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Chemical Constituents from the Stem Bark of *Garcinia urophylla* Scort. ex King and their Antioxidant and Acetylcholinesterase Inhibitory Activities

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Members of the Clusiaceae family, including the *Garcinia* genus, are well-known for their diverse array of bioactive compounds, such as polyisoprenylated benzophenones, xanthones, and biflavonoids. This study focused on investigating the phytochemicals extracted from the stem bark of *Garcinia urophylla* and evaluating their antioxidant and acetylcholinesterase (AChE) inhibitory activities. The extraction was carried out using a cold maceration method, applying a polarity gradient of solvents: hexane, ethyl acetate, and methanol. The isolated phytochemicals were purified using chromatographic techniques, and their structures were elucidated through spectroscopic analyses (IR, NMR, and MS) and comparison with existing literature. The antioxidant activity of the compounds was assessed using the DPPH radical scavenging assay, while their AChE inhibitory activity was evaluated using the Ellman method. The isolation process successfully identified eight compounds: α-mangostin (**1**), β-mangostin (**2**), β-amyrin (**3**), lupeol (**4**), lupeol acetate (**5**), ferulic acid (**6**), syringic acid (**7**), and caffeic acid (**8**). Among these, compound (**1**) exhibited the most potent activity, with IC_{50} values of 15.2 µg/mL for DPPH radical scavenging and 18.0 µg/mL for AChE inhibition. These findings provide valuable insights into the potential nutraceutical and pharmaceutical applications of *Garcinia* species and their bioactive constituents.

Keywords: *Garcinia urophylla*; xanthone; mangostin; antioxidant; acetylcholinesterase

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Phytochemicals, naturally occurring compounds found in plants, are widely recognized for their antioxidant and acetylcholinesterase (AChE) inhibitory activities, which contribute to their therapeutic potential. Antioxidants neutralize reactive oxygen species (ROS) and free radicals, reducing oxidative stress, a major contributor to cellular aging and the development of various diseases, including neurodegenerative disorders like Alzheimer's disease (AD). By protecting lipids, proteins, and DNA from oxidative damage, phytochemicals with antioxidant properties play a crucial role in maintaining cellular health and preventing chronic diseases. In addition, many phytochemicals exhibit acetylcholinesterase inhibitory activity, which is particularly relevant for the treatment of AD. AChE inhibitors prevent the breakdown of acetylcholine, a neurotransmitter essential for memory and cognitive function, thereby enhancing cholinergic signaling in the brain. This dual action, antioxidant and AChE inhibition makes phytochemicals valuable candidates for the development of drugs targeting neurodegenerative diseases, offering both protective and therapeutic benefits [1-3].

Clusiaceae is a family of plants found in tropical and subtropical regions as shrubs and trees. Their fruits, barks, leaves, roots, flowers, latex, and branches are candidates for studies to identify the bioactive or

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therapeutic compounds contained in them, based on their use by local populations endemic to the regions in which they grow for the treatment of diseases [4]. *Garcinia* is the largest genus of the Clusiaceae family comprising of 390 species. These polygamous trees or shrubs are mainly distributed in tropical Asia, Polynesia, and Africa [5]. Due to their valuable pharmacological significance, numerous phytochemical and biological studies have been conducted on *Garcinia* species to date, confirming their traditional functions from a modern scientific point of view and developing their new pharmacological actions. The extracts of this genus are rich in polycyclic, polyprenylated acylphloroglucinols, xanthones, polyphenols, and flavonoids, all of which possess a wide range of pharmacological activities such as antiviral, antitumor, and anti-inflammatory properties. In recent decades, considerable studies have been reported about the pharmacological activity and association with traditional uses of *Garcinia* genus [5]. For instance, the anti-inflammatory properties of *G. multifora* [6], *G. mangostana* [7], *G. cowa* [8], and *G. oblongifolia* [9] were reported, which support the ethnomedicinal use of the plants to treat various inflammatory conditions, such as stomatitis, asthma and ulcerative colitis. However, some *Garcinia* species for medicinal use still need to be investigated for the validation and analysis of their chemical constituents. *G. urophylla* is locally known as 'kandis hutan' in Peninsular Malaysia. This plant is a small fruiting tree (3-10 m tall), usually scattered throughout the hills. The native range of this species is Peninsula Malaysia to Sumatera. The fruits are used to treat stomachache and the leaves are used to treat fever [10]. Previously, two new xanthones were isolated and characterized from the dichloromethane fraction of the leaves of this species [11]. Recently, we have reported the chemical components of the leaf oil of *G. urophylla* [12]. Analysis of the essential oil revealed the presence of eighteen components, accounting for 99.9% of the total oil. The major components of essential oil were β-caryophyllene (56.2%), α-humulene (26.3%), and α-gurjunene (6.3%). The cytotoxicity of essential oil also exhibited cytotoxicity against three cancer cell lines which are HepG2, MCF7, and A549 with the IC_{50} values of 71.5, 56.2, and 68.5 μg/mL, respectively. As part of our ongoing search for bioactive compounds from *Garcinia* species, we have investigated the phytochemicals present in the stem bark of *G. urophylla* and assessed their antioxidant and acetylcholinesterase activities.

