed (on Spectra MAX-340). The percentage inhibitory activity by the samples were determined against a DMSO blank and calculated using the following formula:

$$Inhibition\ (\%) = 100 - \frac{OD\ test\ compound}{OD\ control} \times 100$$

The IC_{50} of the compounds as calculated using EZ-Fit windows-based software (Perrella Scientific Inc. Amherst, USA). To compare the inhibitory activities of the compounds, allopurinol was used as standard. The reaction for each compound was performed in triplicate.

Results and Discussion

The MeOH extract of the stem bark and the trunk of *C. klaineanus* was fractionated and purified on silica gel and sephadex LH-20 columns, successively, to afford six known compounds.

Compound **6** was isolated as yellow crystals. It reacted positively to the Molish test, indicating its glycoside nature. The molecular formula $C_{29}H_{32}O_{17}$, implying 14 degrees of unsaturation, was deduced from NMR and its HR-ESIMS which showed the pseudo-molecular ion peak at *m/z* = 675.15465 [M+Na]+ (calcd. for $C_{29}H_{32}O_{17}$ + Na^+, 675.15686). The ultraviolet spectrum in MeOH exhibited absorption bands at λ_{max} 256, 360 nm, suggesting an ellagic acid derivative skeleton (El-Toumy et al., 2003, Pakulski et al., 1996). The IR spectrum of 6 showed absorptions for hydroxy groups (3431 cm^{-1}) and lactone functions (1751 cm^{-1}), aromatic C=C groups (1600 cm^{-1}), and glycosidic C–O bonds (1091 cm^{-1}). The broad band decoupled ^{13}C NMR of compound 6 displayed 29 carbon signals, which were sorted by DEPT and HSQC experiments into twelve methine groups [including two aromatic methine carbons at δ_C 107.5 (C-5′) and 111.7 (C-5), and ten oxymethine carbons], one oxymethylene at δ_C 60.3, twelve quaternary carbons [including two lactone carbons at δ_C 158.2 (C-7) and 158.3 (C-7′)], three methoxy groups and one methyl group at δ_C 18.0 (C-6‴)] (Table I). Its ^1H NMR spectrum exhibited two deshielded singlets, characteristic of an ellagic acid moiety at δ_H 7.65 (1H, s, H-5′) and 7.84 (1H, s, H-5) (6), ten oxymethine and two oxymethylene protons at δ_H 3.18 (1H, t, *J* = 9.4 Hz, H-4‴), 3.25 (1H, t, *J* = 8.8 Hz, H-4″), 3.37 (1H, brs, H-2‴), 3.47 (2H, m, H-3″ and H-6″), 3.55 (1H, brt, *J* = 8.8 Hz, H-5″), 3.64 (2H, m, H-2″ and H-6″), 3.72 (2H, m, H-3‴ and H-5‴), 5.26 (1H, d, *J* = 1.3 Hz, H-1‴) and 5.46 (1H, d, *J* = 7.5 Hz, H-1″) indicating the presence of two sugar units in 6 (6). The spectrum also exhibited three methoxy groups protons at δ_H 4.01 (3H, s, 4′-MeOH), 4.05 (3H, s, 3′-MeOH) and 4.11 (3H, s, 3-MeOH) as well as six hydroxy protons. The two sugar units obtained from the acid hydrolysis were identified as glucose and rhamnose by comparing with standard sugar samples on thin layer chromatography plates using *n*-BuOH-Me_2CO-H_2O (4: 5: 1) solvent system as well as the carbon and proton chemical shifts and the coupling constant pattern of the protons of the two sugar units compare to those of literature (Soh et al., 2009, El-Toumy et al., 2003).

