Triterpenoid saponins and other glycosides from the stems and bark of Jaffrea xerocarpa and their biological activity

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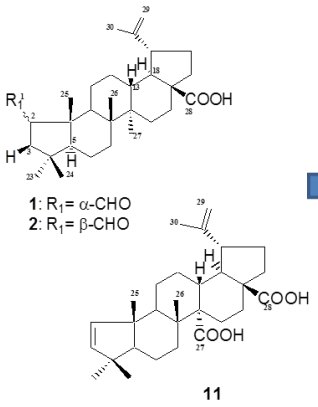
Jaffrea xerocarpa



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Antioxidant activity of the hydromethanolic extract (EC_{50} = 73 µg/mL)

Antimicrobial activity of **1** against *S. aureus* and *E. faecalis* (MIC 4 µg/mL)

Cytotoxic activity against a KB cell line of **11** (IC_{50} 2.6 µM) and **1**-**2** and **12** (IC_{50} from 7.0 to 8.5 µM)

Highlights

► Five dammarane triterpenoid glycosides were isolated from *Jaffrea xerocarpa*.

► Three *nor*lupane triterpenoids were isolated from *Jaffrea xerocarpa*.

► The hydromethanolic extract showed good antioxidant activity (DPPH assay)

► A *nor*lupane triterpenoid showed antibacterial activity.

► Three lupane triterpenoids showed good cytotoxic activity against KB cells.

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ABSTRACT:

Six previously undescribed triterpenoid saponins and two previously undescribed *nor*lupane triterpenes were isolated with five known saponins, three known lupane derivatives, 17,20-didehydro-20-deoxyjujubogenin**,** rutin, (±) 3α-*O*-*β*-D-glucopyranosyl-lyoniresinol, (±) 4-*O*-*β*-D-glucopyranosyl-maesopsin, three phenol glycosides, and uridine from the stems and bark of *Jaffrea xerocarpa* (Baill.) H. C. Hopkins & Pillon (= Basionym *Alphitonia xerocarpus* Baill.) (Rhamnaceae), an endemic tree of New Caledonia. The chemical structures of the purified compounds were identified by nuclear magnetic resonance and mass spectrometry. The isolated compounds were tested for their antioxidant, antityrosinase, antibacterial and cytotoxic activities. The aqueous methanol extract showed antioxidant activity (DPPH assay) due to the presence of rutin and other phenolic compounds. Three lupane triterpenes showed good cytotoxic activities against KB cells line (IC_{50} from 7.7 to 8.5 µM). The previously undescribed 2*α*-formyl-A(1)norlup-20(29)-en-28-oic acid showed antibacterial activity against *Staphylococcus aureus* and *Enterococcus faecalis* with both MIC values of 4 µg/mL.

Keywords: *Jaffrea xerocarpa*, *Alphitonia xerocarpus*; Rhamnaceae; stem; bark; triterpenoids; dammarane; *nor*lupane

INTRODUCTION

New Caledonia is a global biodiversity "hotspot", characterized by 77.8 % of endemic plants. The flora comprises 126 families of dicotyledons, with 490 genera and 2491 species from which 2108 species (84.5% of the total) and 77 genera (15.7%) are endemic. The 77 endemic genera belong to 36 families and comprise 366 species, only 17.3% of the total (Morat *et al.*, 2012). Ten species are included in the Rhamnaceae family (Munzinger *et al.* 2016), three of which are *Alphitonia* species (*A. neocaledonica* (Schltr.) Guillaumin, *A. xerocarpus* Baill. and *A. erubescens* Baill.) (Guillaumin, 1948). Recently, molecular phylogenetic and morphological data show that two of these species firstly described by Baillon (1876), *A. xerocarpus* and *A. erubescens*, are misplaced. Consequently, a new genus, *Jaffrea* H. C. Hopkins & Pillon, was described, and these species were renamed as *Jaffrea xerocarpa* (Baill.) H. C. Hopkins & Pillon and *Jaffrea erubescens* (Baill.) H. C. Hopkins & Pillon (Hopkins *et al.*, 2015). Members of this new genus have a conical hypanthium, petals somewhat incurved at anthesis, a thick disc that is either ± lumpy or annular but not or only partly covering the semi-inferior ovary, and fruits that are ovoid-ellipsoid, strongly beaked and tardily dehiscent. *Jaffrea* can be distinguished from *Alphitonia* s.s., in which the seeds often persist on the receptacle after dehiscence (Hopkins *et al.*, 2015). *Jaffrea xerocarpa* (Baill.) H. C. Hopkins & Pillon (= Basionym *Alphitonia xerocarpus* Baill) is a shrub or small forest tree widely distributed on the main island of Grande Terre, growing on the ultramafic substrates of New Caledonia at an altitude of 800-900 meters (Baillon, 1876). In a continuation of the study of New-Caledonian species (Muhammad *et al.*, 2015, Muhammad *et al.*, 2016), we investigated the specialized metabolite profile of *J. xerocarpa* stem and bark. A recent study on *Alphitonia neocaledonica* leaves and fruits showed the presence of flavonoids, betulinic acid, alphitolic acid, corosolic acid, and (+) gallocatechin (Lin *et al.*, 1995, Muhammad *et al.*, 2015). A previous study on *A. xerocarpus* (= *J. xerocarpa*) leaves from New-Caledonia, showed the presence of thirteen triterpenoid saponins, two *nor*lupane triterpenoids (ceanothic acid and 29-hydroxyceanothic acid) and four flavonoids (Muhammad *et al.*, 2016). The triterpenoid saponins are lupane or dammarane saponins, including derivatives of jujubogenin and 16,17-*seco*dammarane. In addition *in vitro* cytotoxic, anti-inflammatory, and antimicrobial activity (Dzuback *et al.*, 2006, Muhammad *et al.*, 2015, Muhammad *et al.*, 2016) of the isolated compounds were measured. Turning our attention to the stems and bark of *J. xerocarpa*, a further eight previously undescribed (**1-8**), and sixteen known (**9-24**) compounds were found, eight of which (**10-11**, **13-18**), were previously isolated from the leaves (Muhammad *et al.*, 2016). The radical scavenging ability of the extracts was investigated, as well as the tyrosinase inhibitory activity, the cytotoxic activity against KB cells and the antibacterial activity of some of the isolated compounds.

2. Results and discussion

The powdered bark of *Jaffrea xerocarpa* was macerated and extracted successively with petroleum ether and EtOAc and then refluxed with a mixture of CH_3OH-H_2O (8:2) to give three extracts. The EtOAc extract was fractionated by silica gel column chromatography to give a previously undescribed lupane triterpene (**1**) as the major compound, together with the known ceanothic acid (**10**) (Kundu *et al.*, 1989, Jou *et al.*, 2004), ceanothenic acid (**11**) (Jou *et al.*, 2004, Ji *et al.*, 2012) previously isolated from the leaves (Muhammad *et al.*, 2016), and alphitolic acid (**12**) (Lee *et al.*, 2003) (Fig. 1).

The powdered stems of *J. xerocarpa* were refluxed with a mixture of CH_3OH-H_2O (8:2) to give the aqueous methanol extract. This extract was subjected to multiple chromatographic steps over silica gel and RP-C_{18} yielding eight previously undescribed compounds (**1**-**8**) with the aglycon of compounds **4**-**8**, the 17,20-didehydro-20-deoxyjujubogenin (**9**), isolated for the first time alone, and fifteen known compounds (**10**-**24**). All compounds were identified by extensive spectroscopic methods including 1D- (^{1}H and ^{13}C) and 2D-NMR (COSY, TOCSY, *J*-modulated HSQC, HMBC and ROESY) experiments as well as HRESIMS analysis and by comparison with spectral data from the literature values for the known compounds. The known compounds from the stems were identified as three lupane triterpenes, ceanothic acid (**10**) (Kundu *et al.*, 1989), ceanothenic acid (**11**) (Jou *et al.*, 2004), and alphitolic acid (**12**) (Lee *et al.*, 2003), five saponins previously isolated from the leave, 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-[*β*-D-glucopyranosyl-(1→3)]-α-L-arabinopyranosyljujubogenin (**13**) (Okamura *et al.*, 1981), 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-[4-*O*-(sodium sulfonato)-*β*-D-glucopyranosyl-(1→3)]-*α*-L-arabinopyranosyljujubogenin (**14**) (Muhammad *et al.*, 2016), 3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→3)-[*α*-L-rhamnopyranosyl-(1→2)]-*α*-L-arabinopyranosyljujubogenin (**15**) (Wang *et al.*, 2013), 3-*O*-*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-*α*-L-arabinopyranosyljujubogenin (**16**) (Muhammad *et al.*, 2016), and 3-*O*-*α*-L-arabinopyranosyl-(1→6)-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→3)-[*α*-L-rhamnopyranosyl-(1→2)]-*α*-L-arabinopyranosyljujubogenin (**17**) (Muhammad *et al.*, 2016) (Fig. 1), and rutin (**18**) (Lallemand *et al.*, 1977, Li *et al.*, 2008), a flavonoid also previously isolated from the leaves (Muhammad *et al.*, 2016). Other specialized metabolites isolated from the stems include three known phenol glycosides, 1-*O*-*β*-D-glucopyranosyl-4-(8-hydroxyethyl)-2-methoxyphenyl (**19**) (Kuo and Shue, 1991), 1-*O*-*β*-D-glucopyranosyl-5-(8-hydroxyethyl)-phenyl (**20**) (Sugiyama and Kikuchi, 1992), and 1-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranosyl-3-methoxy-4-hydroxyphenyl (**21**) (Chang and Case, 2005), a lignan, (±) 3α-*O*-*β*-D-glucopyranosyl-lyoniresinol (**22**), (±)-4-*O*-*β*-D-glucopyranosyl-maesopsine (**23**) (Yoshikawa *et al.*, 1998), and uridine (**24**) (Pretsch *et al.*, 1989) (Fig. 1).

Acid hydrolysis of the aqueous methanol extract afforded four sugar units in the aqueous layer, identified by HPLC analysis on a chiral column (Lopes and Gaspar, 2008, Muhammad *et al.*, 2016), as D-glucose (Glc), D-xylose (Xyl), L-arabinose (Ara) and L-rhamnose (Rha).

