Two xanthones and two rotameric (3→8) biflavonoids from the Cameroonian medicinal plant *Allanblackia floribunda* Oliv. (Guttiferae)

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Abstract

Two xanthones, 2-(3-hydroxy-3,3-dimethyldihydroallyl)-dihydro-6-deoxyisojacareubin (**1**) and dihydro-6-deoxyjacareubin (**2**), and two 3→8 rotameric biflavonoids, (2*R*,3*S*)-volkensiflavone-7-*O*-*β*-acetylglucopyranoside (**3**) and (2*S*,3*S*)-morelloflavone-7-*O*-*β*-acetylglucopyranoside (**4**), together with fifteen known compounds, were isolated from a dichloromethane/methanol (1/1, *v/v*) extract of the bark of the plant *Allanblackia floribunda*. The structures of the new compounds were elucidated by NMR spectroscopy and mass spectroscopic techniques and those of the known ones were deduced by comparison with data reported in the literature. The isolated biflavonoids were obtained as mixtures of conformers exhibiting duplicate NMR signals in solution at 25 °C and their respective absolute configurations were assigned using circular dichroism spectroscopy. Some of the isolated compounds were assessed for their antibacterial and antioxidant properties.

1. Introduction

Allanblackia floribunda Oliver belongs to the Guttiferae family and is widely distributed along the coastal regions of West Africa. In folk medicine, different parts of this plant are either used alone or in combination with other plants for the treatment of several human ailments including upper respiratory tract infections, dysentery, diarrhoea, and toothache.[1] Biological and pharmacological investigations of extracts of this plant have enabled a better understanding of their use in traditional medicine. For instance, the antitumor, radical scavenging, antimycobacterial, antibacterial and antifungal activities of the root bark extract of *A. floribunda* have been reported.[2] Investigations of this plant have led to the isolation of a series of secondary metabolites belonging to different classes including triterpenoids,[3] benzophenones,[4] xanthones[5] and biflavonoids.[2,4] Some of these compounds exhibit a wide range of biological and pharmacological activities including antioxidant, antitumor,[2] cytotoxic,[5] anti-inflammatory, antimicrobial and antifungal activities,[6,7] as well as HIV inhibitory activity.[8] As part of our continuing search for biologically active compounds from Cameroonian medicinal plants,[9–12] we have investigated the bark of *A. floribunda* for its minor secondary metabolites and herein report the isolation, structural elucidation and biological activities of two new xanthones (**1** and **2**) and two new rotameric biflavonoids (**3** and **4**).

2. Results and Discussion

The dichloromethane/methanol extract of the stem bark of *A. floribunda* was subjected to repeated column chromatography to give several fractions, which were further purified over sephadex LH–20 and preparative HPLC to yield a total of nineteen compounds of which compounds **1**–**4** were unknown.
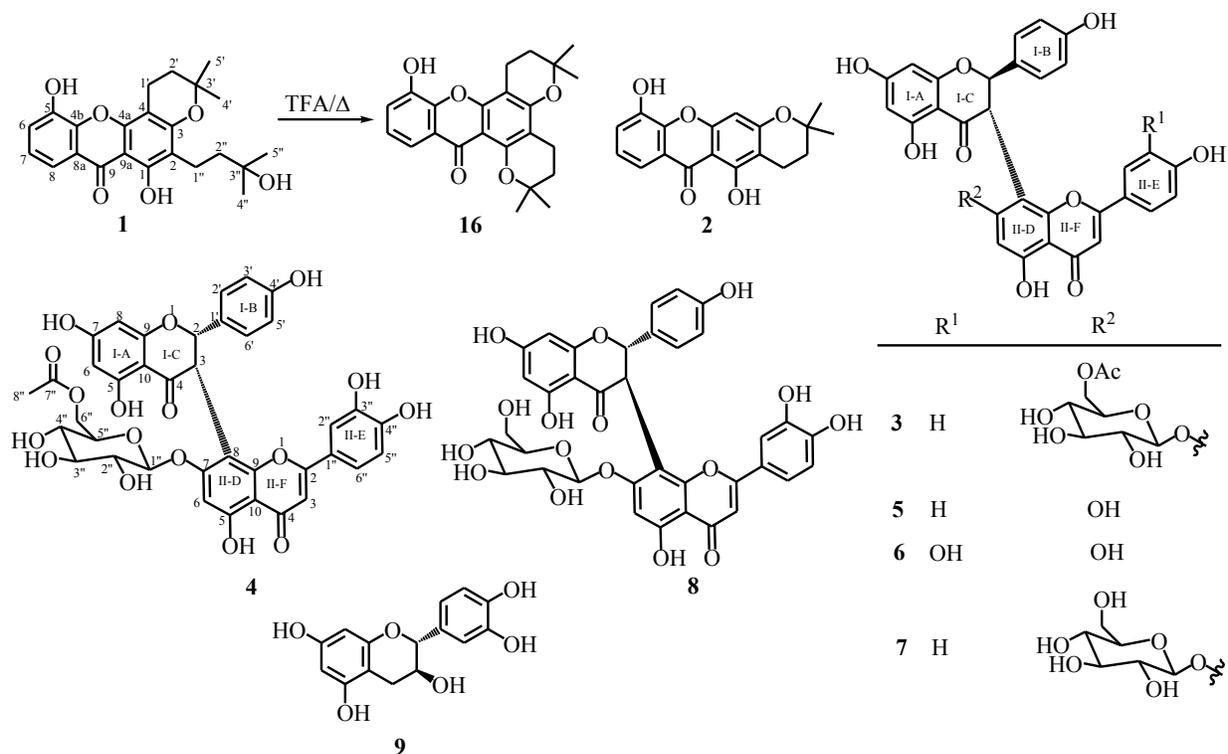
Figure 1. Chemical structures of compounds **1−9** and **16**.

Compound **1** was obtained as a yellow solid (m.p. 186–187 °C), which reacted positively to FeCl₃ reagent. Its molecular formula $C_{23}H_{26}O_6$ was established from the positive ion mode HRESIMS, which showed ion clusters [M+H]⁺ at *m/z* 399.1802 (calcd. for $C_{23}H_{27}O_6$: 399.1808). The IR spectrum showed strong absorptions for hydroxyl and conjugated carbonyl groups at 3400 and 1640 cm⁻¹ respectively, while the UV spectrum exhibited characteristic absorption bands of xanthones at λ_{max} 234 and 250 nm.[13] The ¹H NMR spectrum of compound **1** displayed an ABC spin system at δ_H 7.73 (*dd*, 1.8; 7.9 Hz), 7.27 (*dd*, 1.8; 7.9 Hz) and 7.22 (*pseudo t*, 7.9 Hz) assignable to a 1,2,3-trisubstituted benzene ring. The 2,2-dimethyldihydropyran moiety was deduced from the signals at δ_H 2.93 and 1.76 (2H each, *t*, 7.9 Hz) and δ_H 1.37 (6H, *s*) which was further confirmed by the ¹³C NMR spectrum with resonances at δ_C 72.7, 41.3, 29.8 and 16.4. This 2,2-dimethyldihydropyran group was attached at C-3 and C-4 of the xanthone skeleton of compound **1** as illustrated by HMBC correlations (Fig. 2) observed between the methylene protons at δ_H 2.93 (H-1') with the carbon signals at δ_C 158.8 (C-3); 152.0 (C-4a); 107.9 (C-4); 72.7 (C-3′) and 41.3 (C-2′). On the other hand, the presence of a 3-hydroxy-3-methylbutyl group was also deduced by the presence of signals at δ_H 2.77 and 1.77 (2H each, *t*, 7.0 Hz) and 1.40