EXPERIMENTAL

Plant Material

The stem bark of *G. urophylla* was collected from Fraser Hill, Pahang, in January 2023 and identified by Shamsul Khamis from Universiti Kebangsaan Malaysia (UKM). A voucher specimen (SA30-39) was deposited in the UKM Herbarium. The collected samples were thoroughly washed to remove excess sand and other impurities, then air-dried under shade at room temperature.

General Experimental Procedures

A cold extraction method was employed to extract the phytochemicals from the dried stem bark using solvents of varying polarity (n-hexane, ethyl acetate, and methanol). Column chromatography (CC) was conducted using Merck silica gel 60 (70-230 mesh) as the stationary phase. Thin layer chromatography (TLC) analysis was performed on Merck precoated silica gel F_{254} plates (0.22 mm thickness) to detect and monitor the presence of compound samples. The spots were visualized under UV light (254 and 365 nm) and by spraying with vanillin sulfuric acid in methanol, followed by heating. Melting points were determined by comparison with literature values. 1D and 2D NMR spectra were recorded on a Bruker Avance 500 MHz spectrometer. Chemical shifts are reported in ppm, with $CDCl_3$ as the solvent, and the residual solvent signal was used as an internal standard. IR spectra were recorded on a Perkin Elmer ETR and 1600 series spectrophotometer using KBr discs or NaCl discs for thin films. Mass spectral data were obtained using an Orbitrap Exploris 240 Mass Spectrometer.

Extraction and Isolation

The dried stem bark (500 g) of *G. urophylla* were extracted consecutively by cold extraction with *n*-hexane (5 L), EtOAc (5 L), and MeOH (5 L), respectively. The *n*-hexane extract (GUBH – 3.0 g), EtOAc (GUBE – 10.1 g), and MeOH (GUBM – 5.0 g) were fractionated by CC on silica gel 70-230 mesh. The GUBH was purified by CC to give four major fractions (GUBH 1-4). The fraction GUBH-1 was purified by preparative thin layer chromatography (prep-TLC) to afford compound (**5**) (Hex:$CHCl_3$ 80:20). Meanwhile, the fraction GUBH-2 was purified by CC followed by washing with cold hexane to afford compound (**2**) (Hex:$CHCl_3$ 40:60). The ethyl acetate extract was fractionated using CC on silica gel 70-230 mesh to give five major fractions (GUBE 1-5). The GUBE-1 was purified by preparative thin layer chromatography (prep-TLC) to afford compound (**1**) (Hex:$CHCl_3$ 80:20). The GUBE-2 and 3 were combined and further purified by CC to afford compound (**6**) (Hex:$CHCl_3$ 60:40), compound (**7**) (Hex:$CHCl_3$ 50:50), and compound (**8**) (Hex:$CHCl_3$ 40:60). The GUBM was purified by CC to give four major fractions (GUBM1-4). Fractions GUBM-1 and 2 were purified by CC, followed by washing with diethyl ether to yield compound (**3**) (Hex: $CHCl_3$ 80:20) and compound (**4**) (Hex:$CHCl_3$ 70:30), respectively.

