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SECONDARY METABOLITES FROM THE RHIZOMES OF *Alpinia officinarum*

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The traditional system of medicinals consists of a large number of plants with various medicinal and pharmacological properties and hence represents a priceless resource of new bioactive molecules. *Alpinia officinarum* Hance (Zingiberaceae), also known as lesser galangal, is indigenous to Southeast China and Indochina and the plains of West Bengal, Assam, and Eastem Himalayas in India [1]. It is a perennial herb with thick, creeping reddish-brown rhizomes, lineolate acuminate ornamental leaves, and showy white flowers in racemes. It has been used conventionally both in Ayurvedic and Chinese medicine since the very early times and in Europe since the Middle Ages [1]. The rhizome has been used in China for relieving stomachache, treating colds, invigorating the circulatory system, and reducing swelling. The dry root and rhizome have been used for their antioxidant, antidiarrheal, anti-emetic, analgesic, anti-inflammatory, and anticoagulation effects [2]. In continuation of some studies on the chemotaxonomy and biologically active metabolites from *Zingiberaceous* plants [3–11], a methanol extraction of the rhizomes of *A. officinarum* afforded two phenylalkanoids, [6]-shogaol [12] and [6]-gingerol [12], two diarylheptanoids, hexahydrocurcumin [13] and gingerenone A [14], and five benzenoids, *p*-hydroxybenzoic acid [15], vanillin [15], vanillic acid [15], syringaldehyde [15], and ferulic acid [15]. All of these compounds were isolated for the first time from this species.

In this study, we found that the major compound was gingerenone A (Gin A). Gin A was found to elicit concentration-dependent growth impediment. The death of these cells induced by Gin A was apoptotic in nature, exhibiting a concentration-dependent increase in subfraction G_1 [16]. The crude extracts and the deposited compound (Gin A) show roughly the same effect.

The rhizomes of *A. officinarum* were collected from Taoyuan County, Taiwan, in June 2017. Plant material was identified by Dr. Fu-Yuan Lu (Department of Forestry and Natural Resources College of Agriculture, National Chiayi University). A voucher specimen was deposited at the Department of Medical Technology, School of Medical and Health Sciences, Fooyin University, Kaohsiung, Taiwan. The rhizomes (3.24 kg) of *A. officinarum* were extracted repeatedly with MeOH (5 L × 3) at room temperature for 24–48 h. The MeOH extract was dried and evaporated to leave a viscous residue (112.4 g). The residue was placed on a silica gel column (3.5 kg, 70–230 mesh) and eluted with CH_2Cl_2 and gradually enriched with MeOH to afford 10 fractions. Part of fraction 2 (25.8 g) was subjected to silica gel chromatography (1.4 kg, 70–230 mesh) by eluting with *n*-hexane–acetone (80:1) and enriched gradually with acetone to furnish four fractions (2-1–2-4). Fraction 2-2 (11.2 g) was further purified on a silica gel column using *n*-hexane–acetone mixtures to obtain [6]-shogaol (0.8 g). Part of fraction 3 (14.7 g) was subjected to silica gel chromatography (1.3 kg, 70–230 mesh) by eluting with *n*-hexane–acetone (100:1) and enriched with acetone to furnish three fractions (3-1–3-3). Part of fraction 3-2 (7.1 g) was further purified on a silica gel column using *n*-hexane–acetone mixtures to obtain [6]-gingerol (0.6 g). Part of fraction 4 (22.3 g) was subjected to silica gel chromatography (1.3 kg, 70–230 mesh) by eluting with *n*-hexane–acetone (100:1) and enriched with acetone to furnish five fractions (4-1–4-5). Part of fraction 4-1 (12.3 g) was further purified on a silica gel column using *n*-hexane–acetone mixtures to obtain gingerenone A (1.3 g). Part of fraction 4-3 (4.2 g) was further purified on a silica gel column using *n*-hexane–acetone mixtures

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to obtain hexahydrocurcumin (0.4 g). Part of fraction 5 (10.2 g) was subjected to silica gel chromatography (0.8 kg, 70–230 mesh) by eluting with *n*-hexane-acetone (70:1) and enriched with acetone to furnish six fractions (5-1–5-6). Part of fraction 5-1 (2.5 g) was further purified on a silica gel column using CH_2Cl_2–MeOH mixtures to obtain vanillin (11 mg), vanillic acid (15 mg), and ferulic acid (7 mg). Part of fraction 5-3 (2.5 g) was further purified on a silica gel column using CH_2Cl_2–MeOH mixtures to obtain *p*-hydroxybenzoic acid (10 mg) and syringaldehyde (8 mg).

[6]-Shogaol, brown oil. UV (λ_{max}, nm): 224, 280. IR (ν_{max}, cm^{-1}): 3400, 2930, 2860, 1660, 1610, 1514, 1035, 980. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.88 (3H, t, J = 6.5, H-10), 1.25–1.51 (6H, m, H-7–9), 2.19 (2H, m, H-6), 2.79–2.88 (4H, m, H-1, 2), 3.86 (3H, s, 3′-OCH_3), 5.55 (1H, br.s, OH), 6.08 (1H, dt, J = 16.0, 1.4, H-4), 6.66–6.88 (4H, m, H-5, 2′, 5′, 6′).

