An Antimicrobial Biphenyl Derivative from *Garcinia bancana* Miq.

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From the methanol extract of the twigs and leaves of *Garcinia bancana* Miq., one new biphenyl derivative (1), was isolated and characterized along with nine known compounds; garcinol, isogarcinol, (-)-mellein, 8-hydroxy-6-methoxy-3-n-pentylisocoumarin, blumenol C, O-β-D-glucoside, quercetin 3-O-α-L-rhamnopyranoside, kaempferol 3-O-α-L-rhamnopyranoside, lupeol and stigmasterol. Their structures were determined by analysis of 1D and 2D NMR data and comparison of spectral data and physical data with those previously reported. The antibacterial activity against Staphylococcus aureus was evaluated. Garcinol showed the lowest minimum inhibition concentration (MIC) at 16 μg/ml while compound 1 exhibited weaker activity with MIC value of 64 μg/ml.

Key words *Garcinia bancana*; biphenyl; prenylated benzophenone; isocoumarin; flavone rhamnoside; antibacterial activity

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**Table 1.** 1H-, 13C-NMR and DEPT Spectral Data of Compound 1 (500 MHz and 125 MHz in CDCl3)

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<th>Position</th>
<th>1H</th>
<th>13C</th>
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<td>124.5</td>
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<td>3-OMe</td>
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mined as [1,1’-biphenyl]-2-(3-methyl-2-butenyl)-3-methoxy-4,4’,5,6-tetraol.

All compounds other than compound 3 were tested for antibacterial activity against methicillin-resistant Staphylococcus aureus (MRSA).11) Results were in agreement with the previous investigation showing that garcinol showed the most remarkable activity with minimum inhibition concentration (MIC) value of 16 μg/ml while isogarcinol and the biphenyl derivative (1) exhibited weaker activity with MIC values of 32 and 64 μg/ml, respectively. Others gave weak activity with the same MIC value (>128 μg/ml). These results were in agreement with the previous investigation which indicated that garcinol gave lower MIC value than isogarcinol against MRSA.11)

Experimental

General Experimental Procedures  Infrared spectra (IR) were obtained on a Perkin Elmer Spectrum GX FT-IR system. 1H- and 13C-NMR spectra were recorded on a Varian UNITY INOVA 500 MHz spectrometer using tetramethylsilane (TMS) as internal standard. EI and HR-EI mass spectra were measured on Thermo Finnigan MAT95XL spectrometer. Thin-layer chromatography (TLC) and precoated TLC were performed on silica gel 60 GF254 (Merck). Light petroleum had bp 40—60 °C.

Plant Material  The leaves and twigs of G. bancana were collected at the Pru Tao Daeng Wildlife Sanctuary, Narathiwat, Thailand, in June 2000. The plant (a voucher specimen No. VR0001) was identified by Professor Puangien Siriraksa, Department of Biology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla.

Isolation  The crude MeOH extract (88 g) obtained from the twigs of G. bancana was subjected to column chromatography eluted with a gradient system of CHCl3–MeOH to afford 12 fractions. Fraction 2 (520 mg, eluted with 100% CHCl3) was further purified on Sephadex LH20 column chromatography using MeOH as eluent to yield 5 subfractions. The third subfraction (83.7 mg) gave lupeol (13.5 mg) with acetic anhydride in pyridine at room temperature. Further separation of fraction 2 (106 mg) was subjected to column chromatography eluted with 20—50% MeOH–CHCl3, upon standing at room temperature. Fraction 7 (2.03 g, eluted with 3% MeOH–CHCl3) was subjected to column chromatography on reverse phase C18 silica gel using 60% MeOH–H2O as eluent to afford 3 subfractions. Acetylation of the second subfraction (5.7 mg) with acetic anhydride in pyridine at room temperature gave tetraacetate of 4 (3.5 mg) after purification on silica gel column chromatography using CHCl3 as eluent. The crude MeOH extract (48.4 g) obtained from the leaves of G. bancana was divided into two portions by dissolving in n-hexane. The n-hexane insoluble portion (46.74 g) was further dissolved in CHCl3, to afford the CHCl3 insoluble part (44.20 g). This insoluble part (2 g) was then subjected to column chromatography on silica gel using a mixture of CHCl3, MeOH and water in a ratio of 65:25:3 as eluent to yield 4 fractions. The third fraction (220 mg) was subjected to Semi-preparative HPLC (μBondapak C-18 precoated column, 10 μm, 25×10 mm, Waters) with gradient elution (20% MeOH–H2O up to 100% MeOH within 35 min, flow rate 10 ml/min, UV 254 nm) to yield 7 subfractions. Quercetin 3-O-α-rhamnoside (68.5 mg) and kaempferol 3-O-α-rhamnoside (25 mg) were obtained from the fourth and sixth subfractions, respectively.

Compound I: Colourless viscous liquid. 1H-NMR (CDCl3, 500 MHz) and 13C-NMR (CDCl3, 125 MHz), see Table 1. IR (neat) cm–1: 3391, 1600, UV λmax (MeOH) nm (log ε): 208 (4.64), 250 (4.09). HR-EI-MS m/z 316.1311 (Calcd for C18H20O5 [M]+: 316.1346). EI-MS m/z 316 [M]+, 301, 300, 286, 245, 243, 230, 229, 225, 213, 69.

Antibacterial Activity  Minimum inhibitory concentrations (MICs) were determined by agar microdilution method.10) The test substances were dissolved in DMSO (Merck, Germany). Serial two-fold dilutions of the test substances were mixed with melted Mueller–Hinton agar (Difco) in the ratio of 1:100 in microtiter plates with flat-bottomed wells (Nunc, Germany). Final concentration of the test substances in agar was ranged from 128—0.03 μg/ml. Methicillin-resistant Staphylococcus aureus (MRSA) was used as test microorganism which was isolated from the Songklanakarin Hospital, Thailand. The strain was maintained in the laboratory of the Department of Microbiology, Faculty of Science, Prince of Songkla University. Inoculum suspensions of 107 CFU/ml (10 μl) were spotted on agar-filled wells. The inoculated plates were incubated at 35 °C for 18 h. MICs were recorded by reading the lowest substance concentration that inhibited visible growth. Vancomycin was used as positive control drug.

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References