Spectral Data

α-Mangostin (1): yellow solid (22 mg); m.p: 180–182°C; IR (NaCl): 3440 (OH), 1620 (C=O), 1570

(C=C) , 1250 (C–O); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.79 (3H, s, H-20), 1.79 (3H, s, H-11), 1.86 (3H, s, H-15), 1.87 (3H, s, H-19), 3.47 (2H, d, J = 5.0 Hz, H-11), 3.83 (3H, s, 7-OCH_3), 4.11 (2H, d, J = 5.0 Hz, H-16), 5.27 (1H, t, J = 7.5 Hz, H-17), 5.30 (1H, t, J = 7.5 Hz, H-12), 6.33 (1H, s, H-4), 6.34 (1H, s, 6-OH), 6.50 (1H, s, 3-OH), 6.85 (1H, s, H-5), 13.78 (1H, s, 1-OH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 17.9 (C-19), 18.2 (C-15), 21.4 (C-11), 25.8 (C-14), 25.9 (C-20), 26.5 (C-16), 62.8 (7-OCH_3), 93.3 (C-4), 101.6 (C-5), 103.6 (C-9a), 108.5 (C-2), 112.1 (C-8a), 121.5 (C-12), 123.1 (C-17), 132.0 (C-18), 135.4 (C-13), 137.0 (C-8), 142.6 (C-7), 154.6 (C-6), 155.0 (C-4a), 155.7 (C-10a), 160.6 (C-1), 161.6 (C-3), 182.0 (C-9); EIMS: *m/z* 411 [M^+, $C_{24}H_{28}O_6$].

β-Mangostin (2): yellow solid (18 mg); m.p: 174–176°C; IR (NaCl): 3445 (OH), 1618 (C=O), 1565 (C=C), 1245 (C–O);^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.72 (3H, s, H-15), 1.82 (3H, s, H-20), 1.86 (3H, s, H-14), 1.86 (3H, s, H-19), 3.37 (2H, d, J = 7.0 Hz, H-11), 3.83 (3H, s, 7-OCH_3), 3.93 (3H, s, 3-OCH_3), 4.12 (2H, d, J = 7.0 Hz, H-16), 5.26 (1H, d, J = 7.0 Hz, H-12), 5.27 (1H, t, J = 7.0 Hz, H-17), 6.37 (1H, s, H-4), 6.38 (1H, s, 6-OH), 6.86 (1H, s, H-5), 13.44 (1H, s, 1-OH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 17.8 (C-19), 18.2 (C-15), 21.3 (C-11), 25.8 (C-14), 26.7 (C-20), 31.2 (C-16), 62.0 (7-OCH_3), 88.8 (C-4), 101.5 (C-5), 103.8 (C-9a), 111.5 (C-2), 112.3 (C-8a), 122.3 (C-12), 123.2 (C-17), 131.7 (C-18), 132.0 (C-13), 137.0 (C-8), 142.5 (C-7), 155.6 (C-6), 154.4 (C-4a), 155.3 (C-10a), 159.9 (C-1), 163.5 (C-3), 181.9 (C-9); EIMS: *m/z* 425 [M^+, $C_{25}H_{28}O_6$].

β-Amyrin (3): white solid (12 mg); m.p: 197–198°C; IR (NaCl): 3440 (OH), 2925, 2855, 1465 (C-H); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.71 (1H, m, H-5), 0.77 (3H, s, H-23), 0.81 (3H, s, H-28), 0.85 (3H, s, H-29/H-30), 0.92 (3H, s, H-25), 0.94 (3H, s, H-26), 0.98 (3H, s, H-24), 1.11 (3H, s, H-27), 1.30-1.53 (2H, m, H-6), 1.49-1.55 (2H, m, H-1), 1.52-1.55 (2H, m, H-2), 1.59 (2H, m, H-19), 1.66 (2H, m, H-21), 1.84 (2H, m, H-11), 1.89 (1H, m, H-18), 1.95 (1H, m, H-9), 3.20 (1H, dd, J = 4.4, 11.5 Hz, H-3), 5.16 (1H, t, J = 3.5 Hz, H-12); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 15.7 (C-23), 15.8 (C-25), 17.0 (C-26), 18.5 (C-6), 23.7 (C-11), 23.9 (C-30), 26.2 (C-27), 26.3 (C-15), 27.1 (C-16), 27.4 (C-2), 28.3 (C-24), 28.6 (C-28), 31.3 (C-20), 32.7 (C-17), 32.8 (C-7), 33.5 (C-29), 34.9 (C-22), 37.1 (C-10), 37.3 (C-21), 38.7 (C-1), 38.9 (C-4), 40.2 (C-8), 41.9 (C-14), 47.0 (C-19), 47.4 (C-9), 47.8 (C-18), 55.3 (C-5), 79.2 (C-3), 121.9 (C-12), 145.4 (C-13); EIMS: *m/z* 426 [M^+, $C_{30}H_{50}O$].