Compound **1** was obtained as a white amorphous powder. The positive HRESIMS spectrum of **1** showed a pseudomolecular ion peak at *m/z* 477.3339 ([M+Na] $^{+}$, calcd for $C_{30}H_{46}O_3Na$, 477.3345) corresponding to the molecular formula $C_{30}H_{46}O_3$. The ^{1}H NMR spectrum of **1** showed signals of a lupane triterpene characterized by six tertiary methyl groups at δ_H 0.96 (6H, H_3-26 and H_3-27), 0.97 (H_3-24), 0.99 (H_3-25), 1.12 (H_3-23), and 1.70 (H_3-30), an exomethylene group at δ_H 4.64 (d, J = 2.2, 1.5 Hz, H_a-29) and 4.75, d, J = 2.2 Hz, H_b-29), and an aldehyde group at δ_H 9.84 (d, J = 3.5 Hz). Its ^{13}C NMR spectrum exhibited 30 carbon signals including an aldehyde group (δ_C 205.3), a carboxyl group (δ_C 181.9), and an exomethylene (δ_C 109.9 and 150.2) (Table 1). Analysis of the COSY, *J*-modulated HSQC and HMBC spectra and comparison of these data with the literature revealed that the spectroscopic data of **1** were similar to those of zizyberanalic acid (Kundu *et al.*, 1989). The only difference lay in the absence of a hydroxyl group attached to C-3. This was readily confirmed by COSY correlations between the proton signal of the aldehyde (δ_H 9.84, H-1) and the proton signal at δ_H 2.58 (dd, J = 7.8, 3.5 Hz, H-2) and between H-2 and the methylene protons H-3 at δ_H 1.81 (dd, J = 14.5, 7.8 Hz), and 1.91 (dd, J = 14.5, 0.9 Hz). Furthermore, the HMBC spectrum exhibited correlations between the aldehyde proton H-1 and the carbons C-2 (δ_C 61.5) and C-3 (δ_C 39.1), and from C-3 to H_3-23 and H_3-24. These data were similar to zizyberanal acid possessing an aldehyde at C-1 and no oxygenation at C-3 (Guo *et al.*, 2009). The relative configuration of C-2 for **1** was further suggested by the ROESY spectrum, wherein rOe effects were displayed between H-2 and the β-axial methyl H-24 and H-25, indicating the β-orientation of H-2 and the α-orientation of the aldehyde group as in zizyberanal acid (Guo *et al.*, 2009). Thus the structure of compound **1** was deduced as 2α-formyl-A(1)*nor*lup-20(29)-en-28-oic acid.

Compound **2** had the same molecular formula $C_{30}H_{46}O_3$ as **1** [HRESIMS: *m/z* 477.3329 [M+Na]$^{+}$, calcd for $C_{30}H_{46}O_3Na$, 477.3345]. The ^{1}H NMR and ^{13}C NMR spectra of **2** showed signals very similar to those of compound **1**. The differences lay in the coupling constants and chemical shifts of signals on the ring A suggesting another stereochemistry for the aldehyde group (Table 1). The relative configuration of C-2 for **2** was deduced from rOe effect between H-1 (δ_H 9.73 (d, J = 4.7 Hz)), and H-25, indicating the β-orientation of H-1 as in zizyberanalic acid (Kundu *et al.*, 1989). Full assignments of the proton and carbon resonances of compound **2** were achieved by analysis of the COSY, *J*-modulated HSQC and HMBC spectra. Thus compound **2** is 2β-formyl-A(1)*nor*lup-20(29)-en-28-oic acid.

Compound **3** had the molecular formula $C_{54}H_{86}O_{25}$ deduced from the positive HRESIMS spectrum [*m/z* 1157,5349 [M+Na]$^{+}$, calcd for $C_{54}H_{86}O_{25}Na$, 1157,5356]. The ^{1}H NMR spectrum of the aglycone of **3**

showed signals of a lupane triterpenoid characterized by six tertiary methyl groups (δ_H 0.94, 1.01, 1.02, 1.09, 1.10 and 1.71), an exomethylene group (δ_H 4.61 and 4.73, each *brs*), and an oxymethine (δ_H 4.10, *brs*). Its ^{13}C NMR spectrum exhibited 30 carbon signals including two carboxyl groups (δ_C 174.5 and 177.4), an exomethylene (δ_C 108.8 and 150.4), and an oxymethine (δ_C 84.5) (Table 2). Analysis of the COSY, *J*-modulated HSQC and HMBC spectra and comparison of these data with the literature revealed that the aglycone was ceanothic acid (Jou *et al.*, 2004). The shielded chemical shift of C-28 suggested a monodesmosidic saponin. Analysis of the ^{1}H and ^{13}C NMR spectra of **3** revealed the presence of four anomeric protons at δ_H 5.62 (d, *J*=7.9 Hz), 5.04 (d, *J*=7.4 Hz), 4.73 (d, *J*=7.8 Hz), and 4.59 (d, *J*=7.8 Hz) correlated in the *J*-modulated HSQC spectrum with anomeric carbons at δ_C 92.0, 101.2, 103.4 and 103.4, respectively (Table 2). Analysis of the COSY, TOCSY and *J*-modulated HSQC spectra of **2** allowed complete assignment of the four glycosidic proton and carbon systems leading to five *β*-D-glucopyranose units (Agrawal, 1992) (Table 2). The anomeric configurations were confirmed by the rOe effects observed between the α-axial protons H-1/H-3 and H-1/H-5 in each sugar unit. The three anomeric carbons at δ_C 101.2 and 103.4 indicated that these carbons were involved in ether linkages while the anomeric carbon at δ_C 92.0 (δ_H 5.62) is linked by an ester bond. This was confirmed by the HMBC correlations between Glc-H-1' (δ 5.62) and C-28 (δ 174.5). Other HMBC correlations between Glc-H-1" (δ 5.04) and Glc-C-2' (δ 76.4), Glc-H-1"' (δ 4.73) and Glc-C-2" (δ 81.2), and Glc-H-1"" (δ 4.59) and Glc-C-3" (δ 86.2) revealed the sequence of the tetrasaccharide moiety with the second glucose unit disubstituted in positions C-2" and C-3". These data compared to the pentaglucoside ceanothic acid, 28-*O*-*β*-D-glucopyranosyl-(1→6)-[*β*-D-glucopyranosyl-(1→3)]-[*β*-D-glucopyranosyl-(1→2)]-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosylceanothic acid, isolated from the leaves (Muhammad *et al.*, 2016), showed the loss of a glucopyranosyl moiety at C-6" of the second glucose unit in **3**. Thus the structure of saponin **3** was deduced as 28-*O*-*β*-D-glucopyranosyl-(1→3)-[*β*-D-glucopyranosyl-(1→2)]-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosylceanothic acid.

Comparison of the ^{1}H NMR and ^{13}C NMR spectra of compounds **4-8** with compound **9** indicated that they all possess the same genin (**9)**, 17,20-didehydro-20-deoxyjujubogenin, glycosylated at C-3 (δ_C 88.2) (Pawar *et al.*, 2007) (Table 3). The ^{1}H- and ^{13}C-NMR spectra of **9** were similar to those of the aglycone of bacoside A_6, a saponin from *Bacopa monnieri* (Pawar *et al.*, 2007). The ^{1}H NMR spectrum of the aglycone **9** showed signals of a dammarane triterpenoid, characterized by seven tertiary methyl groups (δ_H 0.80, 0.90, 0.99, 1.13, 1.67, 1.72 and 1.76), a vinyl proton (δ_H 5.19, *dt*, *J*=8.4, 1.4 Hz), two oxygen-bearing methines [δ_H 3.15 (*dd*, *J*=11.4, 5.0 Hz), 4.71 (*ddd*, *J*=11.5, 7.9, 3.4 Hz)], and an oxygen-bearing methylene group [δ_H 4.08 (*brs*)]. Its ^{13}C NMR and *J*-modulated HSQC spectra exhibited signals for seven

methyl groups [δ_C 14.7 (C-29), 15.2 (C-19), 16.2 (C-21), 17.0 (C-27), 17.8 (C-30), 24.4 (C-26), and 27.2 (C-28)], two oxymethine carbons [δ_C 78.0 (C-3) and 69.0 (C-23)], four olefinic carbons [δ_C 124.1 (C-20), 124.4 (C-24), 133.6 (C-17), and 135.5 (C-25)], an oxymethylene carbon [δ_C 66.1 (C-18)], and an acetal carbon [δ_C 106.8 (C-16)] (Table 3). The shielded nature of C-3 (δ_C 78.0) suggested a free hydroxyl at the C-3 position. Analysis of the COSY, *J*-modulated HSQC and HMBC spectra confirmed its identity as 17,20-didehydro-20-deoxyjujubogenin (Pawar *et al.*, 2007). This is the first report of the isolation of this aglycone though three of its saponins, including bacoside A_6, have recently been identified in an extract of *Bacopa monnieri* (Nuengchamong *et al*, 2016).

Compound **4** had the molecular formula $C_{41}H_{64}O_{12}$ [HRESIMS: *m/z* 771.4286 $[M+Na]^+$, calcd for $C_{41}H_{64}O_{12}Na$, 771.4295]. The ^1H and ^{13}C NMR spectra of **4** showed supplementary signals assignable to the sugar units with their anomeric protons at δ_H 4.30 (d, *J*=7.4 Hz), and 4.57 (d, *J*=7.7 Hz) (Table 4) correlated in the *J*-modulated HSQC spectrum with anomeric carbons at δ_C 105.7, and 104.0, respectively (Table 5). Analysis of the COSY and *J*-modulated HSQC spectra of **4** allowed complete assignment of proton and carbon systems of a pentose and a hexose, identified as a terminal *β*-D-glucopyranose (δ_H 4.57) and an *α*-L-arabinopyranose characterized by its equatorial proton H-4 ($J_{H\text{-}3'\text{-}H\text{-}4'}$ = 3.3 Hz) substituted on the hydroxyl at C-3' (δ_C 82.4) (Agrawal, 1992) (Tables 4 and 5). The HMBC correlations between Glc-H-1" (δ 4.57)/Ara-C-3', Ara-H-1' (δ 4.30)/C-3 (δ 89.1), and the rOe correlations between Glc-H-1"/Ara-H-3', Ara-H-1'/H-3 revealed the linkage of the disaccharide moiety. Thus, the structure of saponin **4** was identified as 3-*O*-*β*-D-glucopyranosyl-(1→3)-*α*-L-arabinopyranosyl-17,20-didehydro-20-deoxyjujubogenin (Fig. 1).

