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Ceramide, cerebroside and triterpenoid saponin from the bark of aerial roots of *Ficus elastica* (Moraceae)

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ABSTRACT

Three compounds, ficusamide (**1**), ficusoside (**2**) and elasticoside (**3**), were isolated from the bark of aerial roots of *Ficus elastica* (Moraceae), together with nine known compounds, including four triterpenes, three steroids and two aliphatic linear alcohols. The chemical structures of the three compounds were established by extensive 1D and 2D NMR spectroscopy, mass spectrometry and by comparison with published data. The growth inhibitory effect of the crude extract and isolated compounds was evaluated against several microorganisms and fungi. The cytotoxicity against human cancer cell lines was also assessed. Ficusamide (**1**) displayed a moderate *in vitro* growth inhibitory activity against the human A549 lung cancer cell line and a strong activity against *Staphylococcus saprophyticus*, while elasticoside (**3**) showed a potent activity on *Enterococcus faecalis*.

1. Introduction

The genus *Ficus* (Moraceae) is represented by 750 species, mostly distributed in tropical regions. About 100 species are reported from Africa and over 600 from Asia and Australia (Friis, 1989). This genus is present in Cameroon with 13 subgenera and 99 species. As far as their applications are concerned, many *Ficus* species are used as food and medicine in Traditional Chinese Medicine although their uses, however, have originated in the Middle East (Bankeu et al., 2010). Plants of the genus *Ficus* are known as sources of biologically active compounds such as ficin, 3-keto-urs-12-ene, α-amyrin, α-amyrin acetate, β-amyrin, β-amyrin acetate, boswellonate, β-sitosterol, β-sitosterone, stigmasterol, lupeol and lupenone (Pistelli et al., 2000; Peraza et al., 2002; Djemgou et al., 2009).

The latex of some species of *Ficus* is used in traditional folk medicine for its antihelmintic property in Central and South America (de Amorin et al., 1999). This parasiticidal activity was attributed to the presence of ficin (Pistelli et al., 2000). The latex of *Ficus elastica* has been analyzed for its phytochemicals as an intermediate energy source (Augustus and Seiler, 2011). However, to the best of our knowledge, the bark of aerial roots of *F. elastica* Roxb. ex Hornem. has not yet been studied phytochemically before. In our investigation for the search of bioactive constituents of *F. elastica*, we examined the $CHCl_3$/MeOH 1:1 extract of aerial roots bark. The present paper deals with the isolation and the

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characterization of two sphingolipids ficusamide (**1**), ficusoside (**2**) and a triterpenoid saponin elasticoside (**3**). In order to shed some light on biological activities, the antimicrobial and anticancer properties of the isolates were evaluated.

2. Results and discussion

The $CHCl_3$/MeOH 1:1 crude extract of bark of *F. elastica* aerial roots was separated using different chromatographic techniques affording a mixture of *n*-alkanes (NMR spectroscopy), friedelin (Mahato and Kundu, 1994; de Carvalho et al., 1998), friedelinol (Mahato and Kundu, 1994; de Carvalho et al., 1998), a mixture of linear aliphatic primary alcohols with *n*-dotriacontanol as the major compound (NMR spectroscopy and GC), a mixture of linear fatty acids with tetracosanoic acid as major compound (NMR spectroscopy and GC), phytosterols with stigmasterol and β-sitosterol (de Carvalho et al., 1998), betulinic acid (Parnali et al., 1999), ursolic acid (Werner et al., 2003), and sitosteryl 3-*O*-β-D-glucopyranoside (Parwaiz et al., 2010). In addition, three products, namely ficusamide (**1**), ficusoside (**2**) and elasticoside (**3**) were isolated. Their structures were elucidated by extensive spectroscopic methods including 1D (^{1}H and ^{13}C) and 2D NMR experiments as well as EIMS analysis and comparison with reported data.

2.1. Characterisation of ficusamide (*1*)

Compound **1** was isolated as a colorless solid (m.p. 120–121 °C). The positive HRESI-MS analysis showed a pseudo-molecular ion at *m/z* 682.6372 ($[M+H]^+$), consistent with the formula $C_{42}H_{84}NO_5$ (calc. 682.6372, 1.2 ppm), which accounted for two degrees of unsaturation. Subsequently, the structure was fully elucidated by ^{1}H and ^{13}C NMR spectroscopy. The ^{13}C NMR spectrum revealed a signal at δ_C 51.1 (C–N) and a carbonyl signal at δ_C 175.8, suggesting the presence of an amide group. The ^{1}H NMR spectra (Table 1) of **1** indicated a broad signal in the range of δ_H 1.22–1.35 (brs, CH_2 group) and a triplet at δ_H 0.90 (6H; *J* = 6.3, two terminal Me groups) assigned to two long aliphatic chains. A signal at δ_H 5.42 (2H, m) suggested a double bond. Thus, the two unsaturations of **1** could be readily assigned to the double bond and a carbonyl group. Finally, the presence of two carbinol H-atoms at δ_H 3.77 (dd, *J* = 10.6, 4.4) and 3.72 (dd, *J* = 10.6, 4.4) made compound **1** consistent with the structure of a ceramide (Natori et al., 1994).

Further ^{1}H NMR analysis confirmed the presence of six protons geminal to hydroxy groups at δ_H 4.06 (m, H-2′), 3.77 (dd, *J* = 10.6, 4.4), 3.72 (dd, *J* = 10.6, 4.4), 3.57 (m, H-2) and 3.56 (m, H-3). The IR spectrum showed the absorption band at 3610 cm^{-1} for hydroxyl group(s). Further confirmation of hydroxy groups was made by a peracetylation reaction with acetic anhydride, yielding quantitatively the tetraacetate **1a**. A high resolution electrospray ionization mass spectrometry (HRESIMS) of **1a** exhibited a pseudo-molecular ion peak at *m/z* 852.6923 $[M+H]^+$ (calc. 852.6917, 0.7 ppm) consistent with the molecular formula $C_{50}H_{94}NO_9$. Comparison of mass spectra of **1** and **1a** suggested that **1** contains four free hydroxy groups on the backbone. A fifth signal at δ_H 4.13 (m, H-1) was also identified as a methine proton vicinal to nitrogen atom. This result was also corroborated by the ^{13}C NMR spectrum, which showed a signal at δ_C 175.8 attributed to the amide carbonyl, four methines at δ_C 75.2 (CHOH), 72.1 (CHOH), 71.8 (CHOH) and 51.1 (CHNH), and a methylene group at δ_C 60.9 (CHOH).