(6H, *s*) which were further confirmed in the ^{13}C NMR spectrum with resonances at δ_C 72.7, 41.3, 29.8 and 16.4 respectively. Thus, HMBC correlations between the methylene protons at δ_H 2.77 (H-1″) and the carbon signals at δ_C 158.8 (C-3); 103.7 (C-2); 76.2 (C-3″) and 31.7 (C-2″) clearly identified the point of attachment at C-2 of the xanthone skeleton. Based on the above investigations, structure **1** was named 2-(3-hydroxy-3,3-dimethyldihydroallyl)-dihydro-6-deoxyisojacareubin (Fig. 1). The proposed structure was fully supported by HMBC, DEPT and COSY spectra. Key HMBC correlations of compounds **1** are illustrated in Fig. 2. Furthermore, compound **1** was heated at reflux in TFA for 30 min. The resulting product, bispyranoxanthone (**16**) allowed additional confirmation of the proposed structure for **1**.
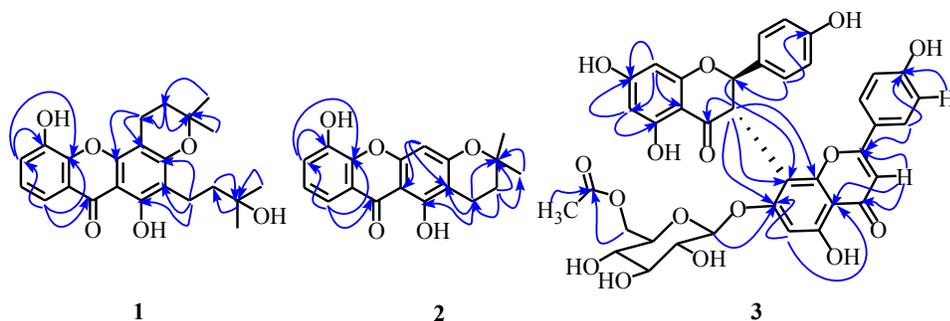

Figure 2. Key HMBC correlations of compounds **1**–**3**.

Table 1. ^{13}C and ^{1}H NMR data of compounds **1** and **16** (CDCl$_3$) at 25 °C.

C and H no.	1		16	
	^{13}C (176 MHz)	^{1}H (500 MHz)	^{13}C (176 MHz)	^{1}H (700 MHz)
	δ_C (m)	δ_H (nH, J in Hz)	δ_C (m)	δ_H (nH, J in Hz)
1	158.2 (C)	-	154.2 (C)	-
2	103.7 (C)	6.19 (1H, s)	105.8 (C)	6.19 (1H, s)
3	158.8 (C)	-	157.8 (C)	-
4	107.9 (C)	-	99.6 (C)	-
4a	152.0 (C)	-	153.9 (C)	-
4b	144.6 (C)	-	143.5 (C)	-
5	145.5 (C)	-	144.1 (C)	-
6	119.9 (CH)	7.27 (dd, 1.8; 7.9)	119.0 (CH)	7.28 (1H, dd, 1.5, 8.0)
7	123.6 (CH)	7.22 (pseudo t, 7.9)	123.8 (CH)	7.23 (1H, pseudo t, 8.0)
8	116.0 (CH)	7.73 (dd, 1.8; 7.9)	117.9 (CH)	7.64 (1H, dd, 1.5, 8.0)
8a	121.0 (C)	-	106.2 (C)	-
9	181.3 (C)	-	176.1 (C)	-
9a	102.6 (C)	-	102.0 (C)	-
1′	16.4 (CH$_2$)	2.93 (2H, t, 7.9)	16.7 (CH$_2$)	2.93 (2H, t, 6.7)
2′	41.3 (CH$_2$)	1.76 (2H, t, 7.9)	31.7 (CH$_2$)	1.93 (2H, t, 6.7)
3′	72.7 (C)	-	76.3 (C)	-
4′	29.8 (CH$_3$)	1.37 (3H, s)	26.9 (CH$_3$)	1.42 (3H, s)
5′	29.8 (CH$_3$)	1.37 (3H, s)	26.9 (CH$_3$)	1.42 (3H, s)
1″	16.2 (CH$_2$)	2.77 (2H, t, 7.0)	17.0 (CH$_2$)	2.65 (2H, t, 6.7)
2″	31.7 (CH$_2$)	1.87 (2H, t, 7.0)	31.5 (CH$_2$)	1.84 (2H, t, 6.7)
3″	76.2 (C)	-	76.0 (C)	-
4″	26.9 (CH$_3$)	1.40 (3H, s)	26.6 (CH$_3$)	1.45 (3H, s)
5″	26.9 (CH$_3$)	1.40 (3H, s)	26.6 (CH$_3$)	1.45 (3H, s)

Compound **2**, named dihydro-6-deoxyjacareubin, was obtained as an orange solid (m.p. 190–192 °C) which also reacted positively to $FeCl_3$ reagent. Its molecular formula $C_{18}H_{16}O_5$ was established from the positive ion mode HRESIMS, which showed ion clusters $[M+H]^+$ at *m/z* 313.1072 (calcd. for $C_{18}H_{17}O_5$: 313.1076). The IR spectrum showed strong absorptions for hydroxyl and conjugated carbonyl groups at 3400 and 1640 cm^{-1} respectively, while the UV spectrum exhibited absorption maxima characteristic of xanthones at λ_{max} 234 and 250 nm.[13] The ^1H and ^{13}C NMR spectra of compound **2** showed similarities with those of compound **1** and additionally displayed two methylene signals at δ_H 2.75 and 1.87 (2H each, *t*, 6.7 Hz) together with a 6-proton singlet at δ_H 1.40 suggesting the presence of a 2,2-dimethyldihydropyran moiety. Furthermore, an ABC spin system at δ_H 7.78 (1H, *dd*, 1.5; 8.0 Hz), 7.32 (*dd*, 1.5; 8.0 Hz) and 7.25 (*pseudo t*, 8.0 Hz) was suggestive of a 1,2,3-trisubstituted benzene ring. The ^{13}C NMR spectrum of **2** displayed 18 carbon signals which were assigned in combination with DEPT and HSQC experiments to two methyls, two methylenes, four methines and ten quaternary carbons, including a carbonyl signal at δ_C 180.7 ppm. The presence of a 2,2-dimethyldihydropyran moiety was further confirmed by signals in the ^{13}C NMR spectrum at δ_C 76.6 (C-3′), 31.7 (C-2′), 26.8 (C-4′/C-5′) and 16.0 (C-1′). The HMBC spectrum proved pivotal in order to attach this 2,2-dimethyldihydropyran moiety to the xanthone skeleton. Thus, correlations between the methylene protons at δ_H 2.75 (H-1′) with the carbon signals at δ_C 160.9 (C-1); 102.6 (C-9a); 76.6 (C-3′) and 31.7 (C-2′) suggested that C-1′ (δ_C 16.0) was attached to C-2 (δ_C 104.6) of the xanthone skeleton. In addition, correlations of the aromatic proton signal at δ_H 6.38 (1H, *s*, H-4) with carbon signals at δ_C 161.8 (C-3); 154.7 (C-4a); 104.6 (C-2) and 102.6 (C-9a) were also crucial in confirming the 2,2-dimethyldihydropyran moiety being attached at positions 2 and 3 of the xanthone skeleton. Moreover, the position of the chelated hydroxyl (δ_H 13.22) group at C-5 was assigned based on the correlations observed between the aromatic proton at δ_H 7.78 (1H, *dd*, 1.5; 8.0, H-8) with the carbonyl group signal at δ_C 180.7. The proposed structure of compound **2** (Fig. 2) was fully supported by HMBC, DEPT and COSY spectra. Its physical and spectroscopic data were consistent with those of a known synthetic compound.[14] To the best of our knowledge, compound **2** is herein reported for the first time from a natural source.