The positive HRESIMS spectrum of compound **5** exhibited two pseudomolecular ions at *m/z* 895.5056 $[M+H]^+$ and *m/z* 917.5215 $[M+Na]^+$, corresponding to a molecular formula $C_{47}H_{74}O_{16}$, and 146 additional molecular weight units relative to **4**, suggesting the presence of an additional deoxyhexose unit. Three anomeric signals were observed in ^1H-NMR and ^{13}C-NMR spectra at δ_H 5.24 (d, *J* =1.5 Hz, δ_C 100.6), 4.52 (d, *J* =7.7 Hz, δ_C 106.8), and 4.51 (d, *J*=7.7 Hz, δ_C 102.9) (Tables 4 and 5). Analysis of COSY, *J*-modulated HSQC, and HMBC experiments identified the three sugars moieties as in **13** (Muhammad *et al.*, 2016), a terminal *β*-D-glucopyranose (δ_H 4.51), a terminal *α*-L-rhamnopyranose (δ_H 5.24) with its methyl signal at δ_H 1.24 (d, *J*=6.2 Hz) and δ_C 16.8, and an *α*-L-arabinopyranose (δ_H 4.52) disubstituted at positions C-2' (δ_C 73.9) and C-3' (δ_C 80.9) (Agrawal, 1992) (Tables 4 and 5). The interglycosidic linkage was confirmed by the HMBC correlations observed between Glc-H-1'"/Ara-C-3', Rha-H-1"/Ara-C-2', and Ara-H-1'/C-3. Thus, the structure of compound **5** is the previously undescribed

3-*O*-*β*-D-glucopyranosyl-(1→3)-[*α*-L-rhamnopyranosyl-(1→2)]-*α*-L-arabinopyranosyl-17,20-didehydro-20-deoxyjujubogenin (Fig. 1).

Compound **6** had the molecular formula $C_{53}H_{84}O_{21}$ [HRESIMS: *m/z* 1079.5399 $[M+Na]^+$, calcd for $C_{53}H_{84}O_{21}Na$, 1079.5403]. The ^1H and ^{13}C NMR spectra and MS data of **6** showed an additional sugar unit, relative to **5**, identified as a hexose unit with its anomeric proton at δ_H 4.77 (d, *J*=7.4 Hz) (Table 4), and anomeric carbon at δ_C 102.0 (Table 5). Analysis of COSY, TOCSY, *J*-modulated HSQC, and HMBC allowed assignment of two *β*-D-glucopyranoses, one terminal (δ_H 4.77) and one substituted at position C-2''' (δ_C 76.5), a terminal *α*-L-rhamnopyranose (δ_H 5.20), and an *α*-L-arabinopyranose (δ_H 4.39) disubstituted at positions C-2' (δ_C 73.9) and C-3' (δ_C 81.7) (Agrawal, 1992) (Tables 4 and 5) as in **15** (Muhammad *et al.*, 2016). The interglycosidic linkages were identical to those observed in saponin **15** with HMBC correlations between Glc-H-1''''/Glc-C-2''', Glc-H-1'''/Ara-C-3', Rha-H-1''/Ara-C-2', and Ara-H-1'/C-3. Thus, compound **6** was identified as 3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→3)-[*α*-L-rhamnopyranosyl-(1→2)]-*α*-L-arabinopyranosyl-17,20-didehydro-20-deoxyjujubogenin (Fig. 1).

Compounds **7** and **8** had the same molecular formula $C_{53}H_{84}O_{21}$, [HRESIMS: *m/z* 1079.5399 $[M+Na]^+$, calcd for $C_{53}H_{84}O_{21}Na$, 1079.5403] and contained 132 additional molecular weight units relative to **6**, suggesting the presence of an additional pentose unit. The ^1H- and ^{13}C-NMR spectra of the sugar part of compounds **7** and **8** were very similar to those of **16** and **17** (Muhammad *et al.*, 2016). Five anomeric signals were observed in ^1H-NMR and ^{13}C-NMR spectra at δ_H 4.34 (*d*, *J* =7.5 Hz, δ_C 104.3), 4.40 (*d*, *J* =6.5 Hz, δ_C 104.7), 4.70 (*d*, *J* =7.5 Hz, δ_C 101.6), 4.89 (*d*, *J* =7.3 Hz, δ_C 102.7) and 5.27 (*d*, *J*=1.5 Hz, δ_C 100.8) for compound **7** and at δ_H 4.36 (*d*, *J* =6.8 Hz, δ_C 104.1), 4.40 (*d*, *J* =6.5 Hz, δ_C 104.7), 4.70 (*d*, *J* =7.5 Hz, δ_C 101.6), 4.88 (*d*, *J* =7.3 Hz, δ_C 102.6) and 5.31 (*d*, *J*=1.3 Hz, δ_C 100.6) for compound **8** (Tables 4 and 5). Analysis of COSY, TOCSY, *J*-modulated HSQC, and HMBC experiments identified the four sugars moieties as in **6** and the supplementary pentose unit was identified as a *β*-D-xylopyranose in compound **7** and an *α*-L-arabinopyranose in **8** (Agrawal, 1992), as in compounds **16** and **17**, respectively (Muhammad *et al.*, 2016). These sugars were attached to the *β*-D-glucopyranose at C-6'''' as suggested by its deshielded signal (δ_C 68.1) (Table 5). The HMBC correlations were observed between Xyl-H-1''''/Glc-C-6'''', Glc-H-1''''/Glc-C-2''', Glc-H-1'''/Ara-C-3', Rha-H-1''/Ara-C-2', and Ara-H-1'/C-3 for compound **7** and between Ara-H-1''''/Glc-C-6'''', Glc-H-1''''/Glc-C-2''', Glc-H-1'''/Ara-C-3', Rha-H-1''/Ara-C-2', and Ara-H-1'/C-3 for compound **8**. Thus, the structure of compound **7** is the previously undescribed 3-*O*-*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→3)-[*α*-L-rhamnopyranosyl-(1→2)]-*α*-L-arabinopyranosyl-17,20-didehydro-20-deoxyjujubogenin, and compound **8** is the previously undescribed 3-*O*-*α*-L-arabinopyranosyl-(1→6)-*β*-D-glucopyranosyl-

(1→2)-*β*-D-glucopyranosyl-(1→3)-[*α*-L-rhamnopyranosyl-(1→2)]-*α*-L-arabinopyranosyl-17,20-didehydro-20-deoxyjujubogenin (Fig. 1).

The antioxidant activity of the EtOAc and hydromethanolic extracts at 200 µg/mL of the stem and bark was 18.5%, 22.6%, 78.5% and 82.5%, respectively. The hydromethanolic extracts were the most active with both an EC_{50} = 73 µg/mL for the DPPH radical scavenging activity. This activity is low considering the presence of rutin (**18**) (EC_{50} = 15.3 µg/mL (Lue *et al*., 2010), maesopsin (EC_{50}=5.3 µg/mL) (Krenn *et al*., 2003) and the phenol glycosides (**19-21**).

The EtOAc extracts of the stem and bark showed no tyrosinase inhibition whereas the hydromethanolic extracts of the stem and bark showed 29.9 % and 49.6 % inhibition, respectively. The four lupane triterpenoids (**1, 10-12**) were tested at 4 mg/mL and their activity was very low with 36.5 %, 28.4 %, 44.8 % and 36.6 % tyrosinase inhibition, respectively.

The cytotoxic activity of the five lupane triterpenoids (**1-2**, **10-12**), the ceanothic acid saponin (**3**), the two dammarane saponins (**5-6**), the (±) lyoniresinol derivative (**22**), and the maesopsin derivative (**23**), against a KB cell line, was measured using a WST-1 proliferation test. The three saponins tested (**3**, **5-6**), (±)-3α-*O*-*β*-D-glucopyranosyl-lyonirésinol (**22**), and (±)-4-*O*-*β*-D-glucopyranosyl-maesopsin (**23**) showed very low cytotoxic activity with growth inhibitions ranging from 2.5 to 13.0 % at 10 µg/mL (See supplementary Information for details). Ceanothic acid (**10**) showed a moderate activity with growth inhibitions of 30.8%. The four lupane triterpenoids (**1**, **2**, **11**, **12**) were the most active with growth inhibitions of 72.9 %, 77.0 %, 79.5% and 79.3 %, respectively, and IC_{50} ranging from 1.2 to 4.0 µg/mL. Ceanothenic acid (**11**) showed good cytotoxic activity (2.6 ± 0.16 µM) and is more active than alphitolic acid (**12**) (8.5 ± 0.25 µM), and the two previously undescribed compounds **1** and **2**, with IC_{50} values of 7.9 ± 0.12 µM and 7.0 ± 0.17 µM, respectively. By comparison of these data, the stereochemistry of the aldehyde group did not affect the activity between **1** and **2**.

The disk diffusion method was used to evaluate the possible antimicrobial activity of the five lupane triterpenes (**1-2**, **10-12**), the ceanothic acid saponin (**3**), the dammarane saponin (**5**), (±) 3α-*O*-*β*-D-glucopyranosyl-lyonirésinol (**22**), and (±) 4-*O*-*β*-D-glucopyranosyl-maesopsin (**23**), against four bacteria including two Gram positive (*S. aureus* and *E. faecalis*) and two Gram negative (*E. coli* and *P. aeruginosa*). Only ceanothenic acid (**11**) and 2α-formyle-A(1)*nor*lup-20(29)-en-28-oic acid (**1**) showed moderate antibacterial activity with inhibition diameters of 10 and 12 for **1** and 16 and 14 for **11** against *S. aureus* and *E. faecalis*, respectively. No inhibition zone was observed with 2β-formyl-A(1)*nor*lup-20(29)-en-28-oic acid (**2**), nor any activity on gram negative bacteria, at the concentrations tested. To further characterize the antibacterial activities of compounds **1** and **11** their MIC values against *S. aureus* and *E. faecalis* were also determined using the microdilution method (Muhammad *et al*., 2016).

Compound **1** showed good antibacterial activity with MIC values of 4 µg/mL against both *S. aureus* and *E. faecalis*, and compound **11** has a moderate activity with MIC values of 8 and 16 µg/mL against *S. aureus* and *E. faecalis*, respectively (See supplementary Information for details).

In conclusion, twenty four compounds, including eight previously undescribed structures, were isolated from the stems and bark of *J. xerocarpa*. The free 17,20-didehydro-20-deoxyjujubogenin (**9**) and its five glycosides (**4-8**), as well as the two lupane derivatives, the 2α/*β*-formyl-A(1)*nor*lup-20(29)-en-28-oic acid (**1-2**) are described for the first time in *Jaffrea* species. Two compounds, zizyberanal and zizyberanalic acid, possessing an aldehyde group at position 1, were isolated from another Rhamnaceae species, *Zizyphus jujuba* (Guo *et al*., 2009). Rutin (**18**), a common flavonoid, and saponins **13-17** were previously isolated from the leaves of *A. xerocarpus (= J. xerocarpa)* (Muhammad *et al*., 2016). Ceanothic acid (**10**), ceanothenic acid (**11**) and alphitolic acid (**12**) are common in *Alphitonia* species and the Rhamnaceae and can be considered as chemotaxonomic markers. This is the first identification in *Jaffrea* species that confirmed the similarity of *Alphitonia* and *Jaffrea* species. The three phenol glycosides (**19-21**) were isolated for the first time from the Rhamnaceae family. The hydromethanolic extract showed antioxidant activity (DPPH essay) due to the presence of phenols. Three lupane triterpenes (**1**, **2**, **12**), showed a good cytotoxic activity against a KB cell line (IC_{50} ranging from 7.0 to 8.5 µM) but were less active than ceanothenic acid (**11**) (IC_{50} 2.6 µM). The previously undescribed 2α-formyl-A(1)*nor*lup-20(29)-en-28-oic acid (**1**) is the major compound of both the AcOEt and hydromethanolic extracts and showed good antibacterial activity against *S. aureus* and *E. faecalis* (MIC 4 µg/mL).