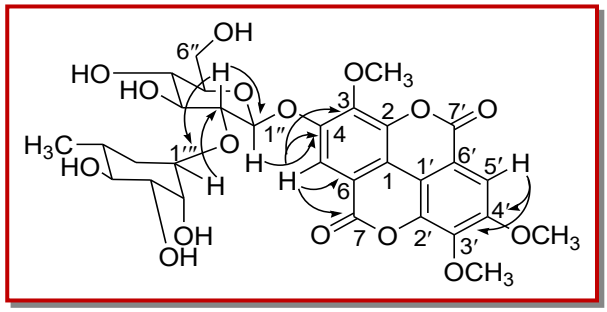

Figure 1: HMBC correlations in compound 6

The configuration of the anomeric protons [5.26 (1H, d, *J* = 1.3 Hz, H-1‴) and 5.46 (1H, d, *J* = 7.5 Hz, H-1″)] were determined based on their coupling constant to be *a*-rhamnose and *β*-glucose, respectively (Soh et al., 2009, El-Toumy et al., 2003). HMBC spectrum was used to determine the junction between the rhamnose and glucose and between the glucose and the ellagic acid moiety (Figure 1). This spectrum was also used to determine the position of the different methoxy groups on the ellagic acid moiety. In fact, cross-peaks were observed in the HMBC spectrum between the anomeric proton of the glucose moiety at δ_H 5.46 (1H, d, *J* = 7.5 Hz, H-1″) and the carbon C-4 (δ_C 151.1), and between the anomeric proton of the rhamnose moiety at δ_H 5.26 (1H, d, *J* = 1.3 Hz, H-1‴) and the carbon C-2″ (δ_C 76.2). In addition, cross-peaks were observed between the three methoxy groups protons at δ_H 4.11 (3H, s, 3-MeOH), 4.05 (3H, s, 3′-MeOH) and 4.01 (3H, s, 4′-MeOH), and carbons C-3 (δ_C 140.8), C-3′ (δ_C 141.1) and C-4′ (δ_C 154.3), respectively. The position of the glucose unit was also supported by the observation of a NOE between protons H-5 (δ_H 7.84) and H-1″ (δ_H 5.46) in the NOESY spectrum. On the basis of the above spectral data and by comparing of these values with those reported in literature, the identity of compound 6 was confirmed as panconoside B which was recently isolated from *Pancovia pedicellaris*, a plant of the same family (Soh et al., 2009).

The structures of the five other known compounds were established as *β*-sitosterol (**1**) (Xiao et al., 2002), umbelliferone (**2**) (Ngadjui et al., 1991), scopoletin (**3**) (Cardona et al., 1992), benjaminamide (**4**) (Simo et al., 2008) and *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (**5**) (Nguyena et al., 2004) by comparing of their spectral and physical data with those published before (Figure 2).

*β-sitosterol (compound **1**)*: Colourless needles, mp 139–140 °C. ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 38.6 (C-1), 30.7 (C-2), 73.8 (C-3), 39.7 (C-4), 141.9 (C-5), 122.9 (C-6), 33.1 (C-7), 33.3 (C-8), 51.7 (C-9), 37.9 (C-10), 22.2 (C-11), 41.1 (C-12), 43.5 (C-13), 58.2 (C-14), 25.3 (C-15), 29.4 (C-16), 57.4 (C-17), 12.3 (C-18), 19.9 (C-19), 37.5 (C-20), 19.3 (C-21), 35.1 (C-22), 27.1 (C-23), 47.3 (C-24), 30.3 (C-25), 20.2 (C-26), 19.4 (C-27), 24.1 (C-28), 12.4 (C-29). EI-MS *m/z* 414 (64), 396 (40), 382 (14), 303 (23), 255 (20), 159 (23), 81 (37), 43 (100).

*Umbelliferone, 7-hydoxycoumarin (compound **2**):* Yellowish solids, mp 231-233 °C. RMN ^1H (500 MHz, DMSO-d_6) δ_H 6.20 (d, *J* = 9.5 Hz, H-3), 7.94 (d, *J* = 9.5 Hz, H-4), 7.53 (d, *J* = 8.8 Hz, H-5), 6.79 (dd, *J* = 8.5, 1.9 Hz, H-6), 6.71 (d, *J* = 1.9 Hz, H-8), 10.57 (7-OH). ^{13}C NMR (125 MHz,

