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Stilbene and new triterpene from *Ficus grevei* Baillon

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Abstract
The hexane and the ethyl acetate extracts of the air-dried stem bark of *Ficus grevei* Baillon endemic Malagasy tree afforded palmitate and stearate of lupeol, 28-formyl-3β-hydroxyurs-12(13)-ene, 28-formyl-3β-hydroxyolean-12(13)-ene, (E)-resveratrol 3-O-ß-glucoside. The structures of the isolated compounds were established by spectroscopic NMR (1D and 2D).

Keywords: *Ficus grevei* Baillon, anti-oxydant, hexane extract, ethyl acetate extract, triterpene, stilbene.

1. Introduction
Species in the *Ficus* genus are acknowledged as a rich source of bioactive secondary metabolites such as flavonoids, stilbenes, triterpenoids and xanthones. [1, 2, 3, 4, 5, 6]
Ficus grevei Baillon is a shrub plant of the Moraceae family. It is an endemic plant of Madagascar. It is a big genus with 800 species distributed mainly in Africa, Asitropical, Australia, and Tropical America of which 100 in continental Africa with 18 species in Madagascar. [7]
A decoction of the stem bark of *Ficus grevei* Baillon is used to treat fever, cough, and dependent of smoking.
According to available literature, no phytochemical research work has been carried out to this species. We now report the isolation of three triterpenes and stilbene from hexane and ethyl acetate extracts of the stem bark of *Ficus grevei* Baillon.

2. Materials and methods
2.1 Plant material
Ficus grevei was collected from Beroy Antsaro North Itampolo region, Toliara, Madagascar, in 2012. This species was identified taxonomically at the Department of Botany, Botanical and Zoological Park of Tsimbazaza, Antananarivo. A voucher specimen was deposited at the Faculty of Sciences of Antananarivo University for the future references.

2.2 General experimental procedures
NMR spectra for all compounds were performed on a Bruker Varian 300 NMR and 600 NMR operating at 300.15/100.6 MHz and 600MHz using $CDCl_3$ or CD_3OD DMSO-d_6 as solvent and TMS as an internal standard. One-dimensional NMR (proton ^1H, carbon ^{13}C) and two-dimensional NMR measurements (including COSY, HSQC, HMBC and ROESY) were performed in order to identify the compounds. Column chromatography (CC) was carried out on silica gel F_{254} (Merck) in glass blades. Thin layer chromatography was performed on precoated TLC plates (Merck, silica 60F254) and visualized by UV light and by spraying with vanillin in H_2SO_4.

2.3 Extraction
Dried and powdered stem barks of *Ficus grevei* Baillon were reduced to a powder with a mechanical grinder. The powder plant material (400 g) was extracted by maceration successively with hexane, dichloromethane and ethyl acetate (3500 ml) for 72 h. The solvent were evaporated under reduced pressure to give respectively hexane (3.35 g), dichloromethane (2.58 g) and ethyl acetate (3.58 g) extracts.

2.5 g of hexane extract were subjected to column chromatography on silica gel (3 X 80 cm), eluting with a gradient hexane – ethyl acetate. 232 fractions of 10 ml each were collected. These fractions were analysed by TLC. The fractions exhibiting an identical form in TLC were combined. Fractions 15 to 20 eluted with hexane – ethyl acetate (90:10) was precipitated with MeOH to give compound 1 (10 mg). Fractions 99 to 120 eluted with hexane – ethyl acetate (0:100) were combined and precipitated with MeOH to obtain a mixture of 2 and 3 (10 mg).

3.5 g of ethyl acetate extract were subjected to column chromatography on silica gel (3 X 80 cm), eluting with a gradient hexane – ethyl acetate. 632 fractions of 10 ml each were collected. These fractions were analysed by TLC. The fractions exhibited an identical form in TLC were combined. Fraction 309-320 eluted with ethyl acetate – MeOH (90:10) was precipitated with hexane to give compound 4 (20 mg).

The purity of each compound was estimated by thin-layer chromatography using different solvent systems.

Transesterification of compound 1

Compound 1 was refluxed in EtOH (15 ml) with 5 ml of NaOH 2 N during 8 hours. The reaction product was extract with sulphuric ether. The organic phase was separated, dried over Na_2SO_4 and evaporate. From the ester 1, stearic acid and palmitic acid were obtained. Addition of HCl (1%) to the remaining aqueous phase, followed by extraction with dichloromethane, yielded lupeol.

