



## Secondary Metabolites from the Leaves of *Aquilaria agallocha*

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### ABSTRACT

Twelve compounds, including three flavonoids, 5-hydroxy-4',7'-dimethoxyflavone (1) [22], luteolin-7,3',4'-trimethyl ether (2) and 5,3'-dihydroxy-7,4'-dimethoxyflavone (3), five benzenoids, methylparaben (4), vanillic acid (5), *p*-hydroxybenzoic acid (6), syringic acid (7), and isovanillic acid (8) and four steroids,  $\beta$ -sitosterol (9), stigmaterol (10),  $\beta$ -sitostenone (11) and stigmasta-4,22-dien-3-one (12) were isolated from the leaves of *Aquilaria agallocha* (Thymelaeaceae). All of these compounds (1-12) were obtained for the first time from the leaves of this plant.

### Keywords

Flavonoids; Benzenoids; Steroids; *Aquilaria agallocha*; Thymelaeaceae

### Academic Discipline And Sub-Disciplines

Pharmacy

### SUBJECT CLASSIFICATION

Natural drug discovery

### TYPE (METHOD/APPROACH)

Experimental

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## INTRODUCTION

*Lignum Aquilariae Resinatum* has been widely used in the treatment of various kinds of pain, cough and anaphylaxis for hundreds of years in Asia, especially in China, Vietnam, and Indonesia. The genus *Aquilaria* (Thymelaeaceae) is widely distributed in Asia. *Aquilaria sinensis* (Lour.) Gilg. is of particular interest economically because it is the principal source of agarwood, one of the most highly valuable forest products currently traded internationally. The leaves of *A. sinensis* (Lour.) Gilg., which widely cultivated in Guangdong, Hainan and Taiwan provinces in China are orally reported to be used locally in trauma-related diseases such as fracture, bruise, etc [1]. Previous phytochemical investigation on Chinese eaglewood revealed characteristic sesquiterpenes and chromone derivatives [2-15], but few reports about the chemical constituents of the leaves of *A. agallocha* [16, 17]. The analgesic and anti-inflammatory activities of the ethanol extract of *A. sinensis* (Lour.) Gilg. Leaves were observed in various experimental models related to nociception and inflammation, so as to provide some evidence for its traditional use. By investigation of interrelated studies, agarwood has significant anticancer activities [18], analgesic and anti-inflammatory activities [19], and anti-depression activities [20, 21]. In continuation of our interest in the phytochemical study of flavonoids in the species *Aquilaria* plant, we report here of twelve compounds isolated from the leaves of *A. agallocha*.

## EXPERIMENTAL

### General experimental procedure

IR, Hitachi 260-30 spectrophotometer; 1D and 2D NMR, Varian (Unity Plus) NMR spectrometer; Low-resolution ESI-MS, API 3000 (Applied Biosystems); High-resolution ESI-MS, Bruker Daltonics APEX II 30e spectrometer; Silica gel 60 for CC and precoated silica gel plates (Merck) were used for TLC, visualized with 10% H<sub>2</sub>SO<sub>4</sub>.

### Plant material

The specimen of *A. agallocha* was collected from Shanshang District, Tainan City, Taiwan in May, 2008. A voucher specimen was identified by Professor Fu-Yuan Lu (Department of Forestry and Natural Resources College of Agriculture, National Chiayi University) and was deposited in the School of Medical and Health Sciences, Fooyin University, Kaohsiung, Taiwan.

### Extraction and isolation

The leaves (1.5 kg) of *A. agallocha* were airdried and extracted repeatedly with MeOH (5 L x 6) at room temperature. The combined MeOH extracts (29.2 g) were then evaporated and further separated into 5 fractions by column chromatography on silica gel (5.1 kg, 70-230 mesh) with gradients of *n*-hexane/CH<sub>2</sub>Cl<sub>2</sub>/acetone/MeOH. Part of fraction 1 (10.2 g) was subjected to silica gel chromatography by eluting with *n*-hexane-acetone (60:1), enriched with acetone to furnish three further fractions (1-1-1-3). Fraction 1-1 (2.5 g) was further purified on a silica gel column using *n*-hexane/acetone mixtures to obtain 5-hydroxy-4',7'- dimethoxy-flavonoid (**1**) (5.9 mg). Part of fraction 1-2 (2.1 g) was subjected to silica gel chromatography, by eluting with *n*-hexane-acetone (60:1), enriched gradually with acetone, to furnish two fractions (1-2-1-1-2-2). Fraction 1-2-1 (0.6 g) was further purified on a silica gel column using *n*-hexane/acetone mixtures to yielded luteolin-7, 3', 4'-trimethyl ether (**2**) (3.7 mg) and 5,3'-dihydroxy-7,4'- dimethoxyflavone (**3**) (3.1 mg). Fraction 1-2-2 (0.6 g) was further purified on a silica gel column using *n*-hexane/acetone mixtures to yielded a mixture of β-sitosterol (**9**) and stigmasterol (**10**) (27.5 mg) and mixture of β-sitosterone (**11**) and stigmasta-4,22-dien-3-one (**12**) (11.2 mg). Part of fraction 2 (6.8 g) was subjected to silica gel chromatography by eluting with *n*-hexane-acetone (45:1), enriched with acetone to furnish two further fractions (2-1-2-2). Fraction 2-1 (3.2 g) was further purified on a silica gel column using *n*-hexane/acetone mixtures to obtain methylparaben (**4**) (5.2 mg), vanillic acid (**5**) (3.7 mg) and *p*-hydroxybenzoic acid (**6**) (4.5 mg). Part of fraction 3 (5.1 g) was subjected to silica gel chromatography by eluting with CH<sub>2</sub>Cl<sub>2</sub>-MeOH (100:1), enriched with MeOH, to furnish two fractions (3-1-3-2). Fraction 3-2 (2.1 g) was further purified on a silica gel column using CH<sub>2</sub>Cl<sub>2</sub>/MeOH mixtures to obtain syringic acid (**7**) (6.5 mg) and isovanillic acid (**8**) (3.1 mg).