The length of the fatty acid chain was determined by MS/MS, which emphasized significant fragment ion peaks at *m/z* 355.3081 $[CH_3(CH_2)_5CH=CH(CH_2)_9CH(OH)CONH=CHCH_2OH]^+$ and 382.3008 $[CH_3(CH_2)_5CH=CH(CH_2)_9CH(OH)CONHCH(CHOH)(CH_2OH)]^+$. The length of the long chain base was elucidated by the characteristic ion at *m/z* 354.3404 $[CH_2=CHCH(OH)CH(OH)(CH_2)_{18}CH_3]^+$. The typical fragment ion at *m/z* 71.0855 was formed by elimination of pentene from $[M]^+$ through McLafferty rearrangement (Pettit et al., 2005; Konga et al., 2001), and also confirmed the position of the double bond in the long fatty chain.

The Δ^{13} double bond was found to be *trans*, as evidenced by the chemical shift of C12′ at δ_C 32.2 and C15′ at δ_C 32.9. Usually the signals of the carbons next to a *trans* double bond appear at δ_C 32–33, while those of a *cis* double bond appear at δ_C 27–28 (Stothers, 1972; Jung et al., 1996). The positions of the hydroxyl groups at C3 and C4 were ascertained by the mass fragmentation pattern (Fig. 1) and especially from ^{1}H–^{1}H COSY (Fig. 1) and HMBC

Table 1
^{1}H (300 MHz) and ^{13}C (75 MHz) NMR data of ficusamide (**1**) in $CDCl_3$/MeOD (1:1) (δ in ppm, *J* in Hz).

Position	δ_H	δ_C	^2J	^3J
CH_2–OH	3.77 (dd, *J* = 4.4, 10.6) 3.72 (dd, *J* = 4.4, 10.6)	60.9	51.1 (C1)	175.8 (C1′), 75.2 (C2)
Long chain base				
1	4.13 (m)	51.1	75.2 (C2), 60.9 (CH_2–OH)	175.8 (C1′)
2	3.57 (m)	75.2	72.1 (C3), 51.1 (C1)	60.9 (CH_2–OH)
3	3.56 (m)	72.1	–	–
4	1.56 (m)	34.5	72.1 (C3), 29.1–29.6 (C5)	–
5–19	1.22–1.35 (br. s)	29.1–29.6	–	–
20	1.22–1.35 (br. s)	31.9	–	–
21	1.22–1.35 (br. s)	22.6	31.9 (C20), 13.6 (C22)	–
22	0.86 (t, *J* = 6.7)	13.6	22.6 (C21)	31.9 (C20)
NH	–	–	–	–
N-acyl moiety				
1′	–	175.8	–	–
2′	4.06 (m)	71.8	175.8 (C1′)	–
3′	1.73 (m)	34.8	71.8 (C2′)	175.8 (C1′)
4′	1.45 (m)	25.9	–	–
5′– 11′	1.22–1.35 (br. s)	29.1–29.6	–	–
12′	1.92 (m)	32.2	129.9 (C13′)	130.5 (C14′)
13′	5.42 (m)	129.9	–	–
14′	5.42 (m)	130.5	–	–
15′	1.77 (m)	32.9	130.5 (C14′)	129.9 (C13′)
16′	1.22–1.35 (br. s)	29.1–29.6	–	–
17′	1.22–1.35 (br. s)	31.9	–	–
18′	1.22–1.35 (br. s)	22.6	31.9 (C17′), 13.6 (C19′)	–
19′	0.86 (t, *J* = 6.7)	13.6	22.6 (C18′)	31.9 (C17′)

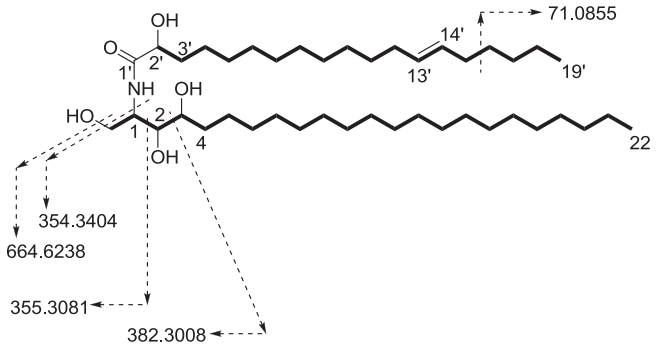

Fig. 1. COSY correlations and MS–MS fragmentation of compound **1**.

spectra (Fig. 2). The long chain base and the fatty acid of **1** correspond to 1-amino-(1-hydroxymethyl)docosane-2,3-diol and 2-hydroxy-13-nonadecenoic acid, respectively. Thus, the structure of ficusamide (**1**) was determined to be 2-hydroxy-*N*-[1-(hydroxymethyl)-2,3-dihydroxy-docosanyl]-13*E*-nonadecenamide. The absolute configuration of the chiral centers at C1, C2, C3, and C2′ could not be established without chemical transformations that would require much more material (Konga et al., 2001). We have named the compound ficusamide after the producing organism, *F. elastica*.

