Taxiphyllin 6′-O-Gallate, Actinidioioside 6′-O-Gallate and Myricetrin 2′-O-Sulfate from the Leaves of Syzygium samarangense and Their Biological Activities

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Three new compounds were isolated from a MeOH extract of the leaves of Syzygium samarangense, one new cyanogenic glucoside, taxiphyllin 6′-O-gallate (1), one new megastigmane glucoside, actinidioioside 6′-O-gallate (2), and one new sulfated flavonoid rhamnoside, myricetrin 2′-O-sulfate (3), together with 14 known compounds, lupeol (4), demethoxymatteucinol (5), cryptostrobin (6), β-sitosterol glucoside (8), 2R-prunasin (9), myriciaphenone A (10), 1-feruloyl-β-o-gluropyranoside (11), (3S,5R,6R,7E,9S)-3,5,6,9-tetrahydroxymegastigma-7-ene (12), guaijaverin (13), myricetin 4′-methyl ether 3-O-α-L-rhamnopyranoside (14), myricetin (15), gallic acid (16) and actinidioioside (17) by comparing their spectroscopic data with those reported in the literature.

Key words Syzygium samarangense; Myrtaceae; taxiphyllin 6′-O-gallate; actinidioioside 6′-O-gallate; myricetrin 2′-O-sulfate

Syzygium samarangense (Blume) Merr. & L. M. Perry (Myrtaceae) (syns. Eugenia javanica L.) is commonly known as wax apple, wax jambu, rose apple and Java apple. Wax apple is widely cultivated throughout Malaysia and also grown throughout southeast Asian countries such as Thailand, Indonesia and Taiwan as well as other tropical countries. The pear-shaped fruits are usually pink, light red or red but may be greenish-white or cream-colored, and are generally crisp, often juicy and refreshing with a subtle sweet taste and aromatic flavor. Wax apple fruit can be eaten raw with salt or cooked as a sauce. The fruit has been used for a wide variety of ailments, including cough, diabetes, dysentery, inflammation and ringworm.1–3

The present study deals with the isolation and structural elucidation of three new compounds (1–3), together with 14 known compounds from the leaves of this plant (Fig. 1).

Results and Discussion

A methanol extract of the leaves of S. samarangense was fractionated by solvent−partitioning. The EtOAc and 1-ButOH-soluble fractions were separated by means of various chromatographic procedures including column chromatography (CC) on silica gel and reversed-phase octadeyl silica gel (ODS) CC, and HPLC, 17 compounds (1–17) being isolated; a new cyanogenic glucoside (1), a new megastigmane glucoside (2) and a new sulfated flavonoid rhamnoside (3), in addition to 14 known compounds, which were identified as lupeol (4), demethoxymatteucinol (5), cryptostrobin (6), β-sitosterol glucoside (8), 2R-prunasin (9), myriciaphenone A (10), 1-feruloyl-β-o-gluopyranoside (11), (3S,5R,6R,7E,9S)-3,5,6,9-tetrahydroxymegastigma-7-ene (12), guaijaverin (13), myricetin 4′-methyl ether 3-O-α-L-rhamnopyranoside (14), myricetin (15), gallic acid (16) and actinidioioside (17) by comparing their spectroscopic data with those reported in the literature.