**5-hydroxy-4',7'-dimethoxy-flavonoid (1)** as in [22], Yellow needles (MeOH), mp 170-173 °C, UV λ<sub>max</sub> 267, 300, 350 nm, IR ν<sub>max</sub> 3300, 1645, 1595 cm<sup>-1</sup>, <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) : δ 3.88 (3H, s, C<sub>7</sub>-OCH<sub>3</sub>), 3.90 (3H, s, C<sub>4</sub>-OCH<sub>3</sub>), 6.37 (1H, *d*, *J* = 2.5 Hz, H-6), 6.48 (1H, *d*, *J* = 2.5 Hz, H-8), 6.58 (1H, s, H-3), 7.01 (2H, *d*, *J* = 8.5 Hz, H-3' and H-5'), 7.84 (2H, *d*, *J* = 8.5 Hz, H-2' and H-6'), 12.81 (1H, *br s*, C<sub>5</sub>-OH), ESI-MS *m/z* : 298 [M]<sup>+</sup>.

**luteolin-7, 3', 4'-trimethyl ether (2)** as in [22], Yellow needles (MeOH), mp 188-190 °C, UV λ<sub>max</sub> 270, 298, 350 nm, IR ν<sub>max</sub> 3300, 1645, 1595 cm<sup>-1</sup>, <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) : δ 3.89 (3H, s, C<sub>7</sub>-OCH<sub>3</sub>), 3.97 (3H, s, C<sub>3</sub>-OCH<sub>3</sub>), 3.99 (3H, s, C<sub>4</sub>-OCH<sub>3</sub>), 6.38 (1H, *d*, *J* = 2.5 Hz, H-6), 6.50 (1H, *d*, *J* = 2.5 Hz, H-8), 6.59 (1H, s, H-3), 6.98 (1H, *d*, *J* = 8.5 Hz, H-5'), 7.35 (1H, *d*, *J* = 2.0 Hz, H-2'), 7.53 (1H, *dd*, *J* = 8.5, 2.0 Hz, H-6'), 12.80 (1H, *br s*, C<sub>5</sub>-OH), ESI-MS *m/z* : 328 [M]<sup>+</sup>.

**5,3'-dihydroxy-7,4'-dimethoxyflavone (3)** as in [24], Yellow needles (MeOH), mp 211-213 °C, UV λ<sub>max</sub> 270, 299, 350 nm, IR ν<sub>max</sub> 3300, 1645, 1595 cm<sup>-1</sup>, <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) : δ 3.89 (3H, s, C<sub>7</sub>-OCH<sub>3</sub>), 4.01 (3H, s, C<sub>4</sub>-OCH<sub>3</sub>), 6.37 (1H, *d*, *J* = 2.4 Hz, H-6), 6.49 (1H, *d*, *J* = 2.4 Hz, H-8), 6.57 (1H, s, H-3), 7.04 (1H, *d*, *J* = 8.4 Hz, H-5'), 7.33 (1H, *d*, *J* = 2.4 Hz, H-2'), 7.49 (1H, *dd*, *J* = 8.4, 2.4 Hz, H-6'), 12.80 (1H, *br s*, C<sub>5</sub>-OH), ESI-MS *m/z* : 314 [M]<sup>+</sup>.