Compound 1: White powders

^{1}H NMR ($CDCl_3$, 600 MHz) δ(ppm): 4.67(H-29β), 4.65(H-29α), 4.46(H-3), 2.36(H-19), 1.69(H-30), 1.03(H-26), 0.94(H-27), 0.85(H-25), 0.84(H-24), 0.83(H-23), 0.78(H-28).

Long chain: 2.28(H-3'), 1.61(H-4'), 1.25(CH_2)$_n$, O.87(CH_3 ter).

^{13}C NMR ($CDCl_3$, 600 MHz) δ (ppm); 151.02 (C-20), 109.36 (C-29), 80.1 (C-3), 55.5 (C-5), 50.34 (C-9), 48.11 (C-18), 48.00 (C-19), 43.19 (C-17), 42.96 (C-14), 40.85 (C-8), 40.00 (C-22), 38.44 (C-1), 38.05 (C-13), 37.90 (C-4), 37.09 (C-10), 34.87 (C-16), 34.22 (C-7), 29.9 (C-21), 27.87 (C-24), 27.44 (C-15), 25.18 (C-12), 23.75 (C-2), 20.95 (C-11), 19.04 (C-30), 18.22 (C-6), 18.01 (C-28), 16.04 (C-26), 16.00 (C-23), 15.98 (C-25), 14.45 (C-27).

Long chain: 174.01 (C-1'), 35.58 (C-2'), 25.10 (C-3'), 30.0 (CH_2)$_n$, 22.80 (CH_2- CH_3 ter), 14.03 (CH_3 ter).

Methylalkanoates a-b: White powders. GC-MS; ():m/z(rel.int)=Rt_a 13.72, 270$[M_a]^+$(10); Rt_b 14.97, 298$[M_b]^+$(4)

Compound 2 and 3: Yellow powders

m/z (rel%): 426 $[M]^+$, ^{1}H NMR spectral data (400.15MHz, $CDCl_3$) 1.5 (H-1), 1.69(H-2α),1.86(H-2β), 3.46(H-3), 5.64(H-6), 1.84(H-7α), 1.96(H-7β), 1.50(H-8), 2.00(H-10), 1.37(H-11α), 1.55(H-11β), 1.34(H-12α), 1.69(H-12β), 1.16(H- 15α), 1.47(H-15β), 1.25(H-16α), 1.37(H-16β), 1.57 (H-18), 1.25(H-19α),1.37(H-19β) 1.22(H-21α), 1.47(H-21β), 0.90(H-22α), 1.53(H-22β), 1.04 (H-23), 1.14(H-24), 0.85(H-25), 1.09(H-26), 1.00 (H-27), 1.16 (H-28), 0.99 (H-29), 0.95 (H-30)

^{13}C NMR spectral data (100.15MHz, $CDCl_3$) 206.3(C-28), 143.46 (C-13 of β-amyrine), 137.9 (C-13 of α-amyrine β), 125.9 (C-12 of α-amyrine), 123.00 (C-12 of β-amyrine), 79.0(C-3), 55.0(C-5),52.4 (C-18), 47.8 (C-17), 47.4(C-9), 42.0(C-14), 40.9(C-8), 38.8(C-4), 38.6(C-1), 38.6(C-19), 38.5(C-20), 32.3(C-7), 30.6 (C-21), 29.7 (C-22), 28.1(C-23), 27.8(C-2), 26.8(C-16), 27.2(C-15), 23.5(C-27), 23.2(C-11), 21.0(C-30), 18.3(C-6), 16.9(C-26), 16.5(C-29), 15.6(C-24), 15.6(C-25).

Compound 4: yellow powders

^{1}H NMR (600 MHz, CD_3OD, ppm): δ 7.37 (d, 2H, J = 8.6 Hz, H2′ and H6′), 7.08 (d, 1H, J = 16.2 Hz, H7), 6.85 (d, 1H, J = 16.2 Hz, H8), 6.77 (d, 2H, J= 8.6 Hz, H3′ and H5′), 6.79 (broad signal, 1H, H2), 6.62 (bs, 1H, H6), 6.45 (bs, 1H, H4), 4.93 (d, J = 7.6 Hz, H1″), 3.94 (bs, 1H, H6″), 3.73 (bs, 1H, H6″), 3.47 (bs, 1H, H2″), 3.43 (bs, 1H, H3 ″), 3.49 (bs, 1H, H4 ″), 3.49(bs, 1H, H5 ″).