Compound (1), [α]D 20 = −51.4, was obtained as an amorphous powder and has the molecular formula C33H32O14N as determined by positive-ion mode high-resolution (HR)-electrospray ionization (ESI)-mass spectrometry (MS). The IR spectrum of 1 showed bands at 3334 and 1698 cm−1 corresponding to hydroxy and carbonyl group absorptions, respectively. The 1H-NMR spectrum (Table 1) exhibited a pair of doublets at δH 6.78 (2H, d, J = 8.6 Hz) and 7.29 (2H, d, J = 8.6 Hz) which were characteristic of a p-disubstituted benzene ring, and a singlet for one oxymethine proton at δH 5.57 (1H, s), along with one anomeric proton signal at δH 4.18 (1H, d, J = 7.4 Hz) for a β-glucopyranosyl moiety. The 13C-NMR spectrum (Table 2) exhibited six signals assignable to a β-glucopyranosyl moiety, six aromatic carbon signals and a methine carbon at δC 68.2, as well as a singlet carbon at δC 119.3, the chemical shift of which suggested a nitrile functional group.9) The presence of the nitrile group was supported by the IR absorption band at 2248 cm−1 for a triple bond and the index of hydrogen deficiency calculated from the positive-ion mode HR-ESI-MS. In accordance with these data, the aglycone was established to be a p-disubstituted aromatic ring linked to the methine carbon, which in turn formed a glycosidic linkage with the anomeric carbon, as well as a linkage with the nitrile functional group. Further NMR analyses involving 1H−1H correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC) and heteronuclear multiple-bond correlation (HMBC) spectroscopies were performed (Fig. 2). In the HMBC spectrum of 1, H-2 (δH 5.57) showed cross peaks with C-1′, C-1 and C-3 (δC 101.0, 119.3 and 124.8, respectively). The signal of the anomeric proton of the glucopyranosyl moiety (δH 4.18) was correlated with C-2 (δC 68.2), confirming the sugar linkage to the hydroxy group at C-2. The presence of glucose was confirmed by HPLC analysis of a hydrolyzate of 1 and the...
absolute configuration of the sugar was simultaneously determined to be in the D-series using a chiral detector. The singlet at δH 7.15 for two aromatic protons observed in 1H-NMR and one carbonyl carbon signal at δC 168.3, and six aromatic carbon signals at δC 110.3×2, 140.0 and 146.6×2 seen in 13C-NMR (Tables 1, 2) corresponded to a galloyl moiety. The location of the galloyl moiety was found to be at C-6' of the glucose unit on the basis of the downfield shifts of C-6' and H-6', as well as the upfield shift of C-5'. The linkage was confirmed by HMBC correlations between H-6'α and 6'β (δH 4.48, 4.32) and C-7' (δC 168.3). Mild alkaline hydrolysis of 1 with CH3ONa gave a glucoside (1a) and methyl gallate (1b) and the NMR spectra of 1a were the same as those of taxiphyllin18 with the R configuration. Thus compound 1 was characterized as taxiphyllin 6'-O-gallate as shown in Fig. 1.

Compound (2), [α]D21 −43.2, was isolated as an amorphous powder and its molecular formula was determined to be C26H38O13 by HR-ESI-MS. The IR spectrum exhibited absorptions for hydroxy groups (3366 cm⁻¹), a conjugated ester carbonyl group (1699 cm⁻¹), and an aromatic ring (1557 cm⁻¹). The UV spectrum also indicated the presence of an aromatic ring (285 nm). In the 1H-NMR spectrum, signals for one doublet and three singlet methyls, one singlet aromatic proton, a pair of trans-olefinic protons and an anomeric proton (δH 4.42) were observed. The 13C-NMR spectrum displayed six signals assignable to a β-glucopyranosyl moiety, and six aromatic signals and one carbonyl carbon signal. The remaining 13 resonances comprised those of four methyls, two methylenes, two methines, a disubstituted trans double bond and three quaternary carbons. From the above evidence, the structure of 2 was expected to be a megastigmane O-β-D-glucopyranoside with a galloyl moiety. The absolute configuration of glucose was determined by HPLC analysis of a hydrolyzate of 2 using a chiral detector. The NMR spectroscopic data for the megastigmane skeleton were essentially the same as those for actinidioionoside (17), a known megastigmane glucoside isolated from Eugenia uniflora.19) The downfield shift of C-6' was expected to be induced by esterification of the galloyl moiety, and the linkage was confirmed by HMBC correlations between H-6'a and 6'b (δH 4.48, 4.32) and C-7'' (δC 168.5) (Fig. 3). On mild alkaline hydrolysis of 2 with CH3ONa, a glucoside (2a) and methyl gallate were obtained. The NMR spectra of 2a were the same as those of 17 and the HPLC retention times of 17 and 2a were found to be the same at 16 min under the same conditions. Therefore, compound 2 was elucidated to be (3S,5R,6R,9R)-3,6,7,9-tetrahydroxymegastigman-7-ene 9-O-β-D-(6'-O-galloyl)glucopyranoside, namely actinidioionoside 6'-O-gallate as shown in Fig. 1.
Compound (3), [α]D$_{20}^0$ = −80.5, was isolated as a pale yellow amorphous powder and its molecular formula was determined to be C$_{21}$H$_{19}$O$_{15}$Na$_2$S by HR-ESI-MS. The $^{13}$C-NMR spectrum showed good similarity to those of myricetrin (15), except for the downfield shift of C-2" (+6.1 ppm) and the upfield shift of C-1" (−2.7 ppm) and C-3" (−1.0 ppm) of the rhamnopyranosyl moiety suggested the attachment of some substituent at this position, which was substantiated by measurement of a mass spectrum. The HR-ESI-MS spectrum showed a single peak at m/z 589.0234 in the positive-ion mode, corresponding to C$_{21}$H$_{19}$O$_{15}$Na$_2$S [M$^{+}$]$_{20}$, showing the attachment of a sulfuryl moiety at the 2"-position. Consequently, the structure of compound 3 was established as myricetrin 2"-O-sulfate (Fig. 4).

