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ABSTRACT:

Ten previously undescribed triterpenoid saponins and a previously undescribed *nor*lupane triterpenoid were isolated, with three known saponins, four known flavonoids, two known lupane derivatives, sitosterol and 6'-heptadecanoyl-3-*O*-*β*-D-glucopyranosylsitosterol from the leaves of *Alphitonia xerocarpus* (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 activity. The aqueous methanol extract showed antioxidant activity (DPPH assay) due to the presence of rutin. Ceanothenic acid showed good cytotoxic activity against a KB cell line (IC_{50} = 2.6 µM) and antibacterial activity against *Staphylococcus aureus* and *Enterococcus faecalis* with MIC values of 8 and 16 µg/mL, respectively. The previously undescribed 29-hydroxyceanothenic acid exhibited moderate cytotoxic activity (IC_{50} = 10 µM), good antibacterial activity against *S. aureus* (MIC = 4 µg/mL) and moderate antibacterial activity against *E. faecalis* (MIC =16 µg/mL).

Keywords: *Alphitonia xerocarpus*; Rhamnaceae; leaves; triterpenoids; *seco*dammarane; *nor*lupane; flavonoids

INTRODUCTION

New Caledonia is well known for its high biodiversity with a global endemic area of 74.3%. This high endemicity is partly due to the multiplicity of geological substrates, with one-third of the total area covered by ultramafic rocks. Erosion and alteration of these rocks have led to nutrient depletion and soil enrichment with heavy metals resulting in the development of a unique flora. In New Caledonia, the Rhamnaceae family is represented by a half-dozen genera and a total of ten species, of which half are endemic. The genus *Alphitonia* is the best represented, with three endemic species (*A. neocaledonica* (Schltr.) Guillaumin, *A. xerocarpus* Baill. and *A. erubescens* Baill.) (Guillaumin, 1948). This genus is composed of approximately 20 species growing in tropical regions of Southeast Asia, Oceania and Polynesia (Correa *et al*., 2010, Richardson *et al.*, 2000). A range of phenolic compounds has already been identified from various *Alphitonia* species (Branch *et al.*, 1972, Guise *et al.*, 1962, Jou *et al*., 2004). More precisely, flavonoids have been identified in the leaves (Lin *et al.*, 1995) and fruit (Muhammad *et al.*, 2015) of *A. neocaledonica*. Lupane triterpenoids, including ceanothic acid, betulin, betulinic acid and alphitolic acid, have also been detected in several *Alphitonia* species (Branch *et al.*, 1972, Guise *et al.*, 1962, Jou *et al.*, 2004, Muhammad *et al.*, 2015, Setzer *et al.*, 2004) and showed *in vitro* cytotoxic, anti-inflammatory, and antimicrobial activity (Dzuback *et al.*, 2006). In addition, various dammarane saponins, including derivatives of jujubogenin (Kimura *et al.*, 1981, Renault *et al.*, 1997) and 16,17-*seco*dammarane (Yoshikawa *et al.*, 1996) have been isolated from the Rhamnaceae and *Alphitonia* species (Li *et al.*, 1994).

Alphitonia xerocarpus Baill (Rhamnaceae) is a small forest tree growing on the mountains in the south of New Caledonia at an altitude of 800-900 meters (Baillon, 1876). The leaves are tough, completely hairless, alternate and elliptical (3x6 cm) with rounded corners (Schlechter, 1907). In a continuation of the study of New-Caledonian *Alphitonia* species (Muhammad *et al.*, 2015), we investigated the secondary metabolite profile of *Alphitonia xerocarpus* leaves. In this study, eleven previously undescribed (**1**-**11**) and eleven known (**12**-**22**) compounds were isolated. Flavonoids have been reported to possess antioxidant (Ko et al. 2011) and antityrosinase activity (Parvez et al. 2007) while triterpenoids from *Alphitonia* species have shown cytotoxic and antimicrobial activity (Dzuback *et al.*, 2006). In this study, the radical scavenging ability of the extracts was determined. The tyrosinase inhibitory activity of the flavonoids as well as the cytotoxic activity (against KB cells) and the antibacterial activity of some of the triterpenoids were also investigated.

2. Results and discussion

The powdered leaves of *Alphitonia xerocarpus* were 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 and RP-C_{18} column chromatography to give a previously undescribed *nor*lupane triterpenoid (**1**) along with the known betulin (**20**) (Guo *et al.*, 2011, Siddiqui *et al.*, 1988), ceanothenic acid (**12**) (Jou *et al.*, 2004), the major component (6 %), sitosterol (**21**) (Kovganko *et al.*, 2000, McCarthy *et al.*, 2005), and 6'-heptadecanoyl-3-*O*-*β*-D-glucopyranosylsitosterol (**22**) (Gutierrez-Lugo *et al.*, 2005).

The aqueous methanol extract was subjected to multiple chromatographic steps over silica gel and RP-C_{18} yielding ten previously undescribed compounds (**2**-**11**) with three known saponins, 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-[4-*O*-(sodium sulfonato)-*β*-D-glucopyranosyl-(1→3)]-*α*-L-arabinopyranosyljujubogenin (**13**), the sodium salt of a kwown saponin (Maciuk *et al.*, 2004), 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-[*β*-D-glucopyranosyl-(1→3)]-*α*-L-arabinopyranosyljujubogenin (**14**) (Okamura *et al.*, 1981) and 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) and four known flavonoids, rutin (**16**) (Lallemand *et al.*, 1977, Li *et al.*, 2008), kaempferol 3-*O*-rutinoside (**17**) (Park *et al.*, 2008), 3-*O*-*α*-L-arabinopyranosyl-(1→2)-*α*-L-rhamnopyranosylkaempferol (**18**) (Muzitano *et al.*, 2006), and 3-*O*-*β*-D-xylopyranosyl-(1→2)-*α*-L-rhamnopyranosylkaempferol (**19**) (Bilia *et al.*, 1996, Soicke *et al.*, 1990) (Figure 1). Rutin was the major component of this extract (3.4%) and was isolated from the leaves in 1.16% yield.

All compounds were identified by extensive spectroscopic methods including 1D- (^{1}H and ^{13}C) and 2D-NMR (COSY, *J*-modulated HSQC, HMBC and NOESY) experiments as well as HR-ESI-MS analysis and by comparison with literature spectral data for the known compounds. Acid hydrolysis of the aqueous methanol extract afforded four sugar units in the aqueous layer, identified by HPLC analysis on a chiral column (Lavaud *et al.*, 2015, Lopes and Gaspar, 2008), as D-glucose (Glc), D-xylose (Xyl), L-arabinose (Ara) and L-rhamnose (Rha).

Compound **1** was obtained as a white amorphous powder with the molecular formula $C_{29}H_{42}O_5$ [HRESIMS: *m*/*z* 493.2936 ([M + Na]$^+$, calcd 493.2930]. The ^{1}H NMR spectrum of **1** showed the presence of four tertiary methyl groups (δ_H 0.94, 1.01 (6H), and 1.08), an exomethylene and a disubstituted double bond [δ_H 4.95 (*brs*), 4.99 (*d*, *J*=1.5 Hz), 5.43 (*d*, *J*=5.7 Hz), and 5.96 (*d*, *J*=5.7 Hz)], and an oxymethylene group (δ_H 4.05, and 4.16, each *d*, *J*=14.8 Hz). Its ^{13}C NMR spectrum exhibited signals for 29 carbons (Table S1), including two

carboxyl groups (δ_C 178.6 and 179.3), two double bonds (δ_C 107.7, 140.1, 141.7, and 155.5), and an oxygenated methylene (δ_C 64.9). The spectroscopic data were similar to those of ceanothenic acid (**12**) (Jou *et al.*, 2004). The only difference is in the presence of a hydroxyl group attached to C-29. This was readily confirmed by HMBC correlations from H_2-30 to C-29 and from H_2-29 and H_2-30 to C-19 (δ_C 44.6) and C-20 (δ_C 155.5), and by a long range $^4J_{H\text{-}H}$ COSY correlation from H_2-29 to the exomethylene protons H_2-30. Full assignments of the proton and carbon resonances of compound **1** were achieved by analysis of the COSY, *J*-modulated HSQC and HMBC spectra. Thus compound **1** is 29-hydroxyceanothenic acid. Compound **2** had the molecular formula $C_{60}H_{96}O_{30}$ [HRESIMS: *m/z* 1319.5892 $[M+Na]^+$, calcd for $C_{60}H_{96}O_{30}Na$, 1319.5884]. The ^{1}H NMR spectrum of the aglycone of **2** 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 1). 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 **2** revealed the presence of five anomeric protons at δ_H 5.64 (*d*, *J*=8.3 Hz), 5.04 (*d*, *J*=7.6 Hz), 4.71 (*d*, *J*=7.8 Hz), 4.68 (*d*, *J*=7.8 Hz) and 4.58 (*d*, *J*=7.8 Hz) correlated in the *J*-modulated HSQC spectrum with anomeric carbons at δ_C 91.9, 101.2, 103.3, 103.6 and 103.6, respectively (Table 1). Analysis of the COSY, TOCSY and *J*-modulated HSQC spectra of **2** allowed complete assignment of the five glycosidic proton and carbon systems leading to five β-D-glucopyranose units (Agrawal, 1992) (Table 1). The β-anomeric configurations, deduced from the large coupling constant $J_{H\text{-}1\text{-}H\text{-}2}$ of 7-8 Hz and the ^{13}C NMR chemical shift (Agrawal, 1992), 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 four anomeric carbons between δ_C 101-104 indicated that these carbons were involved in ether linkages while the anomeric carbon at δ_C 91.9 (δ_H 5.64) is linked by an ester bond. This was confirmed by the HMBC correlations between Glc-H-1' (δ 5.64) and C-28 (δ 174.5). Other HMBC correlations between Glc-H-1" (δ 5.04) and Glc-C-2' (δ 75.7), Glc-H-1'" (δ 4.71) and Glc-C-2" (δ 81.3), Glc-H-1"" (δ 4.58) and Glc-C-3" (δ 86.6); and Glc-H-1""" (δ 4.68) and Glc-C-6" (δ 68.7) revealed the sequence of the pentasaccharide moiety with the second glucose unit trisubstituted in positions C-2", C-3" and C-6". Thus the structure of