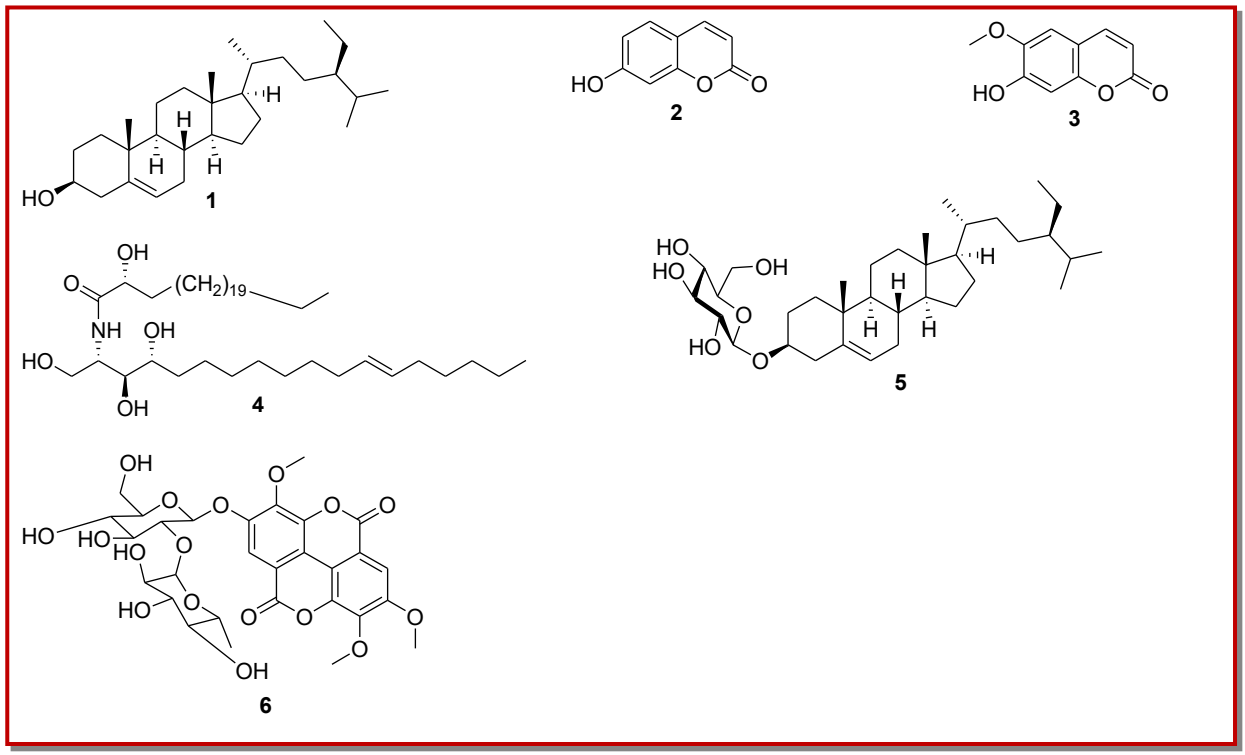

Figure 2: Structures of isolates 1-6

DMSO-d_6) δ_C 161.2 (C-2), 113.1 (C-3), 144.5 (C-4), 111.2 (C-4a), 129.7 (C-5), 111.4 (C-6), 160.4 (C-7), 102.1 (C-8), 155.4 (C-8a). EI-MS *m/z* 78 (31), 105 (23), 134 (100), 162 (87).

*Scopoletin, 7-hydroxy-6-methoxycoumarin (compound **3**):* Yellow crystals, mp 201-203 °C. RMN ^{1}H (500 MHz, DMSO-d_6) δ_H 6.22 (d, *J* = 9.5 Hz, H-3), 7.91 (d, *J* = 9.5 Hz, H-4), 7.21 (s, H-5), 6.78 (s, H-8), 3.82 (s, OMe-6), 10.31 (7-OH). ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 161.1 (C-2), 112.1 (C-3), 144.9 (C-4), 111.0 (C-4a), 110.0 (C-5), 151.6 (C-6), 145.7 (C-7), 103.2 (C-8), 149.9 (C-8a), 56.4 (OMe-6). EI-MS *m/z* 69 (38), 121 (24), 149 (53), 177 (64), 192 (100).