[6]-Gingerol, brown oil. UV (λ_{max}, nm): 280. IR (ν_{max}, cm^{-1}): 3500, 2935, 2860, 1700, 1610, 1516, 1035, 975. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.86 (3H, t, J = 6.2, H-10), 1.23–1.49 (8H, m, H-6–9), 2.51 (2H, m, H-4), 2.67–2.85 (4H, m, H-1, 2), 3.85 (3H, s, 3′-OCH_3), 4.02 (1H, m, H-5), 6.63 (2H, m, H-2′, 6′), 6.80 (1H, d, J = 7.8, H-5′).

Hexahydrocurcumin, brown oil, $[\alpha]_D^{25}$ +5.22° (*c* 1.52, CH_2Cl_2). UV (λ_{max}, nm): 228, 280. IR (ν_{max}, cm^{-1}): 3400, 1700, 1602, 1515, 1030. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.63 (1H, dddd, J = 14.0, 10.1, 6.6, 4.3, H-6a), 1.78 (1H, dddd, J = 14.0, 9.2, 9.2, 5.5, H-6b), 2.51 (1H, dd, J = 17.4, 7.9, H-4a), 2.56 (1H, dd, J = 17.4, 3.0, H-4b), 2.60 (1H, ddd, J = 13.0, 9.2, 6.7, H-7a), 2.70 (2H, t, J = 7.2, H-2), 2.71 (1H, ddd, J = 13.0, 10.1, 5.5, H-7b), 2.82 (2H, t, J = 7.2, H-1), 3.85 (3H, s, 3′-OCH_3), 3.86 (3H, s, 3″-OCH_3), 4.03 (1H, dddd, J = 9.2, 7.9, 4.3, 3.1, H-5), 5.53 (1H, s, OH), 5.55 (1H, s, OH), 6.64 (1H, dd, J = 8.0, 2.0, H-6′), 6.66 (1H, d, J = 2.0, H-2′), 6.67 (1H, dd, J = 8.0, 2.0, H-6″), 6.70 (1H, d, J = 2.0, H-2″), 6.81 (2H, d, J = 8.0, H-5′, 5″).

Gingerenone A, brown oil. UV (λ_{max}, nm): 230, 280. IR (ν_{max}, cm^{-1}): 3430, 1700, 1615, 1515, 1035, 975. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.48 (2H, q, J = 7.2, H-6), 2.70 (2H, t, J = 7.2, H-7), 2.84 (4H, m, H-1, 2), 3.86 (6H, s, 3′, 3″-OCH_3), 5.50 (1H, s, OH), 5.51 (1H, s, OH), 6.11 (1H, d, J = 16.0, H-4), 6.65 (1H, d, J = 2.0, H-2′), 6.66 (1H, dd, J = 8.0, 2.0, H-6′), 6.69 (1H, d, J = 2.0, H-2″), 6.70 (1H, dd, J = 8.0, 2.0, H-6″), 6.82 (1H, d, J = 8.0, H-5′), 6.83 (1H, d, J = 8.0, H-5″).

***p*-Hydroxybenzoic acid**, brown powder ($CHCl_3$). UV (λ_{max}, nm): 250, 285, 290. IR (ν_{max}, cm^{-1}): 3500, 1660, 1590, 1165, 845. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.85 (2H, d, J = 8.6, H-3, 5), 7.96 (2H, d, J = 8.6, H-2, 6).

Vanillin, yellow powder ($CHCl_3$). UV (λ_{max}, nm): 220, 280, 310. IR (ν_{max}, cm^{-1}): 3400, 1670, 1595, 1025. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.95 (3H, s, 3-OCH_3), 6.20 (1H, br.s, OH), 7.09 (1H, d, J = 8.0, H-5), 7.31 (1H, d, J = 2.0, H-2), 7.42 (1H, dd, J = 8.0, 2.0, H-6), 9.77 (1H, s, C<u>H</u>O).

Vanillic acid, colorless needles. UV (λ_{max}, nm): 220, 265, 300. IR (ν_{max}, cm^{-1}): 3600, 1670, 1550, 1215. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.89 (3H, s, 3-OCH_3), 6.80 (1H, d, J = 8.2, H-5), 7.50 (1H, dd, J = 8.2, 2.0, H-6), 7.58 (1H, d, J = 2.0, H-2).

Syringaldehyde, brown needles ($CHCl_3$). UV (λ_{max}, nm): 215, 230, 305. IR (ν_{max}, cm^{-1}): 3300, 1670, 1610, 1585, 1520, 1330. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 3.98 (6H, s, 3, 5-OCH_3), 6.05 (1H, br.s, OH), 7.16 (2H, s, H-2, 6), 9.81 (1H, s, C<u>H</u>O).

Ferulic acid, colorless needles ($CHCl_3$). UV (λ_{max}, nm): 227, 246, 335. IR (ν_{max}, cm^{-1}): 3200 (OH), 1680 (C=O). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.92 (3H, s, OCH_3), 6.37 (1H, d, J = 16.0, H-8), 6.87 (1H, d, J = 8.0, H-5), 7.13 (1H, dd, J = 8.0, 1.6, H-6), 7.32 (1H, d, J = 1.6, H-2), 7.59 (1H, d, J = 16.0, H-7).

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