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Laurentixanthones A and B, antimicrobial xanthones from *Vismia laurentii*

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Abstract

A phytochemical investigation of the constituents of the roots of *Vismia laurentii* has resulted in the isolation of two xanthone derivatives named laurentixanthone A (**1**) (6-hydroxy-3,3-dimethyl-11-(3-methylbut-2-enyl)pyrano[2,3-*c*]xanthen-7(3*H*)-one) and laurentixanthone B (**2**) (1-hydroxy-5,6,7,8-tetramethoxyxanthone), along with 11 known compounds: 1,7-dihydroxyxanthone, vismiaquinone, vismiaquinone B, bivismiaquinone, 3-geranyloxy-6-methyl-1,8-dihydroxyanthraquinone, O^1-demethyl-3′,4′-deoxypsorospermin-3′,4′-diol, 6-deoxyisojacareubin, 1,8-dihydroxy-6-methoxy-3-methylanthraquinone, kaempferol, friedelin and stigmasterol. The structures of compounds were established by means of spectroscopic methods. Furthermore, the compounds were screened for antimicrobial activities in vitro.

Keywords: *Vismia laurentii*; Guttiferae; Xanthones; Anthraquinones; Antimicrobial activities

1. Introduction

The tribe Vismieae belongs to the family of Guttiferae and comprises three genera, *Vismia*, *Harungana* and *Psorospermum*. It is distributed in the tropical and subtropical regions of the world (Hutchinson and Dalziel, 1954). *Vismia laurentii* De Wild is a large shrub or tree which is found in the Centre Province of Cameroon where it is locally called "atondo owse". The bark and roots are employed in decoctions as tonic and febrifugal (Kerharo, 1974; Bamps, 1966, 1970) and is also used in tropical Africa as a remedy for the treatment of skin diseases (such as dermatitis, leprosy, scabies, eczemas) and wounds using coconut as vehicle. Previous chemical studies have reported the presence of triterpenoids, prenylated anthrones, anthraquinones, bianthraquinones, benzophenones, xanthones and lignans from *Vismia* species (Nagem and De Olivera, 1997; Dos Santos et al., 2000; Araújo et al., 1990; Nagem and Alves, 1995; Nagem and Faria De Jesus, 1990; Hussein et al., 2003; Bilia et al., 2000; Botta et al., 1986). Cytotoxic and antifeedant activities have also been reported for various *Vismia* constituents (Cassinelli et al., 1986; Seo et al., 2000). As part of our continuous search for bioactive natural products from Guttiferae family (Nkengfack et al., 2002; Azebaze et al., 2004; Yimdjo et al., 2004), we investigated the constituents of the *V. laurentii*, a plant on which no phytochemical studies has been reported to the best of our knowledge. In the present work, we report the isolation

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and structural elucidation of 13 secondary metabolites from the roots of this plant, two of which are new derivatives, and the results obtained from the evaluation of their antimicrobial properties.

2. Results and discussion

Air-dried and ground roots of *V. laurentii* were extracted at room temperature with a mixture of methanol–dichloromethane (1:1). Separation and purification of this extract by successive silica gel column chromatography (see Section 3) led to the isolation and characterisation of two new xanthone derivatives, named laurentixanthones A (**1**) and B (**2**) along with 11 known secondary metabolites. The known compounds were identified by comparison of their physicochemical and spectroscopic data (^{1}H, ^{13}C NMR, 2D NMR and MS) with those of authentic samples and reference data as: 1-hydroxy-6,7-dimethoxyxanthone (Gunatilaka et al., 1982), 1,7-dihydroxyxanthone (Fujita et al., 1992), vismiaquinone (Goncalves and Mors, 1981), vismiaquinone B (Miraglia et al., 1981), bivismiaquinone (Hussein et al., 2003), 3-geranyloxy-6-methyl-1,8-dihydroxy anthraquinone (Botta et al., 1983), O^1-demethyl-3′,4′-deoxypsorospermin-3′,4′-diol (Abou-Shoer et al., 1988), 6-deoxyisojacareubin (Wu et al., 1998), 1,8-dihydroxy-6-methoxy-3-methylanthraquinone (Goncalves and Mors, 1981), kaempferol (Kouam et al., 1993), friedelin (Gunatilaka et al., 1982) and stigmasterol (Alan et al., 1996).