2.2. Characterisation of ficusoside (*2*)

Compound **2** was isolated as a colorless solid (m.p. 149–150 °C). The positive HRESI-MS analysis showed a pseudo-molecular ion at *m/z* 884.6856[M+H]$^+$, consistent with the formula $C_{48}H_{94}NO_{10}$ (calc. 884.6842, 1.6 ppm). The existence of the two signals at δ_C 50.8 (C–N) and 176.1 (C–O) in the ^{13}C NMR spectrum of **2** suggested the presence of an amide group (Table 2). Compound **2** gave a positive reaction in the Molish test, attesting for the presence of a sugar moiety. The NMR spectra of **2** showed the anomeric center at δ_H 4.30 (1H, d, J = 8.4)/δ_C 103.5, and a set of C-atom signals (δ_C 73.5, 76.4, 70.1, 76.3, and 61.7), consistent with a β-D-glucopyranoside. The value of the coupling constant between H1″ (δ_H 4.30 (1H, d, J = 8.4)) and H2″ (δ_H 3.21 (1H, dd, J = 4.4, 8.4)) corresponded to two *trans* diaxial protons, and suggested a chair conformation of the carbohydrate moiety. The C1″ and C2″ substituents occupied an equatorial position indicating the presence of a β-glycoside.

The ^{1}H NMR spectrum gave additional information on the structure of **2** showing two aliphatic chains as evidenced by an intense signal between δ_H 1.25 and 1.29 (brs), and the triplet at δ_H 0.89 (t, J = 7.6) (δ_C14.0). Moreover, two signals of olefinic protons at δ_H 5.37 (m) and 5.41 (m) matched well with a double bond. The three unsaturations of **2** could thus be readily assigned to a double bond, a carbonyl and a glucopyranosyl group.

The Δ^{10} double bond was found to be *cis*, as evidenced by the chemical shift of C9′ at δ_C 27.5 and C12′ at δ_C 26.3 (Stothers, 1972; Jung et al., 1996). Furthermore, a CH_2O group (δ_H 4.05 (Ha, dd, J = 4.0, 10.4) and δ_H 3.82 (Hb, dd, J = 6.0, 12.0; δ_C 68.8)) and CH–O groups (δ_H 4.02 (dd, J = 3.2, 8.8), δ_H 3.57 (m) and δ_H 3.54 (m); δ_C 72.2, 74.6 and 72.2) were also observed. From the foregoing data, an unsaturated β-D-glucopyranosyl ceramide structure was suggested for compound **2** (Fig. 4). The position of the glucose moiety at C1 was evidenced by the downfield chemical shift observed for the hydroxymethylene carbon at δ_C 68.8 (Kasai et al., 1999) and further confirmed by HMBC spectrum in which the anomeric proton H-C1 correlated with the C-atom C (Ha, Hb).

Pertinent HMBC correlations (Fig. 3) were also observed between H–C2 and C1 and C3, with H–C3 and multiple H-atom signals between δ_H 1.25 and 1.29, which showed cross-peaks with the Me group at δ_H 0.90. H-C3′ displayed correlations with C2′, H–C10′ with C9′, H–C11′ with C12′, H–C9′ with C10′, H–C12′ with C11′, H–C22′ with C21′ and C23′. The COSY spectrum (Fig. 3) established that H-C1 correlated with Ha, Hb, H–C2 and H–C3, H–C2 with H–C3, H–C3 with H–C4, H–C2′ with H–C3′, H–C9′ with H–C10′, H–C11′ with H–C12′H–C21′ with H–C22′ and H–C22′ with H–C23′. The former data suggest that the long chain base side is saturated. The length of the long chain base was determined by the characteristic ions at *m/z* 477.20 [Glc-CHCH(OH)CH(OH)$(CH_2)_{14}CH_3$]$^+$ (Fig. 4). The typical fragment ion at *m/z* 155.13 was formed by elimination of undecene from [M]$^+$, through McLafferty rearrangement. This, together with the correlations described above, also confirmed the position of the double bond in the long fatty chain.

From the foregoing data, the structure of **2** was characterized as 2-hydroxy-N-[1-[(β-D-glucopyranosyloxy)methyl]-2,3-dihydroxy-octadecanyl]-10*Z*-tricosenamide (Fig. 4). However, the absolute configuration of **2** at the chiral centers C1, C2, C3, and C2′ could not be determined.

2.3. Characterisation of elasticoside (*3*)

Compound **3** was isolated as a white solid (m.p. 180–181 °C). The high resolution electrospray ionization mass spectrometry (HRESIMS) (positive-ion mode) exhibited a pseudo-molecular ion peak at *m/z* 812.4806 [M+NH_4]$^+$ (calc. 812.4801, 0.6 ppm), supported a molecular formula of $C_{42}H_{66}O_{14}$, which accounted for ten degrees of unsaturation. Peracetylation of **3** yielded **3a** which was subsequently analyzed by HRESIMS, giving rise to a pseudo-molecular ion peak *m/z* 1106.5542 [M+NH_4]$^+$ (calc. 1106.5537) suggesting molecular formula $C_{56}H_{80}O_{21}$. Comparison of mass spectra of **3** and **3a** indicated the presence of seven free hydroxy groups in **3**. The ESIMSMS (positive-ion mode) of **3a** showed molecular ion peaks at *m/z* 273.0969 ($C_{12}H_{17}O_7$) and *m/z* 331.1026 ($C_{14}H_{14}O_9$), corresponding to the loss of two hexosyl units $C_6H_{11}O_4$ and $C_6H_{11}O_5$, respectively (Fig. 5).

On the basis of the ^{1}H and ^{13}C NMR spectra, compound **3** was identified as an urs-12-ene type pentacyclic triterpene glycoside, confirmed by comparison with NMR data of known urs-12-ene derivatives (Dawidar et al., 1979; Arriaga et al., 1990; Hassanean et al., 1993) (Table 3). Extensive analysis of 1D and 2D NMR spectra indicated the presence of four tertiary methyl groups at δ_H 0.84, 0.89, 0.92 and 1.03, three secondary methyl groups at δ_H 0.91, 0.94 and 1.26 (d, J = 6.3 Hz), an olefinic broad single proton at δ_H 5.64 coupled to a carbon at δ_C 129.6 (C12), a quaternary carbon at δ_C 132.1 (C13), and a oxymethine proton at δ_H 3.12 (dd, J = 3.9,

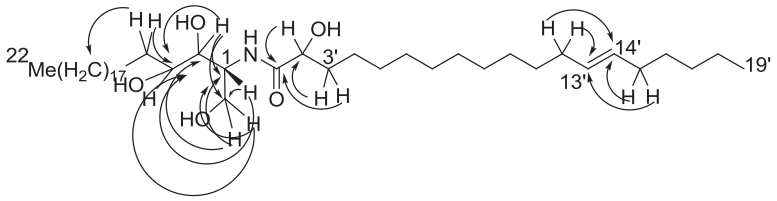

Fig. 2. HMBC correlations of compound **1**.