Table 2. ^{13}C and ^1H NMR data of compound **2** (CDCl$_3$) at 25 °C.

C and H no.	^{13}C (176 MHz) δ_C (m)	^1H (500 MHz) δ_H (nH, J in Hz)
1	160.9 (C)	-
2	104.6 (C)	-
3	161.8 (C)	-
4	94.9 (CH)	6.38 (1H, s)
4a	154.7 (C)	-
4b	144.1 (C)	-
5	144.2 (C)	-
6	119.9 (CH)	7.32 (1H, dd, 1.5, 8.0)
7	123.7 (CH)	7.25 (1H, pseudo t, 8.0)
8	116.9 (CH)	7.78 (1H, dd, 1.5, 8.0)
8a	121.1 (C)	-
9	180.7 (C)	-
9a	102.6 (C)	-
1'	16.0 (CH$_2$)	2.75 (2H, t, 6.7)
2'	31.7 (CH$_2$)	1.87 (2H, t, 6.7)
3'	79.6 (C)	-
4'	26.8 (CH$_3$)	1.40 (3H, s)
5'	26.8 (CH$_3$)	1.40 (3H, s)

Compound **3** was obtained as a yellow solid, and had the molecular formula C$_{38}$H$_{32}$O$_{16}$ as established from low resolution FAB-MS (negative mode) and HRFAB-MS (positive mode) techniques in which the molecular ions appeared at *m/z* 743.3 [M–H]$^+$ and 745.1781 [M+H]$^+$ (calcd. for C$_{38}$H$_{33}$O$_{16}$: 745.1769), respectively. The IR spectrum of compound **3** showed strong absorption bands around 3400 and 1700 cm^{-1} indicating the presence of hydroxyl and carbonyl groups, respectively. Although the prep. HPLC and LC-MS chromatograms indicated this compound to be pure, both its ^1H and ^{13}C NMR spectra exhibited doubled sets of signals, suggesting the presence of two conformers. The ^1H NMR spectrum displayed characteristic signals of a sugar moiety (acetylglucopyranosyl) with the anomeric proton signal at δ_H 5.24 (H-1‴) and a multiplet of non-anomeric protons in the up field region (δ_H 3.70–3.48) along with a two-proton triplet of an oxymethylene entity at δ_H 4.36 ppm. The ^{13}C NMR spectrum displayed signals for 38 carbons, which were sorted by DEPT and HSQC techniques into one methyl, one methylene, nineteen methines and seventeen quaternary carbons, including signals for three carbonyls at δ_C 172.6, 184.0 and 197.3 ppm (Table 4). Compounds with similar spectral data

have been previously reported from the plants of the genus *Garcinia* of the Guttiferae family.[15–18] Therefore, comparison of the spectroscopic data of compound **3** with those described in the literature enabled us to deduce that it was a monoglycosylated biflavonoid derivative presenting doubled NMR signals. It is noteworthy that the doubled signals in the NMR spectra of biflavonoids at room temperature may be due to the fact that the molecules adopt different conformations[16] which arise from the restricted free rotation of the 3→8 interflavanyl bond.[17] Nevertheless, these NMR signals can be merged into a single set of signals at higher temperatures because under these conditions, the molecules do not exist in a preferred stable conformation.[19] However, the structural elucidation of compound **3** based on its NMR data recorded at ambient temperature was not straightforward.

In the ^{1}H NMR spectrum, except for a pair of doublets at δ_H 5.96 and 5.97 (1H each, *d*, 1.6 Hz, H-6, H-8) assignable to two *meta*-coupled protons of ring I-A of the flavanone moiety of both the major and minor conformers, the ^{1}H NMR resonances of the major conformer were mostly observed in the deshielded aromatic region (δ_H 6.40–7.80) whereas, signals appearing in the up-field regions were assigned to the sugar moiety. The ^{1}H NMR spectrum of compound **3** also displayed characteristic signals of flavones at δ_H 6.45 (1H, *s*, H-II-3) and δ_H 5.71 and 5.33 (1H each, *d*, 12.0 Hz, H-I-2, H-I-3) indicating the presence of both moieties in the molecule. The coupling constant of 12.0 Hz indicates a biaxial (*trans*) configuration of the two protons of ring I-C. In addition, characteristic signals for two separate AA'BB' systems were observed at δ_H 7.73 and 6.91 (2H each, *d*, 8.8 Hz, H-3″/5″, H-2″/6″) for one system and δ_H 7.02 and 6.34 (2H each, *d*, 8.4 Hz, H-2′/6′, H-3′/5′) assignable to the two 1,4-disubstituted aromatic ring systems of the flavone (ring II-E) and flavanone (ring I-B) moiety, respectively. The correlations of these sets of protons *viz*. H-2″/6″ with H-3″/5″and H-2′/6′with H-3′/5′ were fully supported by their ^{1}H–^{1}H COSY correlations (see ESI) both for the major and the minor conformers. A one proton singlet assignable to H-6 of each of the flavone units was evident in the ^{1}H NMR spectrum. Interestingly, signals attributable to an acetyl group were observed in the ^{1}H and ^{13}C NMR spectra at $\delta_{H/C}$ 1.95/20.7 (CH$_3$) and 172.7 (CH$_3$$\underline{C}$=O). The complete assignment of the two conformers of compound **3**, as presented in Tables 3 and 4 respectively, was facilitated by the HSQC and HMBC spectra. The acetylglucopyranosyl moiety was linked at position C-7 of the flavone unit and deduced by correlations observed in the HMBC spectrum between the anomeric proton at δ_H 5.24 with carbon signals at δ_C 161.6 (C-7) and δ_C 100.0 (C-6) of ring D (Fig. 2).