saponin **2** was deduced as 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. Compound **3** has a molecular formula $C_{42}H_{68}O_{14}$ [HRESIMS: *m/z* 819.4529 $[M+Na]^+$; calcd for $C_{42}H_{68}O_{14}Na$, 819.4507]. The ^{1}H NMR spectrum of the aglycone of **3** showed signals of a dammarane triterpenoid, characterized by seven tertiary methyl groups (δ_H 0.87, 0.91, 1.06, 1.07, 1.21, 1.65 and 1.71), a vinyl proton (δ_H 5.22, *tq*, *J*=7.3, 0.7 Hz), two oxygen-bearing methines [δ_H 3.19 (*dd*, *J*=7.3, 4.8 Hz), 4.14 (*t*, *J*=6.6 Hz)], and an oxygen-bearing methylene group [δ_H 3.96 (*dd*, *J*=8.3, 1.6 Hz), 3.99 (*d*, *J*=8.3 Hz)]. Its ^{13}C NMR and *J*-modulated HSQC spectra exhibited signals for seven methyl groups [δ_C 15.4 (C-19), 15.5 (C-29), 16.5 (C-27), 17.8 (C-30), 22.1 (C-21), 24.5 (C-26), and 27.0 (C-28)], two oxymethine carbons [δ_C 89.1 and 94.1], two olefinic carbons [δ_C 120.7 (C-24) and 132.6 (C-25)], an oxymethylene carbon [δ_C 65.4], an acetal carbon [δ_C 117.5] and a quaternary oxygenated carbon [δ_C 75.1] (Table 2). These data suggested that compound **3** had the same genin as jujuboside IV (Wang *et al.*, 2013). Analysis of the COSY, *J*-modulated HSQC and HMBC spectra confirmed its identity as 16*β*,22*R*:16*α*,18-diepoxydammar-24-ene-3*β*,20*R*-diol* (Maciuk *et al.*, 2004, Wang *et al.*, 2013). The deshielded nature of C-3 (δ_C 89.1) suggested a monodesmosidic saponin. The ^{1}H and ^{13}C-NMR spectra revealed the presence of two sugar units with anomeric protons at δ_H 4.42 (*d*, *J* =7.8 Hz) and 4.35 ppm (*d*, *J*=7.8 Hz) and the corresponding carbons at δ_C 104.8 and 106.7 (Table 1). Analysis of COSY and *J*-modulated HSQC spectra allowed assignment of two *β*-D-glucopyranose units, one terminal (δ_H 4.42) and the second substituted on the hydroxyl at C-6' (δ_C 69.9) (Agrawal, 1992). The HMBC correlations between Glc-H-1" (δ 4.42)/Glc-C-6', Glc-H-1' (δ 4.35)/C-3 (δ 89.1), and the rOe correlations between Glc-H-1"/Glc-H-6', Glc-H-1'/H-3 revealed the linkage of the disaccharide moiety. Thus, the structure of saponin **3** was identified as 3-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl-16*β*,22*R*:16*α*,18-diepoxydammar-24-ene-3*β*,20*R*-diol (Figure 1).

Comparison of the ^{1}H NMR and ^{13}C NMR spectra of compounds **4-5** with the known compounds **13-15** indicated that they all possess the same genin, jujubogenin, glycosylated at C-3 (δ_C 89.6) (Maciuk *et al.*, 2004).

***The correct biogenetic numbering system for dammaranes, as listed in the Dictionary of Natural Products, is used in this paper (Connolly and Hill, 1992). Methyl groups 18 and 30 are wrongly numbered in the paper by Wang et al. 2013 and many other articles. Thus, C-18 and C-30 should be interchanged to be conforming to the IUPAC nomenclature*.

Compounds **4** and **5** were isolated as a mixture 6:4, inseparable by chromatography on RP-18 and silica gel. They had the same molecular formula $C_{58}H_{94}O_{26}$, [HRESIMS: *m/z* 1229.5923 $[M+Na]^+$; calcd for $C_{58}H_{94}O_{26}Na$, 1229.5931] and contained 132 additional molecular weight units relative to jujuboside I (**15**), suggesting the presence of an additional pentose unit (Wang *et al.*, 2013). The ^{1}H- and ^{13}C-NMR spectra of **4** and **5** were very similar to those of jujuboside I (**15**) with signals assignable to jujubogenin and five sugars moieties. Five anomeric signals were observed in ^{1}H-NMR and ^{13}C-NMR spectra at δ_H 4.34 (*d*, *J* =7.5 Hz, δ_C 104.4), 4.39 (*d*, *J* =6.5 Hz, δ_C 104.7), 4.69 (*d*, *J* =7.8 Hz, δ_C 101.6), 4.89 (*d*, *J* =6.9 Hz, δ_C 102.7) and 5.26 (*d*, *J*=1.5 Hz, δ_C 100.8) for compound **4** and at δ_H 4.36 (*d*, *J* =6.8 Hz, δ_C 104.1), 4.39 (*d*, *J* =6.5 Hz, δ_C 104.7), 4.69 (*d*, *J* =7.8 Hz, δ_C 101.6), 4.89 (*d*, *J* =6.9 Hz, δ_C 102.6) and 5.31 (*d*, *J*=1.5 Hz, δ_C 100.6) for compound **5** (Table 3). Analysis of COSY, TOCSY, *J*-modulated HSQC, and HMBC experiments identified four sugars moieties as in jujuboside I (**15**) (Wang *et al.*, 2013), two *β*-D-glucopyranoses from the anomeric protons at δ_H 4.69 and 4.89, one substituted at position C-6"" (δ_C 68.0), a terminal *α*-L-rhamnopyranose (δ_H 5.26) with its methyl signal at δ_H 1.25 (d, *J*=6.2 Hz) and δ_C 16.8, and an *α*-L-arabinopyranose (δ_H 4.39) disubstitued at positions C-2' (δ_C 74.7) and C-3' (δ_C 81.1) (Agrawal, 1992) (Table 3). The supplementary pentose unit was identified as a *β*-D-xylopyranose in compound **4** and an *α*-L-arabinopyranose in **5** (Agrawal, 1992). These sugars were attached to the *β*-D-glucopyranose at C-6"" as suggested by its deshielded signal (δ_C 68.0) (Table 3). The interglycosidic linkage was established by the HMBC correlations 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 **4** 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 **5**. Thus, the structure of compound **4** 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-arabinopyranosyljujubogenin, and compound **5** 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-arabinopyranosyljujubogenin (Figure 1).

Compound **6**, a white amorphous powder, has the molecular formula $C_{42}H_{68}O_{15}$ [HRESIMS *m/z* 835.4463 $[M+Na]^+$: calcd for $C_{42}H_{68}O_{15}Na$, 835.4456]. The ^{1}H- and ^{13}C-NMR spectra of the aglycone were very similar to those of jujubogenin. The main difference was the presence of an additional secondary hydroxyl group [δ_H 3.01 (s), δ_C 73.8] (Table 2) which was readily located at C-22 by a COSY correlation between H-22 and H-23 and by HMBC correlations from H-22 to C-17, C-20, C-21, C-23 and C-24. The small coupling constant between H-22

and H-23 and the rOe effect observed between the equatorial Me-21 and H-22 indicated that the C-22 hydroxyl was *α*-oriented (axial) (Figure 2). Thus the genin of compound **6** is the previously undescribed 22*α*-hydroxyjujubogenin, which is glycosylated at the C-3 position (δ_C 89.1). The ^{1}H-, ^{13}C-NMR, COSY and *J*-modulated HSQC spectra revealed the presence of a terminal *β*-D-glucopyranose and a C-6' substituted *β*-D-glucopyranose as in compound **3** (Agrawal, 1992) (Table 4). The HMBC correlations between Glc-H-1"/Glc-C-6' and Glc-H-1'/C-3 confirmed the linkage of the disaccharide moiety. Thus, saponin **6** is 3-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl-22*α*-hydroxyjujubogenin (Figure 1).

The ^{1}H-NMR and ^{13}C-NMR spectra of compound **7** were very similar to those of compound **6** with the same disaccharide chain attached to C-3 of 22α-hydroxyjujubogenin but they indicated that it was a 1:1 mixture of esters at C-6" (Table 4). Both components of the mixture possessed the same molecular formula $C_{51}H_{74}O_{17}$ [HRESIMS: *m/z* 981.4822 $[M+Na]^{+}$; calcd for $C_{51}H_{74}O_{17}Na$, 981.4824]. The ^{1}H- and ^{13}C-NMR spectra readily revealed the presence of *Z*- and *E*-*p*-coumaroyl groups (El Sohly *et al.*, 1999) (Table 4). The HMBC correlations from the ester carbonyl at δ_C 166.7 to the *Z*-vinyl protons [δ_H 5.84 and 6.91 (each *d*, *J*=12.8 Hz)] and to Glc-H-6" [4.24 (*dd*, *J*=11.9, 5.8 Hz) and 4.53 (*dd*, *J*=11.9, 2.1 Hz)] indicated that the structure of compound **7a** is 3-*O*-[6-*O*-(Z-*p*-coumaroyl-*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranosyl-22α-hydroxyjujubogenin. Other HMBC correlations from the ester carbonyl at δ_C 167.7 to the *E*-vinyl protons [δ_H 6.40 and 7.68 (each *d*, *J*=16.1 Hz)] and to Glc-H-6" [δ_H 4.29 (*dd*, *J*=11.9, 5.7 Hz) and 4.60 (*dd*, *J*=11.9, 2.2 Hz)] confirmed that the structure of compound **7b** is 3-*O*-[6-*O*-(*E*-*p*-coumaroyl-*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranosyl-22*α*-hydroxyjujubogenin (Figure 1).

The ^{1}H-NMR and ^{13}C-NMR spectra of compound **8** were very similar to those of compound **7** and again showed that it was a mixture of compounds esterified at position C-6" (Table 4). Both components had the same molecular formula $C_{53}H_{78}O_{19}$ [HRESIMS: *m/z* 1041.5024 $[M+Na]^{+}$; calcd for $C_{53}H_{78}O_{19}Na$, 1041.5035]. In the ^{1}H-NMR and COSY spectra, signals for two sets of two methoxy groups [δ_H 3.92 (*s*, 6H) and δ_H 3.89 (*s*, 6H)], two aromatic protons [δ_H 6.98 (*s*, 2H) and δ_H 7.33 (*s*, 2H)], two vicinal vinyl protons [*E* δ_H 6.47 and 7.67 (each *d*, *J*=15.9 Hz) and *Z* δ_H 5.89 and 6.90 (each *d*, *J*=13.0 Hz)] (Table 4) suggested the presence of sinapoyl esters in a ratio of 1:3 (*Z*:*E*). The ^{13}C-NMR and HSQC spectra exhibited signals for two ester carbonyls [δ_C 166.6 and 167.6], four vinyl carbons [δ_C 114.3, 115.3, 144.7 and 146.0], two quaternary aromatic carbons [δ_C 125.1 and 125.5], six oxygenated aromatic carbons [δ_C 137.7, 138.4, 147.2 (2C) and 148.1 (2C)], four aromatic methines [δ_C 105.1 (2C), and 108.7 (2C)] and four methoxy groups [δ_C 55.5 (2C), and 55.4 (2C)], consistent with a mixture of *Z*- and *E*- sinapoyl groups

(Xu *et al.*, 2010) (Table 4). HMBC correlations as above confirmed that compound **8** was a mixture of 3-*O*-[6-*O*-(*E*,*Z*)-sinapoyl-*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranosyl-22*α*-hydroxyjujubogenin (Figure 1).

Analysis of 1D and 2D NMR spectra of compounds **9-11** indicated that they were dammarane *O*-glycosides with identical disaccharides attached to C-3 of the genin. They therefore differed in the aglycone part. The three compounds were identified as 16,17-*seco*-dammarane derivatives, arising from a cleavage of the C-16-C-17 bond, migration of CH_3-21 from C-20 to C-17 and oxidation of C-20 and C-26 (Yoshikawa *et al.*, 1996).