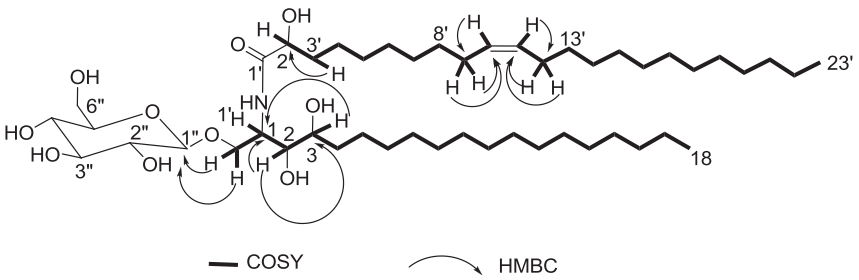

Fig. 3. Important HMBC and COSY correlations of compound **2**.

Table 2
^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data of ficusoside (**2**) in $CDCl_3$/MeOD (1:1) (δ in ppm, J in Hz).

Position	δ_H	δ_C	^2J	^3J
CH_2–Glc	4.05 (dd, J = 6.0, 12.0) 3.82 (dd, J = 4.0, 10.4)	68.8	–	103.5 (C1″)
Long chain base				
1	4.27 (m)	50.8	68.8 (CH_2–Glu)	–
2	3.57 (m)	74.6	72.2 (C3), 50.8 (C1)	–
3	3.54 (m)	72.2	–	–
4	1.32–1.41 (m)	25.4	–	–
5–15	1.25–1.29 (br. s)	29.6–29.9	–	–
16	1.25–1.29 (br. s)	32.1	–	–
17	1.25–1.29 (br. s)	22.9	32.1 (C16), 14.0 (C18)	–
18	0.89 (t, J = 7.6)	14.0	22.9 (C17)	32.1 (C16)
NH	8.59	–	–	–
N-acyl moiety				
1′	–	176.1	–	–
2′	4.02 (dd, J = 3.2, 8.8)	72.2	–	–
3′	1.52–1.74 (m)	34.7	72.2 (C2′)	–
4′–8′	1.25–1.29 (br. s)	29.6–29.9	–	–
9′	2.06 (m)	27.5	129.9 (C10′), 29.6–29.9 (C8′)	–
10′	5.37 (m)	129.9	27.5 (C9′)	–
11′	5.41 (m)	130.5	26.3 (C12′)	–
12′	1.98 (m)	26.3	130.5 (C11′)	–
13′–20′	1.25–1.29 (br. s)	29.6–29.9	–	–
21′	1.25–1.29 (br. s)	32.1	–	–
22′	1.25–1.29 (br. s)	22.9	32.1 (C21′), 14.0 (C23′)	–
23′	0.89 (t, J = 7.6)	14.0	22.9 (C22′)	32.1 (C21′)
Sugar				
1″	4.30 (d, J = 8.4)	103.5	–	76.4 (C3″), 68.8 (CH_2–Glu)
2″	3.21 (dd, J = 4.4, 8.4)	73.5	103.5 (C1″), 76.4 (C3″)	–
3″	3.41 (m)	76.4	–	–
4″	3.32 (m)	70.1	–	73.5 (C2″)
5″	3.25 (m)	76.3	–	76.4 (C3″)
6″	3.85 (dd, J = 4.8, 12.0)	61.7	–	70.1 (C4″)
	3.71 (dd, J = 1.6, 12.0)		76.3 (C5″)	–

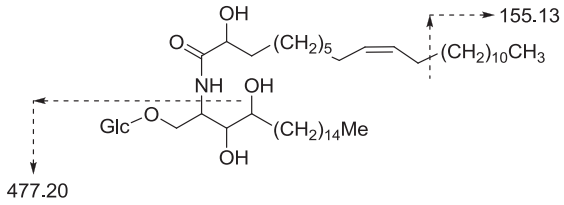

Fig. 4. Important fragmentation of compound **2**.

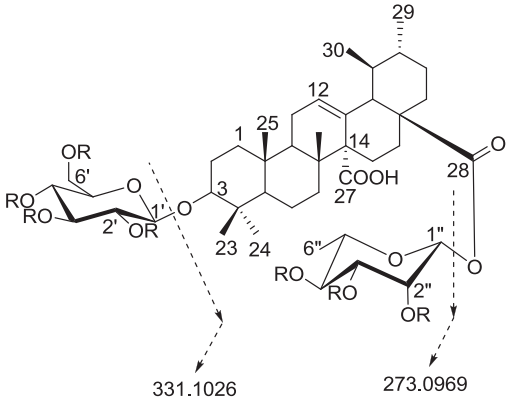

Fig. 5. MS-MS Fragmentation of compound **3a**: R = Ac (**3**: R = H).

11.7 Hz, H-3α). The aglycone on **3** was recognized to be quinovic acid by ^{1}H and ^{13}C NMR analyses (Table 3) using the correlations observed in COSY, HSQC and HMBC spectra. This was in full agreement with literature data (Mahato and Kundu, 1994), in particular for C3 (Pollmanns et al., 1997). Glycosides of quinovic acid display

Table 3
^{1}H (300 MHz) and ^{13}C (75 MHz) NMR data of elasticoside (**3**) in CD_3OD (δ in ppm, J in Hz).