Moreover, the proton signal at δ_H 5.33 (H-3) of the flavanone unit displayed HMBC correlations with carbon signals at δ_C 105.0 (C-8), 156.8 (C-9) and 161.6 (C-7) of the flavone, which suggested that the biflavonoid linkage between the two units was *via* the C-3 and C-8 positions.

The ^1H–^1H NOESY spectrum was useful in determining the spatial interactions of the protons. The protons at δ_H 7.73 (H-2″/6″, ring E) showed cross peaks with those at δ_H 7.02 (H-2′/6′, ring B), 6.66 (H-6, ring D) and 5.33 (H-3, ring C), indicating that these rings are spatially close. In addition a careful study of the NOESY spectrum illustrated that, the protons at δ_H 7.73 (II-2″/6″) of the major conformer displayed correlations with protons δ_H 7.63 (II-2″/6″) of the minor conformer. This abnormal NOE is due to the exchange of conformations of the two conformers,[20] and revealed that the 3→8 biflavonoids are not merely a simple mixture of two conformers but represented a conformationally semi-stable isomeric structure.

The relative configuration of the two stereogenic centres (C-2, C-3) in compound **3** was determined as follows. The large value of the coupling constant (12.0 Hz) between H-2 and H-3 in the ^1H NMR spectrum of the flavanone unit suggested their biaxial (*trans*) orientation. Furthermore, as demonstrated by Gaffield[21] using a CD spectrum, a positive Cotton effect due to the $\pi \rightarrow \pi^*$ transition near 290 nm is more reliable for determining of the stereochemistry at C-2 than the Cotton effect at a higher wavelength (*ca.* 300–340 nm) for the $n \rightarrow \pi^*$ transition. Therefore, since the experimental CD spectrum (see ESI) of compound **3** showed a positive Cotton effect at 292 nm ($\pi \rightarrow \pi^*$ transition), it suggested a 2*β*-orientation of the B-ring and hence a *2R*-absolute configuration at C-2 in the flavanone unit. This, in combination with the above described ^1H-NMR results permitted assignment of the 2*R*,3*S*-configuration to compound **3**. Hence, the structure of **3** was determined to be (2*R*,3*S*)-volkensiflavone-7-*O*-*β*-acetylglucopyranoside.

Compound **4** was obtained as a yellow solid, and had the molecular formula $C_{38}H_{32}O_{17}$ as established by low resolution FAB-MS (negative mode) and HRFAB-MS (positive mode) techniques in which the molecular ions appeared at *m/z* 759.4 [M–H]$^+$ and 761.1732 [M+H]$^+$ (calcd. for $C_{38}H_{33}O_{17}$: 761.1718), respectively. The LC-MS chromatogram showed a single peak indicating this compound to be pure. However, the ^1H and ^{13}C NMR spectra of compound **4** exhibited similarities to those of compound **3** and again presented doubled signals. The chemical structure of compound **4** was deduced by comparison to that of compound **3**. Its molecular ion as shown by FABMS differed by 16 mass units from that of compound **3**, suggesting compound **4**

to have an additional hydroxyl group. Similarities were observed in the spectra of both compounds and particularly for the flavanone moiety. The main differences observed in the ^{1}H-NMR spectra of compounds **3** and **4** are as follows: a pair of one proton doublets in the non-aromatic region observed at δ_H 5.76 and 5.34 assignable to protons H-2 and H-3 of the flavanone unit had a coupling of 7.6 Hz indicating the monoaxial (*cis*) configuration of these protons. In addition, both AA'BB' systems observed in the flavone unit of compound **3** were not observed in **4**. Only one AA'BB' system was observed. The ^{1}H-NMR spectrum of **4** exhibited signals of three distinct protons at 7.28 (1H, *d*, 8.4 Hz, H-2″), δ_H 6.89 (1H, *d*, 8.4 Hz, H-3″) and 7.33 (1H, *s*, H-6″). Besides this, slight differences were also observed in the chemical shifts of some protons as shown in Table 3. The ^{13}C NMR spectrum also showed duplicate signals of 38 carbons which were sorted by DEPT 135, 90 and broad band ^{13}C NMR spectroscopy into one methyl, one methylene, eighteen methines and eighteen quaternary carbons. The complete assignment of ^{13}C and ^{1}H NMR for compound **4** is given in Table 3. The acetylglucopyranosyl moiety was similarly located at C-7 of ring D in the flavone unit as evidenced by the ^{1}H–^{13}C HMBC correlations of the anomeric proton at δ_H 5.24 with C-7 (δ_C 161.5) and C-6 (δ_C 99.9) of the D-ring (Fig. 2).

The absolute configuration about the two asymmetric carbons (C-2, C-3) in compound **4** was determined as follows. The ^{1}H NMR spectral data displayed a smaller *J* value of 7.6 Hz between protons H-2 and H-3 suggesting their monoaxial (*cis*) configuration and thus the absolute configuration in compound **4** could be (2*R*,3*R*) or (2*S*,3*S*).[22] Furthermore, Gaffield[21] reported that, in the CD spectrum of flavanone glycosides possessing racemic aglycones, 2*S*-flavanones generally show a positive Cotton effect at 245–270 nm and a negative Cotton effect at 225–240 nm. The experimental CD spectrum (see ESI) of compound **4** showed a negative Cotton effect at 240 nm, suggesting a *2S*-absolute configuration of C-2 in the flavanone unit. Moreover, the stereochemistry of C-3 relative to C-2 of the flavanone unit was determined by applying Cahn–Ingold–Prelog sequence rules according to which C-3 was assigned as 3*S*. Hence, by considering the known spectral properties, the structure of compound **4** was established as (2*S*,3*S*)-morelloflavone-7-*O*-*β*-acetylglucopyranoside.

Table 3. ^{1}H NMR data of the major ([a]A) and minor ([b]B) conformers of compounds **3** and **4** at 25 °C in CD$_3$OD, 400 MHz.

	Flavanone unit (I)					Flavone unit (II)			
	3		**4**			**3**		**4**	
H no.	[a]A	[b]B	[a]A	[b]B	H no.	[a]A	[b]B	[a]A	[b]B
I-2	5.71 (*d*, 12.0)	5.77 (*d*, 12.0)	5.76 (*d*, 7.6)	5.73 (*d*, 7.6)	II-3	6.45 (*s*)	6.64 (*s*)	6.41 (*s*)	6.41 (*s*)
I-3	5.33 (*d*, 12.0)	4.83 (*d*, 12.0)	5.34 (*d*, 7.6)	5.34 (*d*, 7.6)	II-6	6.66 (*s*)	6.52 (*s*)	6.66 (*s*)	6.66 (*s*)
I-6	5.96 (*d*, 1.6)	5.96 (*d*, 1.6)	5.93 (*d*, 2.0)	5.93 (*d*, 2.0)	II-2″	7.73 (*d*, 8.8)	7.63 (*d*, 8.8)	7.28 (*d*, 8.4)	7.28 (*d*, 8.4)
I-8	5.97 (*d*, 1.6)	5.97 (*d*, 1.6)	5.96 (*d*, 2.0)	5.96 (*d*, 2.0)	II-3″	6.91 (*d*, 8.8)	6.71 (*d*, 8.8)	6.89 (*d*, 8.4)	6.89 (*d*, 8.4)
I-2′	7.02 (*d*, 8.4)	7.11 (*d*, 8.0)	7.05 (*d*, 8.4)	7.12 (*d*, 8.4)	II-5″	6.91 (*d*, 8.8)	6.71 (*d*, 8.8)	-	-
I-3′	6.34 (*d*, 8.0)	6.61 (*d*, 8.4)	6.35 (*d*, 8.4)	6.61 (*d*, 8.4)	II-6″	7.73 (*d*, 8.8)	7.63 (*d*, 8.8)	7.33 (*s*)	7.33 (*s*)