Lupeol (4): white needle (15 mg); m.p: 213–215°C; IR (NaCl): 3435 (OH), 2930, 2860, 1455 (C-H); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.71 (1H, d, J = 9.2 Hz, H-5), 0.77 (3H, s, H-28); 0.80 (3H, s, H-25), 0.94 (3H, s, H-27), 0.96 (3H, s, H-23), 0.98 (3H, s, H-24), 1.00 (3H, s, H-26), 1.67 (3H, s, H-30), 1.95 (2H, m, H-21), 2.36 (1H, dt, J = 11.2, 5.6 Hz, H-19), 3.19 (1H, dd, J = 11.2 and 5.4 Hz, H-3), 4.58 (1H, s, H-29), 4.70 (1H, s, H-29); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.5 (C-27), 15.3 (C-24), 15.9 (C-26), 16.1 (C-25), 18.0 (C-28), 18.3 (C-6), 19.3 (C-30), 20.9 (C-11), 25.1 (C-12), 27.3 (C-23), 27.4 (C-2), 27.9 (C-15), 29.8 (C-21), 34.3 (C-7), 35.5 (C-16), 37.1 (C-10), 38.0 (C-13), 38.7 (C-1), 38.8 (C-4), 40.0 (C-22), 40.8 (C-8), 42.8 (C-14), 43.0 (C-17), 47.9 (C-19), 48.3 (C-18), 50.4 (C-9), 55.3 (C-5), 79.0 (C-3), 109.3 (C-29), 150.9 (C-20); EIMS: *m/z* 426 [M^+, $C_{30}H_{50}O$].

Lupeol acetate (5): white solid (18 mg); m.p: 270–275°C; IR (NaCl): 1735 (C=O), 2925, 2855, 1450 (C–H);^{1}H NMR ($CDCl_3$, 500 MHz): δ 4.66 (1H, br s, H-29), 4.53 (1H, br s, H-29), 4.46 (1H, dd, J = 10.4, 6.0 Hz, H-3), 2.00 (3H, s, H-2′), 2.37 (1H, m, H-19), 1.89 (2H, m, H-21), 1.70 (3H, s, H-30), 0.98 (3H, s, H-26), 0.96 (3H, s, H-27), 0.86 (3H, s, H-28), 0.85 (9H, s, H-23, H-24, H-25), 0.76 (1H, d, J = 10.9 Hz, H-5); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.5 (C-1′), 150.5 (C-20), 109.4 (C-29), 80.7 (C-3), 55.3 (C-5), 50.3 (C-9), 48.2 (C-19), 47.9 (C-18), 42.9 (C-17), 42.8 (C-14), 40.8 (C-8), 39.9 (C-22), 38.4 (C-1), 38.0 (C-13), 37.7 (C-4), 37.0 (C-10), 35.5 (C-16), 34.2 (C-7), 29.8 (C-21), 28.1 (C-23), 27.4 (C-15), 25.0 (C-12), 23.6 (C-2), 21.3 (C-2′), 20.9 (C-11), 19.3 (C-30), 18.2 (C-6), 17.5 (28), 16.5 (C-25), 16.1 (26), 15.9 (C-24), 14.5 (C-27); EIMS: *m/z* 468 [M^+, $C_{32}H_{52}O_2$].

Ferulic acid (6): brown solid (10 mg); m.p: 172–174°C; IR (NaCl): 3430 (OH), 1695 (C=O), 1600, 1510 (C=C);^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.97 (3H, s, 3′-OCH_3), 6.31 (1H, d, J = 15.0 Hz, H-2′), 6.96 (1H, d, J = 8.9 Hz, H-5′), 7.08 (1H, d, J = 1.9 Hz, H-2′), 7.13 (1H, dd, J = 1.9, 8.2 Hz, H-6′), 7.73 (1H, d, J = 15.0 Hz, H-3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 56.0 (3′-OCH_3), 109.5 (C-6′), 114.2 (C-3′), 114.8 (C-2), 123.7 (C-2′), 126.6 (C-1′), 146.8 (C-3), 147.3 (C-5′), 148.5 (C-4′), 171.3 (C-1); EIMS: *m/z* 194 [M^+, $C_{10}H_{10}O_4$].

Syringic acid (7): white crystalline solid (11 mg); m.p: 205–208°C; IR (NaCl): 3350 (OH), 1690 (C=O), 1605, 1505 (C=C); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.95 (6H, s, 3′/5′-OCH_3), 5.97 (1H, s, 4′-OH), 7.38 (2H, s, H-2′/H-6′); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 56.5 (3′/5′-OCH_3), 107.3 (C-2′/C-6′), 120.0 (C-1′), 140.0 (4′-OH), 146.7 (C-3′/C-5′), 170.7 (C-1); EIMS: *m/z* 198 [M^+, $C_9H_{10}O_5$].