Compound **9** had a molecular formula $C_{42}H_{64}O_{15}$ [HRESIMS: *m*/*z* 831.4138 $[M+Na]^+$, calcd for $C_{42}H_{64}O_{15}Na$, 831.4143]. The ^{1}H-NMR spectrum of the aglycone showed signals for four tertiary methyls [δ_H 0.88, 0.90, 1.05, and 1.08], a secondary methyl [δ_H 1.07 (*d*, *J*= 4.0 Hz)] a vinyl methyl [δ_H 1.90 (*brs*)], a vinyl proton [δ_H 7.32 (*dt*, *J*=6.2, 1.7 Hz)], two oxygen-bearing methine protons [δ_H 3.20 (*dd*, *J*=11.7, 3.6 Hz) and 5.39 (*m*)], and two deshielded oxymethylene protons [δ_H 4.39 and 4.49 (each *d*, *J*=10.6 Hz)]. The ^{13}C-NMR spectrum revealed six methyl groups [δ_C 9.1 (C-27), 10.4 (C-21), 15.1 (C-19), 15.6 (C-29), 17.5 (C-30), and 26.9 (C-28)], two ester/lactone carbonyl groups [δ_C 174.8 (C-26), 178.2 (C-16)], a ketone [δ_C 209.3 (C-20)], two olefinic carbons [δ_C 129.4 (C-25) and 149.6 (C-24)], an oxymethylene carbon [δ_C 70.0 (C-18)] and two oxymethine carbons [δ_C 88.5 (C-3) and 77.8 (C-23)] (Table 5). These data show that compound **9** is virtually the same as hovenidulcioside A1 and differs only in the presence of a ketone instead of an acetate at C-20 (Yoshikawa *et al.*, 1995; Yoshikawa *et al.*, 1996). HMBC correlations from H-22, H-17, H-13 and Me-21 to the ketone confirmed its position at C-20. The deshielded chemical shifts of C-17 (δ_C 45.8) and C-22 (δ_C 43.3), relative to hovenidulciogenin A ($\delta_{C\text{-}17}$ 37.7 and $\delta_{C\text{-}22}$ 35.0) (Yoshikawa *et al.*, 1995) are due to the presence of the C-20 ketone. They are in good agreement with the data for the C-20 of 16,17-*seco*-dammarane guoanogenine B (Kennelly *et al.*, 1993). ROe effects between the *β*-axial Me-29/Me-19, Me-19/Me-30, Me-30/H-13 and between the *a*-axial H-3/H-5 and H-5/H-9 confirm the expected stereochemistry (Figure 3). The crystal data of hovenidulcioside A1 (Yoshikawa *et al.*, 1995) suggest rOes from H-18a to Me-21 and from H-13 to H-17. The fact that these are observed supports the assumption that compound **9** has the same side chain stereochemistry as the hovenidulciosides. Definitive proof will require an X-ray crystal structure. The *β*-configuration of H-23 was assigned from the rOe effect between H_a-22 and H-23 and by comparison of the ^{13}C NMR data of C-23 (δ_C 77.8) with those of hovenidulcioside A1 (Yoshikawa *et al.*, 1995). Thus the genin of compound **9** is the previously undescribed (3*β*,17*R*,23*R*) 3-hydroxy-20-oxo-16,17-*seco*-21(20→17)-

*abeo*dammar-24-ene-16,18:26,23-diolide, glycosylated at C-3 (δ_C 88.5). The ^{1}H-, ^{13}C-NMR, COSY and *J*-modulated HSQC spectra revealed the presence of the same disaccharide unit as in hovenidulcioside A1, attached at C-3 (Table 5). Thus saponin **9** is the previously undescribed 3*β*-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyloxy-20-oxo-16,17-*seco*-21(20→17)-*abeo*dammar-24-ene-16,18:26,23-diolide (Figure 1).

Compounds **10** and **11** (Figure 1) have the same molecular formula $C_{42}H_{66}O_{15}$ [HRESIMS: *m/z* 833.4286 $[M+Na]^+$; calcd for $C_{42}H_{66}O_{15}Na$, 833.4299] corresponding to dihydro-derivatives of compound **9**. Analysis of 1D (^{1}H, ^{13}C) and 2D NMR spectra (COSY, *J*-modulated HSQC, and HMBC) indicated that they have the same disaccharide unit as compound **9** and differ only in the terminal γ-lactone of the aglycone. The vinyl carbons have been replaced by two shielded carbons at δ_C 33.7 (C-25) and 34.5 (C-24) in compound **10**, and at δ_C 35.5 (C-25) and 36.2 (C-24) in compound **11**. The C-27 methyl singlet has been replaced by a doublet at δ_H 1.27 (*d*, *J* = 7.3 Hz) (Table 5). These data indicate that both compounds **10** and **11** contain a saturated terminal γ-lactone. In the ROESY spectrum of compound **10** the rOe effect observed between the *β*-oriented protons H_a-22/H-23, and H-23/H-27 indicated a 23,25 *trans*-configuration and suggested a *β*-oriented Me 27 (Figure 4). The ^{3}C NMR chemical shifts of C-27 (δ_C 14.5) and carbons of the terminal γ-lactone were in accordance with the data of hovenidulciogenin B (Yoshikawa *et al.*, 1996). For compound **11**, the rOe effect observed between the *β*-oriented protons H_a-22/H-23, and H-23/H-25 suggested an *α*-oriented methyl 27 (Figure 4). This was readily confirmed by the ^{13}C NMR chemical shifts of C-27 (δ_C 13.7) and carbons of the terminal γ-lactone with the 25-*epi* hovenidulciogenin B (Yoshikawa *et al.*, 1996). Thus, compound **10** is the new (25*S*) 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyloxy-20-oxo-16,17-*seco*-21(20→17)-*abeo*dammarane-16,18:26,23-diolide and compound **11** is (25*R*) 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyloxy-20-oxo-16,17-*seco*-21(20→17)-*abeo*dammarane-16,18:26,23-diolide.

The antioxidant activity of the EtOAc and hydromethanol extracts at 200 µg/mL was 9.6% and 53.4%, respectively. The aqueous methanol extract was the most active with an EC_{50} = 180 µg/mL for the DPPH radical scavenging activity. This activity is low considering the presence of rutin (**16**), the major component in this extract, for which the DPPH radical scavenging activity has already been demonstrated with an EC_{50} = 15.3 µg/mL (Lue et al., 2010).

The aqueous methanol extract showed 10 % inhibition of tyrosinase. The four flavonoids (**16-19**) were tested at 1 mg/mL and no activity was observed, probably due to the glycosylation at

C-3, as observed previously with various flavonoids (Kubo and Kinst-Hori, 1999, Parvez *et al.*, 2007).

The cytotoxic activity of the two *nor*lupane triterpenoids (**1**, **12**), six dammarane saponins (**4-6, 11, 13, 15**) and the ceanothic acid saponin (**2**) against KB cell line, was measured using a WST-1 proliferation test. All tested saponins showed very low cytotoxic activity with growth inhibitions ranging from 6.6 to 22.9 % at 10 µg/mL (Table 6). The two triterpenoids were the most active with 79.5% (IC_{50} of 1.2 ± 0.3 µg/mL) and 58.4% (IC_{50} near 10 µg/mL) growth inhibition, respectively. The IC_{50} values of ceanothenic acid (**12**) showed good cytotoxic activity (2.6 µM) and is approximatively ten times more active than its previously undescribed derivative 29-hydroxyceanothenic acid (**1**).

The disk diffusion method was used to evaluate the possible antimicrobial activity of the three lupane triterpenes (**1**, **12**, **20**), six dammarane saponins (**4-6, 13-15**) and saponin **2** against four bacteria, including two Gram positive (*S. aureus* and *E. faecalis*) and two Gram negative (*E. coli* and *P. aeruginosa*). Only ceanothenic acid (**12**) and 29-hydroxyceanothenic acid (**1**) showed moderate antibacterial activity against *S. aureus* and *E. faecalis* with inhibition diameters of 14 and 16 mm, respectively. Compounds **1** and **12** showed good antibacterial activity against *S. aureus* with MIC values of 4 and 8 µg/mL, respectively and moderate antibacterial activity against *E. faecalis* (both MIC = 16 µg/mL) (Table 7).

In conclusion, twenty two compounds were isolated from the leaves of *A. xerocarpus* including thirteen triterpenoid saponins, two *nor*lupane triterpenoids and four flavonoids. Ten saponins (**2-11**) are previously undescribed compounds, and the genins of saponins **6** and **9-11** are described for the first time. All the flavonoids (**16-19**) have been detected for the first time in *Alphitonia* species. Rutin (**16**) and kaempferol 3-*O*-rutinoside (**17**) are common flavonoids and were previously isolated for example from the fruit of *Ziziphus jujuba* and *Z. spina-christi* fruits (Pawlowska *et al.*, 2008) or *Ziziphus lotus* leaves (Maciuk *et al.*, 2003), members of the Rhamnaceae family. The two other kaempferol flavonoids (**18-19**) were isolated for the first time from the Rhamnaceae family. Rutin is the major compound of the aqueous methanol extract and *A. xerocarpus* can be considered has a new source of rutin. The aqueous methanol extract showed antioxidant activity (DPPH assay) due to the presence of flavonoids.

Ceanothenic acid (**12**), the major compound of the leaves, showed good cytotoxic activity against a KB cell line (IC_{50} = 2.6 µM) and antibacterial activity against *S. aureus* and *E. faecalis* with MIC values of 8 and 16 µg/mL, respectively. The related 29-hydroxyceanothenic acid (**1**) exhibited moderate cytotoxic activity (IC_{50} = 10 µM) and good

antibacterial activity against *S. aureus* (MIC = 4 µg/mL) and moderate antibacterial activity against *E. faecalis* (MIC =16 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). 2D-NMR experiments were performed using standard Bruker microprograms. Chemical shifts (δ) are reported in ppm using the internal solvent resonances at δ_H 3.33 and δ_C 47.6 (CD_3OD). HR-ESI-MS experiments were performed using a hybrid quadrupole/time-of-flight (Q-TOF) instrument, equipped with a pneumatically assisted electrospray ion source operated in the 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.

Preparative and analytical TLC was 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. Analytical and semi-preparative HPLC was 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

Alphitonia xerocarpus Baill. leaves were collected by Pr. Mohammed Nour in September 2009 at the end of the cool season in the ultramafic soil of Bois de Sud, located in southern province. The botanical identification was made at the Laboratoire Insulaire du Vivant et de l'Environnement of the New Caledonia University. A voucher specimen (09NM002) has been deposited in the Herbarium of Noumea (New Caledonia).

3.3 Extraction and isolation

Powdered air–dried leaves of *A. xerocarpus* (150 g) were macerated overnight in 2.5 L of petroleum ether and lixiviated to give 2.8 g of petroleum ether extract after evaporation. The defatted powdered material was then macerated overnight and lixiviated with 2.5 L of EtOAc to afford, after evaporation of the solvent, 3.5 g of EtOAc extract. After drying, the resulting powdered material was refluxed for 3 h with MeOH-H_2O (8:2) (2.5 L). Evaporation under reduced pressure afforded 52 g of a aqueous methanol extract.