	Acetylglucopyranosyl unit			
	3		**4**	
H no.	[a]A	[b]B	[a]A	[b]B
1″	5.24 (*d*, 7.6)	5.24 (*d*, 7.6)	5.24 (*d*, 7.6)	5.24 (*d*, 7.6)
2″	3.56 (*t*. 7.6)	3.56 (*t*. 7.6)	3.56 (*t*. 7.6)	3.56 (*t*. 7.6)
3″	3.70 (*t*, 7.6)	3.70 (*t*, 7.6)	3.70 (*t*, 7.6)	3.70 (*t*, 7.6)
4″	3.47 (*t*, 4.4)	3.47 (*t*, 4.4)	3.47 (*t*, 7.6)	3.47 (*t*, 7.6)
5″	4.19 (*dd*, 12.0; 4.4)	4.19 (*dd*, 12.0; 4.4)	4.17 (*dd*, 12.0; 7.6)	4.17 (*dd*, 12.0; 7.6)
6″a	4.36 (*t*, 12.0)	4.36 (*t*, 12.0)	4.36 (*t*, 11.6)	4.36 (*t*, 11.6)
6″b	4.23 (*t*, 12.0)	4.23 (*t*, 12.0)	4.23 (*dd*, 12.0; 6.8)	4.23 (*dd*, 12.0; 6.8)
8″	1.95 (*s*)	1.95 (*s*)	1.95 (*s*)	1.91 (*s*)

Sets A and B are respectively in an intensity ratio of (~1: 0.85)
[a]A represents the major conformer at 25 °C.
[b]B represents the minor conformer at 25 °C.

Table 4. ^{13}C NMR data of the major ([a]A) and minor ([b]B) conformers of compounds **3** and **4** at 25 °C in CD$_3$OD, 400 MHz.

	Flavanone unit (I)					Flavone unit (II)			
	3		**4**			**3**		**4**	
C no.	[a]A	[b]B	[a]A	[b]B	C no.	[a]A	[b]B	[a]A	[b]B
I-2	82.7 (CH)	84.0	82.8 (CH)	84.0	II-2	166.5 (C)	166.3	166.2 (C)	166.7
I-3	49.7 (CH)	51.1	51.1 (CH)	51.1	II-3	103.6 (CH)	104.4	103.5 (CH)	104.4
I-4	197.3 (C)	198.2	197.3 (C)	198.2	II-4	184.0 (C)	184.1	183.9 (C)	183.9
I-5	169.5 (C)	169.5	165.7 (C)	169.5	II-5	162.3 (C)	162.3	162.6 (C)	162.6
I-6	96.8 (CH)	96.8	97.7 (CH)	96.8	II-6	100.0 (CH)	101.7	99.9 (CH)	99.9
I-7	168.9 (C)	168.9	168.5 (C)	168.5	II-7	161.6 (C)	161.6	161.5 (C)	161.5
I-8	96.6 (CH)	96.6	96.6 (CH)	96.6	II-8	105.0 (C)	105.0	104.8 (C)	103.2
I-9	164.8 (C)	165.0	164.8 (C)	164.8	II-9	156.8 (C)	155.7	155.7 (C)	156.7
I-10	103.2 (C)	103.2	103.2 (C)	103.2	II-10	106.7 (C)	107.4	106.6 (C)	106.6
I-1′	129.3 (C)	130.5	130.4 (C)	130.4	II-1″	122.8 (C)	122.7	123.0 (C)	123.2
I-2′	129.1 (CH)	130.3	129.2 (CH)	130.3	II-2″	129.8 (CH)	129.5	120.8 (CH)	120.1
I-3′	115.4 (CH)	116.0	115.4 (CH)	116.0	II-3″	117.1 (CH)	117.0	116.9 (CH)	116.4
I-4′	159.0 (C)	158.4	158.4 (C)	159.0	II-4″	163.0 (C)	162.7	151.2 (C)	151.2
I-5′	115.4 (CH)	116.0	115.4 (CH)	116.0	II-5″	117.1 (CH)	117.0	146.8 (C)	147.4
I-6′	129.1 (CH)	130.3	129.2 (CH)	130.3	II-6″	129.8 (CH)	129.5	114.3 (CH)	114.5

	Acetylglucopyranosyl unit			
	3		**4**	
C no.	[a]A	[b]B	[a]A	[b]B
1″	101.3 (CH)	102.5	101.2 (CH)	101.2
2″	71.6 (CH)	71.3	71.3 (CH)	71.6
3″	74.7 (CH)	74.7	75.2 (CH)	74.7
4″	78.1 (CH)	77.9	78.1 (CH)	77.9
5″	75.6 (CH)	75.5	75.6 (CH)	75.5
6″	64.5 (CH$_2$)	64.7	64.5 (CH$_2$)	64.7
7″	172.6 (C)	172.8	172.7 (C)	172.7
8″	20.7 (CH$_3$)	20.7	20.7 (CH$_3$)	23.3

Sets A and B are respectively in an intensity ratio of (~1: ~0.8).
[a]A represents the major conformer at 25 °C.
[b]B represents the minor conformer at 25 °C.

The known compounds were identified as volkensiflavone (**5**)[24–26], morelloflavone (**6**),[2,4,15,27] volkensiflavone-7-*O*-*β*-glucopyranoside (**7**),[17] morelloflavone-7-*O*-*β*-glucopyranoside (**8**),[2,17] (–)-catechin (**9**),[28] 1,5-dihydroxyxanthone (**10**),[5,9] 1,7-dihydroxyxanthone (**11**),[2,3,29] allanxanthone A (**12**),[5] 6-deoxyisojacareubin (**13**),[3] hydrocotoin (**14**),[4] betulinic acid (**15**)[30] an inseparable mixture of *β*-sitosterol and stigmasterol[31] and, an inseparable mixture of *β*-sitosteryl- and stigmasteryl-3-*O*-*β*-glucopyranosides.[5] The absolute configurations of compounds **5–8** were determined as (2*R*,3*S*), (2*R*,3*S*), (2*S*,3*R*) and (2*S*,3*R*) respectively. To the best of our knowledge, this is the first time the absolute configurations of these biflavonoids are reported here.