*Benjaminamide, (2S,3S,4R,12E)-2-{[(2R)-2-hydroxytetracosanoyl]amino}octadec-12-ene-1,3,4-triol (compound **4**):* Colorless crystals, mp 141-142 °C, $[\alpha]_D^{30}$ +34 (*c* 0.08, pyridine). IR (KBr) ν_{max} 3200-3500 (OH), 1633 (NH─C=O), ^{1}H NMR (500 MHz, pyridine-d_5) δ_H 8.55 (d, *J* = 9 Hz, NH), 4.69 (dd, *J* = 9.9, 6.6 Hz, H-1a), 4.49 (dd, *J* = 9.9, 6.6 Hz, H-1b), 5.27 (m, H-2), 4.44 (overlapped dd, H-3), 4.18 (overlapped dd, H-4), 2.27 (m, H-5a), 1.90 (m, H-5b and H-6), 1.22 (brs, H-7 to H-10), 2.04 (m, H-11), 5.45 (m, H-12), 5.48 (m, H-13), 2.20 (m, H-14), 1.22 (brs, H-15 to H-17), 0.84 (t, *J* = 7.2 Hz, H-18), 4.55 (brdd, H-2′), 2.17 (m, H-3a′), 1.98 (m, H-3b′), 1.90 (m, H-4′), 1.29 (brs, H-5′ to H-23′), 0.84 (t, *J* = 7.2 Hz, H-24′). ^{13}C NMR (125 MHz, pyridine-d_5) δ_C 62.3 (C-1), 51.7 (C-2), 75.9 (C-3), 72.5 (C-4), 33.9 (C-5), 26.6 (C-6), 29.6-33.9 (C-7 to C-10), 27.5 (C-11), 130.2 (C-12), 130.4 (C-13), 27.9 (C-14), 22.9-33.0 (C-15 to C-17), 14,3 (C-18), 175.7 (C-1′), 72.4 (C-2′), 35.5 (C-3′), 26.7 (C-4′), 29.3-33.0 (C-5′ to C-22′), 22.9 (C-23′), 14.2 (C-24′). (+)HR-FABMS *m/z* 681.6280 (calcd. 681.6271 for $C_{42}H_{83}NO_5$, $[M+H]^+$).

*β-sitosterol-3-O-β-D-glucopyranoside (compound **5**):* Colorless crystals; mp 260-261 °C. ^{13}C NMR (100 MHz, Pyridine-d_5) δ_C 37.4 (C-1), 30.2 (C-2), 78.5 (C-3), 39.3 (C-4), 140.9 (C-5), 121.8 (C-6), 32.1 (C-7), 32.0 (C-8), 50.3 (C-9), 36.9 (C-10), 21.2 (C-11), 39.9 (C-12), 42.4 (C-13), 56.8 (C-14), 24.4 (C-15), 28.4 (C-16), 56.2 (C-17), 11.9 (C-18), 19.0 (C-19), 36.3 (C-20), 19.2 (C-21), 34.2 (C-22), 26.4 (C-23), 46.0 (C-24), 29.4 (C-25), 19.9 (C-26), 19.4 (C-27), 23.3 (C-28), 12.1 (C-29), 102.5 (C-1′), 75.2 (C-2′), 78.3 (C-3′), 71.7 (C-4′), 78.2 (C-5′), 62.8 (C-6′). EI-MS *m/z* 414 (5), 396 (63), 381 (24), 255 (77), 159 (39), 81 (99), 55 (100).

*Panconoside B [3,3′,4′-tri-O-methylellagic acid 4-O-[a-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside] (compound **6**):* Yellow crystals, mp 257-259 °C. UV (MeOH) λ_{max} (logε) 256 (3.72), 360 (2.47). $[\alpha]_D^{20}$ −80.0°(*c* 0.5, MeOH). IR (KBr) ν_{max} 3431 (O─H), 1751 (C=O), 1600 (C=C), 1091 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) spectroscopic data, see Table I. (+)-HR-ESIMS *m/z* 675.15465 (calcd. 675.15686 for $C_{29}H_{32}O_{17}$ + Na^+). EI-MS *m/z* 344 (100), 329 (20), 286 (17).