3. Experimental

3.1 General experimental procedures

Optical rotations were determined in MeOH with a Perkin-Elmer 341 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III 500 spectrometer (^{1}H at 500 MHz and ^{13}C at 125 MHz) or on a Bruker Avance III 600 spectrometer equipped with a cryoprobe (^{1}H at 600 MHz and ^{13}C at 150 MHz). 2D-NMR experiments were performed using standard Bruker microprograms. Chemical shift (δ) were reported in ppm using the internal solvent resonances at δ_H 3.33 and δ_C 47.6 (CD_3OD). HRESIMS experiments were performed using a hybrid quadrupole/time-of-flight (Q-TOF) instrument, equipped with a pneumatically assisted electrospray ion source operated in positive ionization mode (Micromass, Manchester, UK). The samples were introduced by direct infusion in a solution of MeOH at a flow rate

of 5 µL/min. The spray capillary voltage was set at 3500V, and the extraction cone voltage between 30-60V. The source temperature was 80°C and the desolvatation temperature was 100°C.
Analytical TLC were carried out on precoated silica gel 60 F_{254} plates (Merck, Darmstadt, Germany). Spots were visualized after spraying with 50% H_2SO_4 and heating at 100 °C for 1 min. CC was carried out on Kieselgel 60 (63-200 mesh), or LiChroprep RP-18 (40-63 µm) Merck. Flash chromatography was performed on a C_{18} reversed phase (Reveleris C_{18} reversed phase 40 g; CV 45 mL/min; 45 mg-1.35 g sample) or on silica gel 60 (Reveleris silica 40 µm, 12 g; CV 17-36 mL/min; 45 mg-2.4 g sample, or Reveleris silica 40 µm, 4 g; CV 5-18 mL/min; 4 mg-800 mg sample) columns. Analytical and semi-preparative HPLC were performed on a Dionex apparatus equipped with an ASI-100 automated sample injector, a STH 585 column oven, a P580 pump, a diode array detector UVD 340S and the Chromeleon® software version 6.8. Analytical HPLC separations were performed on a prepacked C_{18} reversed phase column Luna (250 x 4.6 mm, 5µm, Phenomenex, France). Semi-preparative HPLC separations were performed on a prepacked C_{18} reversed phase column Luna (250 x 10mm, 5µm, Phenomenex, France). The chromatograms were monitored at 205, 210, 254 and 312 nm.
96-well microplates Greiner® F Bottom (BMG-LABTECH, Champigny sur Marne, France) and a BMG-LABTECH UV-Vis Spectrophotometer Micro-plate reader FLUOstar Omega were used for absorbance measurements in biological assays. DPPH, mushroom tyrosinase (EC 1.14.18.1), L-DOPA, kojic acid (purity 99%), ascorbic acid, and *α*-hederin were purchased from Sigma-Aldrich. WST-1 was obtained from Roche and DMEM F12 was purchased from Gibco-Invitrogen. The KB cell line DSMZ ACC136 was purchased from Interchim®. Deionised water was used to prepare all aqueous solutions.

3.2 Plant material

Jaffrea erubescens (Baill.) H. C. Hopkins & Pillon (Basionym *Alphitonia xerocarpus* Baill.) stems and bark (Rhamnaceae) were collected by Pr. Mohammed Nour in September 2009 at the end of the cool season in southern province (GPS: 22°10' 14,5" S ; 166° 45' 32,66"). The botanical identification was made at the Laboratoire Insulaire du Vivant et de l'Environnement of the New Caledonia University. A voucher specimen (09NM002) was deposited in the Herbarium of Noumea (New Caledonia).

3.3 Extraction and isolation

Powdered air–dried stems of *J. xerocarpa* (200 g) were macerated overnight in 4.0 L of petroleum ether and lixiviated to give 1.6 g of petroleum ether extract after evaporation. The defatted powdered material was then macerated overnight and lixiviated with 4.0 L of EtOAc to afford after evaporation of the solvent 3.8 g of EtOAc extract. After drying, the resulting powdered material was reflux for 3 h with

MeOH-H_2O (8:2) (2.0 L), and then dried under reduced pressure to yield 33.4 g of a hydromethanolic extract.

The EtOAc extract (3.8 g) was purified by silica gel flash chromatography using a gradient of *n*-cyclohexane-$CHCl_3$ (from 8:2 to 0:1) during 30 min, and then $CHCl_3$-EtOAc (from 1:0 to 0:1) during 30 min, to afford 206 fractions. Fractions [1-29] (118 mg) eluted with *n*-cyclohexane-$CHCl_3$ were purified by silica gel flash chromatography using a gradient of *n*-cyclohexane-$CHCl_3$ (from 1:0 to 6:4) during 20 min with a flow rate of 12 mL/min, to afford compound **1** (53.3 mg). Fractions [92-97] (121.8 mg) eluted with $CHCl_3$-EtOAc were purified by silica gel flash chromatography using a gradient of toluene-EtOAc (from 1:0 to 7:3) during 35 min with a flow rate of 15 mL/min. The resulting fractions [23-30] (121.8 mg) eluted with toluene- EtOAc (8:2) were purified by silica gel flash chromatography using a gradient of toluene-EtOAc (from 1:0 to 85:15) during 30 min with a flow rate of 15 mL/min, to afford compound **12** (6.9 mg) in fractions [26-27]. The resulting fractions [20-23] eluted with toluene-EtOAc (85:15) were purified by silica gel flash chromatography using a gradient of toluene-EtOAc (from 1:0 to 93:7) during 30 min with a flow rate of 15 mL/min, to give compounds **12** (3.3 mg) and **10** (3.3 mg). Fractions [135-149] (680 mg), eluted with 100% EtOAc, were purified by silica gel flash chromatography using a gradient of $CHCl_3$-MeOH (from 1:0 to 85:15) during 20 min, to afford compound **11** (6.2 mg) in fractions [36-40].

Powdered air–dried bark of *J. xerocarpa* (500 g) was reflux for 3 h with MeOH-H_2O (8:2) (5 L), and then filtered and dried under reduced pressure to yield 85 g of a hydromethanolic extract. A part of the hydromethanolic extract (80 g) was subjected to vacuum liquid chromatography on silica gel, eluted successively with 1 L of $CHCl_3$-MeOH (1:0, 72:25, 5:5, 25:75 and 0:1), to give fractions I (3.2 g), II (13.5 g), III (23.7 g), IV (15.6 g), and V (8.9 g), respectively.

Fraction I (3 g) was purified by silica gel flash chromatography using a gradient of *n*-cyclohexane-$CHCl_3$ (from 8:2 to 0:1) during 20 min with a flow rate of 35 mL/min, and then $CHCl_3$-EtOAc (from 1:0 to 0:1) during 15 min, to afford 70 fractions. Fractions [9-12] (103.8 mg) eluted with *n*-cyclohexane-$CHCl_3$ were purified by silica gel flash chromatography using a gradient of *n*-cyclohexane-$CHCl_3$ (from 9:1 to 6:4) during 40 min with a flow rate of 13 mL/min, to afford compounds **1** (57.9 mg) in fractions [13-18] and **2** (4.3 mg) in fractions [21-22]. Compound **10** (59 mg) was obtained by precipitation of fraction [49], eluted with 100% EtOAc, in the mixture of $CHCl_3$-MeOH (95:5).

Fraction II (13.5 g) was subjected to vacuum liquid chromatography on silica gel, eluted successively with 1 L of $CHCl_3$-MeOH (1:0, 9:1, 8:2, 5:5 and 0:1), to give fractions II-1 (1.3 g), II-2, (2.8 g), II-3 (3.2 g), II-4 (3.1 g), and II-5 (0.6 g), respectively. Fraction II-3 (3 g) was purified by silica gel column chromatography using a gradient of $CHCl_3$-MeOH (from 1:0 to 75:25). Fractions [44-49] (834 mg),

eluted with $CHCl_3$-MeOH (85:15), were purified by RP-18 column chromatography using a gradient of MeOH-H_2O (from 1:9 to 5:5) to give compounds **19** (6.6 mg), **22** (236 mg), and **24** (5.5 mg). Fractions [53-62] (530 mg), eluted with $CHCl_3$-MeOH (8:2), were purified by RP-18 column chromatography using a gradient of MeOH-H_2O (from 1:0 to 5:5) to give compounds **20** (2.5 mg), **21** (3.4 mg), **22** (11.4 mg), and **23** (25.3 mg).