The EtOAc extract (3.5 g) was fractionated by silica gel column chromatography using a gradient of $CHCl_3$-MeOH (from 1:0 to 6:4), to afford 200 fractions (200 mL each). Fractions [37-99] eluted with $CHCl_3$-MeOH (99.5:0.5) contain compound **21** (2.8 mg). Fractions [53-65] (133 mg), eluted with $CHCl_3$-MeOH (98:2), were separated by silica gel column chromatography using a gradient of $CHCl_3$-MeOH (from 1:0 to 9:1), to afford compound **20** (4 mg). Fractions [118-123] (380.8 mg), eluted with $CHCl_3$-MeOH (85:15), were submitted to a silica gel column chromatography using a gradient of toluene:MeOH (from 1:0 to 7:3), and then the resulting fractions [91-103] were fractionated by RP-18 column chromatography, using a gradient of MeOH-H_2O (6:4 to 1:0), to give compound **22** (2.5 mg). Fractions [124-126] (237.7 mg), eluted with $CHCl_3$-MeOH (8:2), were precipitated into the mixture $CHCl_3$-MeOH (97.5:2.5), to give compound **12** (207 mg). Fractions [151-163] (158.5 mg), eluted with $CHCl_3$-MeOH (6:4), were submitted to a silica gel column chromatography using a gradient of $CHCl_3$-MeOH (from 99:1 to 9:1), and the resulting fractions were subjected to semi-prep HPLC on RP-18 eluting with MeOH-H_2O gradient system (81:19 to 86:14) during 15 min yielding compound **1** (*R*t 9.58 min; 7.5 mg).

A part of the aqueous methanol extract (19 g) was subjected to vacuum liquid chromatography on RP-18, eluting successively with 1 L of MeOH-H_2O (4:6, 6:4, 8:2 and 1:0), to give fractions I (7.74 g), II, (5.9 g), III (3.6 g) and IV (880 mg), respectively. From Fraction I, compound **16** (640 mg) was obtained by precipitation during the process of

methanol evaporation. Fraction II (5 g) was fractionated by silica gel column chromatography using a gradient of $CHCl_3$-MeOH-H_2O (from 1:0:0 to 60:40:7), to afford 128 fractions (200 mL each). Fractions [33-96] (133.6 mg), eluted with $CHCl_3$-MeOH (8:2), were separated by silica gel column chromatography using a gradient of $CHCl_3$-MeOH (9:1 to 8:2), to give compound **14** (13.3 mg) in the fractions [90-103], eluted with $CHCl_3$-MeOH (8:2). The resulting fractions [44-52], eluted with $CHCl_3$-MeOH (85:15), were subjected to semi-prep HPLC on RP-18 eluting with the isocratic mixture CH_3CN-H_2O 0.025% TFA (35:65) during 20 min yielding compounds **9** (*R*t 15.4 min; 2.3 mg), **10** (*R*t 16.6 min; 5.0 mg), and **11** (*R*t 17.5 min; 11.0 mg). Fractions [40-45] (284.1 mg), eluted with $CHCl_3$-MeOH (8:2), were fractionated by silica gel column chromatography using a gradient of toluene-MeOH (9:1 to 7:3). Fractions [40-54], eluted with toluene-MeOH (85:15), were subjected to semi-prep HPLC on RP-18 eluted with an isocratic mixture of CH_3CN-H_2O 0.025% TFA (25:75) during 25 min to afford compounds **17** (*R*t 11.1 min; 8.5 mg), **18** (*R*t 12.9 min; 10.5 mg), and **19** (*R*t 13.1 min; 3.6 mg). Fractions [55-64], eluted with toluene-MeOH (8:2), were further submitted to a silica gel preparative TLC using $CHCl_3$-MeOH-H_2O (70:30:5) as eluent to give 14 mg of compound **7**. Fractions [88-97] (225.6 mg), eluted with $CHCl_3$-MeOH-H_2O (70:30:5), was fractionated by RP-18 column chromatography using a gradient of MeOH-H_2O (from 3:7 to 7:3) to give compound **14** (7.6 mg) in the fractions [71-73]. The resulting fractions [74-79] (18.5 mg), eluted with MeOH-H_2O (4:6), were separated by silica gel preparative TLC using $CHCl_3$-MeOH-H_2O (70:30:5) as eluent to give compound **15** (9 mg). Fractions [115-116] (188.3 mg), eluted with $CHCl_3$-MeOH-H_2O (70:30:5), were fractionated by silica gel column chromatography using a gradient of toluene:MeOH (85:15 to 6:4, v/v). The resulting fractions [43-81], eluted with toluene-MeOH (75:25), were subjected to semi-prep HPLC on RP-18 eluting with a gradient of CH_3CN-H_2O 0.025% TFA (2:8 to 35:65) during 15 min to yield compounds **16** (*R*t 8.2 min; 2.1 mg), and **14** (*R*t 16.9 min; 3.8 mg). Fractions [123-125] (358.7 mg), eluted with $CHCl_3$-MeOH-H_2O (60:40:7), were fractionated by RP-18 column chromatography using a gradient of MeOH-H_2O (from 3:7 to 6:4). Fractions [55-67], eluted with MeOH-H_2O (45:55), were subjected to semi-prep HPLC on RP-18 eluting with an isocratic mixture of CH_3CN-H_2O 0.025% TFA (22:88) during 25 min to give compound **2** (*R*t 19.8 min; 13.1 mg). Fraction III (3 g) was fractionated by silica gel column chromatography using a gradient of $CHCl_3$-MeOH-H_2O (from 1:0:0 to 70:30:5), to afford 82 fractions (135 mL each). Fraction [32] (139.6 mg), eluted with $CHCl_3$-MeOH (8:2), was submitted to a RP-18 column chromatography, using a gradient of MeOH-H_2O (3:7 to 7:3), to give compound **13** (3.5 mg). The resulting fractions [161-182] (25.1 mg), eluted with MeOH-H_2O (55:45), were

subjected to semi-prep HPLC on RP-18 eluted with a gradient of CH_3CN-H_2O 0.025% TFA (39:61 to 4:6) during 15 min yielding compounds **8** (*R*t 6.7 min; 2.1 mg), and **7** (*R*t 8.7 min; 5.5 mg). Fractions [33-34] (178.6 mg), eluted with $CHCl_3$-MeOH (8:2), were fractionated by RP-18 column chromatography using a gradient of MeOH-H_2O (4:6 to 8:2), to give compound **13** (4.0 mg) eluted with $CHCl_3$-MeOH (45:55). The resulting fractions [59-63], eluted with MeOH-H_2O (6:4), were subjected to semi-prep HPLC on RP-18 eluting with an isocratic mixture of CH_3CN-H_2O 0.025% TFA (39:61) during 15 min to afford compounds **8** (*R*t 8.5 min; 4.0 mg), and **3** (*R*t 11.5 min; 2.5 mg). Fractions [35-39] (346.1 mg), eluted with $CHCl_3$-MeOH (8:2), were submitted to a silica gel column chromatography using a gradient of $CHCl_3$-MeOH-H_2O (from 1:0:0 to 70:30:5). The resulting fractions [47-54] (40.5 mg), eluted with $CHCl_3$-MeOH (8:2), were separated by semi-prep HPLC on RP-18 eluting with a gradient of CH_3CN-H_2O 0.025% TFA (35:65 to 45:55) during 15 min to give compound **14** (*R*t 14.2 min; 2.7 mg). Fractions [40-48] (260.1 mg), eluted with $CHCl_3$-MeOH (8:2), were fractionated by RP-18 column chromatography, using a gradient of MeOH-H_2O (4:6 to 7:3), to give compound **13** (98.6 mg) eluted with $CHCl_3$-MeOH (45:55). Fractions [110-125], eluted with MeOH-H_2O (5:5), were separated by silica gel column chromatography using a gradient of $CHCl_3$-MeOH (9:1 to 85:15) to give 3.1 mg of compound **6**. Fractions [137-143], eluted with MeOH-H_2O (6:4), were fractionated by silica gel column chromatography using a gradient of $CHCl_3$-MeOH (1:0 to 7:3) and the resulting fractions [90-137], eluted with $CHCl_3$:MeOH (85:15), were finally submitted to a silica gel prep TLC using $CHCl_3$-MeOH-H_2O 1% TFA (75:25:3) as eluent to afford 7.7 mg of compound **14**. Fractions [56-59] (272.9 mg), eluted with $CHCl_3$-MeOH (7:3), were fractionated by RP-18 column chromatography, using a gradient of MeOH-H_2O (3:7 to 9:1). Fractions [62-108] (37.8 mg), eluted with MeOH-H_2O (3:7), were then submitted to a silicagel prep TLC using $CHCl_3$-MeOH-H_2O 1% TFA (75:25:3) as eluent to afford compounds **14** (12.0 mg) and **15** (10.0 mg). Fractions [142-156] (33.4 mg), eluted with $CHCl_3$-MeOH (5:5), were fractionated by silica gel column chromatography using a gradient of $CHCl_3$:MeOH (1:0 to 70:30, v/v) to give compound **13** (16.0 mg). Fractions [171-174] (35 mg), eluted with MeOH-H_2O (8:2), contained a mixture of compounds **4** and **5** (6 mg), inseparable by silica gel column chromatography.

3.4 Compound characterization

*3.4.1. 29-hydroxyceanothenic acid (**1**)*

White amorphous powder; $[\alpha]_D$ +3.2° (*c* 0.16, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 0.94 (*s*, H-24), 1.01 (*s*, H-23), 1.01 (*s*, H-25), 1.08 (*s*, H-26), 1.26 (*dd*, 11.2-3.6, H-5), 1.39 (*dd*, 12.8-

2.9, H-16a), 1.44 (*m*, H-21a, H-22a), 1.45 (*m*, H-6a), 1.46 (*dd*, 15.3-3.9, H-15a), 1.49 (*m*, H-6b) 1.50 (*m*, H-11a), 1.55 (*dd*, 12.8-4.5, H-12a), 1.60 (*dd*, 12.8-4.9, H-11b), 1.64 (*brd*, 13.4, H-7a), 1.77 (*dd*, 12.6-5.6, H-7b), 1.83 (*t*, 11.3, H-18), 1.89 (*dd*, 12.5-2.9, H-9), 1.94 (*dd*, 10.7-8.3, H-22b), 2.06 (*dt*, 13.5-2.4, H-15b), 2.06 (*m*, H-21b), 2.23 (*dd*, 12.8-5.5, H-12b), 2.37 (*dt*, 12.8-3.2, H-16b), 2.42 (*td*, 12.5-5.3, H-13), 3.03 (*td*, 12.5-4.0, H-19), 4.05 (*d*, 14.8, H-30a), 4.16 (*d*, 14.8, H-30b), 4,95 (*brs*, H-29a), 4,99 (*d*, 1.5, H-29b), 5.43 (*d*, 5.7, H-3), 5.96 (*d*, 5.7, H-2); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 18.5 (C-6), 18.7 (C-26), 20.7 (C-25), 21.8 (C-24), 24.2 (C-11), 27.7 (C-12), 29.1 (C-15), 29.7 (C-23), 33.1 (C-21), 35.4 (C-16), 37.9 (C-22), 38.9 (C-7), 40.8 (C-13), 42.4 (C-8), 44.6 (C-19), 45.7 (C-4), 49.5 (C-9), 51.8 (C-10), 52.8 (C-18), 57.2 (C-17), 61.1 (C-14), 63.8 (C-5), 64.9 (C-29), 107.7 (C-30), 140.1 (C-3), 141.7 (C-2), 155.5 (C-20), 178.6 (C-27), 179.3 (C-28); HRESIMS (positive-ion mode) *m/z* : 493.2936 $[M+Na]^+$ (calcd for $C_{29}H_{42}O_{57}Na$, 493.2930).

*3.4. 2. 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 (**2**)*

White amorphous powder; $[\alpha]_D$ -26.4° (*c* 0.17, CH_3OH); ^{1}H NMR (MeOD-d4, 500 MHz) and ^{13}C NMR (MeOD-d4, 125 MHz), see Table 1; HRESIMS (positive-ion mode) *m/z* : 1319.5892 $[M+Na]^+$ (calcd for : $C_{60}H_{96}O_{30}Na$, 1319.5884).

