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

Dima Muhammad [a], Nathalie Lalun [b], Hélène Bobichon [b], Elisabeth Le Magrex Debar [c], Sophie C. Gangloff [c], Mohammed Nour [d], Laurence Voutquenne-Nazabadioko [a, *]

[a] UMR CNRS 7312, Université de Reims Champagne-Ardenne, Bât. 18, Moulin de la Housse, BP 1039, 51687 Reims, Cedex 2, France
[b] CNRS FRE 3481, Université de Reims Champagne Ardenne, 51 rue Cognacq-Jay, 51095 Reims Cedex, France
[c] Laboratoire de Microbiologie, EA 4691, UFR de Pharmacie, 1 Rue du Maréchal Juin, 51096 Reims Cedex, France
[d] Laboratoire Insulaire du Vivant et de l'Environnement (LIVE), EA 4243, Université de la Nouvelle-Calédonie, BP R4, 98851 Nouméa Cedex, New Caledonia

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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, anti-tyrosinase, 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 2α-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.

1. 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 in curved 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 m (Baillon, 1876). In a continuation of the study of New-Caledonian species (Muhammad et al., 2015, 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

* Corresponding author.
E-mail address: laurence.nazabadioko@univ-reims.fr (L. Voutquenne-Nazabadioko).

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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 (Dzubak et al., 2006; Muhammad et al., 2015, 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- (^1H 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 and Duteil, 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 ^1H 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 ^1H 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 ^1H 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.