An aliquot of fraction IV (1 g) was purified by RP-18 column chromatography using a gradient of MeOH-H_2O (from 4:6 to 9:1) to give 65 fractions (15 mL each). Fractions [9-12] (33.4 mg), eluted with MeOH-H_2O (4:6), were purified by silica gel column chromatography using the mixture $CHCl_3$-MeOH (75:25) to give 10.5 mg of rutin (**18**). Fractions [21-27] (88.6 mg), eluted with MeOH-H_2O (5:5), contained the pure compound **15**. Fractions [40-41] (65 mg), eluted with MeOH-H_2O (6:4), were subjected to semi-prep HPLC on RP-18 eluted with a gradient of CH_3CN-H_2O 0.025% TFA (1:9 to 5:5) during 15 min yielding compound **3** (*R*t 12.8 min; 8 mg). Fractions [42-44] (156 mg), eluted with MeOH-H_2O (6:4), were subjected to semi-prep HPLC on RP-18 eluted with a gradient of CH_3CN-H_2O 0.025% TFA (45:55 to 6:4) during 10 min yielding compound **14** (*R*t 6.8 min; 13 mg). Fractions [50-52] (99.6 mg), eluted with MeOH-H_2O (7:3) contain the mixture of compounds **16** and **17** (6 mg) not separable after silica gel column chromatography and semi-prep HPLC on RP-18. A second aliquot of fraction IV (2 g) was purified by prep- HPLC on RP-18 using a gradient of MeOH-H_2O (from 2:8 to 7:3) at a flow rate of 120 mL/min, to give 67 fractions (150 mL each). Fractions [32-34] (49 mg), eluted with MeOH-H_2O (4:6), were purified by semi-prep HPLC on RP-18 eluted with a gradient of CH_3CN-H_2O 0.025% TFA (18:82 to 22:78) during 10 min to give rutin (**18**) (*R*t 10.4 min; 8.9 mg). Fractions [45-48] (271.6 mg), eluted with MeOH-H_2O (5:5), were purified by silica gel flash chromatography using a gradient of $CHCl_3$-MeOH (from 1:0 to 8:2) during 40 min at a flow rate of 15 mL/min. The resulting fractions [80-85] (271.6 mg) were purified by semi-prep HPLC on RP-18 eluted with a gradient of CH_3CN-H_2O 0.025% TFA (45:55 to 6:4) during 15 min and then (6:4 to 1:0) during 10 min to give the compounds **15** (*R*t 5.4 min; 4.5 mg), **13** (*R*t 6.2 min; 6.7 mg), **6** (*R*t 12.9 min; 7.0 mg), **5** (*R*t 15.3 min; 10.0 mg), **4** (*R*t 18.4 min; 1.3 mg), and **9** (*R*t 19.8 min; 1.6 mg). Fractions [53-55] (156.3 mg), eluted with MeOH-H_2O (4:4), were purified by semi-prep HPLC on RP-18 eluted with a gradient of CH_3CN-H_2O 0.025% TFA (4:6 to 5:5) during 15 min and then (5:5 to 1:0) during 5 min, to give the compounds **16**+**17** (*R*t 6.5 min; 4.0 mg), **13** (*R*t 8.3 min; 6.3 mg), **7** (*R*t 14.9 min; 3.5 mg), and **8** (*R*t 15.2 min; 2.5 mg).

3.4 Compound characterization

*3.4.1. 2α-Formyl-A(1)norlup-20(29)-en-28-oic acid (**1**)*

White amorphous powder; $[\alpha]_D$ +1.5° (*c* 0.83, $CHCl_3$); ^1H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 1; HRESIMS (positive-ion mode) *m/z* : 477.3339 $[M+Na]^+$ (calcd for $C_{30}H_{46}O_3Na$, 477.3345).

*3.4. 2. 2β-Formyl-A(1)norlup-20(29)-en-28-oic acid (**2**)*

White amorphous powder; $[\alpha]_D$ -3.8° (*c* 0.26, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; HRESIMS (positive-ion mode) *m/z* : 477.3329 $[M+Na]^+$ (calcd for $C_{30}H_{46}O_3Na$, 477.3345).

*3.4.3. 28-O-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosylceanothic acid (**3**)*

White amorphous powder; $[\alpha]_D$ +1.7° (*c* 0.23, CH_3OH); ^1H NMR (MeOD-d4, 500 MHz) and ^{13}C NMR (MeOD-d4, 125 MHz), see Table 2 ; HRESIMS (positive-ion mode) *m/z* : 1157.5349 $[M+Na]^+$ (calcd for $C_{54}H_{86}O_{25}Na$, 1157.5356).

*3.4.4. 3-O-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl-17,20-didehydro-20-deoxyjujubogenin (**4**)*

White amorphous powder; $[\alpha]_D$ +18.0° (*c* 0.08, CH_3OH); ^1H NMR (MeOD-d4, 600 MHz) and ^{13}C NMR (MeOD-d4, 150 MHz) of the aglycone part, see Table 3; ^1H NMR (MeOD-d4, 600 MHz) and ^{13}C NMR (MeOD-d4, 150 MHz) of the osidic part, see Tables 4 and 5; HRESIMS (positive-ion mode) *m/z* : 771.4286 $[M+Na]^+$ (calcd for $C_{41}H_{64}O_{12}Na$, 771.4295).

*3.4.5. 3-O-β-D-glucopyranosyl-(1→3)- [α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl-17,20-didehydro-20-deoxyjujubogenin (**5**)*

White amorphous powder; $[\alpha]_D$ -29.7° (*c* 0.19, CH_3OH); ^1H NMR (MeOD-d4, 600 MHz) and ^{13}C NMR (MeOD-d4, 150 MHz) of the aglycone part is identical to compound **4** ± 0.2 ppm; ^1H NMR (MeOD-d4, 600 MHz) and ^{13}C NMR (MeOD-d4, 150 MHz) of the osidic part, see Tables 4 and 5; HRESIMS (positive-ion mode) *m/z* : 917.5215 $[M+Na]^+$ (calcd for $C_{47}H_{74}O_{16}Na$, 917.4875).

*3.4.6. 3-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl-17,20-didehydro-20-deoxyjujubogenin (**6**)*

White amorphous powder; $[\alpha]_D$ +8.9° (*c* 0.11, CH_3OH); ^1H NMR (MeOD-d4, 600 MHz) and ^{13}C NMR (MeOD-d4, 150 MHz) of the aglycone part is identical to compound **4** ± 0.2 ppm; ^1H NMR (MeOD-d4, 600 MHz) and ^{13}C NMR (MeOD-d4, 150 MHz) of the osidic part, see Tables 4 and 5; HRESIMS (positive-ion mode) *m/z* : 1079.5399 $[M+Na]^+$ (calcd for $C_{53}H_{84}O_{21}Na$, 1079.5403).

*3.4.7. 3-O-β-D-xylopyranosyl-(1→6)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl-17,20-didehydro-20-deoxyjujubogenin (**7**)*

White amorphous powder; $[\alpha]_D$ +12.0° (*c* 0.1, CH_3OH); ^1H NMR (MeOD-d4, 600 MHz) and ^{13}C NMR (MeOD-d4, 150 MHz) of the aglycone part is identical to compound **4** ± 0.2 ppm; ^1H NMR (MeOD-d4,

600 MHz) and ^{13}C NMR (MeOD-d4, 150 MHz) of the osidic part, see Tables 4 and 5; HRESIMS (positive-ion mode) *m/z* : 1211.5817 $[M+Na]^+$ (calcd for $C_{58}H_{92}O_{25}Na$, 1211.5825).

*3.4.8. 3-O-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl-17,20-didehydro-20-deoxyjujubogenin (**8**)*

White amorphous powder; $[\alpha]_D$ -4.3° (*c* 0.23, CH_3OH); ^1H NMR (MeOD-d4, 600 MHz) and ^{13}C NMR (MeOD-d4, 150 MHz) of the aglycone part is identical to compound **4** ± 0.2 ppm; ^1H NMR (MeOD-d4, 600 MHz) and ^{13}C NMR (MeOD-d4, 150 MHz) of the osidic part, see Tables 4 and 5; HRESIMS (positive-ion mode) *m/z* : 1211.5817 $[M+Na]^+$ (calcd for $C_{58}H_{92}O_{25}Na$, 1211.5825).

*3.4.9. 17,20-didehydro-20-deoxyjujubogenin (**9**)*

White amorphous powder; $[\alpha]_D$ +10.74° (*c* 0.31, CH_3OH); ^1H NMR (MeOD-d4, 600 MHz) and ^{13}C NMR (MeOD-d4, 150 MHz), see Table 3; HRESIMS (positive-ion mode) *m/z* : 477.3341 $[M+Na]^+$ (calcd for $C_{30}H_{46}O_3Na$, 477.3345).

3.5. Sugar analysis and determination of absolute configuration

1 g of the crude hydromethanolic extract was refluxed with 25 mL of TFA (2N) for 4 h. After extraction with EtOAc (3 × 25 mL), the aqueous layer was neutralized to pH 6 with 50 mM KOH and freeze-dried to provide the monosaccharide residue. The sugar profile was determined by comparison with authentic samples on TLC in MeCOEt-*iso*-PrOH-Me_2CO-H_2O (20:10:7:6). Each sugar was purified by semi-preparative HPLC, on a specific column ROA (250 x 15 mm, T = 35 ° C) eluted isocratically with a solution of H_2O 0.25 µM H_2SO_4 at a flow rate of 3.5 mL/min (Muhammad *et al.*, 2015). After neutralization, each fraction was analyzed by HPLC on an analytical chiral column Chiralpak® ICA, using the mobile phase *n*-hexane-EtOH-TFA (80:20:0.1) isocratically at a flow rate of 0.5 mL/min (Muhammad *et al.*, 2015). Chromatograms were monitored by a refractive index detector RI-410 and identification of the sugars was carried out by comparing the retention times of standard D or L monosaccharide samples (Lopes and Gaspar, 2008). Four sugars were identified as L-rhamnose (α & β) at *R*t 11.7 min, L-arabinose (α & β) at *R*t 14.6-15.5 min, D-xylose (α & β) at *R*t 16.6-18.5 and D-glucose (α & β) at *R*t 18.3–23.2 min.

3.6 DPPH radical scavenging assay

The radical scavenging activity of the EtOAc and hydromethanolic extracts of *J. xerocarpa* stems and bark was determined using the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (Muhammad *et al.*, 2015, 2016) (See supplementary Information for details).

3.7 Tyrosinase inhibitory activity assay

The tyrosinase inhibitory activity of the EtOAc and hydromethanolic extracts of *J. xerocarpa* stems and bark and the four lupane derivatives (**1, 10-12**) was determined against fungi tyrosinase. The assay was performed according to a previously described method using L-DOPA as substrate (Muhammad *et al.*, 2015, 2016) (See supplementary Information for details).

3.8 WST cytotoxicity assay

The cytotoxic activities of compounds **1-3**, **5-6**, **10-12, 22**, and **23** on KB cell lines (ATTC® CCL™-17) were determined by using a colorimetry method based on the cleavage of the WST-1 tetrazolium salt, and using DMEM F12 medium for cells culture (Muhammad *et al.*, 2015). α-hederin was employed as a positive control, which exhibited an IC_{50} value of 5.5 µM under the above conditions (Chwalek *et al.*, 2006) (See supplementary Information for details).

3.9. Disc diffusion antibacterial assay

Disk diffusion was used to screened antibacterial activity of compounds **1-2**, **5**, **10-12**, **22** and **23** against *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (CIP10907) as gram positive bacteria, and *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) as gram negative bacteria (Acebey-Castellon *et al.*, 2011, Muhammad *et al.*, 2016). The antimicrobial gentamicin was used as positive control and tested at 50 µg/disk (See supplementary Information for details).

3.10. Broth diffusion antibacterial assay

The liquid microdilution growth inhibition method (Acebey-Castellon *et al.*, 2011, Yao-Kouassi *et al.*, 2008) was used to determine the MIC values of the actives compounds **1** and **11** against both *S. aureus* and *E. faecalis* as previously described (Muhammad *et al.*, 2016). The experiments were run in triplicate, and each time the MIC values were identical. Gentamicin (25, 12,5, 5, 2,5 µg/mL) was used as inhibition growth positive control in the same conditions.