**methylparaben (4)** as in [25], Colorless needles (CH<sub>2</sub>Cl<sub>2</sub>), mp 130- 131 °C, UV λ<sub>max</sub> 225, 258, 312 nm, IR ν<sub>max</sub> 3400, 2960, 2850, 1690 cm<sup>-1</sup>, <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) : δ 3.89 (3H, s, COOCH<sub>3</sub>), 6.86 (2H, *d*, *J* = 8.8 Hz, H-3 and H-5), 7.95 (2H, *d*, *J* = 8.8 Hz, H-2 and H-6), ESI-MS *m/z* : 152 [M]<sup>+</sup>.



**vanillic acid (5)** as in [26], Colorless needles (MeOH), mp 210-212 °C, UV  $\lambda_{\max}$  220, 265, 300 nm, IR  $\nu_{\max}$  3600, 1670, 1550, 1215  $\text{cm}^{-1}$ ,  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ) :  $\delta$  3.89 (3H, s,  $\text{C}_3\text{-OCH}_3$ ), 6.80 (1H, d,  $J = 8.2$  Hz, H-5), 7.50 (1H, dd,  $J = 8.2, 2.0$  Hz, H-6), 7.58 (1H, d,  $J = 2.0$  Hz, H-2), ESI-MS  $m/z$ : 168  $[\text{M}]^+$ .

***p*-hydroxybenzoic acid (6)** as in [27], Brown powder ( $\text{CH}_2\text{Cl}_2$ ), UV  $\lambda_{\max}$  250, 284, 290 nm, IR  $\nu_{\max}$  3500, 1660, 1590, 1280  $\text{cm}^{-1}$ ,  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ) :  $\delta$  6.85 (2H, d,  $J = 8.6$  Hz, H-3 and H-5), 7.97 (2H, d,  $J = 8.6$  Hz, H-2 and H-6), ESI-MS  $m/z$ : 138  $[\text{M}]^+$ .

**syringic acid (7)** as in [28], Brown needles ( $\text{CH}_2\text{Cl}_2$ ), mp 203-205 °C, UV  $\lambda_{\max}$  212, 235, 310 nm, IR  $\nu_{\max}$  3255, 1670, 1590, 1515  $\text{cm}^{-1}$ ,  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ) :  $\delta$  3.81 (6H, s,  $\text{C}_3\text{-OCH}_3$  and  $\text{C}_5\text{-OCH}_3$ ), 7.37 (2H, s, H-2 and H-6), ESI-MS  $m/z$ : 198  $[\text{M}]^+$ .

**isovanillic acid (8)** as in [28], Colorless needles (MeOH), mp 208-210 °C, UV  $\lambda_{\max}$  215, 260, 292 nm, IR  $\nu_{\max}$  2925, 2852, 1690, 1563, 1278  $\text{cm}^{-1}$ ,  $^1\text{H NMR}$  (400 MHz,  $\text{CD}_3\text{OD}$ ) :  $\delta$  3.92 (3H, s,  $\text{C}_4\text{-OCH}_3$ ), 7.27 (1H, dd,  $J = 8.8, 2.5$  Hz, H-6), 8.01 (1H, d,  $J = 8.8$  Hz, H-5), 8.18 (1H, d,  $J = 2.5$  Hz, H-2), ESI-MS  $m/z$ : 168  $[\text{M}]^+$ .

**$\beta$ -sitosterol (9) and stigmasterol (10)** as in [29], White needles (MeOH), mp 138-140 °C, IR  $\nu_{\max}$  3400, 2900, 1625, 1450  $\text{cm}^{-1}$ ,  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ) :  $\delta$  0.68 (3H, s, H-18), 0.81 (3H, d,  $J = 6.8$  Hz, H-26), 0.84 (3H, d,  $J = 6.8$  Hz, H-27), 0.86 (3H, t,  $J = 7.0$  Hz, H-29), 0.92 (3H, d,  $J = 6.4$  Hz, H-21), 1.01 (3H, s, H-19), 3.53 (1H, m, H-3), 5.02 (1H, dd,  $J = 16.1, 8.3$  Hz, H-22), 5.12 (1H, dd,  $J = 16.1, 8.3$  Hz, H-23), 5.35 (1H, br s, H-6), ESI-MS  $m/z$ : 414  $[\text{M}]^+$ , 412  $[\text{M}]^+$ .

**$\beta$ -sitostenone (11) and stigmasta-4,22-dien-3-one (12)** as in [29], White needles ( $\text{CH}_2\text{Cl}_2$ ), mp 85-86 °C, IR  $\nu_{\max}$  1675, 1620, 1450, 1375  $\text{cm}^{-1}$ ,  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ) :  $\delta$  0.68 (3H, s, H-18), 0.81 (3H, d,  $J = 6.7$  Hz, H-26), 0.84 (3H, s, H-27), 0.86 (3H, d,  $J = 7.0$  Hz, H-29), 0.94 (3H, d,  $J = 6.0$  Hz, H-21), 1.02 (3H, s, H-19), 5.01 (1H, dd,  $J = 16.1, 8.2$  Hz, H-22), 5.12 (1H, dd,  $J = 16.1, 8.2$  Hz, H-23), 5.72 (1H, d,  $J = 1.4$  Hz, H-3), ESI-MS  $m/z$ : 412  $[\text{M}]^+$ , 410  $[\text{M}]^+$ .

