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-β-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 CDCl3 or CD3OD DMSO-d6 as solvent and TMS as an internal standard. One-dimensional NMR (proton 1H, carbon 13C) 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 F254 (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 H2SO4.

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 × 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 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 × 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 exhibiting an identical form in TLC were combined. Fractions 15 to 20 eluted with hexane – ethyl acetate (90:10) were combined and precipitated with MeOH to obtain a mixture of 2 and 3 (10 mg).

Fractions 309-320 eluted with ethyl acetate – MeOH (90:10) were combined and precipitated with MeOH to obtain a mixture of 2 and 3 (10 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 extracted with sulphuric ether. The organic phase was separated, dried over Na2SO4 and evaporated. From the ester 1, stearic acid and palmitic acid were obtained. Addition of HCl (1%) to the mixture of 2 and 3 (10 mg).

The 1H-NMR spectrum of 1 exhibited signals of isopropenyl oxygenated carbons. In HSQC and HMBC spectrum, the δ signals due to seven tertiary methyl groups at 0.78, 0.83, 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 signal for an α-olefinic carbonyl group at δ 158.38 and strong methylene proton signals around δ 1.25 were indicative of the presence of a fatty acid. The existence of oxymethylene (δ 4.48) showed that lupeol was acylated at fatty acid at position 3. Which was also supported by the appearance of 13C-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 δ 1.26. 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.

Methylalkanoates a-b: White powders. GC-MS; (m/z (rel.int)=Rt<sub>b</sub> 13.72, 270[M<sup>+</sup>](10); Rt<sub>b</sub> 14.97, 298[M<sub>b</sub>](4)

Compounds 2 and 3: Yellow powders

\[ \text{Compound 2: } \text{Methylalkanoates a-b: White powders. GC-MS; (m/z (rel.int)=Rt<sub>b</sub> 13.72, 270[M<sup>+</sup>](10); Rt<sub>b</sub> 14.97, 298[M<sub>b</sub>](4)} \]
assigned to (C-28). The presence of aldehyde was corroborated by 1H 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 resveratrol 4 and 5 by silica gel chromatography. The one dimensional 1H 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 assigned 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 13C 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-β-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-β-diglucoside is frequently uncounted in the others species of *Ficus* [15].

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.

**5. References**


