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**SAPONINS AND FLAVONOID GLYCOSIDES FROM THE LEAVES OF *ZIZIPHUS MAURITIANA* LAM. NATIVE OF A FOREST AREA OF IVORY COAST**

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## **Abstract**

*Ziziphus mauritiana* Lam (Rhamnaceae) is traditionally used in the treatment of various ailments. The aim of the present study is to identify the major compounds of the methanol extract of the leaves of *Ziziphus mauritiana* growing in Ivory Coast. The methanol extract of this plant was purified by combining silica gel and RP18 HPLC to give an undescribed compounds, 6''-*O*-malonyl-ziziphus saponin I (**1**) along with nine known compounds from which two saponins, six flavonoids, and one chalcone derivate. The structures of these compounds were elucidated by analysis of 1D- and 2D-NMR spectroscopic data and mass spectrometry (HR-ESI-MS). Apart from zizyphus saponin I (**2**), all the others compounds were isolated for the first time from the leaves of this species.

## **Keywords**

*Ziziphus mauritiana*, Rhamnaceae, Saponin, Flavonoid.

## 1. Introduction

*Ziziphus mauritiana* Lam. (Rhamnaceae) is a thorny shrub and sarmentous, up to 1 to 2 m tall, distributed in the sub-Saharan zone of Africa, from Senegal to Somalia (CEE/END., 1987, Fofie et al., 2018, Sivasankari and Sankaravadiyoo, 2017). It is found in the savannah, central and north regions of Ivory Coast, where it is used in traditional medicine against anemia, hypertonia, nephritis and nervous diseases (Carol et al., 2012). This plant, introduced into the forest areas of Ivory Coast, was used against diabetes, high blood pressure and various forms of inflammation (Adama et al., 2016; Ba., 2005; Bhatia et al., 2010; Cisse et al., 2000; Marwat et al., 2009; Memon et al., 2012; Traore et al., 2008; Yansambou., 2002). The leaves are used in the treatment of diarrhea, abscesses and gonorrhoea, high blood pressure, liver disorders and diabetes (Diallo et al., 2004; Gupta et al., 2012; Koffi et al., 2008; Traore et al., 2008; Yansambou., 2002). Phytochemical investigations carried out to date on *Ziziphus mauritiana* leaves showed that the species consisted of cyclopeptide alkaloids, sterols, triterpene saponins and flavonoids (Akino et al., 1996; Ashraf et al., 2015; Ghasham et al., 2017; Sharma and Kumar, 1982). Recent studies showed that leaf extract of this plant possess antimicrobial and antioxidant activities (Ashraf et al., 2015, Ghasham et al., 2017; Sivasankari and Sankaravadiyoo, 2015), anti-inflammatory activity (Abdallah et al., 2016; Kumar et al., 2017) and cause thrombocytosis and induce kidney tissue damages in rats (Attemene et al., 2017), but no compounds were formerly identified. In addition, the saponin extract of leaves of *Z. mauritiana* possess antidiabetic and antioxidant potential (Dubey et al., 2019). Similarly, less attention has been devoted to *Z. mauritiana* leaves growing in Ivory Coast (Attemene et al., 2017, Fofie et al., 2018). Thus, in the context of this study, we purified the methanol leaf extract of this species in order to identify its chemical components. In this paper, we present the isolation and structural characterization of an undescribed saponin (Fig.1), together with nine known compounds.

## 2. Results and discussion

The MeOH extract of dried and powdered leaves of *Z. mauritiana* was subjected to silica gel column chromatography and prep-HPLC to obtain three triterpene saponins (1-3) from which the compound 1 (Fig. 1), was previously undescribed, and seven phenolic compounds (4-10). Their structures were elucidated on the basis of spectroscopic evidence. Saponins 2 and 3 were identified as ziziphus saponin I (Okamura et al., 1981, Yoshikawa et al., 1992) and lotoside III (Bozicevic et al., 2017) which had been previously reported from leaves of *Z. mauritiana*

(Sharma and Kumar, 1982) and *Z. spina-christi*, respectively. Phenolic compounds (**4-10**) were identified as quercetin 3-*O*-[6''-(3-hydroxy-3-methylglutaroyl)]- $\beta$ -D-glucopyranoside (**4**) (Iwashina et al., 2004), quercetin-3-*O*-rubinobioside (**5**) (Rastrelli et al., 1995), quercetin-3-*O*-rutinoside (**6**) (Rastrelli et al., 1995), quercetin-3-*O*- $\beta$ -D-xylopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranoside (**7**) (Karla et al., 1994), quercetin-3-*O*- $\beta$ -D-xylopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranoside-4'-*O*- $\alpha$ -L-rhamnopyranoside (**8**) (Nawwar et al., 1984), kaempferol-3-*O*-rutinoside (**9**) (Kazuma et al., 2003) and 3',5'-di-*C*- $\beta$ -glucosylphloretin (**10**) (Ogawa et al., 2001).