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References

Acebey-Castellon I., L., Voutquenne-Nazabadioko, L., Doan Thi Mai, H., Roseau, N., Bouthagane, N., Muhammad, D., Le Magrex Debar, E., Gangloff, S.C., Litaudon,M., Sevenet, T., Van Hung, N., Lavaud, C., 2011**.** Triterpenoid saponins from *Symplocos lancifolia*. J. Nat. Prod. 74, 163–168

Agrawal, P.K., 1992**.** NMR spectroscopy in the structural elucidation of oligosaccharides and glycosides. Phytochemistry 31, 3307-3330.

Baillon, H., 1876, Adansonia; recueil d'observations botaniques. Paris.. Vol. 11. 270.

Chang, J., Case, R., 2005. Phenolic glycosides and ionone glycoside from the stem of Sargentodoxa cuneata. Phytochemistry 66, 2752-2758.

Chwalek, M, Lalun, N, Bobichon, H, Ple, K, Voutquenne-Nazabadioko, L., 2006. Structure–activity relationships of some hederagenin diglycosides: haemolysis, cytotoxicity and apoptosis induction. Biochem. Biophys. Acta 1760, 1418–1427

Dzubak, P., Hajduch, M., Vydra, D., Hustova, A., Kvasnica, M., Biedermann, D., Markova, L., Urban, M., Sarek, J., 2006. Pharmacological activities of natural triterpenoids and their therapeutic implications. Nat. Prod. Rep. 23**,** 394-411.

Guo, S., Tang, Y.P., Duan, J.A., Su, S.L., Ding, A.W., 2009. Two new terpenoids from fruits of *Ziziphus jujuba.* Chin. Chem. Lett. 20, 197-200.

Guillaumin, A., Flore de la Nouvelle calédonie. 1948: p. 202-203.

Hopkins, H. C. F., Pillon, Y., Stacy, E. A., Kellermann, J., 2015. *Jaffrea*, a new genus of Rhamnaceae endemic to New Caledonia, with notes on *Alphitonia* and *Emmenosperma*, Kew Bulletin 70:42, DOI 10.1007/S12225-015-9593-6

Ji, C.-J., Zeng, G.-Z., Han, J., He, W.-J., Zhang, Y.-M., Tan, N.-H., 2012. Zizimauritic acids A-C, three novel nortriterpenes from *Ziziphus mauritiana.* Bioorg Med Chem Lett, 22, 6377-80.

Jou, S.J., Chen, C.H., Guh, J.H., Lee, C.N., Lee, S.S., 2004. Flavonol glycosides and cytotoxic triterpenoids from *Alphitonia philippinensis.* J. Chin. Chem. Soc. 51**,** 827-834.

Krenn, L., Presser, A., Pradhan, R., Bahr, B., Papper, D.H., Mayer, K.K., Kopp, B., 2003. Sulfemodin 8-O-β-D-glucoside, a new sulfated anthraquinone glycoside, and antioxidant phenolic compounds from *Rheum emodi.* J. Nat. Prod. 66, 1107-1109.

Kuo, Y.H.Shue, M.J., 1991. New esters, 2-(4-hydroxy-3-methoxyphenyl)ethyl hexa- and octacosanoates from the leaves of *Cinnamomum reticulatum* Hay. J. Chin. Chem. Soc. 38, 65-9.

Kundu, A.B., Barik, B.R., Mondal, D.N., Dey, A.K., Banerji, A., 1989. Zizyberanalic acid, a pentacyclic triterpenoid of *Zizyphus jujuba.* Phytochemistry 28, 3155-8.

Lallemand, J.Y., Duteil, M., 1977. Carbon-13 NMR spectra of quercetin and rutin Org. Magn. Res. 9, 179-80

Lee, S.M., Min, B.S., Lee, C.-G., Kim, K.-S., Kho, Y.H., 2003. Cytotoxic triterpenoids from the fruits of *Zizyphus jujuba.* Planta Med. 69,1051-1054.

Li, Y.-L., Li, J., Wang, N.-L., Yao, X.-S., 2008. Flavonoids and a New Polyacetylene from *Bidens parviflora* Willd. Molecules 13, 1931-1941

Lin, R.C., Bourdy, G., Lacaille-Dubois, M.A., 1995. Flavonoids from *Alphitonia neocaledonica.* Planta Med. 61**,** 197.

Lopes, J.F., Gaspar, E.M.S.M., 2008. Simultaneaous chromatographic separation of enantiomers, anomers and structural isomers of some biologically relevant monosaccharides. J. Chrom. A 1188, 34-42

Lue, B.-M., Nielsen, N.S., Jacobsen, C., Hellgren, L., Guo, Z., Xu, X., 2010. Antioxidant properties of modified rutin esters by DPPH, reducing power, iron chelation and human low density lipoprotein assays. Food Chem. 123, 221-230.

Morat, P., Jaffré, T., Tronchet, F., Munzinger, J., Pillon, Y., Veillon, J.-M., Chalopin, M. 2012. Le référentiel taxonomique Florical et les caractéristiques de la flore vasculaire indigène de la Nouvelle-Calédonie. Adansonia, sér. 3, 34, 179-221. http://dx.doi.org/10.5252/a2012n2a1

Muhammad, D., Hubert, J., Lalun, N., Renault, J.H., Bobichon, H., Nour, M., Voutquenne-Nazabadioko, L. 2015. Isolation of flavonoids and triterpenoids from the fruits of *Alphitonia neocaledonica* and evaluation of their anti-oxidant, anti-tyrosinase and cytotoxic activities. Phytochem Anal. 26, 137-144.

Muhammad, D., Lalun, N., Bobichon, E. Le Magrex Debar , S. C. Gangloff , Nour, M., Voutquenne-Nazabadioko, L. 2016. Flavonoids and triterpenoids from the leaves of *Alphitonia xerocaprus* Baill and their biological activities. Phytochemistry 129, 45-57.

Munzinger, J., Morat, Ph., Jaffré, T., Gâteblé, G., Pillon, Y., Tronchet, F., Veillon, J.-M., M. Chalopin. 2016. FLORICAL: Checklist of the vascular indigenous flora of New Caledonia. vers. 22.IV.2016.

Nuengchamnong, N., Sookying, S., Ingkaninan, K., 2016. LC-ESI-QTOF-MS based screening and identification of isomeric jujubogenin and pseudojujubogenin aglycones in *Bacopa monnieri* extract. J. Pharm. Biomed. Anal. 129, 121-134.*

Okamura, N., Nohara, T., Yagi, A., Nishioka, I., 1981. Studies on the Constituents of Zizyphi Fructus. III. Structures of Dammarane-type Saponins. Chem. Pharm. Bull. 29, 676-683.

Pawar, R.S., Khan, S.I., Khan, I.A., 2007. Glycosides of 20-deoxy derivatives of jujubogenin and pseudojujubogenin from *Bacopa monniera.* Planta Med. 73, 380-383.*

Pretsch, E., Clerc, T., Seibl, J., Simon, W., Tables of spectral data for structure determination of organic compounds1989, Berlin Heidelberg Springer-Verlag

Sugiyama, M.Kikuchi, M., 1992. Studies on the constituents of *Osmanthus species.* X. Structures of phenolic glucosides from the leaves of *Osmanthus asiaticus* Nakai. Chem. Pharm. Bull., 40, 325-326.

Wang, Y., Ding, B., Luo, D., Chen, L.-Y., Hou, Y.-L., Dai, Y., Yao, X.-S., 2013. New triterpene glycosides from Ziziphi Spinosae Semen. Fitoterapia 90, 185-191.

Yao-Kouassi, P.A., Alabdul Magid, A., Richard, B., Martinez, A., Jacquier, M.J., Caron, C., Le Magrex Debar, E., Gangloff, S. C., Coffy, A. A., Zèches-Hanrot, M., 2008. Isoflavonoid glycosides from the roots of *Baphia bancoensis.* J. Nat. Prod. 71, 2073-2076.

Yoshikawa, K., Eiko, K., Mimura, N., Kondo, Y., Arihara, S., 1998. Hovetrichosides C-G, five new glycosides of two auronols, two neolignans, and a phenylpropanoid from the bark of *Hovenia trichocarea.* J. Nat. Prod. 61, 786-790.

*The name 20-deoxyjujubogenin used in these references is wrong. The correct name is 17,20-didehydro-20-deoxyjujubogenin.

Supporting information

1D and 2D NMR spectra of compounds **1-9** can be found in the online version of this article.

