# Flavonoid Glycosides and Other Constituents of *Psorospermum androsaemifolium* Baker (Clusiaceae)

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**Two new flavonoid glycosides, namely 3′-(2″,4″-dihydroxybenzyloxy)acanthophorin B (1b) and β,2,3′,4,4′,6-hexahydroxy-α-(α-L-rhamnopyranosyl)dihydrochalcone (2) were isolated from the leaves of *Psorospermum androsaemifolium* together with quercetin (1), acanthophorin B (1a), α- (3) and β-amyrine (3a), vismiaquinone (4), 12-hentriacontanol and hentriacontane. The structures of these secondary metabolites were established using detailed spectroscopic analysis and by comparison with published data. Compounds 1, 1a, 1b, 2, 3, 3a and 4 showed weak antifungal and antibacterial activities.**

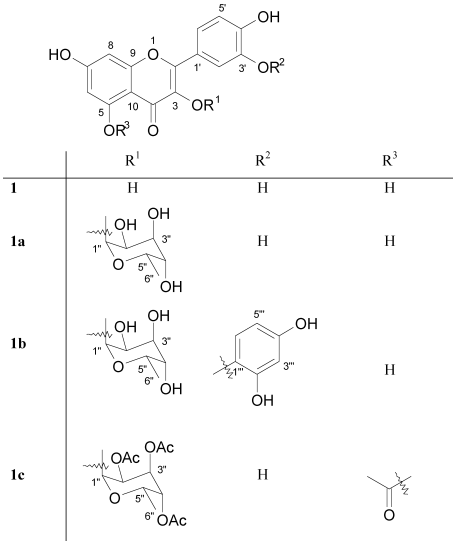
**Key words** *Psorospermum androsaemifolium*; quercetin; dihydrochalcone; antimicrobial

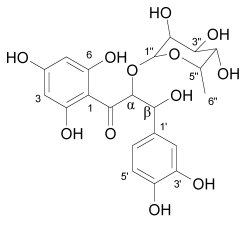
*Psorospermum androsaemifolium* Baker, an endemic plant from Madagascar, locally known as "Tsifady", "Harongampanihy", "Fanerana" or "Hazomafaika" belongs to the Clusiaceae family.[1)] In the traditional medicine it is used as remedy for spiders or scorpions bite and also healing stomach disease.[2,3)] The widespread use of *P. androsaemifolium* in indigenous medicine for different ailments, justified further attempts to isolate and identify active compounds. The present paper deals with the isolation and structural elucidation of one new flavone glycoside and one new dihydrochalcone glycoside including their antimicrobial activities. To the best of our knowledge, no previous phytochemical study has been reported on *P. androsaemifolium*.

## Results and Discussion

The leaves of *P. androsaemifolium* were extracted at room temperature with methanol for 2 d. This extract was concentrated to dryness under vacuum, and after flash chromatography on silica gel using a hexane/ethyl acetate gradient of increasing polarity; fractions A—C were obtained. Repeated column chromatography and preparative TLC afforded quercetin (**1**), acanthophorin B (**1a**), vismiaquinone, α- and β-amyrine, 12-hentriacontanol, hentriacontane and two new flavonoid glycosides: 3′-(2″,4″-dihydroxybenzyloxy)-acanthophorin B (**1b**) and β,2,3′,4,4′,6-hexahydroxy-α-(α-L-rhamnopyranosyl)dihydrochalcone (**2**). The $^1$H- and $^{13}$C-NMR, and MS data of the known compounds were consistent with those reported in the literature.

Compounds **1** and **1a** were isolated as yellow powder in silica gel column (3×40 cm) from fraction A eluted with solvent system dichloromethane/methanol of increasing polarity. The EI mass spectrum of compound **1a** exhibited a molecular ion at *m/z* 448 corresponding to a molecular formula $C_{21}H_{20}O_{11}$. The $^1$H-NMR of **1a** exhibited an ABX system at δ 8.30 (1H, d, *J*=8.4 Hz), 7.96 (1H, dd, *J*=8.4, 2.1 Hz) and 7.21 (1H, d, *J*=2.0 Hz), a doublet of doublet at δ 6.62 and 6.60 with *J*=2.3 Hz were characteristic of *meta* coupling protons for A ring of quercetin/acanthophorin B.[4)] The signals at