Position	δ_H	δ_C
1	1.67	38.8
2	1.76	25.8
3	3.12 (dd, J = 3.9, 11.7)	89.3
4	–	38.7
5	3.85	55.7
6	1.52	18.0
7	1.68	36.8
8	–	39.5
9	2.23	47.1
10	–	36.5
11	1.96	22.6
12	5.64 (br. s)	129.6
13	–	132.1
14	–	56.0
15	1.36	29.7
16	2.07	25.1
17	–	48.4
18	2.31	54.0
19	0.96	38.9
20	1.02	36.9
21	1.31	30.1
22	1.71	35.7
23	1.03 (s)	27.4
24	0.92 (s)	16.8
25	0.84 (s)	15.6
26	0.89 (s)	17.9
27	–	177.9
28	–	176.6
29	0.91	18.3
30	0.94	20.2
3-*O*-Glc		
1′	4.27 (d, J = 6.6)	105.7
2′	3.47	74.0
3′	3.41	77.0
4′	3.30	70.3
5′	3.38	77.3
6′	3.72	61.2
	3.82	
28-*O*-Rha		
1″	5.40 (d, J = 8.1)	94.3
2″	3.48	71.3
3″	3.61	71.9
4″	3.35	72.6
5″	3.39	69.9
6″	1.26 (d, J = 6.3)	17.4

Assignments were based on the COSY, DEPT, HSQC and HMBC experiments. Overlapped proton NMR signals are reported without designated multiplicity.

varied structures among which four groups can be discerned including glycosides having as glycosylation pattern a C3, a C28, a C3/C28 or a C3/C27 (Hassanean et al., 1993). The chemical shifts of C3 (δ_C 89.3) and C28 (δ_C 176.6) (Table 3) indicated that **3** is a bidesmosidic glycoside (Woldemichael and Wink, 2001; Sahu and Achari, 2001) of quinovic acid, with sugar chains linked to C3 and C28 of the aglycone, through an ether and ester bond, respectively.

The ^{1}H NMR spectrum showed in the sugar region two anomeric proton signals at δ_H 4.27 (d, J = 6.6 Hz) and 5.40 (d, J = 8.1 Hz), which were correlated by HSQC experiment to the corresponding carbon atom resonances at δ_C 105.7 and 94.3, respectively. Complete assignments of the resonances of each sugar unit were achieved by extensive 1D and 2D NMR analysis. These data showed the presence of one β-glucopyranosyl (Glc) and one β-rhamnopyranosyl (Rha) units (Table 3). The anomeric protons of Glc and Rha suggested a β-orientation, according to their relatively large values of coupling constants $^{3}J_{H-1,\ H-2}$ (6.6 Hz) and $^{3}J_{H-1'',\ H-2''}$ (8.1 Hz), respectively (Agrawal, 1992). The absolute configurations of these sugar moieties were determined to be D for Glc and L for Rha by comparison of their NMR data (Table 3) with those found in the literature, for similar sugar derivatives (Agrawal, 1992).

In the HMBC spectrum, the correlation between H1″ (δ_H 5.40) of Rha and C28 (δ_C 94.3) of aglycone, suggested that Rha was attached to C28 of the aglycone through an ester linkage. For the oligosaccharide moiety at C3, a correlation was observed between H1′ (δ_H 4.27) of Glc and C3 (δ_C 89.3) of the aglycone. As a result, Glc is attached to C3 of the aglycone through an ether linkage. A peracetylation reaction with acetic anhydride of **3** yielded the heptaacetate **3a**, confirming the presence of seven hydroxy groups in compound **3**, assigned to the three hydroxy groups of Rha and the four hydroxy group of Glc. Thus, the ten unsaturations of the molecular formula could be readily attributed to the double bond, the two carbonyl groups and the seven cycles. Based on the aforementioned evidences, elasticoside (**3**) was established as 3-*O*-[β-D-glucopyranosyl]-quinovic acid-28-*O*-[β-L-rhamnopyranosyl] ester (Fig. 5).

2.4. Biological activities

The $CHCl_3$/MeOH 1:1 crude extract and pure compounds of the aerial root barks of *F. elastica* were screened for their antimicrobial activity using the microbroth dilution method against several microorganisms: gram (+) bacteria (*Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Staphylococcus epidermididis*), filamentous fungi (*Trichophyton rubrum*), yeast (*Candida albicans*) and gram (−) bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella typhi*) (Tables 4 and 5).

The $CHCl_3$/MeOH 1:1 crude extract of the aerial root barks *Ficus elastic* showed rather good bactericidal and bacteriostatic activities. In particular, the activities of crude extract compared to reference antibiotics were better against *S. saprophyticus* and *K. pneumonia,* while the lowest activities were observed against *S. aureus* and *S. epidermididis*. The compounds (**1**, **3**) showed very good antimicrobial activities against all the tested microbial strains, particularly against gram negative bacteria such as *E. coli* and *K. pneumonia*, compared to the reference antibiotic gentamycin. The compound **1** was the most active compound with a MIC of 3 μg/ml and a MMC of 6 μg/ml against *S. Saprophyticus* while the values obtained for the reference gentamycin were of 980 and 1950 μg/ml, respectively (Table 4). Whereas the compound **3** was observed to be notably efficient against *E. faecalis*, with MIC and MMC values of 30 and 60 μg/ml, respectively.

The known compounds isolated from the aerial root barks of *F. elastica* also showed good antimicrobial activities, consistent with the literature (friedelin, friedelinol, stigmasterol and β-sitosterol (Jain et al., 2001), betulinic acid (Perumal and Dharmarajan, 2005), linear fatty acids (Agoramoorthy et al., 2007), mixture of linear aliphatic primary alcohols and sitosteryl 3-*O*-β-D-glucopyranoside (Mbosso et al., 2010). It can be noted that MMC/MIC ratios lower than 4 were obtained with most of the studied samples, suggesting that killing effects could be expected (Carbonnelle et al., 1987). This important antimicrobial activity obtained is consistent with the antimicrobial activity of another *Moraceae*, *Ficus polita* (Kuete et al., 2011).

For examination of *in vitro* growth inhibitory activity on cancer cell lines, the $CHCl_3$/MeOH 1:1 crude extract of bark of *F. elastica* aerial roots, friedelin, ursolic acid, betulinic acid, **1**, **1a** and **3** were analyzed by means of the MTT colorimetric assay as previously reported (Ingrassia et al., 2009; Van Goietsenoven et al., 2010). The MTT assay allows to determine the *in vitro* concentration that decreases by 50% (IC50) the growth of cells cultured in our case for 72 h in the absence (control) or the presence of the extract or the compound of interest. We made use of three human cancer cell lines that display various levels of resistance to pro-apoptotic stimuli, i.e. the U373 glioblastoma (Branle et al., 2002; Ingrassia

Table 4
Antimicrobial activities (Gram (+) bacteria) of total extract and pure compounds isolated from the $CHCl_3$/MeOH 1:1 crude extract of bark of *Ficus elastica* aerial roots.