Compound **1**, laurentixanthone A, m.p. 156–157 °C, was obtained as yellow needles from a mixture of cyclohexane–EtOAc (4:1). It reacted positively to $FeCl_3$ reagent, suggesting the presence of at least one phenolic hydroxyl group in the molecule. Its molecular formula was determined to be $C_{23}H_{22}O_4$ using positive high-resolution (HR) electrospray ionisation time of flight (ESI-TOF) mass spectroscopy which showed a pseudomolecular ion peak $[M+H]^+$ at m/z 363.1589 (calcd. for $C_{23}H_{23}O_4$, 363.1596). The broad-band proton decoupled ^{13}C NMR spectrum of compound **1** (Table 1) showed 22 carbon signals which were attributed by APT and HSQC techniques as four methyls, one methylene, seven methines and 11 quaternary carbons including one carbonyl group (δ_C 181.8), four oxygenated sp^2 carbons, five sp^2, and one sp^3 carbons. The IR spectrum of compound **1** displayed vibration bands characteristic of a chelated hydroxyl group ($\nu_{max} = 3356\ cm^{-1}$), a conjugated and chelated carbonyl group ($\nu_{max} = 1664\ cm^{-1}$) and a C–C double bonds of an aromatic ring ($\nu_{max} = 1650\ cm^{-1}$). These data together with those obtained from the UV spectrum of compound **1** [λ_{max} ($\log\varepsilon$): 250 (4.08), 322 (3.71) nm], were consistent with the presence of a xanthone skeleton (Cardona et al., 1990). In the ^{1}H NMR spectrum (acetone-d_6) (Table 1) of compound **1**, analysed by a combination of ^{1}H–^{1}H COSY

Table 1
^{1}H and ^{13}C NMR data for compounds **1** and **2**

Position	**1** (Acetone-d_6)		**2** ($CDCl_3$)	
	δ_H (mult. J Hz)	δc (APT)	δ_H (mult. J Hz)	δ_C (APT)
1	–	164.1	–	162.0
2	5.67 (1H, *s*)	99.7	6.75 (1H, *dd*, 7.9, 1.8)	110.7
3	–	161.7	7.51 (1H, *t*, 7.9)	136.1
4	–	102.1	6.90 (1H, *dd*, 7.9, 1.8)	106.4
4a	–	152.1	–	155.3
5	–	107.9	–	137.2
6	7.28 (1H, *dd*, 7.8, 1.9)	122.2	–	147.7
7	7.22 (1H, *t*, 7.8)	125.0	–	143.1
8	7.75 (1H, *dd*, 7.8, 1.9)	116.5	–	149.4
8a	–	121.7	–	117.0
4b	–	147.0	–	153.2
9	–	181.8	–	181.6
8b	–	104.2	–	108.8
2′	–	79.1	–	–
3′	5.60 (1H, *d*, 10)	128.1	–	–
4′	6.74 (1H, *d*, 10)	115.7	–	–
5′,6′	1.49 (6H, *s*)	28.4	–	–
1-OH[a]	13.07 (1H, *s*)	–	13.07 (1H, *s*)	–
5-OMe	–	–	3.90 (3H, *s*)	61.6[b]
6-OMe	–	–	3.95 (3H, *s*)	61.7[b]
7-OMe	–	–	3.95 (3H, *s*)	62.0[b]
8-OMe	–	–	4.10 (3H, *s*)	62.8
1′′	3.49 (2H, *d*, 7.9)	23.4	–	–
2′′	5.22 (1H, *t*, 7.9)	125.1	–	–
3′′	–	133.6	–	–
4′′	1.86 (3H, *s*)	26.1	–	–
5′′	1.71 (3H, *s*)	19.3	–	–

[a] Exchangeable in D_2O.
[b] Assignments bearing the same superscript in the same column may be reversed.