Compound **1** was isolated as white amorphous powder. The ESI-HRMS (positive-ion mode) experiment revealed a sodium adduct ion peak  $[M+Na]^+$  at  $m/z$  1021.4992, in agreement with the molecular formula  $C_{50}H_{78}O_{20}Na$  (calcd for  $C_{50}H_{78}O_{20}Na$ , 1021.4984). The molecular ion of **1** was 86 mass units higher than that of **3** ( $m/z$  935.4969  $[M+Na]^+$ ). These mass differences suggested that **1** contained a malonyl group. The fragment ion at  $m/z$  935.4992  $[M-malonyl+Na]^+$  was characteristic of a malonic acid ester (Stein et Zinsmeister, 1990).  $^1H$  and  $^{13}C$  NMR spectra showed the presence of five methyl singulets in the high field region ( $\delta_H$  0.79, 0.85, 0.99, 1.13 and 1.15), a 2-methylpropenyl group ( $\delta_H$  1.68 and 1.72, H<sub>3</sub>-26 and 27;  $\delta_H$  5.18, H-24), two oxygenated quaternary carbons ( $\delta_C$  11.1, C-16; and  $\delta_C$  69.2, C-20), an oxygenated methylene carbon ( $\delta_C$  66.7, C-30), oxygenated methine carbon ( $\delta_C$  69.4, C-23), and a quaternary carbon ( $\delta_C$  54.4, C-14) (Fig. 2). These NMR data of **1** were in good agreement with those of jujubogenin (Qiang et al., 2016). The ROESY correlations between H-17, Me-21 and H-22b, and between H-22a and H-23 as well as the magnitude of the coupling constant between H-13 and H-17 ( $J = 6.5$  Hz) were in agreement with the configuration of D-F rings of jujubogenin (Wang et al., 2013; Yu et al., 2013). The downfield shifts of C-3 ( $\delta$  89.3) suggested that **1** was a monodesmosidic saponin (Qiang et al., 2016).

The  $^1H$  NMR spectrum of **1** exhibited three anomeric proton resonances at  $\delta$  5.47 (1H, brs), 4.48 (1H, d,  $J = 7.6$  Hz) and 4.37 (1H, d,  $J = 7.1$  Hz), which were linked to corresponding carbon at  $\delta$  101.3, 104.3 and 105.3 in the HSQC spectrum, respectively. By  $^1H$ - $^1H$ -COSY and HSQC experiments, the three sugar spin systems were assigned to  $\beta$ -glucopyranose, 6-deoxy- $\alpha$ -talopyranose and  $\alpha$ -arabinopyranose, respectively (Table 1), as in zizyphus saponin I (**2**). The  $\beta$ -glucopyranose was identified by the  $J$  values from H-1''' to H-5''' up to 7 Hz that showed all-trans diaxial interactions. The 6-deoxy- $\alpha$ -talopyranose was identified by the small  $J$  values (brs) of H-1'', H-2'', H-3'' and H-4'' and the presence of the signal of methyl doublet at  $\delta$  1.21 (d,  $J_{H-5/H-6} = 6.6$  Hz) while the  $^{13}C$ -NMR shifts of C-3'' ( $\delta$  66.6) and C-5'' ( $\delta$  67.5) confirmed