Cafeic acid (8): white solid (12 mg); m.p: 194–198°C; IR (NaCl): 3320 (OH), 1685 (C=O), 1600, 1502 (C=C); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.46 (1H, s, H-4′), 3.32 (1H, s, H-3′), 6.12 (1H, d, J = 15.9 Hz, H-2), 6.71 (1H, d, J = 8.2 Hz, H-5′), 6.92 (1H, dd, J = 2.1, 8.1 Hz, H-6′), 6.98 (1H, s, H-2′), 7.37 (1H, d, J = 15.9 Hz, H-3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 115.1 (C-6′), 115.6 (C-3′), 116.2 (C-2), 121.6 (C-2′), 126.2 (C-1′), 145.1 (C-3), 146.0 (C-5′), 148.6 (C-4′), 168.4 (C-1); EIMS: *m/z* 180 [M^+, $C_9H_8O_4$].

DPPH Radical Scavenging Activity

The DPPH free radical scavenging assays of phytochemicals were investigated as a previous method with slight modifications [13, 14]. Stock solutions of each sample was diluted to final concentrations of 100 µM. Then, a total of 3.8 mL of 50 µM DPPH methanolic solution was added to 0.2 mL of each sample solution and allowed to react at room temperature for 30 min. The absorbance of the mixtures was measured at 517 nm. A control was prepared without sample or standard and measured immediately at 0 min. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity, and vice versa. Inhibitions of DPPH radical in percent (I%) were calculated as follows:

$$I\% = [A_{blank} - A_{sample} / A_{blank}] \times 100;$$

where A_{blank} is the absorbance value of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance value of the test compounds. The sample concentration that provides 50% inhibition (IC_{50}) was calculated by plotting the inhibition percentages against concentrations of the sample. All tests were carried out in triplicate and IC_{50} values were reported as means ± SD of triplicate. Ascorbic acid was used as a standard and diluted to the same concentration as the samples.

Acetylcholinesterase Activity

The phytochemicals were evaluated for their acetylcholinesterase activity by assessing their AChE inhibitory effects. The spectrophotometric method was used with modifications [15, 16]. AChE from *Electrophorus electricus* and acetylthiocholine iodide wase used as substrate. The acetylcholinesterase activity was determined using 5,5′-dithiobis(2-nitrobenzoic acid (DTNB). In brief, a 96-well microplate was used to combine 140 µL of sodium phosphate buffer (pH 8.0), 20 µL of DTNB, 20 µL of compounds, and 20 µL of AChE (0.22 U/mL) solution. This mixture was incubated at 25°C for 15 min before adding 10 µL of acetylthiocholine iodide to initiate the reaction. The hydrolysis of acetylthiocholine iodide was measured by monitoring the formation of the yellow 5-thio-2-nitrobenzoate anion at 412 nm using a 96-well microplate reader (Epoch Micro-Volume Spectrophotometer, USA). The inhibition percentage (I%) of AChE was calculated by comparing the reaction rates relative to the blank sample (EtOH in phosphate buffer, pH 8) using the formula: I% = [E - S / E] × 100; where E is the enzyme activity without the test sample and S is the enzyme's activity with the test sample. All tests were carried out in triplicate and IC_{50} values were reported as means ± SD of triplicate. Galantamine was used as a positive control at the same concentration as the compounds.

RESULTS AND DISCUSSION

In this study, we successfully isolated eight compounds from the stem bark of *G. urophylla*, which were characterized as α-mangostin (**1**), β-mangostin (**2**), β-amyrin (**3**), lupeol (**4**), lupeol acetate (**5**), ferulic acid (**6**), syringic acid (**7**), and caffeic acid (**8**). They were all identified by analyzing their spectroscopic data and comparing them with the reported literature. Their chemical structures are shown in Figure 1.

Compound (**1**) was identified as α-mangostin and showed characteristic signals in the NMR spectrum confirming its identity. The presence of prenylated xanthone group was confirmed with the occurrence of doublet signals at δ 3.47 (J = 5.0 Hz) and 4.11 (J = 5.0 Hz) abbreviated to methylene groups of H-11 and H-16, respectively. Besides, one singlet at δ 3.83 (7-OCH_3) which was contributed to one methoxyl group. The presence of this methoxyl group was further confirmed by the corresponding carbon signals in the ^{13}C NMR spectrum at δ 62.8.