and HSQC experiments, an ABC spin system, indicated by two double doublets at δ_H 7.28/δ_C 122.2 (1H, *dd*, $J = 7.8$, 1.9 Hz) and δ_H 7.75/δ_C 116.5 (1H, *dd*, $J = 7.8$, 1.9 Hz) and a triplet at δ_H 7.22/δ_C 125.0 (1H, *t*, $J = 7.8$ Hz), corresponding to a 1,2,3-trisubstituted benzene ring, was observed in addition to a chelated hydroxyl signal at δ_H 13.07 and one-proton singlet at δ_H 5.67/δ_C 99.7 due to an aromatic proton. Furthermore, the ^{1}H and ^{13}C NMR spectra also displayed the presence of two sets of signals. The first set, comprising a six-proton singlet at δ_H 1.49/δ_C 28.4 and two *cis*-olefinic protons (δ_H 5.60/δ_C 128.1 and δ_H 6.74/δ_C 115.7, each *d*, $J = 10.0$ Hz) due to a dimethylchromene bicyclic system. The second set of signals, consisting of a doublet of two protons at δ_H 3.49/δ_C 23.4 (2H, *d*, $J = 7.9$ Hz), a triplet of one proton at δ_H 5.22/δ_C 125.1 (1H, *t*, $J = 7.9$ Hz), a carbon signal at δ_C 133.6, three proton singlet at δ_H 1.86/δ_C 26.1 (3H, Me) and another three proton singlet at δ_H 1.71/δ_C 19.3 (3H, Me), established the presence of a γ,γ-dimethylallyl moiety. A combination of the COSY and HSQC experiments permitted the assignment of all the protonated carbons (Table 1). It remained to establish the positions of the substituents on the xanthone skeleton. This was done by analysis of the HMBC spectrum. In effect, the chelated hydroxyl group at δ_H 13.07 correlated with carbons at δ_C 164.1 (C-1), δ_C 99.7 (C-2) and δ_C 104.2 (C-8b), while the aromatic proton at δ_H 5.67 correlated with quaternary carbons at δ_C 164.1 (C-1), δ_C 161.7 (C-3) and δ_C 104.2 (C-8b). These correlations indicated clearly that the aromatic proton at δ_H 5.67 is located at C-2 position. On the other hand, the *cis*-olefinic proton of the dimethylchromene bicyclic system at δ_H 6.74, showed correlations with the quaternary carbons at δ_C 102.1 (C-4) and δ_C 152.1 (C-4a), while the other *cis*-olefinic proton at δ_H 5.60 was correlated with the quaternary carbons at δ_C 102.1 (C-4) and δ_C 79.1 (C-2′). These results demonstrated clearly that the dimethylchromene moiety was fused in an angular manner to the aromatic ring A of xanthone skeleton bearing the hydroxyl group. The position of the remaining γ,γ-dimethylallyl group was established as follows. Once more, the HMBC spectrum of **1** (Fig. 1), showed that, one of the ABC spin proton at δ_H 7.28 displayed cross-peaks with carbons C-5 (δ_C 107.9) and C-1″ (δ_C 23.4), whereas the allylic proton at δ_H 3.49 of the γ,γ-dimethylallyl group gave cross-peaks with carbons C-4b (δ_C 147.0), C-5 (δ_C 107.9), C-6 (δ_C 122.2) and C-2″ (δ_C 125.1), indicating clearly that the γ,γ-dimethylallyl group was located at the C-5 position. This was further confirmed by the NOESY spectrum which showed on one hand, correlations peaks between H-2 at δ_H 5.67 and the chelated hydroxyl group at δ_H 13.07, on the other hand, between allylic proton H-1″ at δ_H 3.49 and H-6 at δ_H 7.28. On the basis of the above results, the structure of laurentixanthone A (**1**), was determined to be 6-hydroxy-3,3-dimethyl-11-(3-methylbut-2-enyl)pyrano[2,3-*c*]xanthen-7(3*H*)-one.