the  $\alpha$ -configuration of the anomeric carbon (Kim et al., 2006; Okamura et al., 1981). Finally the  $\alpha$ -arabinopyranose unit was identified by the small  $J$  values of H-3'/H-4' (d,  $J_{\text{H}3'/\text{H}4'} = 3.2$  Hz), while the  $^{13}\text{C}$ -NMR shifts of C-3' ( $\delta$  84.0) and C-5' ( $\delta$  65.7) confirmed the  $\alpha$ -configuration of the anomeric carbons (Okamura et al., 1981). The absolute configurations of these sugars were determined as D for glucose and L for arabinose and 6-deoxytalose after acid hydrolysis. In the HMBC spectrum, the anomeric proton signals at  $\delta_{\text{H}}$  4.37 (ara-1), 5.47 (6-deoxy-tal-1), and 4.48 (glc-1) showed crosspeaks with the carbon signals at  $\delta_{\text{C}}$  89.3 (aglycone-C-3), 73.9 (ara-C-2'), and 84.0 (ara-C-3'), respectively (Fig. 2). These signals provided some evidences to determine the linkages between the sugars and the aglycones. These linkages were also confirmed by correlations, in the NOESY spectrum, between aglycone-H-3/ara-H-1', ara-H-2'/H-1'' and ara-H-3'/glc-H-1'''. Additional signals were detected in the  $^{13}\text{C}$  NMR spectrum of compound **1**: a methylene group ( $\delta_{\text{H}}$  3.77,  $\delta_{\text{C}}$  61.5), and signals attributable to carboxylic groups in the DEPT spectrum ( $\delta_{\text{C}}$  168.4 and 170.2) (Table 1). Hence, saponin was esterified with a malonyl moiety. The signals corresponding to the  $\text{CH}_2$ -6 of glucose moiety ( $\delta_{\text{H}}$  4.55 and 4.26/  $\delta_{\text{C}}$  65.0) appeared downfield compared to those in **3** ( $\delta_{\text{H}}$  3.70 and 3.85/  $\delta_{\text{C}}$  62.1), and thereby indicated an attachment of the acyl moiety at this position. The saponin was thus identified as jujubogenin 3- $\beta$ -O-(6'''-O-malonyl)- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 3)-[6-deoxy- $\alpha$ -L-talopyranosyl-(1 $\rightarrow$ 2)]- $\alpha$ -L-arabinopyranoside (**1**), and was named 6'''-O-malonyl-ziziphus saponin I (Fig. 1).

From a chemotaxonomic viewpoint, similar malonyl glycoside with jujubogenin as aglycone, such as **1** were obtained in *Z. spina-christi* (Bozicevic et al., 2017) and *Colubrina retusa* (Li et al., 1999) belonging to Rhamnaceae family. Zizyphus saponin I (**2**) was previously isolated from the leaves of *Z. mauritiana* (Sharma and Kumar, 1982), *Z. jujuba* (Yoshikawa et al., 1992) and *Z. incurva* (Devkota et al., 2013). Lotoside III (**3**) was isolated from the leaves of *Z. spina-christi* (Bozicevic et al., 2017). Acylglycoside-flavonoids such as **4** have been obtained from species such as *Vaccinium* (Ericaceae) (Kaisu et al., 2013) or *Astragalus* (Fabaceae) (Semmar et al. 2002), but were isolated here for the first time in Rhamnaceae family. All flavonoids (**5-9**) and 3',5'-di-C- $\beta$ -glucosylphloretin (**10**) were previously isolated from the fruits of *Z. jujuba*, and *Z. spina-christi* (Pawlowska et al., 2009), **7-9** were also identified in the aerial parts of *Z. incurva* (Devkota et al., 2019), and **3** and **7** were identified by LC/MS in the leaves of *Z. jujuba* (Bozicevic et al., 2017). They are to our knowledge describe here for the first time in *Z. mauritiana* leaves. Therefore, they could be used to establish a relationship between these species.

### 3. Experimental

#### 3.1. General Experimental Procedures

Optical rotations were measured on a Perkin Elmer model 341 polarimeter (589 nm, 20°C). IR spectra were obtained on a Nicolet Avatar 320 FT-IR spectrometer with KBr disks. NMR data were performed in MeOD on Bruker Avance 500 or 600 spectrometer. ESI-HRMS data were gained using a Micromass Q-TOF high-resolution mass spectrometer. Mass spectra were recorded in the positive-ion mode in the range  $m/z$  100-2000, with a mass resolution of 20000 and an acceleration voltage of 0.7 kV. Chromatography Column (CC) was carried out on HP-20 resin (Sigma Aldrich). Flash chromatography was conducted on a Grace Reveleris system equipped with dual UV and ELSD detection using Grace® cartridges (Silica gel or RP-18). HPLC separations were performed on a Dionex apparatus equipped with an ASI-100 autosampler, an Ultimate 3000 pump, a STH 585 column oven, a diode array detector UVD 340S and a Chromeleon software. A prepacked RP-18 column (Phenomenex 250 x 15mm, Luna 12  $\mu$ m) was used for semi-preparative HPLC. The eluting mobile phase consisted of H<sub>2</sub>O with TFA (0.0025%) and CH<sub>3</sub>CN with a flow rate of 5 mL/min and the chromatogram was monitored at 205 and 210 nm. TLC were carried out using silica gel 60 F<sub>254</sub> pre-coated aluminium plates (0.2 mm, Merck). Spots were visualized through developing agent (CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O, 14:6:1) and chromogenic agent (50% aq. H<sub>2</sub>SO<sub>4</sub>) subsequent heating.