Compounds 1–17 were examined for their antibacterial activities, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activities, anti-Leishmania activities and also tumor cell growth inhibitory activities toward A549 by means of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. None of the tested compounds (1–17) showed any significant activity against Mucor racemosus, Staphylococcus aureus (MRSA) and Leishmania major (IC$_{50} > 100 \mu M$) except that compound 6 showed weak activity against M. racemosus (IC$_{50}$: 65.4 ± 2.86 $\mu M$) and L. major (IC$_{50}$: 86.7 ± 4.6 $\mu M$). The results were compared with those of amphotericin B as a positive control (IC$_{50}$: 0.5 ± 0.2 and 0.39 ± 0.5 $\mu M$, respectively. Compound 1 exhibited weak antibacterial activity against S. aureus with an IC$_{50}$ value of 86.9 ± 17.9 $\mu M$ (IC$_{50}$ for oxacillin was 10.3 ± 4.52 $\mu M$). Compounds 1, 3 and 13–16 showed potent DPPH radical scavenging activity (IC$_{50}$: 14.8 ± 1.56, 10.9 ± 0.29, 29.3 ± 2.17, 31.4 ± 3.41, 10.5 ± 0.75 and 9.9 ± 0.55 $\mu M$, respectively) comparable with that of the standard trolox (16.6 ± 2.2 $\mu M$). Compounds 1, 3, 6, 7, 15 and 16 exhibited weak growth inhibitory activity toward A549 (IC$_{50}$ 73.9 ± 10.2, 76.0 ± 11.6, 75.4 ± 13.5, 56.4 ± 16.8, 59.4 ± 10.3 and 43.2 ± 23.2 $\mu M$, respectively) in the MTT cytotoxic assay. The IC$_{50}$ value for standard doxorubicin was found to be 0.53 ± 0.03 $\mu M$.

### Experimental

#### General Experimental Procedures

Optical rotation data were measured on a JASCO P-1030 polarimeter. IR and UV spectra were obtained on Horiba FT-710 Fourier transform infrared and JASCO V-520 UV/Vis spectrophotometers, respectively. $^1$H- and $^{13}$C-NMR spectra were recorded on a JEOL JNM α-400 spectrometer with tetramethylsilane as an internal standard. HR-ESI mass spectra were taken on a LTQ Orbitrap XL mass spectrometer. Silica gel CC was performed on silica gel 60 [(E. Merck, Darmstadt, Germany) 70–230 mesh]. ODS open CC (RPCC) was performed on Cosmosil 75C$_{18}$-OPN (Nacalai Tesque, Kyoto, Japan) (20 × 400 mm, 10 g fractions being collected). HPLC was performed on an ODS column (Inertsil ODS-3; GL Science, Tokyo, Japan) (6 × 250 mm, flow rate: 1.0 ml/min), using a refractive index and/or a UV detector. Precoated silica gel 60F$_{254}$ plates (E. Merck; 0.25 mm in thickness) were used for TLC analyses, with visualization by spraying with a 10% H$_2$SO$_4$ solution in ethanol and heating to around 150°C on a hotplate.