*3.4.3. 3-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-16β,22:16α,18-diepoxydammar-24-ene-3β,20R-diol (**3**)*

White amorphous powder; $[\alpha]_D$ -8.3° (*c* 0.12, CH_3OH); ^{1}H NMR (MeOD-d4, 500 MHz) and ^{13}C NMR (MeOD-d4, 125 MHz), see Tables 1 and 2; HRESIMS (positive-ion mode) *m/z* : 819.4529 $[M+Na]^+$ (calcd for $C_{42}H_{68}O_{14}Na$, 819.4507).

*3.4.5. 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 (**4**)*

White amorphous powder; ^{1}H NMR (MeOD-d4, 500 MHz) and ^{13}C NMR (MeOD-d4, 125 MHz), see Tables 2 and 3; HRESIMS (positive-ion mode) *m/z*: 1229.5923 $[M+Na]^+$ (calcd for $C_{58}H_{94}O_{26}Na$, 1229.5931).

3.4.6. 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 (**5**)*

White amorphous powder; ^{1}H NMR (MeOD-d4, 500 MHz) and ^{13}C NMR (MeOD-d4, 125 MHz) of the aglycone part is identical to compound **4** ± 0.2 ppm; ^{1}H NMR (MeOD-d4, 500 MHz) and ^{13}C NMR (MeOD-d4, 125 MHz) of the osidic part, see Table 3; HRESIMS (positive-ion mode) *m/z* : 1229.5923 $[M+Na]^+$ (calcd for $C_{58}H_{94}O_{26}Na$, 1229.5931).

*3.4.7. 3-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-22α-hydroxyjujubogenin (**6**)*

White amorphous powder; $[\alpha]_D$ -27.8° (*c* 0.18, CH_3OH); ^{1}H NMR (MeOD-d4, 500 MHz) and ^{13}C NMR (MeOD-d4, 125 MHz), see Tables 2 and 4; HRESIMS (positive-ion mode) *m/z* : 835.4463 $[M+Na]^+$ (calcd for $C_{42}H_{68}O_{15}Na$, 835.4456).

*3.4.8. 3-O-[6-O-(trans,cis)-p-coumaroyl-β-D-glucopyranosyl-(1→6)]-β-D-glucopyranosyl-22α-hydroxyjujubogenin (**7**)*

White amorphous powder; $[\alpha]_D$ -15.8° (*c* 0.18, CH_3OH); ^{1}H NMR (MeOD-d4, 500 MHz) and ^{13}C NMR (MeOD-d4, 125 MHz) of the aglycone part is identical to compound **6** ± 0.2 ppm; ^{1}H NMR (MeOD-d4, 500 MHz) and ^{13}C NMR (MeOD-d4, 125 MHz) of the osidic part, see Table 4; HRESIMS (positive-ion mode) *m/z* : 981.4822 $[M+Na]^+$ (calcd for $C_{51}H_{74}O_{17}Na$, 981.4824).

*3.4.9. 3-O-[6-O-(trans,cis)-sinapoyl-β-D-glucopyranosyl-(1→6)]-β-D-glucopyranosyl-22α-hydroxyjujubogenin (**8**)*

White amorphous powder; $[\alpha]_D$ -22.9° (*c* 0.28, CH_3OH); ^{1}H NMR (MeOD-d4, 500 MHz) and ^{13}C NMR (MeOD-d4, 125 MHz) of the aglycone part is identical to compound **6** ± 0.2 ppm; ^{1}H NMR (MeOD-d4, 500 MHz) and ^{13}C NMR (MeOD-d4, 125 MHz) of the osidic part, see Table 4; HRESIMS (positive-ion mode) *m/z* : 1041.5024 $[M+Na]^+$ (calcd for $C_{53}H_{78}O_{19}Na$, 1041.5035).

*3.4.10. 3β-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyloxy-20-oxo-16,17-seco-21(20→17)-abeodammar-24-ene-16,18:26,23-diolide (**9**)*

White amorphous powder; $[\alpha]_D$ -14.1° (*c* 0.14, CH_3OH); ^{1}H NMR (MeOD-d4, 500 MHz) and ^{13}C NMR (MeOD-d4, 125 MHz), see Table 5; HRESIMS (positive-ion mode) *m/z* : 831.4138 $[M+Na]^+$ (calcd for $C_{42}H_{64}O_{15}Na$, 831.4143).

*3.4.11. (25S) 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyloxy-20-oxo-16,17-seco-21(20→17)-abeodammarane-16,18:26,23-diolide (**10**)*

White amorphous powder; $[\alpha]_D$ -46.6° (*c* 0.42, CH_3OH); ^1H NMR (MeOD-d4, 500 MHz) and ^{13}C NMR (MeOD-d4, 125 MHz), see Table 5; HRESIMS (positive-ion mode) *m/z* : 833.4286 $[M+Na]^+$ (calcd for $C_{42}H_{66}O_{15}Na$, 833.4299).

*3.4.12. (25R) 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyloxy-20-oxo-16,17-seco-21(20→17)-abeodammarane-16,18:26,23-diolide (**11**)*

White amorphous powder; $[\alpha]_D$ -38.9° (*c* 0.38, CH_3OH); ^1H NMR (MeOD-d4, 500 MHz) and ^{13}C NMR (MeOD-d4, 125 MHz), see Table 5; HRESIMS (positive-ion mode) *m/z* : 833.4286 $[M+Na]^+$ (calcd for $C_{42}H_{66}O_{15}Na$, 833.4299).

3.5. Sugar analysis and determination of absolute configuration

1 g of the crude aqueous methanol extract was refluxed with 25 mL of TFA (2M) 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 TLC as previously described (Muhammad *et al.*, 2015). The monosaccharide residue (40 mg) was solubilized in H_2O (1 mL) and 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, to give the four sugars. The chromatogram was monitored by a refractive index detector RI-410 (T = 35°C). Each fraction was neutralized with a 50 mM solution of KOH, dried, dissolved in 1 mL pyridine, and filtered to remove potassium sulfate salts. After pyridine evaporation, the sugars were dissolved (0.1-0.5 mg/mL) in a mixture *n*-hexane-EtOH-TFA (70:30:1) and 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. 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 (Gossan *et al.*, 2016; Lavaud *et al.*, 2015, 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 aqueous methanol extracts of *A. xerocarpus* leaves was determined using the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (Muhammad *et al.*, 2015). Briefly, a stock solution of DPPH was prepared at 158 µM in EtOH/H_2O (1:1, v/v). Each sample was dissolved in DMSO (200 µg/mL) and 5 µL were added to the DPPH stock solution (95 µL), in triplicate in 96-well plates. The DPPH$^{\bullet}$ absorbance in each reaction mixture was monitored at $\lambda = 515$ nm. Absorbance measurements were performed at regular interval of 1.5 min for 30 min at 37°C for all samples. The aqueous methanol extract was then tested at 200, 100, 50 and 10 µg/mL to calculate the concentration able to quench 50% of the reaction system (EC_{50}) at 30 min. The EtOH/H_2O (1:1, v/v) solution was used as a blank, the free DPPH solution was used as a negative control and ascorbic acid (5 µg/mL) was used as a positive control. Results are expressed as percentage decrease with respect to control values.

3.7 Tyrosinase inhibitory activity assay

The tyrosinase inhibitory activity of the EtOAc and aqueous methanol extracts of *A. xerocarpus* leaves and the four flavonoids (**16-19**) was determined against mushroom tyrosinase. The assay was performed according to a previously described method using L-DOPA as substrate (Muhammad *et al.*, 2015). Briefly, the tested compounds were dissolved in DMSO 10% and mixed (1:1) with Na-phosphate buffer (PBS, pH 6.8) to obtain a concentration of 4 mg/mL for the extracts or 1 mg/mL for the compounds. Tyrosinase (100 µL; 135 U/mL) was first pre-incubated with the tested compounds (100 µL) at 25 °C for 10 min, and then 100 µL of L-DOPA (0.5 mM, PBS pH 6.8) was added. The enzyme reaction was monitored by measuring the change in absorbance at λ 475 nm (at 25 °C) after 10 min incubation. These solutions were prepared in triplicate in 96-well plates. Kojic acid (1 mM) was used as positive control. The inhibitory percentage of tyrosinase was calculated as follows: % inhibition = $\{[(A - B) - (C - D)]/(A - B)\} \times 100$ (*A*: OD at 475 nm without test substance; *B*: OD at 475 nm without test substance and tyrosinase; *C*: OD at 475 nm with test substance; *D*: OD at 475 nm with test substance, but without tyrosinase).

3.8 WST cytotoxicity assay

The cytotoxic activities of compounds **1-2**, **4-6**, **11-13**, and **15** on KB cell lines (ATTC® CCL™-17) were determined by using a colorimetry method based on the cleavage of the WST-1 tetrazolium salt (Muhammad *et al.*, 2015), and using DMEM F12 medium for cells

culture (Chwalek *et al.*, 2006). The stock solutions of compounds (1 mg/mL) were prepared in DMSO. Sample dilutions were then performed in medium DMEM F12 (1, 2.5, 5, 7.5 or 10 µg/mL). After removal of pre-incubated culture medium, 200 µL of DMEM F12 containing various concentrations of samples were added and further incubated for 48 h at 37 °C. Cell viability was determined by adding WST-1 tetrazolium salt and by measuring the absorbance at λ 450 nm after ≈1 h. Each assay was realized in triplicate in 96-well microplates. A dose–response curve was plotted for each compound, and the concentration giving 50% inhibition (IC_{50}) was calculated by using MSExcel based program. *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).

3.9. Disc diffusion antibacterial assay

Disk diffusion was used to screen antibacterial activity of compounds **1-2**, **4-6**, **12-15**, and **20** against *S. aureus* (ATCC 25923) and *E. faecalis* (CIP10907), for Gram positive, *E.coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853), for gram negative (Acebey-Castellon *et al.*, 2011). 50µL (of the solution at 10 mg/mL in H_2O) were applied in a sterile atmosphere to 8 mm diameter paper disks corresponding to 500µg/disk of each compounds (or 100µg/disk for **14).** After evaporation of the solvent, paper disks were placed in Petri dished of 9 cm diameter containing nutrient Mueller-Hinton agar previously inoculated with 0.2 mL of suspension of bacteria (15 10^7 CFU/mL for *S aureus* and *E. faecalis*; 15 10^6 for *E coli* and *P. aeruginosa*). After 18 hours of incubation at 37°C, the inhibition zone for the active extract was measured (CLSI, 2005). The antimicrobial gentamicine was used as positive control and tested at 50 µg/disk.

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 active compounds **1** and **12** against two standard strains *S. aureus* and *E. faecalis*. Briefly, the mother compound solutions (10 mg/mL) were prepared by dissolving the compound in DMSO. Fifty microliters of each solution was added to 950 µL of Muller-Hinton medium. This was serially diluted 2-fold with Muller-Hinton medium to obtain concentration ranges of 4 to 256 µg/mL. Fifty microliters of each concentration was added in a well (96-well microplate) containing 150 µL of Mueller-Hinton medium and 5 µL of the standard inoculum. The final concentration of DMSO in the

well was less than 5% (preliminary analysis with 5% (v/v) DMSO/Mueller-Hinton medium affected neither the growth of the test organisms nor the change of color due to this growth). The negative control well consisted of 12.5 µL of DMSO, 187.5 µL of Mueller-Hinton medium, and 5 µL of the standard inoculum. The plates were covered with a sterile plate sealer, then agitated and incubated at 37 °C for 18 h. Microbial growth was determined by observing the change of color in the wells. The lowest concentration showing no color change was considered as the MIC. 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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Supporting information

Supporting information can be found in the online version of this article.