In vitro xanthine oxidase inhibitory assay All the isolated compounds and the extract were tested *in vitro* for their inhibitory effects towards the xanthine oxidase enzyme,

Table I

Comparative ^{1}H and ^{13}C NMR spectral data of 6 with those of panconoside B

Position	δ_C	δ_H [m, *J* (Hz)]	δ_C	δ_H [m, *J* (Hz)]
1	113.7		112.6	
2	141.9		141.7	
3	140.8		142.4	
4	151.1		151.6	
5	111.7	7.84 (*s*)	112.2	7.83 (*s*)
6	112.2		114.2	
7	158.2		158.6	
1′	113.7		113.1	
2′	141.2		141.6	
3′	141.1		141.3	
4′	154.3		154.8	
5′	107.5	7.65 (*s*)	108.0	7.65 (*s*)
6′	112.6		113.3	
7′	158.3		158.8	
1″	98.5	5.46 (*d*, *J* = 7.5)	99.0	5.46 (*d*, *J* = 7.5)
2″	76.2	3.64 (*m*)	76.7	3.63 (*m*)
3″	77.2	3.47 (*m*)	77.5	3.46 (*m*)
4″	69.6	3.25 (*t*, *J* = 8.8)	70.1	3.24 (*t*, *J* = 8.8)
5″	77.0	3.55 (*brt*, *J* = 8.8)	77.7	3.56 (*brt*, *J* = 8.8)
6a″	60.3	3.64 (*m*)	60.8	3.65 (*m*)
6b″		3.47 (*m*)		3.48 (*m*)
1‴	100.2	5.26 (*d*, *J* = 1.3)	100.7	5.26 (*d*, *J* = 1.3)
2‴	70.4	3.37 (*brs*)	70.9	3.37 (*brs*)
3‴	70.3	3.72 (*m*)	70.8	3.72 (*m*)
4‴	71.8	3.18 (*t*, *J* = 9.4)	72.3	3.18 (*t*, *J* = 9.4)
5‴	68.5	3.72 (*m*)	69.0	3.70 (*m*)
6‴	18.0	1.11 (*d*, *J* = 6.3)	18.5	1.11 (*d*, *J* = 6.3)
3-OMe	61.3	4.11 (*s*)	62.2	4.11 (*s*)
3′-OMe	61.7	4.05 (*s*)	61.8	4.05 (*s*)
4′-OMe	56.8	4.01 (*s*)	57.2	4.01 (*s*)

which is a target in gouty arthritis and uric acid nephrolithiasis drug research. As depicted in Table II, results showed that, overall, all the pure compounds exhibited weaker xanthine oxidase inhibitory activity compared to the reference drug, allopurinol.

Table II

In vitro* inhibition of xanthine oxidase by natural compounds from *Chythrantus claneianus

	Sample	% Inhibition	IC_{50} ± SEM (µM)[a]
1	*β*-sitosterol	-	ND
2	Umbelliferone	47	>500
3	Scopoletin	52	475 ± 3.7
4	Benjaminamide	11	ND
5	*β*-sitosterol-3-*O*-*β*-D-glucopyranoside	-	ND
6	Panconoside B	82	307±2.3
	MeOH Extract	43	ND
	Allopurinol[b]	98.8	13.7 ± 0.2

[a]IC_{50}: Concentrations that inhibited 50% of enzymes relative to negative control; ND: Not determined; SEM: standard mean error. [b]Standard used for the assay

We found for the first time that panconoside B (**6**) inhibited XO activity. The highest values were obtained with compound **6** (82%) and 3 (52%). The isolates showed IC_{50} values ranging from 307 µM (compound **6**) to 475 µM (compound **3**), whereas the IC_{50} value of the reference drug allopurinol was 13.70 µM.

Lin et al. investigated the effect of the coumarin derivatives on the inhibition of xanthine oxidase (XO) activity, and the structure–activity relationships of these derivatives against xanthine oxiase activity. They reported that scopoletin (**3**) and umbelliferone (**2**) showed lower (IC_{50} values of >100 mM) XO inhibitory activity (Hsiu-Chen et al., 2007).

All constituents of *Conyza bonariensis* (L.) including β-sitosterol (**1**) were tested for their activity towards XOD inhibition. The *in vitro* enzyme assay demonstrated that this compound didn't display XOD inhibitory activity (Kong et al., 2001). In another study 3β-hydroxy-sitosterol from snake fruit (*Salacca edulis* Reinw.) was mentioned to be inactive (Leni et al., 2007). We reported

previously that β-sitosterol (**1**), *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (**5**) and benjaminamide (**4**) were inactive (Gojayev et al., 2011).

It is interesting to note that the active compounds were coumarins and their derivatives. Our results can justify the use of *C. klaineanus* by Cameroonian traditional healers in the treatment of inflammatory diseases such as rheumatism.

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