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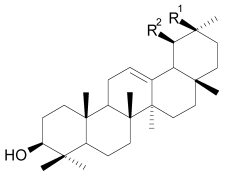
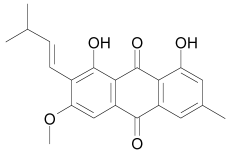

3: $R^1$ = H, $R^2$ = $CH_3$-
3a: $R^1$ = $CH_3$-, $R^2$ = H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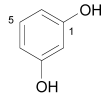
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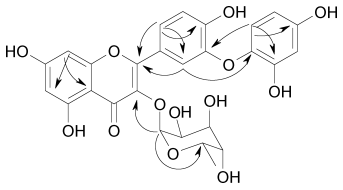

Fig. 1. Selected HMBC Correlations in Compound **1b**

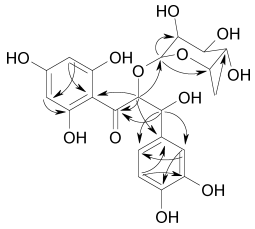

Fig. 2. Selected HMBC Correlations in Compound **2**

$\delta$ 1.03 and between $\delta$ 4.50—3.32 assigned to the rhamnopyranosyl residue and the signal at $\delta$ 5.42 was assigned to the anomeric proton. These data were carried out in AntiBase[5] and the compound **1a** was easily identified as acanthophorin B (**1a**) or quercetin-3-*O*-$\alpha$-L-fucopyranoside. The $^1$H- and $^{13}$C-NMR spectra of compounds **1** and **1a** were nearly superimposable. The only significant difference being the absence of the peaks of rhamnopyranosyl-residue moiety in compound **1**.

Compound **1b** was isolated as yellow powder in fraction B and showed on ESI-HR-MS an $[M+H]^+$ peak corresponding to the molecular formula $C_{27}H_{25}O_{13}$. The $^{13}$C/attached proton test (APT) NMR spectrum showed 27 carbon signals consistent with one methyl, thirteen methine and thirteen quaternary carbon signals.

The $^1$H-NMR spectrum exhibited signals of one methyl group as doublet at $\delta$ 0.95 (d) and the multiplets between $\delta$ 3.40 and 4.24 were characteristic of rhamnopyranosyl-sugar ring.[6] The anomeric proton appeared as doublet at $\delta$ 5.40 ($J$=3.4 Hz), implying the $\alpha$-configuration of the rhamnose.[7] In the aromatic region, an AB system at $\delta$ 6.39 and 6.21 (d, $J$=1.6 Hz) was observed and two ABX system with the first one at $\delta$ 7.42 (1H, dd, $J$=8.1, 2.2 Hz), 7.41 (1H, d, $J$=2.2 Hz) and 6.78 (1H, d, $J$=8.0 Hz) and the second at $\delta$ 7.33 (1H, d, $J$=7.8 Hz), 7.29 (1H, dd, $J$=7.8, 1.9 Hz) and 6.91 (1H, d, $J$=1.9 Hz) were also observed. The comparison of this data with compound **1a** showed an additional 2,4-dihydroxybenzene which was connected at the position 3′ of acanthophorin B according to the HMBC correlation (Fig. 1). The peak at *m/z* 109 in EI-MS also supported the presence of this moiety. The analysis of all these data compared to those of acanthophorin B led to the conclusion that compound **1b** was 3′-(2″,4″-dihydroxybenzyloxy)acanthophorin B or 3′-(2″,4″-dihydroxybenzyloxy)quercetin-3-*O*-$\alpha$-L-fucopyranoside isolated and described here for the first time.

Compound **2** was isolated as yellow powder in fraction C eluted with hexane/ethyl acetate (5 : 2). The (+)-ESI-HR mass spectrum indicated a pseudomolecular ion $[M+H]^+$ at *m/z* 469.40921, corresponding to the molecular formula $C_{21}H_{25}O_{12}$. Comparison of the spectral data of compounds **1a** and **2** indicated the same sugar moiety, which was confirmed by the peak at *m/z* 321 $[M-C_6H_{11}O_4]^+$. The IR spectrum of compound **2** revealed a broad signal at $\nu$ 3415 cm$^{-1}$ indicating free hydroxyl group, and an ester absorptions at $\nu$ 1724 (C=O) and 1247 (C–O) cm$^{-1}$.