	Gram positive bacteria												
			E. faecalis			*S. aureus*			*S. saprophyticus*			*S. epidermididis*		
			MIC	MMC	MMC/MIC	MIC[c]	MMC[c]	MMC/MIC	MIC	MMC	MMC/MIC	MIC	MMC	MMC/MIC
	Concentration (mg/ml)													
FSBR[a]	12.0	50.0	0.39	0.78	2	6.25	12.5	2	0.02	0.05	2.5	6.25	12.5	2
Ficusamide (**1**)	21.0	6.0	0.19	0.38	2	0.75	1.50	2	0.003	0.006	2	0.75	1.50	2
Elasticoside (**3**)	2.00	4.0	0.03	0.06	2	0.50	1.00	2	0.13	0.25	1.92	0.50	1.00	2
Gentamycin[b]	22.0	500.0	0.98	1.95	1.99	1.95	3.91	2	0.98	1.95	1.99	0.49	0.98	2

[a] FSBR: $CHCl_3$/MeOH 1:1 crude extract of bark of *Ficus elastica* aerial roots.
[b] Reference antibiotics (gentamycin for bacteria).
[c] MIC is considered as the lowest concentration of the sample, that inhibits the visible growth of the strain and MMC is considered as the lowest concentration of agent capable of causing the deaths of at least 99.99% of an inoculums.

et al., 2009), the A549 NSCLC (Mathieu et al., 2004; Mijatovic et al., 2006) and the SKMEL-28 melanoma (Mathieu et al., 2009; Van Goietsenoven et al., 2010) cell lines. Betulinic acid and ursolic acid displayed significant *in vitro* growth inhibitory activity in the three cell lines analyzed as already reported by other groups (Bonaccorsi et al., 2008; Huang et al., 2011; Fang et al., 2010; Ahmad et al., 2010). In contrast, friedelin, ficusamide (**1**) and ficusamide Ac (**1a**) displayed weak *in vitro* growth inhibitory activity in one cancer cell line only, i.e. the A549 NSCLC model (Table 6). The $CHCl_3$/MeOH 1:1 crude extract and **3** did not display significant *in vitro* growth inhibitory activity (Table 6). Finally, the compound **2** has not been tested due to lack of product.

3. Concluding remarks

We reported the first phytochemical investigation on the bark of *F. elastica* aerial roots. Three compounds, namely ficusamide (**1**), ficusoside (**2**) and elasticoside (**3**), were isolated and their structures were established. These compounds showed remarkable antibacterial activities against several microorganisms. In particular, ficusamide (**1**) displayed strong antibacterial activity against *S. saprophyticus* and elasticoside (**3**) presented a good activity against *E. faecalis*. To the best of our knowledge, *F. elastica* is not used in traditional medicine in Cameroon while the latex of the plant *Ficus* is generally exploited as anthelmintic. Our study has demonstrated the antimicrobial activity of the bark of *F. elastica* aerial roots and, for some isolated compounds, against bacteria, filamentous fungi and yeast. Standardized extracts of *F. elastica* could be used in traditional medicine for the treatment of wounds and other topical infections. Our results also suggest these compounds as potential leads for natural product-based candidates for further studies.

4. Experimental section

4.1. General experimental procedures

Melting points were determined on an American Optical (Reichert) Forty Stereomicroscope. The ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 and Varian Inova 400 spectrometers, respectively, in $CDCl_3$ and CD_3OD using the residual isotopic solvent $CHCl_3$ and CH_3OH, as references for $\delta_H = 7.26$ ppm, $\delta_C = 77.16$ ppm, $\delta_H = 3.31$ ppm, $\delta_C = 49.00$ ppm. Chemical shifts were measured relatively to tetramethylsilane as internal standard. The following abbreviations are used for spin multiplicity: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet; br, broad. ^1H NMR and ^{13}C NMR spectra were assigned by comparison with NMR spectra of compounds previously described in the literature.

Routine thin-layer chromatography (TLC) was performed on silica gel plates (Silica gel GF254 from VWR), and spots were visualized after treatment with an EtOH solution of phosphomolybdic acid (20%, Aldrich). Flash column chromatography was carried out using silica gel at moderate pressure (spherical particle size 60–200 μm from MP Biomedicals). The following 2D NMR spectra were also acquired as necessary: ^1H, ^1H COSY spectra (^1H, ^1H correlated spectroscopy), HMBC spectra (Heteronuclear Multi Bond connectivity) and HMQC spectra (Heteronuclear Multiple Quantum coherence).

High resolution mass spectra (positive mode) were recorded by direct infusion in a 6520 series electrospray ion source (ESI)-quadrupole time-of-flight (Q-TOF) mass spectrometer (Agilent, Palo Alto, CA, USA). The error between the observed mass and the calculated mass was expressed in ppm. Below 3 ppm, the compounds were considered to have the predicted formula. Optical rotation was measured with a polarimeter (ATAGO AP 100).

The separation of the extracts was screened according to the common phytochemical methods described by Harborne (1973).

4.2. Plant material

The bark of *F. elastica* aerial roots was collected from Yaoundé (Cameroon) in December 2007. Botanical identification was performed at the National Herbarium of Cameroon (NHC) by Mr. Nana, where voucher specimens (No. 65646 HNC) have been deposited.

4.3. Extraction and isolation

Dried bark material of *F. elastica* (1.1 kg) was percolated for 48 h with MeOH at room temperature and the solution afterwards filtered and concentrated under vacuum. The crude $CHCl_3$/MeOH 1:1 extract (100.0 g) was subjected to column chromatography (CC) on silica gel (cyclohexane/EtOAc/MeOH gradient of increasing polarity) to give six fractions N1–N6 on the basis of TLC composition.