Compound **2**, m.p. 112–114 °C, was obtained as brown needles from cyclohexane. It reacted positively to $FeCl_3$ reagent, suggesting the presence of at least one phenolic

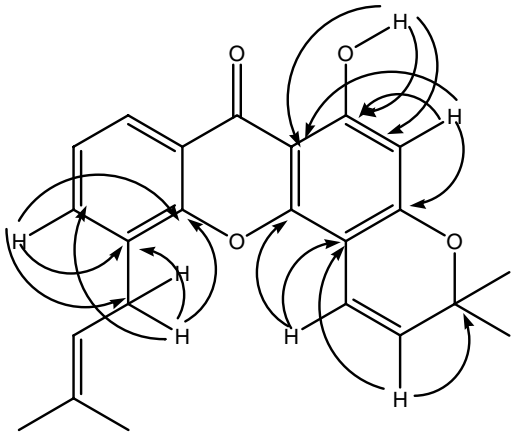


Fig. 1. HMBC correlations of compound **1**.

hydroxyl group in the molecule. Its molecular formula, $C_{17}H_{16}O_7$, was determined by high-resolution (HR) electrospray ionisation time of flight (ESI-TOF) mass spectroscopy which showed in positive mode, a pseudomolecular ion peak $[M+H]^+$ at *m*/*z* 333.0972 (calcd. for $C_{17}H_{17}O_7$, 333.0974) implying 10° of unsaturation. The broad-band proton-decoupled ^{13}C NMR spectrum of compound **2** (Table 1), sorted by APT and HSQC spectra revealed the presence of four methoxyl groups at δ_H 3.90/δ_C 61.6, δ_H 3.95/δ_C 61.7, δ_H 3.95/δ_C 62.0 and δ_H 4.10/δ_C 62.8, three sp^2 methines and 10 sp^2 quaternary carbons, one of which corresponded to a carbonyl group at δ_C 181.6. These data together with those obtained from IR spectrum [ν_{max} (C=O) 1661 $cm^{-1;}$ (ν_{max} (OH) 3212 cm^{-1}] and UV spectrum [λ_{max} (log ε): 221 (4.10), 245sh (4.32), 254 (4.41), 316 (3.85) and 375 (3.70) nm], suggesting that compound **2** possesses a xanthone skeleton in its structure (Cardona et al., 1990). Its ^{1}H NMR spectrum ($CDCl_3$) showed the presence of one proton signal at δ_H 13.07 characteristic of a chelated hydroxyl group located at *peri* position (C-1) to carbonyl group. This spectrum further exhibited typical signals of three vicinal aromatic protons of an ABC spin system characteristic of 1,2,3-trisubstituted benzene ring at δ_H 6.75/δ_C 110.7 (1H, *dd*, $J = 7.9$, 1.8 Hz), δ_H 6.90/δ_C 106.4 (1H, *dd*, $J = 7.9$, 1.8 Hz) and δ_H 7.51/δ_C 136.1 (1H, *t*, $J = 7.9$ Hz) and four three proton singlet confirming the presence of four methoxyl groups in compound **2**. From the above spectroscopic data, it appears that all the four methoxyl

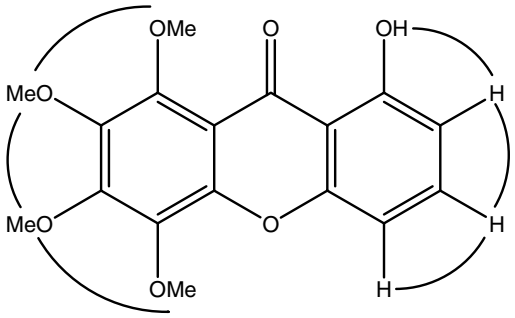
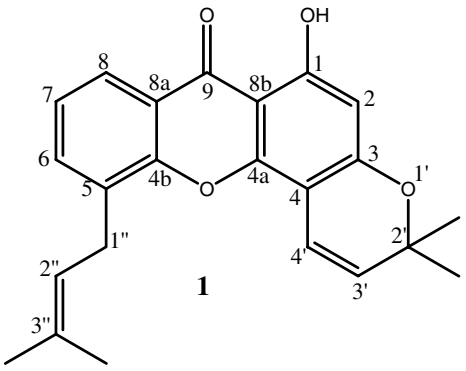
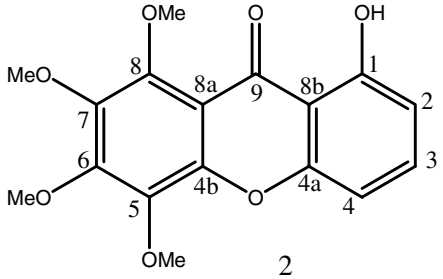


Fig. 2. NOESY correlations of compound **2**.

groups are located on ring B. This was further confirmed by correlations observed on NOESY spectrum between the different methoxyl groups as shown in Fig. 2. Thus, the structure of compound **2** was assigned to be 1-hydroxy-5,6,7,8-tetramethoxyxanthone.