Figure caption

Fig. 1: Structures of isolated compounds **1-17, 19-21** and **23**

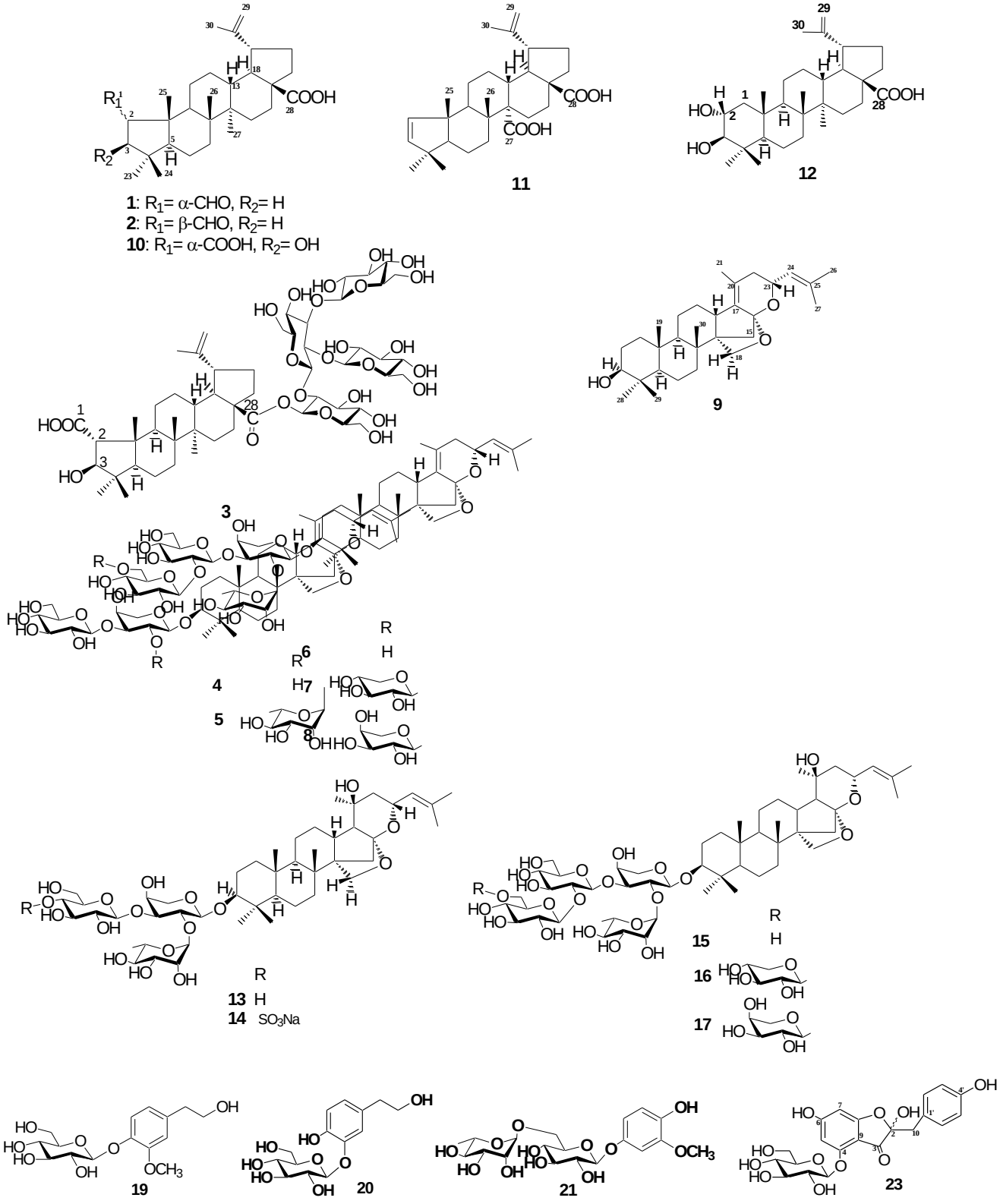

Fig. 1: Structures of isolated compounds **1-17, 19-21** and **23**

Table 1: ^{1}H and ^{13}C-NMR data of compounds **1** and **2**

	1[a]		**2**[b]	
Position	δ_C	δ_H (*m*, *J* in Hz)	δ_C	δ_H (*m*, *J* in Hz)
1	205.3	9.84 (*d*, 3.5)	206.6	9.73 (*d*, 4.7)
2	61.5	2.58 (*dd*, 7.8, 3.5)	64.1	2.25 (*ddd*, 9.8, 4.7, 3.6)
3	39.1	1.81(*dd*, 14.5, 7.8)	40.4	1.61 (*dd*, 13.6, 3.6)
		1.91 (*dd*, 14.5, 0.9)		1.99 (*dd*, 13.6, 9.8)
4	38.3	-	37.7	-
5	58.7	1.25 (*dd*, 9.1, 2.8)	63.1	1.07 (*dd*, 8.9, 2.6)
6	18.5	1.37 (*m*)	17.9	1.40 (*m*)
		1.42 (*m*)		1.40 (*m*)
7	34.0	1.37 (*m*)	34.3	1.40 (*m*)
		1.37 (*m*)		1.40 (*m*)
8	41.6	-	41.7	-
9	44.9	1.73 (*dd*, 12.8, 2.9)	50.1	1.65 (*dd*, 16.3, 3.7)
10	52.0	-	51.1	-
11	23.7	1.51 (*dd*, 9.6, 1.8)	24.3	1.23 (*m*)
		1.65 (*dd*, 13.5, 3.9)		1.50 (*dd*, 13.3, 4.7)
12	24.9	1.09 (*dd*, 12.9, 4.9)	25.1	1.15 (*dd*, 11.7, 5.1)
		1.72 (*m*)		1.71 (*dm*, 5.8)
13	38.6	2.22 (*td*, 12.2, 3.6)	38.3	2.19 (*td*, 12.2, 3.8)
14	42.8	-	42.6	-
15	29.9	1.20 (*dt*, 13.7, 3.2)	29.9	1.21 (*dt*, 13.8, 3.5)
		1.58 (*td*, 12.9, 4.2)		1.59 (*tm*, 13.5)
16	32.2	1.44 (*dd*, 9.8, 3.4)	32.2	1.47 (*dd*, 13.5, 3.3)
		2.30 (*dt*, 12.9, 3.3)		2.30 (*td*, 13.0, 3.3)
17	56.3	-	56.2	-
18	49.2	1.62 (*t*, 11.5)	49.3	1.62 (*t*, 11.5)
19	46.9	3.01 (*td*, 10.9, 4.9)	47.0	3.01 (*td*, 10.8, 5.1)
20	150.2	-	150.1	-
21	30.5	1.43 (*dd*, 10.1, 3.2)	30.5	1.44 (*dd*, 13.1, 3.1)
		2.01 (*m*)		2.01 (*m*)
22	37.1	1.50 (*m*)	37.1	1.50 (*m*)
		1.99 (*dm*, 9.1)		2.00 (*dd*, 11.3, 4.3)
23	32.0	1.12 (*s*)	32.5	1.03 (*s*)
24	26.2	0.97 (*s*)	25.8	0.98 (*s*)
25	19.3	0.99 (*s*)	13.5	1.03 (*s*)
26	16.9	0.96 (*s*)	16.6	0.94 (*s*)
27	14.8	0.96 (*s*)	14.7	1.02 (*s*)
28	181.9	-	181.0	-
29	109.9	4.64 (*dd*, 2.2, 1.5)	110.0	4.63 (*dd*, 2.2, 1.4)
		4.75 (*d*, 2.2)		4.75 (*d*, 2.3)
30	19.3	1.70 (*s*)	19.3	1.70 (*s*)

[a] NMR data were measured in $CDCl_3$ at 600 MHz (^{1}H) and 150 MHz (^{13}C)

[b] NMR data were measured in $CDCl_3$ +CD_3OD at 500 MHz (^{1}H) and 125 MHz (^{13}C)

Table 2: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of **3** in CD_3OD.

Position	δ_C	δ_H (*m*, *J* in Hz)	Position	δ_C	δ_H (*m*, *J* in Hz)
1	177.4	-	**C_{28}-Glc**		
2	65.6	2.50 (*brs*)	**1'**	92.0	5.62 (*d*, 7.9)
3	84.5	4.10 (*brs*)	**2'**	76.4	3.87 (*dd*, 9.6, 7.9)
4	42.9	-	**3'**	77.0	3.80 (*dd*, 9.6, 8.5)
5	56.7	1.70 (*m*)	**4'**	69.3	3.43 (*t*, 9.6)
6	18.3	1.35 (*m*)	**5'**	77.4	3.43 (*m*)
		1.54 (*m*)	**6'**	60.9	3.72 (*dd*, 12.3, 4.7)
7	34.1	1.37 (*m*)			3.86 (*dd*, 12.3, 1.7)
		1.45 (*m*)	**$C_{2'}$-Glc**		
8	41.7	-	**1"**	101.2	5.04 (*d*, 7.4)
9	44.4	1.78 (*dd*, 12.5, 2.5)	**2"**	81.2	3.65 (*t*, 8.0)
10	49.0	-	**3"**	86.2	3.69 (*t*, 8.4)
11	23.2	1.50 (*m*)	**4"**	69.7	3.30 (*m*)
		1.59 (*m*)	**5"**	75.4	3.32 (*m*)
12	25.3	1.09 (*m*)	**6"**	69.9	3.60 (*dd*, 11.6, 6.5)
		1.68 (*m*)			3.93 (*dd*, 11.6, 2.2)
13	38.3	2.25 (*td*, 11.8, 3.6)	**$C_{2''}$-Glc**		
14	42.8	-	**1"'**	103.4	4.73 (*d*, 7.8)
15	30.9	1.13 (*m*)	**2"'**	74.5	3.24 (*t*, 8.4)
		1.59 (*m*)	**3"'**	76.1	3.40 (*t*, 9.0)
16	31.3	1.45 (*m*)	**4"'**	70.0	3.37 (*m*)
		2.57 (*dm*, 12.7)	**5"'**	76.9	3.36 (*m*)
17	56.7	-	**6"'**	61.1	3.77 (*dd*, 12.0, 4.1)
18	49.4	1.65 (*t*, 11.0)			3.99 (*dd*, 12.1, 1.7)
19	46.9	3.02 (*td*, 11.0, 4.8)	**$C_{3''}$-Glc**		
20	150.4	-	**1''''**	103.4	4.59 (*d*, 7.8)
21	30.2	1.38 (*m*)	**2''''**	74.0	3.30 (*t*, 8.4)
		1.90 (*t*, 10.7)	**3''''**	76.8	3.42, (*m*)
22	36.2	1.50 (*t*, 12.5)	**4''''**	70.2	3.30 (*t*, 8.9)
		2.04 (*dd*, 12.4, 8.2)	**5''''**	76.8	3.38, (*m*)
23	30.0	1.10 (*s*)	**6''''**	61.2	3.65 (*dd*, 11.9, 6.7)
24	18.5	0.94 (*s*)			3.92 (*dd*, 11.9, 2.3)
25	17.9	1.09 (*s*)			
26	16.4	1.02 (*s*)			
27	14.0	1.01 (*s*)			
28	174.5	-			
29	18.2	1.71(*s*)			
30	108.8	4.61 (*brs*)			
		4.73 (*brs*)			

Table 3: ^{1}H and ^{13}C NMR data of the aglycone of compounds **4** and **9** in CD_3OD.