The $^{13}$C/APT NMR spectrum showed 21 carbon signals, of which one was assigned to methyl, twelve to methine and eight to quaternary carbon signals. The $^1$H-NMR spectrum exhibited signals of one broad doublet of three protons at $\delta$ 1.19 and the multiplets between $\delta$ 3.30 and 3.70 are characteristic of a rhamnopyranosyl residue.[6] The $\alpha$ and $\beta$ protons of dihydrochalcone appeared as doublets at $\delta$ 5.09 and 4.58 ($J$=5.1 Hz), respectively whose chemical shifts and coupling patterns indicated the presence of –CO–CH(O–)–CH(OH)– group.[8] The $\alpha$-L-rhamnosyl unit was detected at $\delta$ 5.34 ($J$=3.1 Hz).[9] In the aromatic region, an AB system at $\delta$ 5.91 and 5.89 (d, $J$=2.6 Hz) and one ABX system at $\delta$ 6.95 (1H, d, $J$=8.0 Hz), 6.80 (1H, d, $J$=2.0 Hz) and 6.78 (1H, dd, $J$=8.0, 2.0 Hz) were observed. In the HMBC spectrum (Fig. 2), correlations between the H-$\alpha$ signal and carbons 1, 1′, 1′′ and C=O, between the H-$\beta$ signal and carbons 2′, 6′ and C=O, between the H-1′′ and carbons 2′′, 5′′ and C-$\alpha$ as well as the close similarity with the shifts of $\alpha$,$\beta$,2′,3,4,4′,5,6′-octahydroxydihydrochalcone[10] indicated that compound **2** was $\beta$,2,3′,4,4′,6-hexahydroxy-$\alpha$-($\alpha$-L-rhamnopyranosyl)dihydrochalcone, which is described here for the first time.

The acetylation of **1b** afforded compounds **1c** and **5**. Their structures were confirmed by EI, ESI-HR-MS, IR and $^1$H- and $^{13}$C-NMR spectra (see Experimental).

The antifungal and antibacterial activities of all the compounds isolated from this plant were determined using the agar diffusion method with 6 mm paper disks loaded with 40 $\mu$g of each compound. Compounds **1**, **1a** and **1b** showed activities against *Bacillus subtilis* (11, 13, 14 mm inhibition diameter), *Staphylococcus aureus* (12, 17, 15 mm), *Streptomyces viridochromogenes* (Tü 57) (15, 16, 20 mm), and *Escherichia coli* (12, 10, 11 mm), respectively. $\beta$,2,3′,4,4′,6-Hexahydroxy-$\alpha$-($\alpha$-L-rhamnopyranosyl)dihydrochalcone (**2**) showed weak activities against *Mucor miehi* (13 mm), and *Escherichia coli* (11 mm). Vismiaquinone (**4**) was weakly active against *Candida albicans* (14 mm) and hentriacontane was weakly active against *Staphylococcus aureus* (13 mm). $\alpha$- (**3**) and $\beta$-Amyrine (**3a**) showed weak activity against *Bacillus subtilis* (12, 13 mm) and *Candida albicans* (14, 11 mm), respectively.

## Experimental

**Materials and Methods** NMR spectra were measured on Varian Unity 300 (300.145 MHz) and Varian Inova 500 (499.876 MHz) spectrometers. ESI mass spectra were recorded on a Finnigan LCQ with quaternary pump Rheos 4000 (Flux Instrument). ESI HR mass spectra were recorded on a Bruker FTICR 4.7 T mass spectrometer. EI mass spectra were recorded on a Finnigan MAT 95 spectrometer (70 eV) with perfluorkerosene as a reference substance for HR-EI-MS. IR spectra were recorded on a Perkin-Elmer 1600 Series FT-IR spectrometer from films. Flash chromatography was carried out on silica gel (230—400 mesh). *Rf* values were measured on Polygram SIL G/UV254 (Macherey-Nagel & Co.) and melting and decomposition points were measured with the melting point apparatus of Electrothermal and were not corrected.

**Plant Material** The leaves of *Psorospermum androsaemifolium* Baker were collected in April 2005 in the eastern rain forest of Madagascar. The plant was authenticated at the botanical and zoological park of Tsimbazaza Antananarivo Madagascar where a voucher specimen is deposited.