1



2

Pure isolated metabolites **1** and **2** were evaluated for their antibacterial and antifungal potential against a wide range of microorganisms: Gram-positive, Gram-negative bacteria and yeasts. As shown in Table 2, compounds **1** and **2** exhibited both antibacterial and antifungal effects. Nevertheless, pronounced activity (MIC lower than 78.12 μg/ml) was observed against a limited number of microorganisms (30%) for compound **2**, whereas compound **1** appeared to be more active. A pronounced activity was observed against 60% of the tested microbial strains. However, the inhibitory effect of the two compounds against some of the tested pathogens was equal or more important than that of the reference drugs (Table 2). *Streptococcus faecalis* and *Candida gabrata* were, respectively, the most sensitive pathogens to the effects of compounds **1** and **2**.

3. Experimental

3.1. General experimental procedures

All melting points were measured on a Büchi apparatus and are uncorrected. The UV spectra were obtained on a Kontron Uvikon 932 spectrophotometer. The IR spectra were recorded on a Nicolet Impact 400D FT-IR spectrometer. The ^{1}H and ^{13}C NMR data (including HSQC, HMBC, NOESY and ^{1}H–^{1}H COSY) were measured on a Bruker

Table 2
Minimum inhibition concentration (μg/ml and μM) of **1**, **2** and reference antibiotics

Microorganisms	Tested samples					
	1		**2**		RA[a]	
Gram-negative bacteria						
Citrobacter freundii	–	–	–	–	4.88	9.0
Enterobacter aerogens	–	–	–	–	9.76	18
Enterobacter cloacae	39.06	107.9	–	–	4.88	9.0
Escherichia coli	–	–	–	–	1.22	2.25
Klebsiella pneumoniae	–	–	–	–	2.44	4.5
Morganella morgani	9.76	26.99	–	–	2.44	4.5
Proteus mirabilis	39.06	107.9	–	–	2.44	4.5
Proteus vulgaris	–	–	–	–	1.22	2.25
Pseudomonas aeruginosa	–	–	4.88	14.70	4.88	9.0
Shigella dysenteriae	4.88	13.49	–	–	2.44	4.5
Shigella flexneri	4.88	13.49	19.53	58.83	2.44	4.5
Salmonella typhi	–	–	–	–	2.44	4.5
Gram-positive bacteria						
Streptococcus faecalis	1.22	3.37	–	–	4.88	9.0
Staphylococcus aureus	–	–	–	–	4.88	9.0
Bacillus cereus	78.12	215.8	78.12	235.3	2.44	4.5
Bacillus megaterium	2.44	6.74	–	–	4.88	9.0
Bacillus stearothermophilus	4.88	13.49	–	–	4.88	9.0
Bacillus subtilis	4.88	13.49	2.44	7.35	2.44	4.5
Yeasts						
Candida albicans	78.12	215.8	19.53	58.83	4.88	5.21
Candida gabrata	2.44	6.74	0.61	1.84	4.88	5.21

No effect of the DMSO used as dilution solvent was observed on the tested microbial strains.
–, Not determined because MIC was greater than 78.12 μg/ml.
[a] RA: reference antibiotics (Gentamycin for bacteria, Nystatin for yeast).

DRX-400 instrument operating at 400 MHz for ^1H and 100 MHz for ^{13}C. The chemical shifts (δ) are reported in ppm downfield from tetramethylsilane (TMS) using TMS or the solvent signal as standard. The HRESI-TOF-MS and ESI-TOF-MS/MS were obtained in the positive ion mode on a API QSTAR pulsar mass spectrometer. Analytical thin layer chromatography (TLC) was performed on Kieselgel $60F_{254}$ precoated Al sheet (0.2 mm layer thickness, Merck) and the spots were detected by ultraviolet irradiation (254, 366 nm) and by spraying with 10% H_2SO_4 reagent.