Fraction N1 (0.34 g) was subjected to CC on silica gel eluted with cyclohexane to yield a mixture of *n*-alkanes (7.5 mg). Fraction N2 (10.23 g) was submitted to CC with a gradient of cyclohexane/EtOAc yielding friedelin (10 mg) at cyclohexane/EtOAc 98:02, friedelinol (15 mg) at cyclohexane/EtOAc 94:06, a mixture of linear aliphatic primary alcohols with *n*-dotriacontanol (9 mg) as the major compound, a mixture of linear fatty acids(6 mg) with tetracosanoic acid as major compound (identified according to NMR spectroscopy and GC) and a mixture of phytosterols (43 mg) with stigmasterol and β-sitosterol at cyclohexane/EtOAc 90:10, betulinic acid (56 mg) at cyclohexane/EtOAc 85:15 and ursolic acid (10 mg) at cyclohexane/EtOAc 80:20.

Fraction N3 (2 g) was subjected to CC on silica gel eluted with cyclohexane/EtOAc to afford ursolic acid (6 mg) at cyclohexane/EtOAc 78:22. Fraction N4 (1 g) was subjected to CC on silica gel eluted with $CHCl_3$/MeOH gradient to yield ficusamide (**1**) (13 mg) at $CHCl_3$/MeOH 95:05 and sitosteryl 3-*O*-β-D-glucopyranoside

Table 5
Antimicrobial activities (filamentous fungi, yeast and Gram (−) bacteria) of total extract and pure compounds isolated from the $CHCl_3$/MeOH 1:1 crude extract of bark of *Ficus elastica* aerial roots.

	Filamentous fungi					Yeast			Gram (−) bacteria								
	T. rubum					*C. albicans*			*E. coli*			*K. pneumoniae*			*S. typhimurium*		
	MIC[c]		MMC[c]		MMC/MIC	MIC	MMC	MMC/MIC	MIC	MMC	MMC/MIC	MIC	MMC	MMC/MIC	MIC	MMC	MMC/MIC
	Concentration (mg/ml)																
FSBR[a]	12.0	50.0	3.13	6.25	2	3.13	6.25	2	6.25	6.25	1	0.78	1.56	2	0.50	1.00	2
Ficusamide (**1**)	21.0	6.0	1.50	1.50	1	0.75	1.50	2	0.19	0.38	2	0.38	0.75	1.80	0.75	1.50	2
Elasticoside (**3**)	2.00	4.0	0.50	0.50	1	0.50	1.00	2	0.50	0.50	1	0.25	0.50	2	0.50	1.00	2
Nystatin[b]	22.0	267.0	–	–	–	3.91	7.81	2	–	–	–	–	–	–	–	–	–
Gentamycin[b]	22.0	500.0	–	–	–	–	–	–	7.81	15.63	2	7.81	15.63	2	0.12	0.24	2
Clotrimazole[b]	–	500.0	0.49	0.98	2	–	–	–	–	–	–	–	–	–	–	–	–

[a] FSBR: $CHCl_3$/MeOH 1:1 crude extract of bark of *Ficus elastica* aerial roots.
[b] Reference antibiotics (nystatin for yeast, gentamycin for bacteria and clotrimazole for filamentous fungi).
[c] MIC is considered as the lowest concentration of the sample, that inhibits the visible growth of a microbe and MMC is considered as the lowest concentration of agent capable of causing the deaths of at least 99.99% of an inoculums.

Table 6
In vitro growth inhibitory activity of extract and compounds isolated from the $CHCl_3$/MeOH 1:1 crude extract of bark of *Ficus elastica* aerial roots in a panel of 3 cancer cell line cells.

#	U373 (glioma)	A549 (lung)	SKMEL-28 (melanoma)	Mean IC_{50}
Human cancer cell lines(IC_{50} in vitro growth inhibitory concentrations (μM)[a]				
Ficusamide (**1**)	>100	79	>100	>93
Ficusamide Ac (**1a**)	>100	96	>100	>99
Elasticoside (**3**)	>100	>100	>100	>100
IC_{50} in vitro growth inhibitory concentrations (μg/ml)[a]				
$CHCl_3$/MeOH 1:1 crude extract of bark of aerial roots of *Ficus elastica*	>100	>100	>100	>100

[a] The IC_{50} *in vitro* growth inhibitory concentrations were determined using the MTT colorimetric assay. The human cell lines analyzed include the U373 (ECACC code 89081403) glioma, the A549 (DSMZ code ACC107) NSCLC and the SKMEL-28 (ATCC code HTB-72) melanoma models. The IC_{50} concentration represents the concentration of extract or compound needed to decrease by 50% cell population growth after having cultured the cells for 72 h in the absence (control) or the presence of the extract or the compound of interest.

(30 mg) at $CHCl_3$/MeOH 90:10. Fraction N5 (12.29 g) was subjected to CC on silica gel eluted with $CHCl_3$/MeOH gradient to afford sitosteryl 3-*O*-*β*-D-glucopyranoside (20 mg) and ficusoside (**2**) (3 mg) at $CHCl_3$/MeOH 90:10 and elasticoside (**3**) (16 mg) at $CHCl_3$/MeOH 85:15. Fraction N6 (15.88 g) obtained by elution with MeOH was a complex mixture.

4.4. Ficusamide (*1*)

Colorless solid, m.p. 120–121 °C. $[\alpha]_D^{24}$:+16.3($CHCl_3$; *c* 0.4). ^1H NMR, (300 MHz, $CDCl_3/CD_3OD$ 1:1) and ^{13}C NMR spectra, (75 MHz, $CDCl_3/CD_3OD$ 1:1) see Table 1; (+)-HRESI-MS: *m/z* 682.6383 $[M+H]^+$ (calc. 682.6372, 1.2 ppm for $C_{42}H_{84}NO_5$).

4.5. Acetylation of *1*

Dry pyridine (0.5 ml) and Ac_2O (0.5 ml) were added to compound **1** (3 mg) and left overnight. The usual workup yielded **1a** (3.8 mg) which was identified without ambiguity with ^1H NMR and HRESIMS.