**Hydrolysis Experiments** Acid hydrolysis of **1b** (4.0 mg) was performed using 1% aq. HCl at 95 °C for 120 min and usual workup afforded quercetin (**1**, 1.5 mg) and rhamnose (1 mg). For rhamnose detection, the final

aqueous acidic mixture was extracted with mixture of ethyl acetate, then the aqueous layer was neutralized for determination of the released sugar moiety using silica gel plates with EtOAc–$H_2O$ (3 : 1), giving *Rf* value of 0.7. Anisaldehyde/sulphuric acid was employed as a spray for colour (brown) detection of the rhamnose-sugar.

**Extraction and Isolation** Air dried powdered leaves of *Psorospermum androsaemifolium* (400 g) was extracted with MeOH at room temperature during 48 h. After removing the solvent by evaporation under reduced pressure, the crude extract (67 g) was chromatographed on silica gel using hexane/ethyl acetate in increasing polarity. A total of three main fractions [A—C] were collected and evaluated for their antimicrobial activity *in vitro*. All these fractions showed no antimicrobial activity.

Fraction A (5.0 g) eluted with $CH_2Cl_2$–MeOH in increasing polarity using PTLC and Sephadex LH-20 for purification yielded quercetin (**1**, 2.7 mg),[11] acanthophorin B (**1a**, 13.4 mg) and vismiaquinone (**4**, 15.0 mg).[12]

Fraction B (13.0 g) was chromatographed on silica gel and eluted with a mixture of hexane/ethyl acetate in increasing polarity to yield 3′-(2″,4″-dihydroxybenzyloxy)acanthophorin B (**1b**, 20.3 mg), α- (**3**, 7.1 mg) and β-amyrine (**3a**, 3.4 mg).[13]

Fraction C (17.0 g) was chromatographed on silica gel and eluted using hexane/ethyl acetate. A total of 35 fractions (of *ca.* 300 ml each) were collected and combined on the basis of TLC analysis leading to two main series I—II. Series I (4 g) [fractions 1—16] was eluted with a mixture of hexane/ethyl acetate (5 : 2) to deliver β,2,3′,4,4′,6-hexahydroxy-α-(α-L-rhamnopyranosyl)dihydrochalcone (**2**, 2.4 mg). Series II (1.5 g) [fractions 17—35] was column chromatographed on silica gel and eluted with $CH_2Cl_2$–MeOH (3 : 1) to yield 12-hentriacontanol (1.9 mg)[14] and hentriacontane (4.7 mg).[15]

**3′-(2″,4″-Dihydroxybenzyloxy)acanthophorin B or 3′-(2″,4″-Dihydroxybenzyloxy)quercetin-3-*O*-α-L-fucopyranoside (1b)** Yellow powder. mp 141—143 °C. *Rf*=0.31 (hexane/25% ethyl acetate). $^1$H-NMR (400 MHz, $CD_3OD$) δ: 7.42 (1H, dd, *J*=8.1, 2.2 Hz, H-6′), 7.41 (1H, d, *J*=2.2 Hz, H-2′), 7.33 (1H, d, *J*=7.8 Hz, H-6‴), 7.29 (1H, dd, *J*=7.8, 1.9 Hz, H-5‴), 6.91 (1H, d, *J*=1.9 Hz, H-3‴), 6.78 (1H, d, *J*=8.0 Hz, H-5′), 6.39 (1H, d, *J*=1.6 Hz, H-8), 6.21 (1H, d, *J*=1.6 Hz, H-6), 5.40 (1H, d, *J*=3.4 Hz, H-1″), 4.24 (1H, dd, *J*=3.3, 1.5 Hz, H-2″), 3.75 (1H, dd, *J*=9.3, 1.5 Hz, H-3″), 3.43 (1H, m, H-5″), 3.33 (1H, dd, *J*=9.3, 5.7 Hz, H-4″), 0.95 (1H, d, *J*=5.6 Hz, H-6″). $^{13}$C-NMR (100 MHz, $CD_3OD$) δ: 179.7 (s, C-4), 164.9 (s, C-7), 163.3 (s, C-5), 159.4 (s, C-2), 158.6 (s, C-9), 151.4 (s, C-4‴), 149.8 (s, C-4′), 146.6 (s, C-1‴), 146.5 (s, C-3′), 146.1 (s, C-2‴), 136.3 (s, C-3), 123.9 (d, C-5‴), 123.0 (d, C-6′), 122.9 (s, C-1′), 117.8 (d, C-3‴), 117.0 (d, C-2′), 116.4 (d, C-5′), 115.8 (d, C-6‴), 105.9 (s, C-10), 103.6 (d, C-1″), 99.8 (d, C-6), 94.8 (d, C-8), 73.3 (d, C-5″), 72.1 (d, C-3″), 72.0 (d, C-4″), 71.9 (d, C-2″), 17.7 (q, C-6″). IR (film) cm$^{-1}$: 3440, 2924, 2366, 1693, 1651, 1602, 1565, 1504, 1462, 1436, 1394, 1356, 1289, 1275, 1195, 1118, 1086, 1059, 994, 887, 813, 750, 719, 670. (+)-ESI HR-MS *m/z*: 557.47196 ([M+H]$^+$, Calcd 557.47187 for $C_{27}H_{25}O_{13}$). EI MS *m/z* (%): 556 (M$^+$, 9), 447 (15), 426 (9), 218 (100), 203 (33), 189 (22), 161 (10), 146 (13), 135 (19), 119 (16), 109 (18), 97 (26), 83 (37), 69 (40), 57 (42). $[\alpha]_D^{27}$ −23 (*c*=0.9, MeOH).