Compound (**2**) was identified as β-mangostin, it was closely resembled to the ^{1}H NMR spectrum of compound (**1**) by replacement of one hydroxyl group at C-3 with a methoxyl group. These assignments were further confirmed by the HMQC spectrum, which displayed correlations between the methoxyl signal at δ 3.93 with their carbon signal at δ 55.8.

Compound (**3**) was identified as β-amyrin, which exhibited characteristic signals in the NMR spectra, confirming its structure. A proton of H-12 was identified as having a distinctive double of the doublet at δ 5.20 (J = 3.7 Hz), indicating the presence of an olean-12-ene structure. A methine proton of H-3 was observed at δ 3.25, which appeared as the doublet of doublet signal indicating the presence of at least one hydroxyl group in the structure. This methine proton's coupling constant (J = 4.5 and 11.1 Hz) suggests the hydroxyl group must be oriented axially.

Compound (**4**) was identified as lupeol, displaying characteristic signals in its NMR spectrum, revealing the presence of seven methyl groups represented as singlets at δ 0.75 (H-28), 0.78 (H-25), 0.82 (H-27), 0.93 (H-23), 0.96 (H-24), 1.02 (H-26) and 1.67 (H-30). In addition, a doublet of doublets at δ 3.18 (J = 11.2 and 5.4 Hz) was attributable to the oxymethine, H-3. Besides, it also showed two singlets at δ 4.56 and 4.68 representing the non-equivalent protons of an exocyclic double bond, H-29a/H-29b, respectively.

Compound (**5**) was identified as lupeol acetate, which exhibited characteristic signals in its NMR spectra, confirming the presence of a proton signal at δ 2.06 and 4.48 were assignable to acetyl and oxymethine at H-2′ and H-3 respectively. Two broad singlet proton signals representing the exocyclic double bond protons were observed at δ 4.71 (H-29a) and 4.59 (H-29b). These proton signals differ from those of lupeol (**4**) by the presence of additional

acetyl groups and a downfield oxymethine proton at δ 2.06 (H-2′) and 4.48 (H-3), respectively.

Compound (**6**) was identified as ferulic acid, it exhibited a singlet at δ 3.93 indicative of a methoxyl group. Aromatic proton signals were identified as a doublet at δ 6.93 (J = 8.8 Hz, H-5′) and δ 7.08 (J = 1.9 Hz, H-2′), along with a doublet of doublets at δ 7.10 (J = 2.0 and 8.1 Hz, H-6′). Additionally, a doublet was observed at δ 7.69 and δ 6.30, corresponding to the olefinic protons H-3 and H-2, respectively. The *trans*-configuration of these protons was confirmed by a coupling constant of 15.0 Hz.

Compound (**7**) was identified as syringic acid, it was almost identical with the ^{1}H NMR spectrum of compound (**6**). The differences between both spectra were the replacement of the methoxyl group with a hydroxyl group at C-3′. The hydroxyl signal was confirmed by the appearance of a broad singlet at δ 3.32.

Compound (**8**) was identified as caffeic acid, displaying characteristic signals in its NMR spectra, confirming the presence of a singlet signal at δ 7.32 that corresponded to meta-coupled protons of H-2 and H-6. In addition, another singlet signal appeared at δ 3.88 attributed to two methoxyl groups at C-3 and C-5. The ^{13}C NMR spectrum revealed the presence of nine carbons. The methoxy group carbon signal was distinctly assigned at δ 56.4 (3/5-OCH_3), while the carbonyl carbon was observed at δ 170.7 (C-1′).

Compounds (**1**) and (**2**) were isolated previously from G. cowa [17] and *G. mangostana* [18]. Besides, compounds (**3**) and (**4**) were isolated previously from *G. hanburyi* [19] and *G. vilersiana* [20], whereas compound (**5**) from *G. hombroniana* [21]. In addition, compound (**6**) and (**8**) was found from *G. mangostana* [22], while compound (**7**) was from *G. indica* [23].