Figures captions :

Figure 1: structures of isolated compounds **1**-**19**

Figure 2: Key rOe effects and HMBC correlations of ring E in compound **6**

Figure 3: Key rOe effects, COSY and HMBC correlations of compound **9**

Figure 4: Key rOe effects on the ring E of compounds **10** and **11**

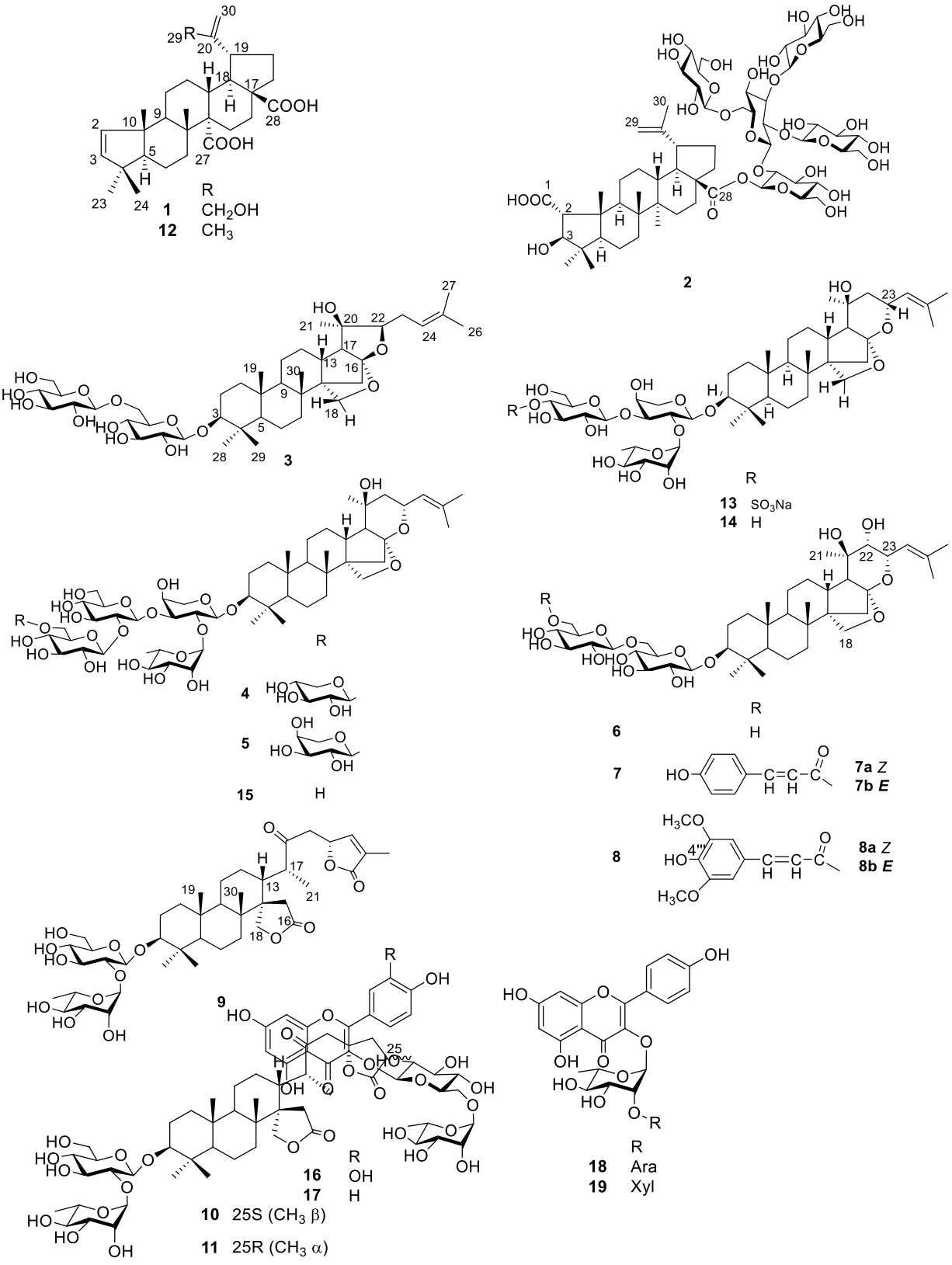

Figure 1: Structures of isolated compounds **1-19**

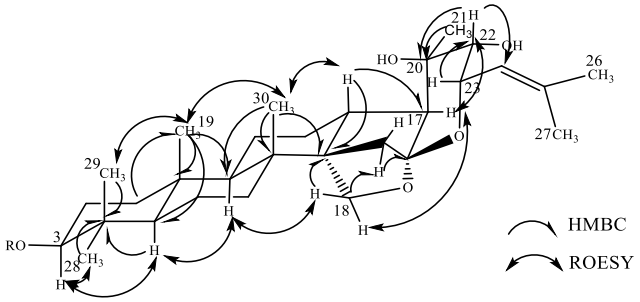

Figure 2: Key rOe effects and HMBC correlations of ring E in compound **6**

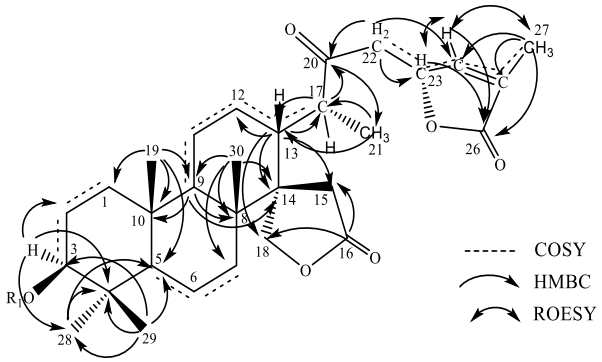

Figure 3: Key rOe effects, COSY and HMBC correlations of compound **9**

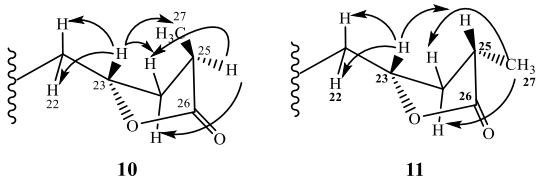

Figure 4: Key rOe effects on the ring E of compounds **10** and **11**

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

	2			**2**		**3**	
Position	**δ_C**	**δ_H (*m*, *J* in Hz)**	**Position**	**δ_C**	**δ_H (*m*, *J* in Hz)**	**δ_C**	**δ_H (*m*, *J* in Hz)**
1	177.4	-	**C_{28}-Glc**			**C_3-Glc**	
2	65.6	2.50 (*brs*)	**1'**	91.9	5.64 (*d*, 8.3)	106.7	4.35 (*d*, 7.8)
3	84.5	4.10 (*brs)*	**2'**	75.7	4.07 (*t*, 8.8)	75.6	3.21 (*t*, 8.5)
4	42.9	-	**3'**	77.1	3.76 (*dd*, 9.6, 8.8)	78.2	3.34 (*t*, 9.1)
5	56.7	1.70 (*m*)	**4'**	69.3	3.5 (*t*, 9.6)	71.7	3.34 (*m*)
6	18.3	1.35 (*m*)	**5'**	77.6	3.42 (*m*)	77.0	3.46 (*m*)
		1.54 (*m*)	**6'**	61.0	3.76 (*m*)	69.9	3.81 (*dd*, 11.8, 5.7)
7	34.1	1.37(*m*)			3.86 (*dd*, 12.5, 2.2)		4.12 (*dd*, 11.8, 2.3)
		1.45 (*m*)	**$C_{2'}$-Glc**			**$C_{6'}$-Glc**	
8	41.7	-	**1"**	101.2	5.04 (*d*, 7.6)	104.8	4.42 (*d*, 7.8)
9	44.4	1.78 (*dd*, 12.5, 2.5)	**2"**	81.3	3.68 (*m*)	75.2	3.23 (*t*, 8.5)
10	49.0	-	**3"**	86.6	3.68 (*m*)	78.0	3.38 (*t*, 8.8)
11	23.2	1.50 (*m*)	**4"**	69.6	3.32 (*m*)	71.6	3.30 (*t*, 8.8)
		1.59 (*m*)	**5"**	75.7	3.58 (*ddd*, 9.8, 7.8, 2.4)	78.0	3.28 (*m*)
12	25.3	1.09 (*m*)	**6"**	68.7	3.74 (*dd*, 9.9, 7.8)	62.8	3.69 (*dd*, 11.8, 5.4)
		1.68 (*m*)			4.17 (*dd*, 10.0, 2.5)		3.89 (*dd*, 11.8, 2.2)
13	38.3	2.25 (*td*, 11.8, 3.6)	**$C_{2''}$-Glc**				
14	42.8	-	**1"'**	103.3	4.71 (*d*, 7.8)		
15	30.9	1.13 (*m*)	**2"'**	74.4	3.24 (*t*, 8.2)		
		1.59 (*m*)	**3"'**	76.1	3.40 (*t*, 8.9)		
16	31.3	1.45 (*m*)	**4"'**	70.0	3.37 (*t*, 9.0)		
		2.57 (*dm*, 12.7)	**5"'**	77.0	3.36 (*m*)		
17	56.7	-	**6"'**	61.3	3.77 (*dd*, 11.8, 6.0)		
18	49.4	1.65 (*t*, 11.0)			3.99 (*d*, 11.8)		
19	46.9	3.02 (*td*, 11.0, 4.8)	**$C_{3''}$-Glc**				
20	150.4	-	**1""**	103.6	4.58 (*d*, 7.8)		
21	30.2	1.38 (*m*)	**2""**	74.0	3.30 (*t*, 9.5)		
		1.90 (*t*, 10.7)	**3""**	76.9	3.42 (*t*, 9.0)		
22	36.2	1.50 (*t*, 12.5)	**4""**	70.3	3.31 (*m*)		
		2.04 (*dd*, 12.4, 8.2)	**5""**	76.8	3.39 (*m*)		
23	30.0	1.10 (*s*)	**6""**	61.3	3.64 (*dd*, 11.9, 6.7)		
24	18.5	0.94 (*s*)			3.93 (*dd*, 11.8, 2.0)		
25	17.9	1.09 (*s*)	**$C_{6''}$-Glc**				
26	16.4	1.02 (*s*)	**1""'**	103.6	4.68 (*d*, 7.8)		
27	14.0	1.01 (*s*)	**2""'**	73.9	3.22 (*t*, 9.0)		
28	174.5	-	**3""'**	76.4	3.49 (*t*, 9.3)		
29	108.8	4.61 (*brs*)	**4""'**	70.3	3.31 (*t*, 8.2)		
		4.73 (*brs*)	**5""'**	76.3	3.37 (*m*)		
30	18.2	1.71 (*s*)	**6""'**	61.4	3.69 (*dd*, 9.7, 5.6)		
					3.87 (*dd*, 11.8, 2.0)		

Table 2: ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data of the genin of compounds **3**, **4** and **6** in CD_3OD.