#### 3.2. Plant Material

The leaves of *Z. mauritiana* were collected in April 2017 in Sinfra (Ivory Coast) (Authors must give the geographic coordinates of the harvesting area), and identified by comparing with those found in literature. Further proof on these leaves was made by experts of the Centre National de Floristique of Félix HOUPHOUËT-BOIGNY University, Abidjan-Cocody. A voucher specimen (SSC N°1) was deposited at the Centre National de Floristique.

#### 3.3. Extraction and isolation

Dried and powdered leaves (500g) of *Ziziphus mauritiana* were extracted successively in a Soxhlet with solvents by increasing polarity (CHCl<sub>3</sub>, MeOH). The MeOH extract (5g) was separated by a flash chromatography over silica gel using CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O (9:1:0 to 0:0:10, each 3L) as eluant, to give five fractions (S<sub>1</sub>-S<sub>5</sub>). Fraction S<sub>3</sub> (82.3 mg) was purified by flash chromatography over RP-18, eluted with H<sub>2</sub>O/CH<sub>3</sub>CN (9:1 to 1:9, in 30 min) to afford three

sub-fractions (A<sub>1</sub>-A<sub>3</sub>). The purification by semi-preparative HPLC of sub-fraction A<sub>1</sub> (38.6 mg) led to compound **9** (2.2 mg). Fraction S<sub>4</sub> (1621.3 mg) was purified by preparative HPLC over RP-18 (12 $\mu$ m), eluted by a gradient system of 20-30% CH<sub>3</sub>CN in H<sub>2</sub>O during 60 min, to afford compounds **2** (120.4 mg), **3** (17.2 mg), **5-6** (53.2 mg) and **7** (9.3 mg). Fraction S<sub>5</sub> (1588.5 mg) was purified by preparative HPLC over RP-18 (12 $\mu$ m), eluted by a gradient system of 20-30% CH<sub>3</sub>CN in H<sub>2</sub>O during 45 min, to give five sub-fractions (B<sub>1</sub>-B<sub>5</sub>). Sub-fraction B<sub>4</sub> (308.2 mg) was purified by flash chromatography over silica gel, eluted with CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O (8:2:0 to 14:6:1), to yield three sub-fractions (C<sub>1</sub>-C<sub>3</sub>). Sub-fraction C<sub>2</sub> (161.2 mg) was purified by a semi-preparative HPLC using a gradient from 27-45% CH<sub>3</sub>CN in H<sub>2</sub>O during 10 min, then isocratic elution at 45% CH<sub>3</sub>CN for 20 min to yield 1.2 mg of compound **1** (Rt 29.5 min). Sub-fraction B<sub>3</sub> (97.2 mg) was submitted to a flash chromatography over silica gel, eluted with CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O (7:3:0 to 14:6:1) in 35 min, to afford compound **10** (14.1 mg) and four sub-fractions (D<sub>1</sub>-D<sub>4</sub>). Sub-fraction D<sub>3</sub> (18.5 mg) was purified by semi-preparative HPLC using a gradient from 27-45% CH<sub>3</sub>CN in H<sub>2</sub>O during 10 min, then isocratic elution at 45% CH<sub>3</sub>CN for 22 min to yield 2.0 mg of compound **4** (Rt 15.5 min) and 3.7 mg of compound **8** (Rt 26.2 min).

#### 3.4. Acid hydrolysis

An aliquot of ziziphus saponin I (**2**) (40 mg) containing the same sugars as compound **1** was refluxed with 5 mL of 2 N TFA for 4 h. After cooling, the reaction mixture was extracted with EtOAc (3 x 5 mL) to remove aglycones, and the water-soluble layer was evaporated to dryness (20 mg). Two sugars were identified as glucose, and arabinose by comparison with authentic samples on TLC over silica gel (CH<sub>3</sub>COOEt:CH<sub>3</sub>COOH:CH<sub>3</sub>OH:H<sub>2</sub>O, 65:25:15:15), with a third sugar similar in R<sub>f</sub> to rhamnose attributed to 6-deoxy-talose. The purification of sugars was achieved by prep. TLC with the same solvent to afford 6-deoxy-talose [6.4 mg, R<sub>f</sub> = 0.73,  $[\alpha]^{20}_D$  -16 (c 0.58, H<sub>2</sub>O)]; arabinose [2.8 mg, R<sub>f</sub> = 0.59,  $[\alpha]^{20}_D$  +43 (c 0.25, H<sub>2</sub>O)] and glucose [4.6 mg, R<sub>f</sub> = 0.48,  $[\alpha]^{20}_D$  +21 (c 0.42, H<sub>2</sub>O)]. The absolute configurations of these sugars were determined as D for glucose and L for arabinose and 6-deoxytalose.