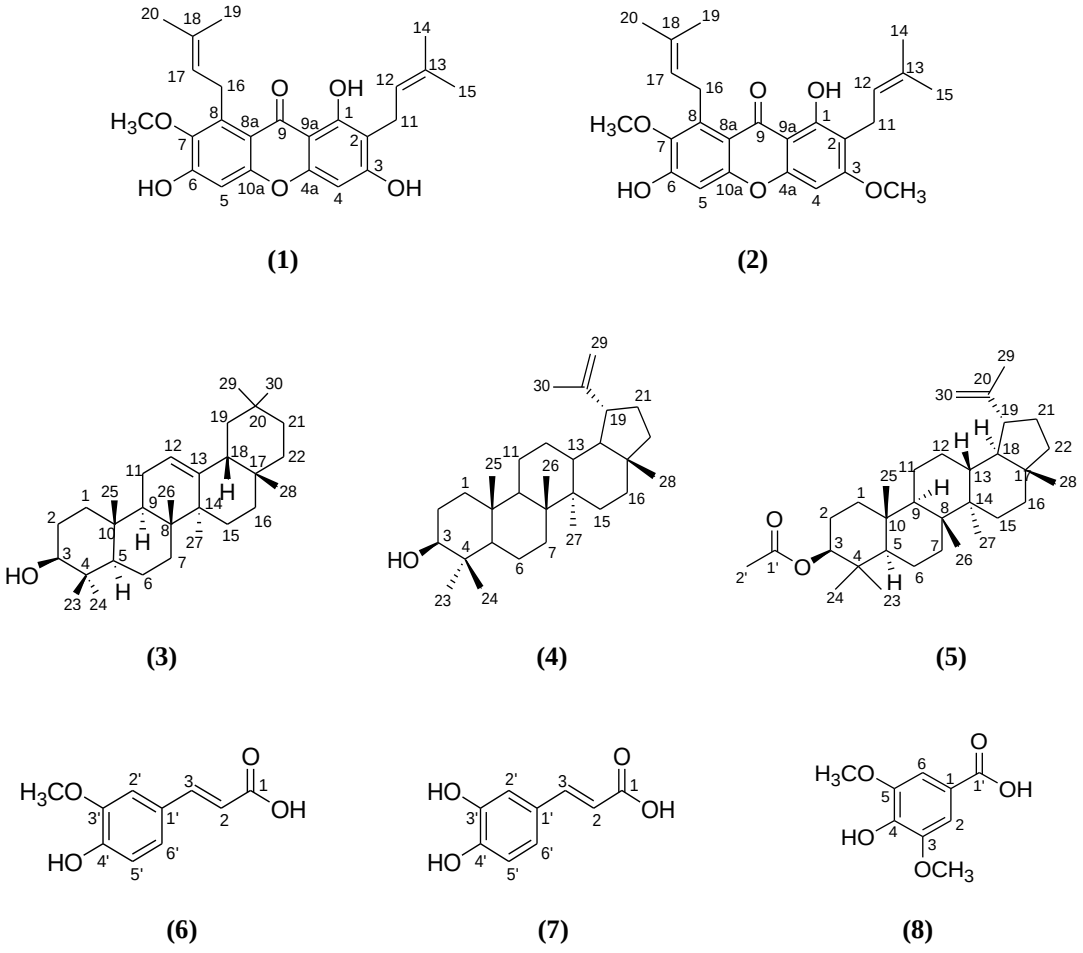

Figure 1. Chemical structures of isolated compounds from *G. urophylla*

Table 1. DPPH radical scavenging and acetylcholinesterase activities isolated phytochemicals.

Compounds	DPPH Radical Scavenging		Acetylcholinesterase	
	Inhibition (%) at 100 µM	IC_{50} (µM)	Inhibition (%) at 100 µM	IC_{50} (µM)
α-Mangostin (**1**)	85.5 ± 0.2	15.2 ± 0.5	78.3 ± 1.0	18.0 ± 1.0
β-Mangostin (**2**)	80.7 ± 0.6	18.5 ± 1.2	66.9 ± 1.2	25.7 ± 1.2
β-Amyrin (**3**)	23.4 ± 1.0	>100	34.5 ± 1.5	>100
Lupeol (**4**)	20.8 ± 0.5	>100	29.4 ± 1.0	>100
Lupeol acetate (**5**)	22.6 ± 0.6	>100	30.2 ± 1.2	>100
Ferulic acid (**6**)	65.3 ± 1.0	40.5 ± 0.5	42.7 ± 0.8	>100
Syringic acid (**7**)	55.8 ± 0.8	62.7 ± 1.2	50.8 ± 0.3	92.6 ± 1.0
Caffeic acid (**8**)	72.2 ± 1.2	28.4 ± 1.5	62.4 ± 0.8	76.3 ± 0.7
Ascorbic acid	92.8 ± 0.2	3.5 ± 0.2	-	-
Galantamine	-	-	85.5 ± 1.1	0.9 ± 0.5