3.2. Plant material

The roots of *V. laurentii* De Wild were collected in March 2004 at Mbalmayo, in the Centre Province of the Republic of Cameroon and identified by Mr. Nana (plant taxonomist) of *National Herbarium*, Yaounde. A voucher specimen (No. 1882/SRFK) documenting the collection has been deposited at the *National Herbarium*, Yaounde, Cameroon.

3.3. Extraction and isolation

Air-dried powder of the roots of *V. laurentii* (2 kg) was exhaustively extracted at room temperature for 48 h using a mixture of methanol/dichloromethane (1:1) by maceration. The suspension was filtered and the filtrates concentrated under reduce pressure to give 100 g of brown crude residue. This residue was subjected to flash column chromatography on silica gel (Merck, 230–400 mesh) and eluted with cyclohexane, cyclohexane/EtOAc (4:1), cyclohexane/EtOAc (1:1) and EtOAc to give four fractions labelled A (20 g), B (35 g), C (18 g) and D (10 g), respectively. Fraction B (35 g) was further subjected to column chromatography on silica gel (Merck, 70–230 mesh) and eluted with a cyclohexane/AcOEt mixture of increasing polarity. One hundred fractions of 100 ml each were collected and analysed by TLC using cyclohexane–CH_2Cl_2 (7:3) as mobile phase. Fractions 1–25, eluted with cyclohexane afforded three compounds which were identified as: 1,8-dihydroxy-3-geranyloxy-6-methylanthraquinone (300 mg), friedelin (25 mg) and stigmasterol (35 mg). Fractions 27–47, eluted with a mixture of cyclohexane/EtOAc (9:1) gave a brown residue (1.3 g) which was subjected to further column chromatography to afford laurentixanthone B (**2**) (25 mg) from sub-fractions 10–25. From sub-fractions 26–29, we obtained 1,8-dihydroxy-3-methoxy-6-methylanthraquinone (25 mg) and sub-fractions 30–40 afforded bivismiaquinone (40 mg). Finally, fractions A (20 g) eluted with cyclohexane/EtOAc (4:1) also afforded brown residue (3.5 g) which was also subjected to column chromatography to give 1,7-dihydroxyxanthone (18 mg), from sub-fractions 10–25, laurentixanthone A (**1**) (50 mg) from sub-fractions 27–37. While, sub-fractions 38–47, 50–60, 65–70, 80–100 and 108–120 were left to crystallize at room temperature to afford kaempferol (16 mg), vismiaquinone (70 mg), vismiaquinone B (50 mg), O^1-demethyl-3′,4′-deoxypsorospermin-3′,4′-diol (12 mg) and 1-hydroxy-6,7-dimethoxyxanthone (10 mg), respectively.

3.3.1. Laurentixanthone A (*1*)

Yellow needles, m.p. 156–157 °C. UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 250 (4.08), 260 (3.97), 322 (3.71) and 379 (3.42). IR ν_{max}^{MeOH} 3356, 1664, 1650, 1215, 1113 cm^{-1}. ^1H and ^{13}C NMR (see Table 1). ESI-TOF-MS *m/z* 363 $[M+H]^+$. HRESI-MS *m/z* 363.1589 (calcd. for $C_{23}H_{22}O_4$+H, 363.1596). MS/MS *m/z* (rel. int.): 347 $[M-CH_3]^+$(53), 299 $[M-C_5H_9]^+$(60), 211 $[M-CH_3-C_7H_4O_3]^+$(47), 188 $[M-CH_3-C_{10}H_7O_2]^+$(35).

3.3.2. Laurentixanthone B (*2*)

Brown needles, m.p. 112–114 °C. UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 221 (4.10), 245 sh (4.32), 254 (4.41), 316 (3.85) and 375 (3.70). IR ν_{max}^{MeOH}: 3212, 2930, 2845, 1661, 1647, 1245, 1145 cm^{-1}. ^1H and ^{13}C NMR (see Table 1). ESI-TOF-MS *m/z* 333 $[M+H]^+$. HRESI-MS *m/z* 333.0972 (calcd. for $C_{17}H_{16}O_7$+H, 333.0974).