### Microorganisms

The microorganisms used in this study...
M. racemousus (IFM52680) and S. aureus (JCM20624) were purchased from RIKEN BRC (Japan) and Medical Mycology Research Center, Chiba University, respectively.

Plant Material The leaves of S. samarangense were collected in May 2010 from Aswan Botanical Garden, Egypt. A voucher specimen of the plant was deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Minia University, Egypt (Mn-Ph-Cog-Ss).

Extraction and Isolation Air-dried powdered leaves (1.30 kg) of S. samarangense were extracted with methanol (5 L×5) till exhaustion followed by concentration under reduced pressure to yield a viscous gummy material. This residue (223 g) was dissolved in 500 mL of water and defatted with n-hexane (1 L×5). The aqueous layer was evaporated to remove a trace amount of the organic solvent, and then extracted with EtOAc and 1-BuOH, successively (1 L×5 each). The EtOAc and 1-BuOH fractions were concentrated under reduced pressure to give 21.5 g and 13.0 g of residues, respectively. The remaining aqueous layer was concentrated to furnish a water-soluble fraction (80.0 g).

The EtOAc fraction (21.5 g) was subjected to silica gel CC (600 g) (60×550 mm). The column was eluted initially with n-hexane (5 L), and then with an n-hexane–EtOAc gradient mixture, 500 mL fractions being collected. Similar fractions were combined, which afforded 20 fractions. The fourth fraction E-4 gave 4 (207 mg). The fifth fraction E-5 afforded 5 (161 mg). The sixth fraction E-6 produced 6 (81.2 mg). The seventh fraction E-7 yielded compound 7 (51.4 mg). Fraction E-18 (961 mg) was purified on RPCC, affording five fractions. The second fraction E-18-2 (116 mg) was purified by HPLC (30% MeOH) to produce compound 1 (10.9 mg).

Fraction E-19 (5.88 g) was rechromatographed over silica gel (200 g) (30×620 mm), using CHCl3–MeOH gradient system, 200 mL fractions being collected and similar fractions were combined to yield eight fractions. The fourth fraction E-19-4 gave 8 (85.5 mg). Fraction E-19-6 was subjected to RPCC, which gave ten fractions. The third fraction E-19-6-3 (16.1 mg) was purified by HPLC (30% MeOH) to produce compound 2 (6.50 mg). The fourth fraction E-19-6-4 (51.6 mg) was purified by HPLC (40% MeOH) to afford 11 (4.66 mg) and 12 (17.6 mg). The sixth fraction E-19-6-6 (160.8 mg) was purified by HPLC (40% MeOH) to produce 13 (2.66 mg) and 14 (10.3 mg). The seventh fraction E-19-7 gave 15 (18.8 mg).

The 1-BuOH fraction (13.0 g) was chromatographed on silica gel (400 g) (50×500 mm), using a CHCl3–MeOH gradient system and 200 mL fractions being collected and similar fractions were combined to yield 14 fractions. Fraction B-8 (1.13 g) was purified on RPCC, affording six fractions. The second fraction B-8-2 gave 16 (84.5 mg). Fraction B-12 (1.26 g) was rechromatographed over silica gel (75 g), (20×500 mm), using CHCl3–MeOH gradient system, 100 mL fractions being collected and the similar fractions were combined to yield five fractions. The third fraction B-12-3 (75.9 mg) was purified by HPLC (30% MeOH) to produce 17 (22.3 mg) and 2 (6.50 mg). Fraction B-14 (915 mg) was rechromatographed over
silica gel (50 g), (20×350 mm), using CHCl₃–MeOH gradient system, 100 mL fractions being collected and the similar fractions were combined to yield six fractions. The sixth fraction B-12-6 (202 mg) was purified by HPLC (30% MeOH) to give 3 (27.9 mg).

**Taxiphyllin 6′-O-Gallate (1)** Amorphous powder; [α]D²⁰ −51.4 (c=0.72, MeOH); IR νmax (film) cm⁻¹: 3334, 2946, 2842, 2248, 1698, 1610, 1513, 1450, 1342, 1318, 1227, 1026, 766; UV νmax (MeOH) nm (log e): 219 (4.26), 267 (3.93), 335 (3.65); ¹H-NMR (400 MHz, CDCl₃); Table 1; ¹³C-NMR (100 MHz, CDCl₃); Table 2; HR-ESI-MS (positive-ion mode) m/z: 486.1004 [M+Na]⁺ (Calcd for C₁₀₂H₁₃₁O₃₃Na: 486.1012).