In this study, the antioxidant and acetylcholinesterase inhibitory activities of eight compounds were evaluated to assess their potential as bioactive molecules. The results are shown in Table 1. The antioxidant activities of the compounds were evaluated using the DPPH assay, which measures the ability of compounds to scavenge free radicals. The results showed a significant variation in radical scavenging ability across the tested compounds. α-Mangostin (**1**) and β-mangostin (**2**) exhibited strong antioxidant activities, with scavenging percentages of 85.5% and 80.7%, respectively, at 100 µM. These results suggest that xanthones, such as α- and β-mangostin, are potent antioxidants, likely due to their phenolic structure, which facilitates electron donation and free radical stabilization. This aligns with previous studies showing that mangostin derivatives possess robust antioxidant activity, attributed to their ability to neutralize free radicals efficiently [24]. Caffeic acid (**8**) and ferulic acid (**6**) also showed considerable antioxidant activity, with 72.2% and 65.3% radical scavenging at 100 µM, respectively. These compounds belong to the class of phenolic acids, which are known for their antioxidant potential due to the hydroxyl groups on their aromatic rings. Syringic acid (**7**), another phenolic compound, exhibited moderate activity with 55.8% DPPH scavenging at 100 µM. Its antioxidant potency is lower than that of caffeic and ferulic acids, which may be due to structural differences or steric hindrance affecting its electron-donating capacity [25]. β-Amyrin (**3**), lupeol (**4**), and lupeol acetate (**5**), which are triterpenoids, showed significantly lower antioxidant activity, with DPPH scavenging percentages of 23.4%, 20.8%, and 22.6%, respectively. These compounds have fewer hydroxyl groups compared to the phenolic compounds, which likely contributes to their weaker radical scavenging activity. The presence of fewer functional groups that can donate electrons might explain the reduced effectiveness in scavenging DPPH radicals. These findings are consistent with literature reports indicating that triterpenes generally show weaker antioxidant properties compared to polyphenolic compounds [26].

Acetylcholinesterase (AChE) inhibition is a critical mechanism for the treatment of neuro-degenerative diseases such as Alzheimer's disease. In this study, the acetylcholinesterase inhibitory activity was assessed using the Ellman method. α-Mangostin (**1**) and β-mangostin (**2**) were the most effective inhibitors, with inhibition rates of 78.3% and 66.9% at 100 µM, respectively. The high AChE inhibitory activities of these compounds can be attributed to the presence of xanthone and related functional groups in their structures, which have been previously reported to possess neuroprotective effects. The xanthone core could interact with the active site of the enzyme, blocking acetylcholine hydrolysis and enhancing cholinergic function [27]. Syringic acid (**7**) and caffeic acid (**8**), also showed notable AChE inhibition of 50.8% and 62.4% at 100 µM. This moderate activity is consistent with reports that certain phenolic acids can inhibit AChE, though they may be less potent than other standard inhibitors like donepezil. However, ferulic acid (**6**) demonstrated weaker acetylcholinesterase inhibition (42.7% at 100 µM), which may be due to its less optimal binding to the enzyme's active site [28]. Furthermore, β-amyrin (**3**), lupeol (**4**), and lupeol acetate (**5**) showed minimal AChE inhibitory activity, with inhibition percentages below 35%. This result is consistent with studies on triterpenoids, which often exhibit weak to no significant activity against acetylcholinesterase. The absence of hydroxyl or other functional groups capable of interacting effectively with the enzyme may explain their low inhibitory activity [29].

CONCLUSION

In the present study, the phytochemical investigation of the stem bark of *G. urophylla* yielded two xanthones, three phenolics, and three triterpenes. Additionally, the compounds tested exhibit varied antioxidant and acetylcholinesterase inhibitory activities. α-Mangostin and β-mangostin showed promising dual activity in both antioxidant and acetylcholinesterase inhibition assays, making them strong candidates for further development as potential neuroprotective agents. Caffeic acid and ferulic acid also demonstrated moderate activity in both assays, while the triterpenoids (β-amyrin, lupeol, lupeol acetate) showed weaker activities overall. These findings suggest that compounds with phenolic or xanthone structures are more likely to possess potent antioxidant and AChE inhibitory effects, which may be valuable for therapeutic applications in neurodegenerative diseases. Further studies, including in vivo experiments and structural modifications, could enhance the bioactivity of these compounds.

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