3.4. Antimicrobial assays

3.4.1. Microbial strains

A total of 20 microbial cultures belonging to six Gram-positive bacterial species (*Bacillus cereus*, *Bacillus megaterium*, *Bacillus substilis*, *Bacillus stearothermophilus*, *Staphylococcus aureus*, *Streptococcus faecalis*), 12 Gram-negative bacteria (*Escherichia coli*, *Shigella dysenteriae*, *Proteus vulgaris*, *Proteus mirabilis*, *Shigella flexneri*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Morganella morgani*, *Enterobacter aerogens*, *Citrobacter freundii*, *Enterobacter cloacae*) and two yeasts from *Candida* species (*Candida albicans* and *Candida gabrata*) were used in this study. Three Bacillus species were provided by ''l'institut Appert de Paris'', *Bacillus cereus* by the A.F.R.C Reading Laboratory of Great Bretain. The other strains were clinical isolated from patients in the Centre Pasteur de Yaoundé-Cameroun (health institution). They were then maintained on agar slant at 4 °C in the LMP where the antimicrobial tests were performed. The strains were activated at 37 °C for 24 h on nutrient agar (NA) (bacteria) or NA supplemented with Bromocresol purple (*Bacillus* strains), Sabouraud glucose agar (fungi). The nutrient broth (NB) was used to determine, respectively, the sensitivity and the minimal inhibition concentration (MIC) of all samples against the tested pathogens.

3.4.2. MIC determination: microdilution method

The MICs of compounds **1**, **2** and reference antibiotics (RA) were evaluated against the pathogens. The inocula of microorganisms were prepared from 12 h both culture and the suspensions were adjusted to 0.5 Mc Farland turbidity. The tested samples were first dissolved in dimethylsulfoxide (DMSO), then in NB to the highest dilution of 78.12 μg/ml. Then, serial twofold dilutions were made in a concentration ranged from 0.075 to 78.12 μg/ml in the

96 wells microplate. The final concentration of DMSO was less than 1% v/v. MIC values of the tested samples against pathogens were determined based on the microdilution method (Zgoda and Porter, 2001). Gentamycin (Bacteria) and Nystatin (Yeasts) diluted prior in water were used as reference antibiotics. Negative control was made with DMSO.

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References

Abou-Shoer, M., Boettner, F.E., Chang, C.J., Cassady, J.M., 1988. Antitumour and cytotoxic xanthones of *Psorospermum febrifugum*. Phytochemistry 27, 2795–2800.

Alan, M.S., Choppra, N., Ali, M., Niwa, M., 1996. Oleanen and stigmasterol derivatives from *Ambroma augusta*. Phytochemistry 41, 1197–1200.

Araújo, F.W.L., Souza, M.P., Filho, R.B., 1990. Vismiaefolic acid, a new triterpene from *Vochysia vismiaefolia*. J. Nat. Prod. 53, 1436–1440.

Azebaze, A.G.B., Meyer, M., Bodo, B., Nkengfack, A.E., 2004. Allanxanthone B, a polyisoprenylated xanthone from the stem bark of *Allanblackia monticola* staner L.C. Phytochemistry 65, 2561–2564.

Bamps, P., 1966. Note sur les Guttiféracées d'Afrique Tropicale. Jard. Bot. Nat. Bruxelles 36, 315–319.

Bamps, P., 1970. Flore du Congo, du Rwanda et du Burundi. Jard. Bot. Nat. Bruxelles, 24–31.

Bilia, A.R., Yusuf, A.W., Braca, A., Keita, A., Morelli, I., 2000. New prenylated anthraquinones and xanthones from *Vismia guineensis*. J. Nat. Prod. 63, 16–21.

Botta, B., Delle, M.F., Delle, M.G., Marini, B.G., Oguaka, J.U., 1983. 3-Geranyloxy-6-methyl-1,8-dihydroxy-anthraquinone and vismiones C, D and E from *Psorospermum febrifugum*. Phytochemistry 22, 539–542.

Botta, B., Delle, M.G., Delle, M.F., Marini, B.G., Menchini, F., 1986. Vismione H and prenylated xanthones from *Vismia guineensis*. Phytochemistry 25, 1217–1219.

Cardona, M.L., Fernandez, I., Pedro, J.R., Serrano, A., 1990. Xanthones from *Hypericum reflexum*. Phytochemistry 29, 3003–3006.