**Actinidiocinoiside 6′-O-Gallate (2)** Amorphous powder; [α]D²⁰ −43.2 (c=0.34, MeOH); IR νmax (film) cm⁻¹: 3366, 2931, 1699, 1649, 1615, 1557, 1540, 1058, 1568, 1339, 1221, 1070, 1028, 669; UV νmax (MeOH) nm (log e): 213 (3.66), 285 (3.16), 304 (3.39), 351 (3.65), 364 (3.78); ¹H-NMR (400 MHz, CDCl₃); Table 1; ¹³C-NMR (100 MHz, CDCl₃); Table 2; HR-ESI-MS (positive-ion mode) m/z: 589.2197 [M+Na]⁺ (Calcd for C₁₀₂H₁₃₁O₃₃Na: 589.2205).

**Myricetin 2′-O-Sulfate (3)** Pale yellow amorphous powder; [α]D²⁰ −80.5 (c=1.85, MeOH); IR νmax (film) cm⁻¹: 3294, 2982, 2942, 1653, 1606, 1502, 1447, 1202, 1164, 1042, 988, 841; UV νmax (MeOH) nm (log e): 225 (4.05), 258 (4.03), 348 (3.99); +NaOAc 238 (4.16), 265 (4.13), 353 (3.93), 397 (3.86); +AlCl₃ 232 (4.06), 269 (4.03), 417 (4.08); +AlCl₃/HCl 225 (4.06), 269 (4.07), 393 (3.87); +NaOAc 231 (4.11), 268 (4.03), 363 (3.93); ¹H-NMR (400 MHz, CDCl₃); Table 1; ¹³C-NMR (100 MHz, CDCl₃); Table 2; HR-ESI-MS (positive-ion mode) m/z: 589.0234 [M−H+2Na]⁺ (Calcd for C₁₀₂H₁₃₁O₃₃Na₂S: 589.0235).

**Analysis of the Sugar Moiety** About 1 mg of compounds 1–3 were hydrolyzed with 1 mL HCl (1.0 mL) at 80°C for 2 h. The reaction mixtures were neutralized with Amberlite IRA96SB (OH⁻), and then partitioned with an equal amount of EtOAc. The water layers were analyzed for their sugar components. The sugars were determined by HPLC on an amino column [Shodex Asahipak NH₂-P-50 4E (4.6×250 mm), CH₃CN–H₂O (3:1), 1 mL/min], using a chiral detector (JASCO OR-2090 plus), in comparison with authentic D-glucose and L-rhamnose. Compounds 1 and 2 gave a peak for D-glucose at a retention time of 7.17 min with a positive rotation sign. Compound 3 gave a peak for L-rhamnose at a retention time of 5.12 min with a negative rotation sign.

**Mild Alkaline Hydrolysis** About 5.9 mg of compound 1 in MeOH (450 μL) was added to 1 mL CH₃ONa (50 μL), followed by standing at 20°C for 4 h. The reaction mixture was neutralized with Amberlite IR-120B (H⁺) and then evaporated to dryness. The residue was partitioned with CHCl₃ (2 mL)–H₂O (2 mL) to afford a taxiphyllin (1a) (2.3 mg) from the H₂O-soluble fraction and methyl gallate (1b) (1.0 mg) from the CHCl₃-soluble fraction.

In a similar manner to as for 1, compound 2 (2.2 mg) gave a megastigmane glucoside (actinidiocinoiside) (2a=17) (1.0 mg) and methyl gallate (2b) (0.4 mg).

**HPLC Analysis** Megastigmane glucoside (2a), obtained by mild alkaline hydrolysis, was analyzed by HPLC [Inertsil ODS-3; GL Science, Tokyo, Japan (6×250 mm, flow rate: 1.0 mL/min), using a refractive index detector] with MeOH–H₂O (3:7, v/v) which gave a peak at 16 min, which was the same retention time as that of 17.