4.6. Ficusamide tetra acetate (*1a*)

Colorless solid, m.p. 52–53 °C. $[\alpha]_D^{24}$:+10.7 ($CHCl_3$; *c* 0.2). ^1H NMR, (300 MHz, $CDCl_3$): δ = 0.90 (*t*, *J* = 6.3 Hz, 6H, 19′-H, 23-H), 1.21–1.36 (br. *s*, 52H, 5′-11′-H, 17′-H, 18′-H, 5–21-H), 1.62 (*m*, 2H, 4′-H), 1.85 (*m*, 4H, 3′-H, 15′-H), 1.97 (*m*, 2H, 4-H), 2.09 (*m*, 2H, 12′-H), 2.05–2.13 (12H, *s*, 4-OAc), 4.03 (*dd*, *J* = 3.0, 11.7 Hz, 1H, CH_2(a)OH), 4.36 (*dd*, *J* = 6.3, 11.7 Hz, 1H, CH_2(b)OH), 4.47 (*m*, 1H, 3-H), 4.97 (*dd*, *J* = 2.7, 12.0 Hz, 1H, 2′-H), 5.12 (*m*, 1H, 2-H), 5.39 (*m*, 1H, 1-H), 6.62 (*m*, 2H, 13′-14′-H); (+)-HRESIMS: *m/z* 852.6923 $[M+H]^+$ (calc. 852.6917 for $C_{42}H_{84}NO_5$). 3.29 (m, 1 H, 4″-H), 3.37 (m, 1 H, 2″-H), 3.41 (m, 1 H, 5″-H).

4.7. Ficusoside (*2*)

Colorless solid, m.p. 149–150 °C. $[\alpha]_D^{24}$: −7.0 ($CHCl_3$; *c* 0.22). ^1H NMR, (400 MHz, $CDCl_3/CD_3OD$ 1:1) and ^{13}C NMR spectra, (75 MHz, $CDCl_3/CD_3OD$ 1:1) see Table 2; (+)-HRESIMS: *m/z* 884.6856 $[M+H]^+$(calc. 884.6842, 1.6 ppm for $C_{48}H_{94}NO_{10}$).

4.8. Elasticoside (*3*)

Colorless solid, m.p.180–181 °C. $[\alpha]_D^{24}$:+29.1 CH_3OH; *c* 0.35). ^1H NMR, (300 MHz, CD_3OD) and ^{13}C NMR spectra, (75 MHz, CD_3OD) see Table 3; (+)-HRESIMS: *m/z* 812.4806 $[M+NH_4]^+$ (calc. 812.4801, 0.6 ppm for $C_{42}H_{66}O_{14}$).

4.9. Antimicrobial assay using agar diffusion test

Nine microorganisms were tested among which 4 Gram positive, 3 Gram negative bacteria specimens, one yeast and one filamentous fungi. The Gram positive bacteria included: *E. faecalis*, *S. aureus*, *S. Saprophyticus*, *S. epidermididis* isolated from urine and pus samples obtained from patients visiting the Tiko CDC Hospital. The Gram negatives bacteria comprised: *E. coli*, *K. pneumonia*, and *Salmonella typhimurium*, all obtained from urine and faecies specimen collected from patients. Yeast: *C. albicans* was isolated from vaginal swab. Filamentous fungi: *T. rubrum* was isolated from skin foot specimen.

The minimum inhibitory concentration (MIC), considered as the lowest concentration of the sample, that inhibits the visible growth of a microbe, was determined by the microbroth dilution method (Carbonnelle et al., 1987; Berghe and Vlietinck, 1991) in Mueller Hinton or Sabouraud broth supplemented with 10% glucose and 0.5% phenol red. For susceptibility testing, in a first step Mueller Hinton broth (50 μl) was distributed from the first to the twelfth

microwell. Dry extract and pure compounds were initially dissolved in DMSO (20%) (100 μl) and subsequently in Mueller Hinton broth, to reach a final concentration of 1000 and 1600 μg/ml. These solutions (50 μl) were added to the first well of each microtiter line. Successive dilutions were done by transferring the mixture/solution (50 μl) from the first to the eleventh well. An aliquot (50 μl) was discarded from the eleventh tube. The twelfth tube served as growth control since no sample (extract, pure compounds, or reference antibiotics) was added. A microbial suspension (50 μl, 10^5 colony forming units/ml), obtained from an overnight growth at 37 °C was added to each well. The final concentration of the extract adopted to evaluate the antimicrobial activity ranged from 25,000 to 2.4 μg/ml and for pure compounds from 2,000 to 0.98 μg/ml. Tests were incubated aerobically at 37 °C for 24 h before being read. The MIC was considered as the lowest concentration of the sample that prevented visible growth or changed in color from red to yellow due to the formation of acidic metabolites corresponding to microbial growth.

The minimum microbicidal concentration (MMC), considered as the lowest concentration of agent capable of causing the deaths of at least 99.99% of an inoculums was also determined. After the MIC determination, an aliquot (10 μl) from each microwell presenting no visible growth was inoculated on fresh drug-free Mueller Hinton agar plates and incubated at 37 °C for 24 h Plates showing no growth indicated bactericidal effect of the fraction (sensitive) (Berghe and Vlietinck, 1991). Each experiment was performed in triplicate.

4.10. In vitro growth inhibition activity towards human cancer cell lines

The histological types and origin of the three cancer cell lines under study are detailed in the legend of Table 4. The cells were cultured in RPMI (Invitrogen, Merelbeke, Belgium) media supplemented with 10% heat inactivated fetal calf serum (Invitrogen). All culture media were supplemented with 4 mM glutamine, 100 μg/mL gentamicin, and penicillin–streptomycin (200 U/mL and 200 μg/mL) (Invitrogen).

The overall growth level of these human cancer cell lines was determined using the colorimetric MTT (3-[4,5]-dimethylthiazol-2yl-diphenyl tetrazolium bromide, Sigma, Belgium) assay as detailed previously (Ingrassia et al., 2009; Van Goietsenoven et al., 2010). Each experimental condition was performed in six replicates.

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