Results of the antibacterial and antioxidant assays of some of the isolated compounds are presented in Table 5. In these assays, compounds **5** and **6** (see experimental) showed weak antibacterial activity against the gram negative bacterium *Proteus mirabilis* with MIC values of 64 µg/mL while compound **6** weakly inhibited *Enterococcus faecalis* (MIC = 64 µg/mL). However, their glycosylated derivatives (**3** and **4**) did not display any notable activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis*. Although xanthones have been reported to have good antibacterial activity,[32] the two new isolated xanthones (**1** and **2**) did not display any inhibitory activity against the tested bacteria. Furthermore, compounds **4**, **6**, **7** and **9** displayed remarkable DPPH radical scavenging activities with IC_{50} values of 9.15, 4.84, 9.99, and 4.23 µg/mL respectively. These findings were in agreement with previously reported antioxidant properties of flavonoids.[33] It is noteworthy that these same four compounds exhibited greater antioxidant activity than the standard, ascorbic acid and could thus be considered as leads for the development of novel antioxidant drugs. Taken in the context of this particular investigation, these results support the use of this plant in folk medicine.

Table 5. Antibacterial and antioxidant activities of some isolated compounds.

| Compounds | Antibacterial activity MIC (µg/mL) | | | | | Antioxidant activity IC$_{50}$ (µg/mL) |
	EC	PA	SA	EF	PM	
1	-	-	-	-	-	> 100
2	-	-	-	-	-	> 100
3	-	128	128	-	-	429.53±0.23
4	-	-	-	-	128	9.15±0.47
5	128	128	128	128	64	71.92±0.35
6	128	128	128	64	64	4.84±0.62
7	-	-	-	-	128	> 100
8	128	-	-	-	128	9.99±0.15
9	-	-	-	-	-	4.23±0.52
10	128	-	-	-	-	> 100
11	128	-	-	-	-	> 100
12	-	-	-	-	-	27.07±0.09
13	-	-	-	-	-	> 100
14	-	-	-	-	-	> 100
15	-	-	-	-	-	> 100
16	-	-	-	-	-	> 100
Ascorbic acid	nd	nd	nd	nd	nd	10.55±0.37
Ciprofloxacin	2	0.5	1	4	2	nd

- = >128 µg/mL; EC= *Escherichia coli* (ATCC 25922)*;* PA= *Pseudomonas aeruginosa* (ATCC 27853)*;*
SA= *Staphylococcus aureus* (ATCC BAA1026); EF= *Enterococcus faecalis (*ATCC 29212);
PM = *Proteus mirabilis* (isolate); nd = not determined.

3. Conclusion

The phytochemical study of *A. floribunda* Oliver yielded four new compounds; two xanthones and two flavanone–flavone dimers which were isolated as an inseparable mixture of two conformers. NMR analyses of the flavanone–flavone dimers displayed doubled signals in their ^{1}H and ^{13}C spectra and were consistent with earlier reported data for 3→8 rotameric biflavonoids.[17] The isolated compounds did not display any notable antibacterial activity, while some exhibited antioxidant properties. Thus the new biflavonoid (**4**) displayed good antioxidant activity with an IC$_{50}$ value of 9.15 µg/mL and further modification of its structure might lead to potentially useful antioxidant agents. The antioxidant activities of the isolated compounds may explain the usefulness of the extracts of this plant in folk medicine.

4. Experimental section

4.1 General experimental procedures

Silica gel 60 (0.230–0.400 mm) and (0.040–0.063 mm) was used as adsorbents for flash and column chromatography respectively. The semi-pure compounds were finally purified successively over Sephadex LH-20 (bead size 25–100 μm, Sigma-Aldrich) with CH_2Cl_2–MeOH (1/1, v/v), reverse (JAIGEL–ODS H80, serial no. 209963) and normal (JAIGEL–SIL, D-60-10, serial no. 051300228) phase preparative HPLC. The specifications of these columns were 250 mm length×20 mm inner diameter; $4\mu m$ particle size and 80 Å pore size. Merck TLC plates (silica gel 60 F_{254}) were used for the detection of the purity of compounds. Melting points were determined with Gallekamp apparatus. UV spectra were recorded using a PerkinElmer Lambda 25 UV/Vis spectrometer. IR spectra were recorded in KBr on a PerkinElmer 2000 FT-IR spectrophotometer. EI/ESI mass spectra were recorded on an Agilent 5975C MSD and Thermo Finnigan MAT95XL mass spectrometers at the Hussein Ebrahim Jamal Research Institute of Chemistry (HEJ–RIC) of the International Centre for Chemical and Biological Sciences (ICCBS), University of Karachi (UOK), Karachi Pakistan. The NMR experiments of pure compounds were performed in different deuterated solvents depending on their solubility, using Bruker Ascend 400, 500 and 600 MHz (^{1}H and ^{13}C) spectrometers equipped with a Bruker 5 mm Broadband probe (depending on the amount). Chemical shifts (δ) in ppm are referenced to tetramethylsilane (TMS) at 0.00 ppm for ^{1}H and ^{13}C. Coupling constants are expressed in hertz (Hz).

4.2. Plant material

The stem bark of *A. floribunda* Oliver was collected in November 2014 in the Kye-Ossi, Ntem valley Division in the South region of Cameroon. The sample was identified by Mr. Victor Nana, a retired botanist at the National Herbarium of Cameroun, Centre region, where a voucher specimen was deposited as voucher No: 52904/HNC.

4.3. Extraction and isolation

The air-dried and ground stem bark (2.2 kg) of *A. floribunda* was extracted at room temperature with a mixture of CH_2Cl_2/MeOH (1/1, *v/v*) for 48 h and concentrated to a viscous black residue (556 g). A part of this residue (550.0 g) was then subjected to flash chromatographic separation over a silica gel (230–400 mesh) column using a stepwise gradient of *n*-Hex/EtOAc (ranging from 0 to 100% of EtOAc, *v/v*), followed by a gradient of EtOAc/MeOH (ranging from 9/1 to 8/2, *v/v*), to afford a total of 58 fractions (*f*r₁–*f*r₅₈) of ca.