**Antimicrobial Susceptibility Assay** Susceptibility tests were performed using a broth microdilution assay according to the National Committee for Clinical Laboratory Standards reference methods. Assays were performed using Mueller–Hinton broth (Difco). The bacterial inocula were adjusted to yield a density of 5×10⁸ colony forming units/mL. Samples were diluted directly in 96-well microtiter plates by serial two-fold dilution using a multi channel pipette. The microtiter plates were incubated during 24 h at 35°C and were read using a Molecular Device Versamex Tunable Microplate Reader at 620 nm as well as by visual observation. The MIC₅₀ value was determined as a 50% decrease in the optical density. Amphotericin B and oxacillin were used as positive controls.

**Anti-Leishmania Assay** The anti-Leishmaniacidal activity of the isolated compounds was determined using the colorimetric MTT assay. Medium 199 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 100 μg/mL of kanamycin was used as the cell culture medium. The test compounds were dissolved in dimethyl sulfoxide (DMSO) and then added to the wells of 96-well microtiter plates to the final concentration of 1%. L. major cells (2×10⁵ cells/well) were cultured in a CO₂ incubator at 25°C for 72 h and then a MTT solution was added to each well, followed by incubation overnight at 25°C. The absorbance was measured at 540 nm using a microplate reader. Amphotericin B was used as a positive control.

The inhibition % was calculated using the following equation:

\[
\text{% inhibition} = 1 - \frac{(A_{\text{sample}} - A_{\text{blank}})}{(A_{\text{control}} - A_{\text{blank}})} \times 100
\]

where \( A_{\text{control}} \) is the absorbance of the control reaction mixture (containing DMSO and all reagents, except for the test compounds). IC₅₀ was determined as the concentration of sample required to inhibit the formation of MITT formazan by 50%.

**DPPH Radical Scavenging Activity** The absorbance with various concentrations of the test compounds dissolved in MeOH (100 μL) in 96-well microtiter plates was measured at 515 nm as \( A_{\text{blank}} \). Then, a 200 μM DPPH solution (100 μL) was added to each well, followed by incubation at room temperature for 30 min. The absorbance was measured again as \( A_{\text{sample}} \).

The % inhibition was calculated using the following equation:

\[
\text{% inhibition} = 1 - \frac{(A_{\text{sample}} - A_{\text{blank}})}{(A_{\text{control}} - A_{\text{blank}})} \times 100
\]

where \( A_{\text{control}} \) is the absorbance of the control reaction mixture containing DMSO and all reagents, except for the test compound. IC₅₀ was determined as the concentration of sample required to inhibit the formation of the DPPH radical by 50%.

**Human Cancer Cell Growth Inhibition Assay** This assay was performed using a human lung cancer cell line (A549) and the viability was estimated by means of the colorimetric MTT assay. Dulbecco’s modified Eagle’s medium supplemented with 10% heat-inactivated FBS and 100 μg/mL of kanamycin was used as the cell culture medium. The test compounds were dissolved in DMSO and then added to the wells of 96-well microtiter plates to the final concentration of 1%. A549 cells (5×10⁴ cells/well) were cultured in a 5% CO₂ incubator at 37°C for 72 h and then a MTT solution was added to each well and the plates were incubated for a further 1.5 h. Then the formazan precipitates were dissolved in DMSO and the optical density value for each well was measured at
540 nm with a microplate reader. Doxorubicin was used as a positive control.

The cell growth inhibition was calculated using the following equation:

\[
\text{% inhibition} = \left(1 - \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}} - A_{\text{blank}}} \right) \times 100
\]

where \( A_{\text{control}} \) is the absorbance of the control reaction mixture containing DMSO and all reagents except for the test compound. IC\textsubscript{50} was determined as the concentration of sample required to inhibit the formation of MTT formazan by 50\%.

Acknowledgments The authors are grateful for access to the superconducting NMR instrument, UV and ESI-MS at the Analytical Center of Molecular Medicine, the Analysis Center of Life Science and the Natural Science Center for Basic Research and Development of the Graduate School of Biomedical and Health Sciences, Hiroshima University.

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