**2″,3″,4″,5-Tetraacetoxyacanthophorin B or 2″,3″,4″,5-Tetraacetoxyquercetin-3-*O*-α-L-fucopyranoside (1c)** 3′-(2″,4″-Dihydroxybenzyloxy)-acanthophorin B (**1b**, 7 mg) was dissolved in pyridine (1 ml) and acetanhydride (1 ml). The solution was stirred for 5 h at 70 °C. Hydrolysis and usual work-up gave **5** (1.5 mg, 4%) and **1c** (3 mg, 47%) as an yellow solid. Melting point: 136—138 °C. *Rf*=0.48 (hexane/25% ethyl acetate). $^1$H-NMR (400 MHz, $CD_3OD$) δ: 7.30 (1H, d, *J*=2.5 Hz, H-2′), 7.32 (1H, dd, *J*=8.5, 2.5 Hz, H-6′), 6.95 (1H, d, *J*=8.5 Hz, H-5′), 6.40 (1H, d, *J*=2.5 Hz, H-8), 6.21 (1H, d, *J*=2.5 Hz, H-6 ), 5.62 (1H, d, *J*=3.0 Hz, H-1″), 5.50 (1H, dd, *J*=3.1, 1.9 Hz, H-2″), 5.28 (1H, dd, *J*=9.6, 1.8 Hz, H-3″), 4.29 (1H, dd, *J*=9.6, 5.3 Hz, H-4″), 3.42 (1H, m, H-5″), 2.15 (3H, s, Ac-5), 2.05 (3H, s, Ac-4″), 2.01 (3H, s, Ac-3″), 1.95 (3H, s, Ac-2″), 0.89 (3H, d, *J*=5.3 Hz, H-6″). $^{13}$C-NMR (100 MHz, $CD_3OD$) δ: 178.1 (s, C-4), 170.6 (s, C=O, Ac-5), 170.5 (s, C=O, Ac-2″), 170.4 (s, C=O, Ac-4″),170.2 (s, C=O, Ac-3″), 165.1 (s, C-7), 162.1 (s, C-5), 158.5 (s, C-2), 157.7 (s, C-9), 149.0 (s, C-4′),146.0 (s, C-3′), 134.0 (s, C-3), 122.2 (d, C-6′), 121.9 (s, C-1′), 115.6 (d, C-2′), 115.5 (d, C-5′), 104.9 (s, C-10), 99.0 (d, C-1″), 98.0 (d, C-8), 93.9 (d, C-6), 70.5 (d, C-5″), 69.5 (d, C-4″), 69.3 (d, C-3″), 68.7 (d, C-2″), 20.1 (q, $CH_3$-5), 19.5 (q, $CH_3$-2″, 3″, 4″), 16.6 (q, $CH_3$-6″). IR (film) cm$^{-1}$: 3363, 1743, 1652, 1603, 1501, 1442, 1365, 1199, 1164, 1801, 1043, 965, 809, 785, 642. (+)-ESI HR-MS *m/z*: 617.41021 ([M+H]$^+$, Calcd 617.41074 for $C_{29}H_{29}O_{15}$). EI-MS *m/z* (%): 616 ([M]$^+$, 30), 574 (44), 490 (10), 302 (100), 286 (13), 268 (12), 263 (29), 194 (21), 178 (9), 143 (15), 124 (8), 110 (14), 94 (23), 79 (30), 68 (31), 57 (26), 43 (60).