## RESULTS AND DISCUSSION

These observations provide useful information for potential chemopreventive drug design. The MeOH extract of its leaves were subjected to solvent partitioning and chromatographic separation to afford twelve pure substances. Twelve compounds, including three flavonoids, 5-hydroxy-4',7-dimethoxy-flavonoid (1) [22], luteolin-7,3',4'-trimethyl ether (2) [23] and 5,3'-dihydroxy-7,4'-dimethoxyflavone (3) [24], five benzenoids, methylparaben (4) [25], vanillic acid (5) [26], *p*-hydroxybenzoic acid (6) [27], syringic acid (7) [28], and isovanillic acid (8) [28] and four steroids,  $\beta$ -sitosterol (9) [29], stigmasterol (10) [29],  $\beta$ -sitostenone (11) [29] and stigmasta-4,22-dien-3-one (12) [29] were isolated from the leaves of *A. agallocha*. All of these compounds (1-12) were obtained for the first time from the leaves of this plant.

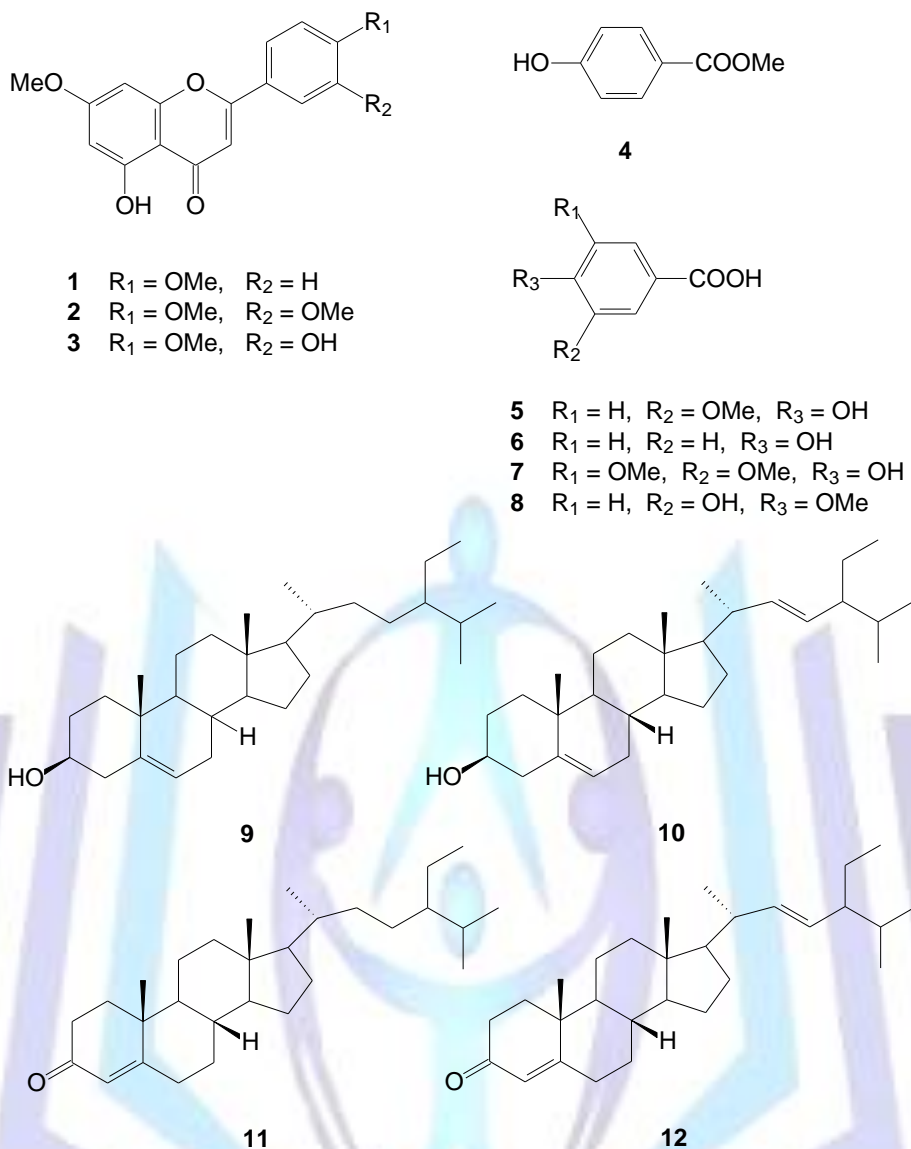


Fig 1: The structure of isolated compounds

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