^{13}C NMR (125 MHz, CD_3OD, ppm): δ 158.3 (C3), 158.2 (C5), 156.9 (C4′), 140.0 (C1), 128.4 (C1′), 128. 7 (C8), 127.54 (C2′ and C6′), 125.03 (C7), 114.89 (C3′ and C5′), 106.3 (C6), 105.6 (C2), 102.47 (C4), 100.80 (C1″), 76.7 (C5″), 76. 7(C3″), 73.59 (C2″), 70.22 (C4″), 61.25 (C6″).

3. Results and discussion

Structures of isolated compounds

Compound 1

Hexanic extract was fractioned by column chromatography on silica gel to afford compounds 1, 2 and 3 (figure 1). The structure elucidation of these compounds was based on spectral techniques as 1D and 2D NMR. The structures of isolated compounds were identified as lupeol fatty acid ester 1[8] (lupeol palmitate 1a and lupeol stearate 1b), 28-formyl-3β-hydroxyurs-12(13)-ene 2 and 28-formyl-3β-hydroxyolean-12(13)-ene 3, after comparing with a previous data.

The ^{1}H-NMR spectrum of 1 exhibited signals of isopropenyl protons at δ 1.69 (3H, s), 4.56 (1H, s) and 4.66 (1H, s) and signals due to seven tertiary methyl groups at δ 0.78, 0.83, 0.84, 0.85, 0.99, 1.01, 1.03 and 1.69, each broad singlet, which are reminiscent of a lupeol-type triterpene [9]. Additionally, a terminal methyl signal at δ 0.87 and strong methylene proton signals around δ 1.25 were indicative of the presence of a fatty acid. The existence of oxymethine (δ 4.48) showed that lupeol was acylated of fatty acid at position 3. Which was also supported by the appearance of ^{13}C-signals due to a ester carbonyl group at δ 173.2, a long-chain of methylene groups at δ 22.8—29.9 and a terminal methyl group at δ14.03 [10]. Transesterification of compound 1 yielded lupeol and mixture of fatty acids which were characterized by GC analysis. The fatty acid was estimated to be composed of C16 and C18 by GC-MS. Therefore compound 1 is a mixture of lupeol 3-hexadecanoate 1a and lupeol 3-octadecanoate 1b.

Compounds 2 and 3

Fractions 99-120 of the hexane extract of the stem bark of *Ficus grevei* Baillon yielded mixtures of triterpenes which exhibited a single spot when analysed by TLC in several solvents.

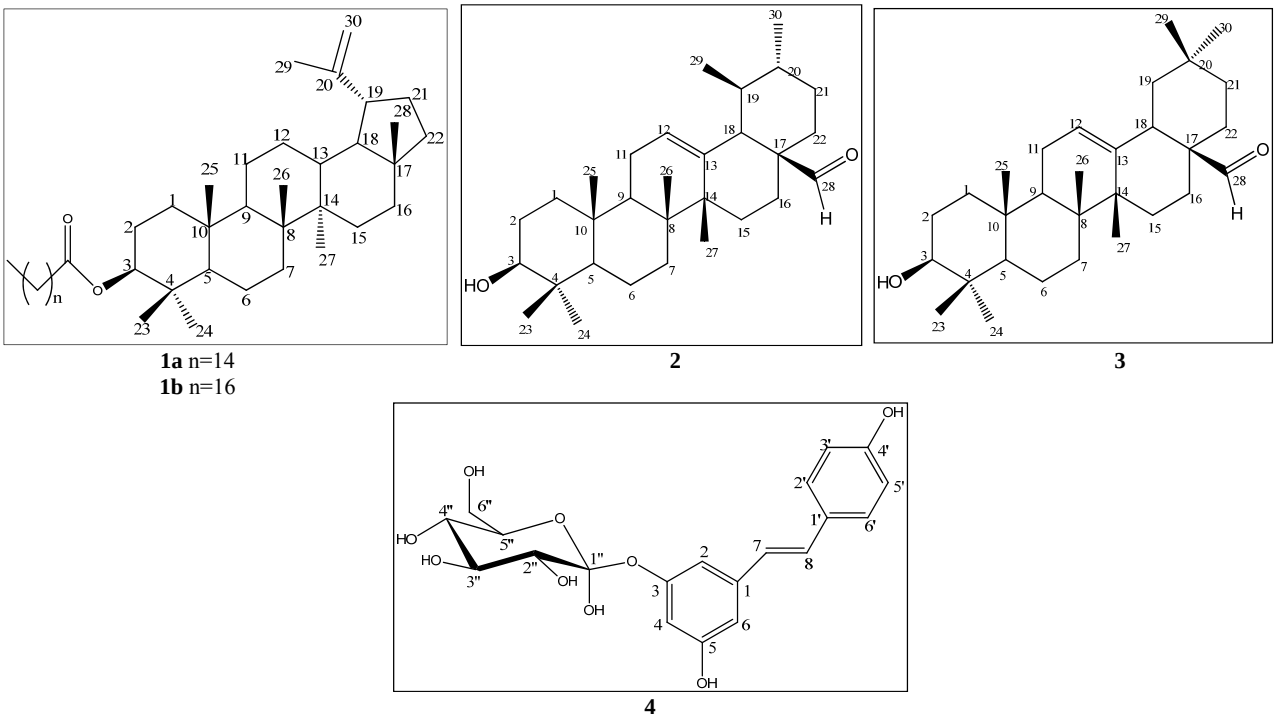
The ^{13}C-NMR spectra of these fractions showed signals indicating a triterpene mixture. In particular, it was observed four signals for olefinic carbons, being two tertiary (δ 123.00, δ 125.98) and two quaternary carbons (δ 143.46, δ 137.88). Comparison with literature data suggested the presence of triterpenes type urs-12(13)-ene [11] (δ125.98, δ137.88), olean-12-ene [12] (δ 123.00, δ143.46). Also it was observed a signal at δ 79.00, typical of an alcool triterpene, and a signal for an aldehyde carbonyl (δ 206.30). There were no others signals for oxygenated carbons. In HSQC and HMBC spectrum, the proton at δ_H 2.22/52.48 ppm (position 18) correlated with the carbons at δ_C 47.88 (C-17), δ_C 29.70 (C-22), δ_C 206.32