1000 mL per fraction. Fraction fr_{15} (eluted with n-Hex/EtOAc 9/1) precipitated to give compound **10** (100 mg, yellow solid). Further precipitation of the remaining aliquot of this same fraction gave an inseparable mixture of phytosterols (180 mg, white powder). Fraction fr_{22} (n-Hex/EtOAc 8/2, v/v) precipitated to afford compound **12** (6 mg, yellow solid). Fractions fr_{48}, fr_{50}, fr_{51} and fr_{53} obtained with pure EtOAc, were mass-precipitated to give four different yellow amorphous powders labelled **Y₅** (0.5 g), **Y₆** (3.4 g), **Y₇** (2.1 g) and **Y₈** (0.4 g) respectively. All four impure powders were subsequently separately purified over reverse phase preparative HPLC (column JAIGEL–ODS H80) using a mixture of acetonitrile/water: 1/1 + 0.08% of TFA as eluent, with the following settings: UV sensitivity 0.05; RI sensitivity 50; initial & final flow rates: 3 & 4 mL/min; initial & final pressures: 47 & 64 psi. Consequently, compounds **5** (75 mg), **6** (100 mg), **7** (25 mg) and **8** (105 mg) were eluted with retention times of 22, 18, 14 and 12 min respectively. All 58 fractions (fr_1–fr_{58}) from the flash chromatography were combined into 4 main fractions (A–D) on the basis of their TLC analyses. Fraction A (fr_1–fr_7: 850 mg) obtained with pure n-Hex as eluent, consisted of fatty acids and was not investigated further. Fraction B (fr_8–fr_{33}: 38.7 g) obtained with n-Hex/EtOAc (8/2–4/6, v/v), was subjected to further column chromatography over silica gel (0.040–0.063 mm) and eluted with a gradient of n-Hex/EtOAc (9.5/0.5–0/10, v/v) to produce 100 fractions (B_1–B_{100}) of ca. 500 mL each which were combined on the basis of TLC analysis. The first 28 fractions from fraction B (B_1–B_{28}), were eluted with a mixture of n-Hex/EtOAc (9.5/0.5, v/v) and combined on the basis of their TLC profiles into two sub-fractions. The first sub-fraction b_{15}–b_{20} (200 mg) was rechromatographed over Sephadex LH-20 (CH_2Cl_2/MeOH (1/1, v/v) and then over normal phase prep. HPLC, using an isocratic mode solvent of n-Hex/EtOAc (8.8/1.2, v/v), to afford compound **14** (2.5 mg, orange oil) with a retention time of 44 min. The second sub-fraction b_{21}–b_{28} (150 mg) showed a complex mixture of oils and was not further investigated. The second series of fractions from fraction B (B_{29}-B_{40}) was eluted with n-Hex/EtOAc (9/1, v/v) and combined, then was further chromatographed and eluted on normal phase preparative HPLC, using an isocratic mode of solvent n-Hex/EtOAc (8.5/1.5, v/v) to afford compound **2** (0.7 mg, orange solid), at a retention time of 20 min. The third series of fractions from fraction B (B_{41}-B_{43}) (30 mg) was eluted with n-Hex/EtOAc (8/2, v/v), then rechromatographed and eluted on normal phase preparative HPLC, using an isocratic mode of solvent n-Hex/EtOAc (7/3, v/v) to afford compound **1** (12 mg, yellow solid), at a retention time of 25 min. The fourth series of fractions from fraction B (B_{44}-B_{45}) (88 mg) was

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rechromatographed over Sephadex LH-20 (CH$_2$Cl$_2$/MeOH (1/1, *v/v*), to afford compound **11** (18 mg, yellow solid). The fifth series of fractions from fraction B (B$_{52}$–B$_{59}$) (10 mg) was further chromatographed and eluted on normal phase preparative HPLC, using an isocratic mode of solvent *n*-Hex/EtOAc (7.5/2.5, *v/v*) to afford compound **13** (1.8 mg, yellow solid), at a retention time of 44 min. From the sixth series of fractions from fraction B (B$_{60}$–B$_{77}$), a white solid compound **15** (60 mg, white neat solid) was obtained. The last series of fractions obtained from fraction B (B$_{78}$–B$_{100}$) eluted with *n*-Hex/EtOAc (7/3–2/8, *v/v*) was found to be a complex mixture of compounds and was not further investigated.

Fraction C (*f*r$_{34}$–*f*r$_{57}$: 137 g), eluted with *n*-Hex/EtOAc 3/7–1/9, *v/v*) from the flash chromatography was shown to contain the already purified compounds **5**; **6** and **8** and therefore was not further investigated.

A part of fraction D (*f*r$_{58}$: 80 g; pure EtOAc) was subjected to further chromatographic separations over sephadex LH-20 using CH$_2$Cl$_2$/MeOH (1/1) as eluent to afford eighteen sub-fractions (D$_1$–D$_{18}$). Sub-fraction D$_9$ (1.8 g) was subjected to silica gel column chromatography and eluted with an isocratic system of *n*-Hex/EtOAc (6/4, v/v) to afford a mixture of glycosylated sterols (105 mg). Sub-fractions D$_5$ (150 mg), D$_{16}$ (200 mg) and D$_{17}$ (300 mg) were separately purified on a reverse phase prep. HPLC using isocratic solvents to afford compounds **9** (5 mg, orange oil), **3** (15 mg, yellow solid), and **4** (18.8 mg, yellow solid) respectively.

4.3.1. *2-(3-hydroxy-3,3-dimethyldihydroallyl)-dihydro-6-deoxyisojacareubin* (**1**). Yellow solid from Hex/EtOAc 7/3, m.p. 186–187 °C; UV (MeOH) – λ_{max} nm (PDA): 234, 250, 269, 330 nm; HRESIMS *m/z* 399.1802 (calcd. for C$_{23}$H$_{27}$O$_6$: 399.1808). For ^{1}H and ^{13}C NMR data, see Table 1.

4.3.2. *dihydro-6-deoxyjacareubin* (**2**). Orange solid from Hex/EtOAc 8.5/1.5, m.p. 190–192 °C; UV (MeOH) – λ_{max} nm (PDA): 234, 250, 269, 330 nm; HRESIMS [M+H]$^+$ at *m/z* 313.1072 (calcd. for C$_{18}$H$_{17}$O$_5$: 313.1076). For ^{1}H and ^{13}C NMR data, see Table 2.

4.3.3. *(2R,3S)-volkensiflavone-7-O-β-acetylglucopyranoside* (**3**). Yellow solid from EtOAc fraction; m.p. 241–243 °C; $[\alpha]^{25}_D$ = 0° (*c* = 0.067, MeOH); UV (MeOH) – λ_{max} nm (log $\mathcal{E}$): 229 (3.91), 292 (3.80), 324 (3.73); IR (KBr): ν_{max} = 3418, 2927, 1728, 1645, 1604, 1510, 1451, 1370, 1243, 1172, 1082, 834, 742, 622, 526 cm^{-1}; HRFESIMS *m/z* 745.1781 [M+H]$^+$ (calcd. for C$_{38}$H$_{33}$O$_{16}$: 745.1769). For ^{1}H and ^{13}C NMR data, see Tables 3 & 4.

4.3.4. (2S,3S)-morelloflavone-7-O-β-acetylglucopyranoside (**4**). Yellow solid from EtOAc; m.p. 245–247 °C; $[\alpha]^{20}_D = 0°$ (*c* = 0.046, MeOH); UV (MeOH) – λ_{max} nm (log $\mathcal{E}$): 222 (4.31), 230 (4.39), 287 (4.36), 291 (4.36), 344 (4.18); IR (KBr): ν_{max} = 3384, 1726, 1643, 1603, 1515, 1452, 1369, 1262, 1169, 1082, 834, 741, 630, 559, 526 cm^{-1}; HRESIMS *m/z* 761.1732 [M+H]$^+$ (calcd. for $C_{38}H_{33}O_{17}$: 761.1718). For ^{1}H and ^{13}C NMR data, see Tables 3 and 4.