Cassinelli, G., Geroni, C., Botta, B., Delle, M.F., Delle, M.G., 1986. Cytotoxic and antitumor activity of vismiones isolated from *Vismieae*. J. Nat. Prod. 49, 929–931.

Dos Santos, M.H., Nagem, T.J., Da silva, M.C., Silva, L.G.F., 2000. Xathones from *Vismia latifolia*. J. Braz. Chem. Soc. 8, 537–539.

Fujita, T., Da, L., Ueda, S., Takeda, Y., 1992. Xanthones from *polygala tenuifolia*. Phytochemistry 31, 3397–4000.

Goncalves, M. De L.S., Mors, W.B., 1981. Vismiaquinone, A Δ^1-isopentenyl substituted anthraquinone from *Vismia reichardtiana*. Phytochemistry 20, 1947–1950.

Gunatilaka, A.A.L., De Silva, A.M.Y.J., Sotheeswaran, S., 1982a. Minor xanthones of *Hypericum mysorense*. Phytochemistry 21, 1751–1753.

Gunatilaka, A.A.L., Nanayakkara, N.P.D., Sultanbawa, M.U.S., Balasubramaniam, S., 1982b. Friedelin, D: a-friedo-olean-3, 21-dione and 21α-hydroxy-D: a-friedo-olean-3-one from *Kokoona zeylanica*. Phytochemistry 21, 2061–2063.

Hussein, A.A., Bozzi, B., Correa, M., Capson, T.L., Kursar, T.A., Coley, P.D., Solis, P.N., Gupta, M.P., 2003. Bioactive constituents from three *Vismia* species. J. Nat. Prod. 66, 858–860.

Hutchinson, J., Dalziel, J., 1954. "Flora of west tropical Africa (revised by Keay, R.W)", vol. 1, Whitefriars, London, p. 288.

Kerharo, J.O., 1974. Pharmacopée Sénégalaise Traditionnelle. Vigot-Frères, Paris, p. 485.

Kouam, Mpondo, T.G., Lavaud, C., Massiot, G., Nuzillard, J.M., Connolly, J.D., Rycroft, D.S., 1993. Bipendensin, an unusual phenolic acetal from *Afzelia bipendensis*. Nat. Prod. Lett. 3, 299–303.

Miraglia, M.C.M., Mesquita, A.A.L., Varejaro De Jesus, M.C., Gottlieb, O.R., Gottlieb, H.E., 1981. Anthraquinones from *Vismia* species. Phytochemistry 20, 2041–2042.

Nagem, T.J., Alves, N.L., 1995. Constituents of *Vismia magnoliaefolia*. Fitoterapia 66, 278.

Nagem, T.J., De Olivera, F.F., 1997. Xanthones and other constituents of *Vismia parviflora*. J. Braz. Chem. Soc. 8, 505–508.

Nagem, T.J., Faria De Jesus, T., 1990. Quinonoid and other constituents of *Vismia martiana*. Phytochemistry 29, 3362–3364.

Nkengfack, A.E., Mkounga, P., Fomum, Z.T., Meyer, M., Bodo, B., 2002. Globulixanthones A and B, two new cytotoxic xanthones with isoprenoid groups from the root bark of *Symphonia globulifera*. J. Nat. Prod. 65, 734–736.

Seo, E., Wani, M.C., Wall, M.E., Navarro, H., Mukherjee, R., Farnsworth, N.R., Kinghorn, A.D., 2000. New bioactive aromatic compounds from *Vismia guianensis*. Phytochemistry 55, 35–42.

Wu, Q.L., Wang, S.P., Du, L.J., Yang, J.S., Xiao, P.G., 1998. Xanthones from *Hypericum japonicum* and *H. Henryi*. Phytochemistry 49, 1395–1402.

Yimdjo, M.C., Azebaze, A.G.B., Nkengfack, A.E., Meyer, M., Bodo, B., 2004. Antimicrobial and cytotoxic agents from *Calophyllum inophyllum*. Phytochemistry 65, 2789–2795.

Zgoda, J.R., Porter, J.R., 2001. A convenient micro-dilution method for screening natural products against bacteria and fungi. Pharmaceut. Biol. 39, 221–225.