#### 3.5. 6'''-O-Malonyl-zizyphus saponin I (**1**)

White amorphous powder;  $[\alpha]^{24}_D$  = -50.6 (C = 0.002 ; MeOH) ; IR (KBr)  $\nu_{max}(cm^{-1})$  : 3415, 2942, 1678, 1384, 1206, 1137, 1066, 1066, 980 ; <sup>1</sup>H and <sup>13</sup>C data , see [Table 1](#); ESI-HRMS *m/z* : 1021.4992 [M+Na]<sup>+</sup>(calcd for C<sub>50</sub>H<sub>78</sub>O<sub>20</sub>Na, 1021.4984).



## Disclosure statement

The authors declare no conflict of interest.

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## Appendix A. Supplementary Material

Supplementary data associated with this article can be found in the online version.

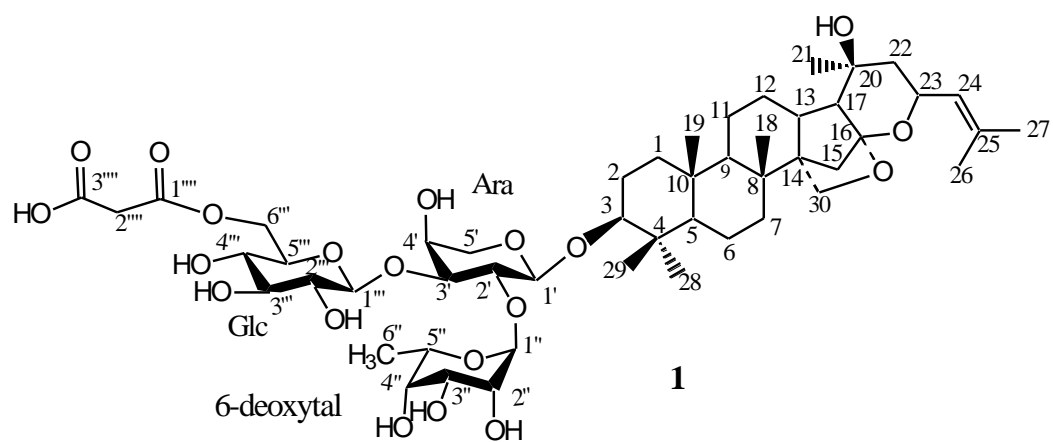
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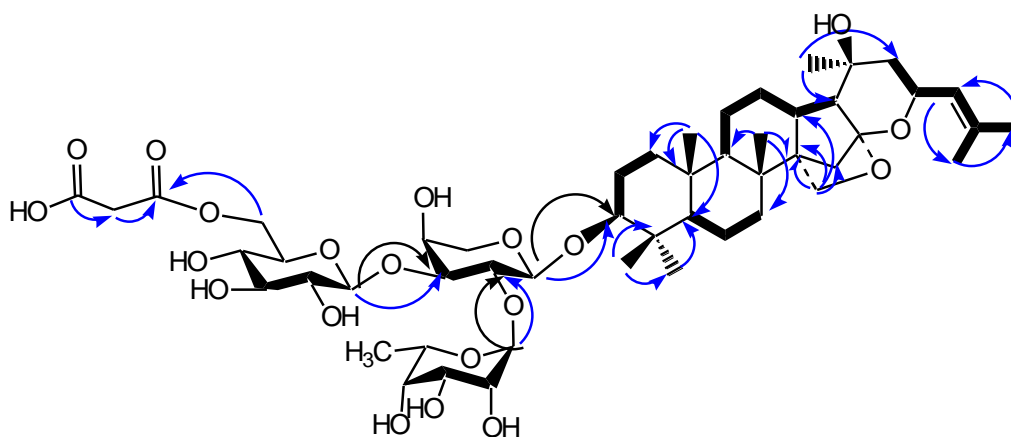
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**Fig 1.** Structure of compound **1**



**Fig 2.** Key COSY (-), key HMBC (->) and key ROESY (->) correlations of **1**