	3		**4**		**6**	
Position	**δ_C**	**δ_H (*m*, *J* in Hz)**	**δ_C**	**δ_H (*m*, *J* in Hz)**	**δ_C**	**δ_H (*m*, *J* in Hz)**
1	38.4	0.99 (*t*, 11.8)	40.0	0.98 (*m*)	38.3	1.01 (*td*, 14.0, 3.1)
	-	1.73 (*dd*, 11.3, 1.3)		1.71 (*brd*, 11.2)	-	1.72 (*brd*, 13.3)
2	25.8	1.67 (*t*, 9.5)	27.3	1.71 (*m*)	25.8	1.69 (*brd*, 16.4)
	-	1.94 (*dd*, 13.4, 3,8*)*		1.84 (*dd*, 14.9, 5.2)	-	1.95 (*dd*, 15.3, 3.7)
3	89.1	3.19 (*dd*, 7.3, 4.8)	89.6	3.15 (*dd*, 11.6, 4.4)	89.1	3.19 (*dd*, 7.1, 4.7)
4	39.0	-	40.5	-	39.0	-
5	55.8	0.77 (*dd*, 12.0, 1.6)	57.5	0.75 (*brd*, 11.1)	55.9	0.78 (*brd*, 11.1)
6	17.7	1.52 (*brd*, 13.3)	19.1	1.53 (*brd*, 14.8)	17.7	1.52 (*brd*, 11.1)
	-	1.59 (*t*, 14.0)		1.59 (*tl*, 10.5)	-	1.58 (*t*, 9.8)
7	35.5	1.48 (*m*)	36.9	1.49 (*d*, 11.9)	35.5	1.46 (*m*)
	-	1.57 (*m*)		1.56 (*tl*, 10.5)	-	1.56 (*t*, 10.9)
8	37.5	-	38.5	-	37.1	-
9	52.3	0.79 (*dd*, 13.1, 2.4)	54.1	0.90 (*dd*, 10.0, 3.9)	52.7	0.92 (*dd*, 11.1)
10	36.8	-	38.3	-	36.9	-
11	20.8	1.47 (*dd*, 13.8, 7.3)	22.5	1.51 (*brd*, 12.8)	21.1	1.49 (*d*, 11.8)
	-	1.65 (*m)*	-	1.66 (*dd*, 12.8, 2.4)	-	1.69 (*dd*, 17.3, 5.3)
12	26.9	1.67 (*m*)	29.2	1.71 (*m*)	27.9	1.72 (*brd*, 16.0)
	-	1.89 (*m*)	-	1.87 (*d*, 12.8)		1.85 (*dd*, 13.1, 5.6)
13	36.9	2.43 (*m*)	38.0	2.51 (*m*)	36.1	2.55 (*m*)
14	56.2	-	54.6	-	53.4	-
15	37.3	1.41 (*dd*, 8.9, 1.4)	37.1	1.20 (*d*, 8.6)	35.6	1.19 (*dd*, 8.9, 1.7)
		1.69 (*d*, 8.9)		2.09 (*dd*, 8.6, 1.6)		2.11 (*d*, 8.9)
16	117.5	-	111.4	_	109.7	_
17	61.6	1.81 (*dd*, 7.3, 1.3)	54.4	1.03 (*dd*, 7.2, 1.4)	48.1	1.35 (*d*, 8.5)
18	65.4	3.96 (*dd*, 8.3, 1.6)	66.9	3.97 (*d*, 7.2)	65.5	3.95 (*d*, 7.0)
	-	3.99 (d, 8.3)		4.05 (*d*, 7.2)	-	4.07 (*d*, 7.0)
19	15.4	0.91 (*s*)	16.9	0.90 (*s*)	15.4	0.91 (*s*)
20	75.1	-	69.4	-	70.9	-
21	22.1	1.21 (*s*)	29.6	1.16 (*s*)	24.2	1.21 (*s*)
22	94.1	4.14 (*t*, 6.6)	45.4	1.40 (*dd*, 13.8, 11.1)	73.8	3.01 (*s*)
	-	-		1.49 (*brd*, 10.2)	-	-
23	27.3	2.29 (*tl*, 6.6)	69.7	4.70 (*td*, 9.8, 2.0)	70.1	4.85 (*d*, 8.4)
-	-	-	-	-	-	-
24	120.7	5.22 (*tq*, 7.3, 0.7)	126.3	5.18 (*dq*, 8.3, 1.4)	122.1	5.46 (*dq*, 8.4, 1.7)
25	132.6	-	136.7	-	136.1	-
26	24.5	1.71 (*s*)	25.8	1.74 (*s*)	24.5	1.78 (s)
27	16.5	1.65 (*s*)	18.4	1.71 (*s*)	17.1	1.72 (s)
28	27.0	1.07 (*s*)	28.5	1.03 (*s*)	27.0	1.07 (s)
29	15.5	0.87 (*s*)	17.1	0.88 (*s*)	15.5	0.87 (s)
30	17.8	1.06 (*s*)	19.2	1.16 (*s*)	17.8	1.17 (*s*)

Table 3: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of the osidic part of compounds **4** and **5** in CD_3OD.

	4		**5**	
	δ_C	**δ_H (*m*, *J* en Hz)**	**δ_C**	**δ_H (*m*, *J* en Hz)**
$C_{3'}$-Ara				
1'	104.7	4.39 (*d*, 6.5)	104.7	4.39 (*d*, 6.5)
2'	75.0	3.89 (*t*, 9.4)	74.7	3.89 (*t*, 9.4)
3'	81.1	3.86 (*m*)	81.1	3.86 (*m*)
4'	68.7	4.07 (*m*)	68.7	4.07 (*m*)
5'		3.55 (*dd*, 12.6, 2.5)		3.55 (*dd*, 12.6, 2.5)
	64.7	3.87 (*dd*, 12.6, 3.4)	64.7	3.87 (*dd*, 12.6, 3.4)
$C_{2''}$-Rha				
1"	100.8	5.26 (*d*, 1.5)	100.6	5.31 (*d*, 1.5)
2"	70.5	4.07 (*m*)	70.5	4.03 (*m*)
3"	70.8	3.73 (*dd*, 9.1, 3.5)	70.8	3.73 (*dd*, 9.4, 2.7)
4"	72.5	3.43 (*t*, 8.4)	72.5	3.43 (*t*, 8.4)
5"	68.7	3.97 (*m*)	68.7	3.97 (*m*)
6"	16.8	1.25 (*d*, 6.2)	16.8	1.25 (*d*, 6.4)
$C_{3'''}$-Glc				
1"'	101.6	4.69 (*d*, 7.8)	101.6	4.69 (*d*, 7.8)
2"'	80.2	3.70 (*dd*, 8.6, 7.8)	80.1	3.70 (*dd*, 8.6, 7.8)
3"'	76.9	3.63 (*t*, 8.6)	76.9	3.63 (*t*, 8.6)
4"'	69.8	3.04 (*t*, 9.0)	69.8	3.04 (*t*, 9.0)
5"'	76.1	3.46 (*m*)	76.1	3.46 (*m*)
6"'		3.68 (*dd*, 11.8, 5.4)		3.68 (*dd*, 11.8, 5.4)
	61.1	3.87 (*dd*, 11.8, 3.0)	61.1	3.87 (*dd*, 11.8, 3.0)
$C_{2''''}$-Glc				
1""	102.7	4.89 (*d*, 6.9)	102.6	4.89 (*d*, 6.9)
2""	73.8	3.43 (*dd*, 8.8, 6.9)	73.8	3.43 (*dd*, 8.4, 6.9)
3""	76.6	3.38 (*t*, 8.8)	76.6	3.38 (*t*, 8.8)
4""	69.5	3.47 (*t*, 8.9)	69.5	3.47 (*t*, 8.9)
5""	75.8	3.46 (*m*)	75.8	3.46 (*m*)
6""	68.0	3.79 (*dd*, 11.3, 4.4)	68.0	3.79 (*dd*, 11.3, 4.4)
		4.16 (*dd*, 11.3, 2.7)		4.16 (*dd*, 11.3, 2.7)
$C_{6''''}$-xyl			**Ara**	
1""'	104.4	4.34 (*d*, 7.5)	104.1	4.36 (*d*, 6.8)
2""'	73.3	3.32 (*dd*, 9.5, 7.5)	71.0	3.66 (*dd*, 8.9, 6.8)
3""'	76.6	3.38 (*t*, 9.5)	72.6	3.61 (*dd*, 8.9, 3.4)
4""'	69.7	3.53 (*td*, 9.5, 5.5)	68.3	3.83 (*m*)
5""'	65.6	3.25 (*t*, 9.9)	65.5	3.61 (*dd*, 9.0, 3.4)
		3.89 (*dd*, 9.9, 2.6)		3.89 (*dd*, 9.0, 2.6)

Table 4: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of the osidic part of compounds **6-8** in CD_3OD