assigned to (C-28). The presence of aldehyde was corroborated ^{1}H NMR that showed a singulet at 9.60. In addition also signals for oxymethine (δ 3.25) and olefinic protons of 2 (δ 5.17), 3 (δ 5.18) were observed. Thus the components of these fractions were identified as 28-formyl-3β-hydroxyurs-12(13)-ene 2 and 28-formyl-3β-hydroxyolean-12(13)-ene 3.

Compound 4

The ethyl acetate extracts of the stem bark of *Ficus grevei* Baillon yielded resveratrols 4 and 5 by silica gel chromatography. The one dimensional ^{1}H NMR data of 4 showed characteristic signals of a resveratrol structure. Indeed the resonance set, between δ 6.74 and 7.36 ppm, composed of three systems of two olefinic and seven aromatic protons, was typical for resveratrol [13]. Furthermore the resonance set, between δ 3.7 and 4.0 ppm, was attributed to β-glycosyl unit linked to an aromatic ring and one doublet at δ 4.94 ppm (2H, J=7.2 Hz) was attributed to the anomeric proton. The assignment of the resveratrol moiety as trans was due to the presence of the coupling constant of the olefinic proton signals at δ 6.89 and 7.04 (each, J=16.4 Hz). The presence of glucose moiety was corroborated by ^{13}C NMR that showed for oxymethine at 73.59, 70.22, 76.7, 100.8 (anomeric carbon) and oxymethylene at 61.25. In addition also observed signals for aromatic carbons (δ 102.47 to 158.2) other two olefinic carbons at (δ 125.03) and (δ 128.7).

Finally, NMR data indicated that compound 4 was (E)-resveratrol 3-O-ß-glucoside previously identified in *Vitis vinifera* [14]. To our acknowledgment, this compound is identified for the first time in the *Ficus* genus but (E)-resveratrol 3, 5-O-ß-diglucoside is frequently uncounted in the others species of *Ficus* [15].



1a n=14
1b n=16

2

3

4

Fig 1: Compounds isolated from hexanic and ethyl acetate extract of *Ficus grevei* Baillon

All these compounds (triterpenes and stilbene) present potential biological properties. Stilbenes are very interesting for their biological properties, such as potential antioxidative activity on human low density proteins, antimicrobial activity, inhibition of human platelet aggregation and as cancer-chemopreventive natural products. [16, 17]

4. Conclusion

This work has demonstrated that hexanic extract of *Ficus grevei* Baillon is rich in triterpene such the other species of *Ficus* genus. The abundant presence of stilbene is detected in ethyl acetate extract.

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