4.3.5. *Conversion of* (**1**) *to* (**16**)

Compound **1** (10 mg) was dissolved in trifluoroacetic acid (2 mL) and was heated under reflux for 30 min. The resulting mixture was separated and purified by prep. HPLC with *n*-Hex/EtOAc (7/3, *v/v*) to afford compound **16** (7.4 mg, 74%), at a retention time of 22 min. Compound **16** was obtained as a pale yellow oil and identified as 1,5-dihydroxy-1,2,3,4-bis(2,2-dimethyldihydropyrano)xanthone. HRESIMS [M+H]$^+$ at *m/z* 381.1702 (calcd. for $C_{23}H_{25}O_5$: 381.1702). For ^{1}H and ^{13}C NMR data, see Table 1.

4.3.6. *Assays for antibacterial and antioxidant activities*

The antibacterial activity was evaluated using the broth microdilution method by determining the minimum inhibitory concentration (MIC) values against the five bacteria strains viz., *Escherichia coli* (ATCC 25922)*; Pseudomonas aeruginosa* (ATCC 27853)*; Staphylococcus aureus (*ATCC BAA1026)*; Enterococcus faecalis (*ATCC 29212)*; Proteus mirabilis* (isolate). The antioxidant capacity of the compounds was evaluated based on the principle of scavenging the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical.

Conflict of Interest

The authors declare no conflict of interest.

References and notes

1. Raponda-Walker, A.; and Sillans, R. "Les Plantes Utiles du Gabon". Paul Lechevalier, **1961**, Paris VI.

2. Kuete, V.; Azebaze, A.G.; Mbaveng, A.; Nguemfo, E.L.; Tshikalange, E.T.; Chalard P.; and Nkengfack, A.E. *Pharm. Biol*. **2011**, *49*, 57–65.

3. Azebaze, A.G.B.; Ouahouo, B.M.W.; Vardamides, J.C.; Valentin, A.; Kuete, V.; Acebe L.; Beng, V.P.; Nkengfack, A.E.; and Meyer, M. *Nat. prod. Res*. **2008**, *22*, 333–341.

4. Locksley, H.D.; and Murray, I.G. *J. Chem. Soc*. **1971**, 1332–1340.

5. Nkengfack, A.E.; Azebaze, G.A.; Vardamides, J.C.; Fomum, Z.T.; and van Heerden, F.R. *Phytochemistry* **2002**, *60*, 381–384.

6. Nagem, T.J.; and Peres V. *Phytochemistry* **1997**, *44*, 199–214.

7. Peres, V.; Nagem, T.J.; and de Oliveira, F.F. *Phytochemistry* **2000**, *55*, 683–710.

8. Blunt, J.W.; Boswell, J.L.; Boyd M.; Cardellina, II. J.H.; and Fuller R.W. *J. Nat. Prod*. **1999**, *62*, 130–132.

9. Kouam, S.F.; Yapna, D.B.; Krohn, K.; Ngadjui, B.T.; Ngoupayo, J.; Choudhary, M.I.; and Schulz, B. *J. Nat. Prod*. **2007**, *70*, 600−603.

10. Kouam, S.F.; Njonkou, Y.L.N.; Kuigoua, G.M.; Ngadjui, B.T.; Green, I.R.; Schulz, B.; and Krohn, K. *Phytochem. Lett*. **2010**, *3*, 185–189.

11. Tchamgoue, J.; Hafizur, M.R.; Tchouankeu, J.C.; Kouam, F.S.; Adhikari, A.; Hameed, A.; Green, R.I.; and Choudhary, M.I. *Phytochem Lett*. **2016**, *17*, 181–186.

12. Happi, M.G.; Kouam, S.F.; Talontsi, F.M.; Hartmut, L.; Zühlke, S.; Ngadjui, T.B.; and Spiteller, M. *Fitoterapia* **2018**, *124*, 17−22.

13. Ngouela, S.; Zelefack, F.; Lenta, B.N.; Ngouamegne, E.T.; Tchamo, D.N.; Tsamo, E.; and Connolly, J.D. *Nat Prod Res*. **2005**, *19*, 685–688.

14. Locksley, H. D.; Quillinan, A. J.; and Scheinmann, F. *J. Chem. Soc*. **1971**, 3804–38140.

15. Muharni; Elfita; and Amanda. *Indo J. Chem*. **2011**, *11*, 169–173.

16. Messi, B.B.; Ndjoko-Ioset, K.; Hertlein-Amslinger, B.; Lannang, M.A.; Nkengfack, A.E.; Wolfender, J.-L.; Hostettmann, K.; and Bringmann G. *Molecules* **2012**, *17*, 6114-6125.

17. Jamila N.; Khairuddean M.; Khan S.N.; and Khan N. *Magn. Reson. Chem*. **2014**, 52, 345–352.

18. Ito, T.; Yokota, R.; Watarai, T.; Mori, K.; Oyama, M.; Nagasawa, H.; Matsuda, H.; and Iinuma, M. *Chem. Pharm. Bull.* **2013**, *61*, 551–558.

19. Li, X-C.; Joshi, A.S.; Tan, B.; ElSohly, H.N.; Walker, L.A.; Zjawiony, J.K.; and Ferreira, D. *Tetrahedron* **2002**, *58*, 8709–8717.

20. Hatano, T.; and Hemingway, R.W. *J. Chem. Soc. Perkin Trans*. **1997**, *2*, 1035–1043.

21. Gaffield, W. *Tetrahedron* **1970**, *26*, 4093–4108.

22. Hosoi, S.; Shimizu, E.; Ohno, K.; Yokosawa, R.; Kuninaga, S.; Coskun, M.; and Sakushima, A. *Phytochem. Anal*. **2006**, *17*, 20–24.

24. Herbin, G.A.; Jackson, B.; Locksley, H.D.; Scheinmaim F.; and Wolstenholme, W.A. *Phytochemistry* **1970**, *9*, 221.

25. Joshi, B.S.; Kamat, V. N.; and Viswanathan, N. *Phytochemistry* **1970**, *9*, 881–888.

26. Sukpondma, Y.; Rukachaisirikul, V.; and Phongpaichit, S. *J. Nat. Prod.* **2005**, *68*, 1010–1017.

27. Hutadilok-Towatana, N.; Kongkachuay, S.; and Mahabusarakam, W. *Nat. Prod. Res.*, **2007**, *21*, 655–662.

28. Jin, W.; and Tu, P.-F. *J. Chromatogr.* **2005**, 1019*, 241.

29. Azebaze, A.G.B.; Teinkela, J.E.M.; Nguemfo, E.L.; Valentin, A.; Dongmo, A.B.; and Vardamides, J.C. *Afri Health Sci.* **2015**, *15*, 835–40.

30. Okoli, B.J.; Ndukwe, G.I.; Habila, J.D.; Lawson, L.; and Jummai, A.T. *Int. J. Chem. St*. **2016**, *4*, 55–62.

31. Luhata, L.P.; and Munkombwe, N.M. *JIPBS.* **2015**, *2*, 88–95.

32. Fouotsa, H.; Lannang, A.M.; Dzoyem, J.P.; Tatsimo, S.J.; Neumann, B.; Mbazoa C.D.; Razakarivony, A.A.; Nkengfack, A.E.; Eloff, J.N.; and Sewald N. *Planta Med*. **2015**, *81*, 594–599.

33. Pietta P.G. *J. Nat. Prod*. **2000**, *63*, 1035–1042.