**1,3-Dihydroxybenzene (5)** Yellow powder. $^1$H-NMR (400 MHz, $CD_3OD$) δ: 6.43 (1H, d, *J*=2.1 Hz, H-2), 6.78 (1H, dd, *J*=8.5, 2.1 Hz, H-6), 7.41 (1H, t, *J*=8.5 Hz, H-5), 7.42 (1H, dd, *J*=8.5, 2.1 Hz, H-4). $^{13}$C-NMR (100 MHz, $CD_3OD$) δ: 159.7 (s, C-1, C-3), 136.3 (d, C-6), 110.5 (d, C-4, C-6), 104.2 (d, C-2). EI-MS *m/z* (%): 110 ([M]$^+$, 100), 94 (56), 79 (23), 56 (15), 43 (17).

**β,2,3′,4,4′,6-Hexahydroxy-α-(α-L-rhamnopyranosyl)dihydrochalcone (2)** Yellow powder. mp 150—152 °C. *Rf*=0.52 (hexane/25% ethyl acetate). $^1$H-NMR (400 MHz, $CD_3OD$) δ: 6.95 (1H, d, *J*=8.0 Hz, H-5′), 6.80 (1H, d, *J*=2.0 Hz, H-2′), 6.78 (1H, dd, *J*=8.0, 2.0 Hz, H-6′), 5.91 (1H, d, *J*=2.6 Hz, H-5), 5.89 (1H, d, *J*=2.6 Hz, H-3), 5.34 (1H, d, *J*=3.1 Hz, H-1″), 5.09 (1H, d, *J*=5.1 Hz, H-α), 4.58 (1H, d, *J*=5.1 Hz, H-β), 3.65 (1H, dd, *J*=3.0, 1.5 Hz, H-2″), 3.55 (1H, dd, *J*=8.8, 1.5 Hz, H-3″), 3.40 (1H, dd, *J*=8.8, 5.0 Hz, H-4″), 3.35 (1H, m, H-5″), 1.19 (3H, br d, H-6″). $^{13}$C-NMR (100 MHz, $CD_3OD$) δ: 196.0 (s, C=O), 168.7 (s, C-4), 165.5 (s, C-2), 164.1 (s, C-6), 147.4 (s, C-4′), 146.7 (s, C-3′), 129.2 (s, C-1′), 120.5 (d, C-6′), 116.3 (d, C-5′), 115.5 (d, C-2′), 102.6 (s, C-1), 102.2 (d, C-1″), 97.7 (d, C-3), 96.3 (d, C-5), 84.0 (d, C-β), 78.6 (d, C-α), 73.8 (d, C-5″), 71.2 (d, C-4″), 71.1 (d, C-3″), 70.5 (d, C-2″), 17.9 (q, C-6″). IR (film) cm$^{-1}$: 3415, 2943, 2466, 1724, 1639, 1622, 1575, 1462, 1463, 1394, 1326, 1247, 1159, 1118, 1096, 1075, 996, 888, 719, 677. (+)-ESI HR-MS *m/z*: 469.40921 ([M+H]$^+$, Calcd 469.40954 for $C_{21}H_{25}O_{12}$). EI-MS *m/z* (%): 468 ([M]$^+$, 17), 436 (12), 410 (15), 290 (78), 274 (13), 256 (22), 210 (30), 188 (9), 148 (10), 119 (16), 109 (7), 96 (26), 86 (12), 58 (17), 44 (31). $[\alpha]_D^{27}$ −17.4 (*c*=0.18, MeOH).

**Antimicrobial Assay** Agar diffusion tests were performed in the usual manner[16] with *Bacillus subtilis* and *Escherichia coli* (on peptone agar), *Staphylococcus aureus* (Bacto nutrient broth), *Streptomyces viridochromogenes* (M test agar), the fungi *Mucor miehei* and *Candida albicans* (Sabouraud agar), and three microalgae (*Chlorella vulgaris*, *Chlorella sorokiniana* and *Scenedesmus subspicatus*).

The test substances were dissolved in chloroform/methanol (87 : 13) azeotrope and paper disks (φ 6 mm) were impregnated with each 40 μg pro disk using a 100 μl syringe, dried for 1 h under sterile conditions and placed on the pre-made agar test plates. Plates with bacteria and fungi were kept in an incubator at 37 °C for 12 h, micro algae plates were kept for 3 d at room temperature in a day light incubator. The diameter of inhibition zones was measured.

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