	4[a]		**9**[b]	
Position	δ_C	δ_H (*m*, *J* in Hz)	δ_C	δ_H (*m*, *J* in Hz)
1	38.6	0.98 (*td*, 14.2, 5.0)	38.3	0.99 (*td*, 13.2, 3.9)
		1.71 (*m*)		1.74 (*ddd*, 12.3, 4.3, 2.2)
2	26.0	1.73 (*dl*, 16.9, 5.9)	26.6	1.60 (*ddd*, 11.6, 6.3, 3.7)
		1.87 (*dd*, 18.5, 4.6)		1.64 (*dd*, 13.3, 3.8*)*
3	88.2	3.14 (*dd*, 11.9, 4.7)	78.0	3.15 (*dd*, 11.4, 5.0)
4	39.1	-	38.7	-
5	56.1	0.78 (*dm*, 11.2)	55.6	0.76 (*dd*, 11.3 , 2.4)
6	17.7	1.53 (*dm*, 9.5)	17.8	1.55 (*dd*, 9.6, 3.6)
		1.63 (*dd*, 13.5, 8.2)		1.63 (*m*)
7	34.9	1.52 (*dd*, 10.6, 2.5)	34.9	1.52 (*dd*, 13.3, 2.6)
		1.63 (*dd*, 13.5, 8.2)		1.62 (*dd*, 12.0, 1.3)
8	36.7	-	36.7	-
9	52.5	0.91 (*dd*, 12.9, 5.8)	52.4	0.92 (*dd*, 13.1, 3.3)
10	36.8	-	37.1	_
11	20.7	1.54 (*dd*, 11.4, 2.5)	20.7	1.54 (*dd*, 16.7, 3.8)
		1.70 (*dd*, 9.4, 1.5)		1.70 (*m)*
12	25.3	1.75 (*dd*, 8.0, 6.3)	25.3	1.74 (*dd*, 13.2, 2.1)
		2.21 (*ddd*, 16.1, 9.6, 4.6)		2.23 (*ddd*, 13.5, 5.0, 2.3)
13	41.3	2.87 (*d*, 10.6)	41.4	2.87 (*dm*, 11.7)
14	53.4	-	53.5	-
15	39.9	1.38 (*d*, 9.2)	39.9	1.38 (*d*, 9.2)
		1.80 (*d*, 9.2)		1.80 (*d*, 9.3)
16	106.8	-	106.8	-
17	133.5	-	133.6	-
18	66.1	4.08 (*brs*)	66.1	4.08 (*brs*)
19	15.4	0.90 (*s*)	15.2	0.90 (*s*)
20	124.1	-	124.1	-
21	16.2	1.67 (*s*)	16.2	1.67 (*s*)
22	35.6	4.70 (*dd*, 20.4, 10.1)	35.6	1.81 (*dt*, 18.6, 3.4)
		1.93 (*ddd*, 20.1, 4.6, 2.8)		1.92 (*ddd*, 11.2, 3.6, 1.2)
23	69.0	4.70 (*ddd*, 15.8, 8.1, 3.5)	69.0	4.71 (*ddd*, 11.5, 7.9, 3.4)
				-
24	124.4	5.20 (*dt*, 8.5, 1.4)	124.4	5.19 (*dt*, 8.4, 1.4)
25	136.5	-	135.5	-
26	24.4	1.76 (s)	24.4	1.76 (*s*)
27	17.0	1.72 (s)	17.0	1.72 (*s*)
28	27.0	1.05 (s)	27.2	0.99 (*s*)
29	15.7	0.89 (s)	14.7	0.80 (*s*)
30	17.8	1.12 (*s*)	17.8	1.13 (*s*)

[a] NMR data were measured in CD_3OD at 600 MHz (^{1}H) and 150 MHz (^{13}C)
[b] NMR data were measured in CD_3OD at 500 MHz (^{1}H) and 125 MHz (^{13}C)

Table 4: ^{1}H NMR data of the osidic part of compounds **4-8** in CD_3OD.

Position	**4**[a] δ_H (*m*, *J* in Hz)	**5**[b] δ_H (*m*, *J* in Hz)	**6**[b] δ_H (*m*, *J* in Hz)	**7**[a] δ_H (*m*, *J* in Hz)	**8**[a] δ_H (*m*, *J* in Hz)
C-3-Ara					
1'	4.30 (*d*, 7.4)	4.52 (*d*, 7.7)	4.39 (*d*, 6.5)	4.40 (*d*, 6.5)	4.40 (*d*, 6.5)
2'	3.73 (*dd*, 9.4, 7.4)	3.90 (*dd*, 8.5, 7.5)	3.90 (*dd*, 8.9, 6.5)	3.90 (*dd*, 9.2, 6.5)	3.91 (*dd*, 8.7, 6.5)
3'	3.66 (*dd*, 9.4, 3.3)	3.88 (*dd*, 8.5, 3.3)	3.88 (*t*, 9.1)	3.87 (*t*, 9.2)	3.87 (*dd*, 9.0, 2.7)
4'	4.05 (*m*)	4.04 (*m*)	4.07 (*m*)	4.07 (*m*)	4.07 (*m*)
5'	3.57 (*dd*, 12.6, 1.3)	3.52 (*dd*, 11.9, 1.4)	3.57 (*dd*, 12.5, 1.6)	3.55 (*dd*, 12.5, 1.4)	3.55 (*dd*, 12.5, 1.8)
	3.87 (*dd*, 12.5, 2.5)	3.89 (*dd*, 11.9, 2.5)	3.86 (*dd*, 12.5, 2.6)	3.87 (*dd*, 12.5, 3.0)	3.87 (*dd*, 12.5, 3.0)
C-2'-Rha					
1"		5.24 (*d*, 1.5)	5.20 (*d*, 1.6)	5.27 (*d*, 1.4)	5.31 (*d*, 1.3)
2"		3.93 (*dd*, 3.3, 1.7)	3.94 (*dd*, 3.3, 1.7)	4.06 (*m*)	4.03 (*dd*, 3.2, 1.7)
3"		3.73 (*dd*, 9.7, 3.6)	3.72 (*dd*, 9.7, 3.7)	3.73 (*dd*, 9.5, 3.4)	3.74 (*dd*, 9.6, 3.4)
4"		3.41 (*t*, 9.6)	3.44 (*t*, 9.8)	3.43 (*t*, 9.5)	3.44 (*t*, 9.6)
5"		3.88 (*m*)	3.37 (*dq*, 9.8, 6.3)	3.97 (*m*)	3.97 (*dq*, 9.6, 6.3)
6"		1.24 (*d*, 6.2)	1.24 (*d*, 6.3)	1.25 (*d*, 6.2)	1.25 (*d*, 6.2)
C-3'-Glc					
1"'	4.57 (*d*, 7.7)	4.51 (*d*, 7.7)	4.66 (*d*, 7.3)	4.70 (*d*, 7.5)	4.70 (*d*, 7.5)
2"'	3.31 (*m*)	3.31 (*dd*, 8.9, 7.6)	3.62 (*dd*, 9.1, 7.2)	3.71 (*t*, 8.1)	3.71 (*dd*, 9.0, 7.5)
3"'	3.40 (*t*, 8.9)	3.40 (*t*, 8.8)	3.61 (*t*, 9.1)	3.63 (*dd*, 9.0, 8.1)	3.64 (*t*, 9.0)
4"'	3.36 (*t*, 8.9)	3.36 (*t*, 8.7)	3.39 (*t*, 9.0)	3.40 (*t*, 9.0)	3.40 (*t*, 9.6)
5"'	3.31 (*m*)	3.33 (*d*, 8.2)	3.32 (*m*)	3.34 (*m*)	3.34 (*m*)
6"'	3.86 (*dd*, 11.6, 5.2)	3.70 (*dd*, 12.0, 2.1)	3.68 (*dd*, 11.9, 5.5)	3.69 (*dd*, 11.9, 5.6)	3.69 (*dd*, 12.0, 5.2)
	3.70 (*dd*, 11.4, 2.8)	3.86 (*dd*, 12.0, 2.1)	3.88 (*dd*, 11.9, 2.3)	3.86 (*dd*, 8.8, 3.0)	3.86 (*dd*, 12.0, 2.5)
C-2"'-Glc					
1""			4.77 (*d*, 7.4)	4.89 (*d*, 7.3)	4.88 (*d*, 7.3)
2""			3.39 (*m*)	3.43 (*t*, 8.9)	3.43 (*t*, 8.8)
3""			3.39 (*m*)	3.38 (*t*, 9.0)	3.40 (*t*, 9.0)
4""			3.39 (*t*, 8.6)	3.45 (*t*, 8.9)	3.42 (*t*, 9.2)
5""			3.31 (*m*)	3.39 (*m*)	3.47 (*m*)
6""			3.68 (*dd*, 11.9, 5.5)	3.79 (*dd*, 11.4, 4.6)	3.80 (*dd*, 11.4, 5.6)
			3.91 (*dd*, 12.0, 2.6)	4.16 (*dd*, 11.4, 1.5)	4.16 (*dd*, 11.4, 2.4)
C-6""				**Xyl**	**Ara**
1""'				4.34 (*d*, 7.5)	4.36 (*d*, 6.8)
2""'				3.31 (*t*, 8.6)	3.65 (*dd*, 9.1, 6.9)
3""'				3.38 (*t*, 9.0)	3.61 (*dd*, 9.1, 3.4)
4""'				3.52 (*ddd*, 10.6, 9.0, 5.4)	3.83 (*td*, 3.4, 2.5)
5""'				3.25 (*dd*, 11.4, 10.5)	3.61 (*dd*, 11.4, 3.4)
				3.90 (*dd*, 11.4, 5.4)	3.90 (*dd*, 11.3, 2.5)

[a] NMR data were measured in CD_3OD at 600 MHz (^{1}H) and 150 MHz (^{13}C)
[b] NMR data were measured in CD_3OD at 500 MHz (^{1}H) and 125 MHz (^{13}C)

Table 5: ^{13}C NMR data of the osidic part of compounds **4-8** in CD_3OD

Position	**4**[a]	**5**[b]	**6**[b]	**7**[a]	**8**[a]
	δ_C	δ_C	δ_C	δ_C	δ_C
C-3-Ara					
1'	105.7	106.8	104.6	104.7	104.7
2'	70.7	73.9	73.9	75.0	74.7
3'	82.4	80.9	81.7	80.9	81.0
4'	68.1	67.2	69.3	69.0	68.8
5'	65.2	63.3	65.1	64.7	64.7
C-2'-Rha					
1"		100.6	99.8	100.8	100.6
2"		70.7	70.9	70.5	70.5
3"		70.7	70.8	70.8	70.8
4"		72.4	72.5	72.5	72.4
5"		68.9	68.7	68.7	68.7
6"		16.6	16.4	16.8	16.7
C-3'-Glc					
1"'	104.0	102.9	104.4	101.6	101.6
2"'	76.5	73.7	76.5	80.2	80.1
3"'	76.3	70.7	74.4	76.9	76.9
4"'	69.8	69.8	69.4	69.8	69.7
5"'	73.9	70.7	77.2	76.6	76.6
6"'	61.0	61.0	60.8	61.1	61.1
C-2"'-Glc					
1""			102.0	102.7	102.6
2""			82.4	73.8	73.8
3""			76.8	76.7	76.7
4""			69.7	69.6	69.9
5""			76.6	75.8	75.9
6""			61.1	68.1	68.1
C-6""				**Xyl**	**Ara**
1""'				104.3	104.1
2""'				73.3	71.0
3""'				76.1	72.6
4""'				69.8	68.2
5""'				65.6	65.5

[a] NMR data were measured in CD_3OD at 600 MHz (^1H) and 150 MHz (^{13}C)
[b] NMR data were measured in CD_3OD at 500 MHz (^1H) and 125 MHz (^{13}C)