	6		*trans*-**7**		*cis*-**7**		*trans*-**8**		*cis*-**8**	
	δ_C	**δ_H (*m*, *J* en Hz)**	**δ_C**	**δ_H (*m*, *J* en Hz)**	**δ_C**	**δ_H (*m*, *J* en Hz)**	**δ_C**	**δ_H (*m*, *J* en Hz)**	**δ_C**	**δ_H (*m*, *J* en Hz)**
C_3-Glc										
1'	105.3	4.35 (*d*, 7.8)	105.2	4.34 (*d*, 7.8)	105.1	4.34 (*d*, 7.9)	105.1	4.34 (*d*, 7.9)	105.1	4.34 (*d*, 7.9)
2'	74.2	3.21 (*t*, 8.1)	74.1	3.21 (*dd*, 9.1, 7.8)	74.1	3.21 (*dd*, 9.1, 7.9)	74.3	3.21 (*dd*, 9.0, 7.9)	74.3	3.21 (*dd*, 9.0, 7.9)
3'	76.7	3.35 (*t*, 8.3)	76.7	3.37 (*t*, 9.1)	76.7	3.37 (*t*, 9.1)	76.7	3.37 (*t*, 9.0)	76.7	3.37 (*t*, 9.0)
4'	70.1	3.32 (*t*, 8.3)	70.3	3.32 (*m*)	70.3	3.32 (*m*)	70.3	3.30 (*t*, 9.2)	70.3	3.30 (*t*, 9.2)
5'	75.6	3.46 (*td*, 7.7, 2.2)	75.2	3.48 (*m*)	75.2	3.48 (*m*)	75.2	3.49 (*m*)	75.2	3.49 (*m*)
6'	68.4	3.81 (*dd*, 11.7, 5.7)	68.9	3.82 (*dd*, 11.7, 5.8)	68.7	3.80 (*dd*, 11.7, 6.0)	68.7	3.82 (*dd*, 11.7, 5.9)	68.7	3.82 (*dd*, 11.7, 5.9)
		4.12 (*dd*, 11.7, 1.7)		4.09 (*dd*, 11.7, 2.0)		4.07 (*dd*, 11.7, 3.2)		4.09 (*dd*, 11.7, 1.9)		4.09 (*dd*, 11.7, 1.9)
$C_{6'}$-Glc										
1"	103.4	4.42 (*d*, 7.8)	103.3	4.43 (*d*, 7.7)	103.4	4.40 (*d*, 7.8)	103.3	4.43 (*d*, 7.8)	103.3	4.43 (*d*, 7.8)
2"	73.8	3.22 (*t*, 8.4)	73.7	3.25 (*dd*, 9.0, 7.7)	73.7	3.25 (*dd*, 9.0, 7.8)	73.8	3.26 (*dd*, 9.2, 7.8)	73.8	3.26 (*dd*, 9.2, 7.8)
3"	76.6	3.38 (*t*, 8.8)	76.5	3.39 (*t*, 9.0)	76.5	3.39 (*t*, 9.0)	76.5	3.40 (*t*, 9.2)	76.5	3.40 (*t*, 9.2)
4"	70.2	3.30 (*t*, 8.9)	70.3	3.38 (*m*)	70.3	3.31 (*m*)	70.2	3.40 (*t*, 9.2)	70.2	3.40 (*t*, 9.2)
5"	76.6	3.29 (*m*)	73.9	*m*)	73.9	3.51(*m*)	73.9	3.52 (*m*)	73.9	3.52 (*m*)
6"	61.4	3.69 (*dd*, 11.9, 5.4)	63.2	4.29 (*dd*, 11.9, 5.7)	63.2	4.24 (*dd*, 11.9, 5.8)	63.1	4.30 (*dd*, 11.9, 5.7)	63.4	4.25 (*dd*, 12.0, 6.3)
		3.89 (*dd*, 11.9, 2.1)		4.60 (*dd*, 11.9, 2.2)		4.53 (*dd*, 11.9, 2.1)		4.61 (*dd*, 11.9, 2.1)		4.52 (*dd*, 12.0, 2.2)
$C_{6'''}$-				*trans*-*p*-coumaroyl		*cis*-*p*-coumaroyl		*trans*-sinapoyl		*cis*-sinapoyl
1"'			125.7	-	126.2	-	125.1	-	125.5	
2"'			129.9	7.51 (*d*, 8.7)	132.5	7.70 (*d*, 8.7)	105.1	6.98 (*s*)	108.7	7.33 (*s*)
3"'			115.5	6.84 (*d*, 8.7)	114.6	6.80 (*d*, 8.7)	148.1	-	147.2	-
4"'			160.0	-	158.8	-	138.4	-	137.7	-
5"'			115.5	6.84 (*d*, 8.7)	114.6	6.80 (*d*, 8.7)	148.1	-	147.2	-
6"'			129.9	7.51 (*d*, 8.7)	132.5	7.70 (*d*, 8.7)	105.1	6.98 (*s*)	108.7	7.33 (*s*)
β -7"'			145.5	7.68 (*d*, 16.1)	144.0	6.91 (*d*, 12.8)	146.0	7.67 (*d*, 15.9)	144.7	6.90 (*d*, 13.0)
α -8"'			113.6	6.40 (*d*, 16.1)	114.9	5.84 (*d*, 12.8)	114.3	6.47 (*d*, 15.9)	115.3	5.89 (*d*, 13.0)
9"'			167.7		166.7	-	167.6		166.6	-
3"'-OCH_3			-	-	-	-	55.5	3.92 (*s*)	55.5	3.89 (*s*)
5"'-OCH_3			-	-	-	-	55.5	3.92 (*s*)	55.5	3.89 (*s*)

Table 5: ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data of compounds **9-11** in CD_3OD.

	9		**10**		**11**	
Position	**δ_C**	**δ_H (*m*, *J* en Hz)**	**δ_C**	**δ_H (*m*, *J* en Hz)**	**δ_C**	**δ_H (*m*, *J* en Hz)**
1	38.3	1.02 (*t*, 13.6)	38.3	1.01 (*td*, 12.6, 3.7)	38.3	1.03 (*td*, 15.9, 6.5)
		1.72 (*brd*, 10.8)		1.72 (*brd*, 11.5)		1.72 (*brd*, 13.4)
2	25.9	1.70 (*t*, 14.5)	25.9	1.69 (*t*, 12.1)	25.9	1.69 (*t*, 14.2)
		1.99 (*brd*, 10.7)		1.99 (*dd*, 13.8, 3.1)		1.99 (*dd*, 14.4, 4.1)
3	88.5	3.20 (*dd*, 11.7, 3.6)	88.5	3.19 (*dd*, 11.4, 4.0)	88.5	3.20 (*dd*, 11.5, 4.3)
4	38.9	-	38.9	-	38.9	-
5	55.2	0.83 (*brd*, 14.5)	55.2	0.83 (*brd*, 10.9)	55.2	0.83 (*brd*, 11.6)
6	17.7	1.55 (*brd*, 7.0)	17.7	1.49 (*m*)	17.7	1.54 (*dt*, 12.1, 3.8)
		1.70 (*brd*, 14.5)		1.56 (*m*)		1.69 (*m*)
7	34.1	1.47 (*brd*, 11.3)	34.2	1.48 (*dd*, 15.1, 7.3)	34.2	1.47 (*dd*, 17.3, 4.6)
		1.58 (*m*)		1.58 (*dd*, 13.9, 4.2)		1.58 (*dd*, 9.2, 3.4)
8	40.9	-	40.9	-	40.8	-
9	52.6	0.74 (*brd*, 11.8)	52.7	0.73 (*dd*, 12.5, 2.8)	52.6	0.75 (*dd*, 12.5, 2.6)
10	36.5	-	36.5	-	36.5	-
11	20.1	1.52 (*m*)	20.0	1.49 (*dd*, 15.6, 3.9)	20.1	1.47 (*dd*, 12.8, 4.2)
		1.62 (*m*)		1.61 (*m*)		1.63 (*brd*, 12.2)
12	23.5	1.32 (*m*)	23.6	1.30 (*brd*, 10.4)	24.2	1.29 (*dd*, 13.4, 4.4)
		1.58 (*brd*, 12.7)		1.58 (*brd*, 13.8)		1.67 (*brd*, 16.7)
13	37.2	2.43 (*td*, 13.0, 3.5)	37.3	2.42 (*td*, 13.0, 3.3)	37.5	2.48 (*td*, 14.2, 4.4)
14	51.9	-	51.9	-	51.9	-
15	33.7	2.23 (*d*, 18.8)	33.8	2.24 (*d*, 19.1)	33.7	2.18 (*d*, 19.1)
		2.74 (*d*, 18.8)		2.74 (*d*, 18.9)		2.71 (*d*, 19.0)
16	178.2	-	178.3	-	178.4	-
17	45.8	2.61 (*m*)	45.8	2.60 (*m*)	45.9	2.61 (*m*)
18	70.0	4.39 (*d*, 10.6)	70.1	4.39 (*d*, 10.6)	70.3	4.37 (*d*, 11.0)
		4.49 (*d*, 10.6)		4.49 (*d*, 10.6)		4.49 (*d*, 11.0)
19	15.1	0.90 (*s*)	15.1	0.89 (*s*)	15.2	0.89 (*s*)
20	209.3	-	210.3	-	210.7	-
21	10.4	1.07 (*d*, 4.0)	10.6	1.07 (*d*, 7.5)	11.6	1.10 (*d*, 7.1)
22	43.3	2.87 (*d*, 8.6)	45.2	2.82 (*d*, 17.2, 5.1)	45.4	2.86 (*dd*, 17.6, 4.6)
		2.93 (*d*, 7.4)		3.06 (*dd*, 17.2, 8.2)		3.09 (*dd*, 17.6, 7.5)
23	77.8	5.39 (*m*)	74.8	5.01 (*m*)	74.6	4.83 (*m*)
24	149.6	7.32 (*dt*, 6.2, 1.7)	34.5	2.14 (*dd*, 14.3, 6.4)	36.2	1.64 (*brd*, 13.0)
		-		2.22 (*td*, 8.7, 4.6)		2.61 (*ddd*, 13.5, 7.6, 4.7)
25	129.4	-	33.7	2.83 (*m*)	35.5	2.80 (*ddd*, 13.8, 6.7, 5.4)
26	174.8	-	180.8	-	180.3	-
27	9.1	1.90 (*brs*)	14.5	1.27 (*d*, 7.3)	13.7	1.24 (*d*, 7.3)
28	26.9	1.08 (*s*)	26.9	1.08 (*s*)	26.9	1.08 (*s*)
29	15.6	0.88 (*s*)	15.6	0.88 (*s*)	15.6	0.88 (*s*)
30	17.5	1.05 (*s*)	17.5	1.05 (*s*)	17.4	1.06 (*s*)
C_3-Glc						
1'	104.2	4.43 (*d*, 7.6)	104.2	4.42 (*d*, 7.5)	104.2	4.42 (*d*, 7.6)
2'	77.5	3.42 (*dd*, 8.9, 7.6)	77.5	3.42 (*dd*, 8.8, 7.5)	77.5	3.43 (*dd*, 9.0, 7.6)
3'	78.1	3.48 (*t*, 8.9)	78.1	3.48 (*t*, 8.8)	78.1	3.48 (*t*, 9.0)
4'	70.6	3.30 (*t*, 9.2)	70.7	3.31 (*t*, 9.4)	70.6	3.30 (*t*, 9.1)
5'	76.3	3.24 (*m*)	76.2	3.24 (*m*)	76.3	3.24 (*m*)
6'	61.4	3.68 (*dd*, 11.9, 5.6)	61.4	3.68 (*dd*, 11.8, 5.5)	61.4	3.68 (*dd*, 11.7, 5.5)
		3.86 (*dd*, 11.9, 2.2)		3.86 (*dd*, 11.8, 1.7)		3.86 (*dd*, 11.7, 2.3)
$C_{2'}$-Rha						
1"	100.4	5.39 (*d*, 1.3)	100.4	5.39 (*d*, 1.4)	100.4	5.39 (*d*, 1.8)
2"	70.6	3.97 (*m*)	70.7	3.97 (*m*)	70.6	3.97 (*m*)
3"	70.7	3.76 (*dd*, 9.5, 3.4)	70.8	3.76 (*dd*, 9.5, 3.4)	70.7	3.76 (*dd*, 9.6, 3.3)
4"	72.6	3.40 (*t*, 9.6)	72.6	3.40 (*t*, 9.7)	72.6	3.40 (*t*, 9.6)
5"	68.6	3.99 (*m*)	68.6	3.99 (*m*)	68.6	3.99 (*m*)
6"	16.6	1.23 (*d*, 6.7)	16.6	1.23 (*d*, 6.1)	16.6	1.23 (*d*, 6.5)

Table 6: KB cells death (%) induced by compounds **1-2, 4-6, 11-13** and **15** at 10 µg/mL and IC_{50} of compound **12**

	cells death % at 10 µg/mL	**IC_{50} (µg/mL)**	**$IC_{50} \pm \sigma$ (µM)**
1	58.4		
2	7.8		
4+5	22.9		
6	6.6		
11	10.5		
12	79.5	1.2 ± 0.3	2.6 ± 0.16
13	16.4		
15	19.6		
***α*-hederin**			5.5 ± 0.11

Table 7: Antimicrobial activities of compounds **1-2, 4-6, 12-15** and **20** by disc diffusion and broth diffusion methods

	Inhibition zone (Ø, mm)				**MIC (µg/mL)**	
Compounds (µg/disc)	*S. aureus*	*E. faecalis*	*E. coli*	*P. aeruginosa*	*S. aureus*	*E. faecalis*
1 (100)	14	14	-	-	4	16
2 (500)	-	-	-	-		
4+5 (500)	-	-	-	-		
6 (500)	-	-	-	-		
12 (500)	16	14	-	-	8	16
13 (500)	-	-	-	-		
14 (500)	-	-	-	-		
15 (500)	-	-	-	-		
20 (500)	-	-	-	-		
Gentamicin	22	22	25	18	5	5