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Research Article

Isolation of tyrosinase inhibitors from *Artocarpus heterophyllus* and use of its extract as antibrowning agent

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A new furanoflavone, 7-(2,4-dihydroxyphenyl)-4-hydroxy-2-(2-hydroxy propan-2-yl)-2, 3-dihydrofuro(3, 2-g)chromen-5-one (artocarpfuranol, 1), together with 14 known compounds, dihydromorin (2), steppogenin (3), norartocarpetin (4), artocarpanone (5), artocarpesin (6), artocarpin (7), cycloartocarpin (8), cycloartocarpesin (9), artocarpetin (10), brosimone I (11), cudraflavone B (12), carpachromene (13), isoartocarpesin (14), and cyanomaclurin (15) were isolated from the wood of *Artocarpus heterophyllus*. Their structures were identified by interpretation of MS, ^{1}H-NMR, ^{13}C-NMR, HMQC, and HMBC spectroscopic data. Among them, compounds 1 – 6 and 14 showed strong mushroom tyrosinase inhibitory activity with IC_{50} values lower than 50 μM, more potent than kojic acid (IC_{50} = 71.6 μM), a well-known tyrosinase inhibitor. In addition, extract of *A. heterophyllus* was evaluated for its antibrowning effect on fresh-cut apple slices. It was discovered that fresh-cut apple slices treated by dipping in solution of 0.03 or 0.05% of *A. heterophyllus* extract with 0.5% ascorbic acid did not undergo any substantial browning reaction after storage at room temperature for 24 h. The antibrowning effect was significantly better than samples treated with the extract (0.03 or 0.05%) or ascorbic acid (0.5%) alone. The results provide preliminary evidence supporting the potential of this natural extract as antibrowning agent in food systems.

Keywords: Antibrowning agent / *Artocarpus heterophyllus* / Furanoflavone / Tyrosinase inhibition

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1 Introduction

It is well established that polyphenol oxidases (tyrosinase)-catalyzed browning reactions can lead to deleterious changes in the appearance and organoleptic properties of food products, resulting in shorter shelf-life. Unfavorable browning of raw fruits, vegetables, and beverages is a major problem in the food industry and is believed to be one of the main causes of quality loss [1]. Application of polyphenol oxidase inhibitors has been one of the most popular and desirable strategies adopted by the food industry to prevent food browning. In the identification of inhibitors for this purpose, many of the challenges pertain to the simultaneous requirements of such inhibitors to be effective and free from health hazards. As a consequence, inhibitors from natural sources represent the most important candidates for researchers to embark on. Kojic acid [2], arbutin, and glabridin [3] are well-known examples. The majority of previous studies on identification of tyrosinase inhibitors were related to the use of a single chemical agent. Recent reports tend to support the use of a combination of chemicals for achieving a synergistic effect. The most common combination has been a potent inhibitor plus ascorbic acid which likely serves as a reducing agent in the action mechanism [4, 5]. Assisted by the availability of a wide range of separation and identification techniques, the academia, and various commercial bodies have been constantly searching for more potent and safe tyrosinase inhibitors to accommodate the evolving demands from customers for ever better quality products.

Artocarpus heterophyllus, mainly grown in the tropical and subtropical regions, has been used in traditional folk medicine in Indonesia against inflammation and malarial fever [6]. Several previous studies reported the characterization of prenylflavonoids from *A. heterophyllus* [7 – 9] and their anti-inflammatory [10], antioxidant [11, 12], melanin-biosynthesis inhibitory [13], and antiplatelet [14]

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activities. In addition, extract of the wood of *A. heterophyllus* was reported to inhibit tyrosinase activity [15, 16]. But the action of its extract in food products has not yet been further examined. Neither has systematic phytochemical analysis been done to characterize the components that contribute to the tyrosinase inhibitory effect of this extract. As part of our continuing search for natural tyrosinase inhibitory agents, activity-guided (tyrosinase inhibition assay) phytochemical analysis was carried out for the wood of *A. heterophyllus*, which led to the isolation of several active compounds, including one new compound. Another aim of this study was to evaluate the potential of *A. heterophyllus* extract as antibrowning agent in practical applications. On the basis of Li *et al.*'s method [5], fresh-cut apple slices were chosen as the food model and antibrowning effect of *A. heterophyllus* extract was assessed by monitoring changes in a^*, b^*, and L^* values of the apple slices after treatment with extract solution relative to the control.

2 Materials and methods

2.1 Plant material

Fresh woods of *A. heterophyllus* were collected from Wenchang County, Hainan Province, P. R. China, in October 2006. A voucher specimen (accession number 20061001) was deposited at the School of Biological Sciences, The University of Hong Kong. Fuji apple cultivar was chosen for investigation because of its popularity among different apple cultivars available on the market. The apples were obtained from a local supermarket and stored at 4°C until used for analysis.

2.2 Chemicals and instruments

Mushroom tyrosinase (3900 u/mg), L-tyrosine, and boric acid were purchased from Sigma Chemical (St. Louis, USA). HPLC grade solvents were purchased from BDH (Poole, UK). Sephadex LH-20 was purchased from GE Healthcare Bio-Sciences AB (Uppsala, Sweden). MCI gel CHP 20P (75 – 150 μ) was purchased from Mitsubishi Chemical (Tokyo, Japan). LiChroprep® RP-18 (40 – 63 μm) was purchased from Merck (Darmstadt, Germany). Silica gel (200 – 300 mesh) for column chromatography was obtained from Qingdao Marine Chemical Company (Qingdao, P. R. China). TLC plates (25 DC-platten Kieselgel 60 F_{254}) were obtained from Merck. Semipreparative HPLC system was carried out on a Waters 600 system equipped with a 2487 dual-wavelength detector, a Masslynx V4.0 software and a Phenomenex Luna C18 (2) column (250 × 21.2 mm, 5 μm). ^{1}H-NMR, ^{13}C-NMR, and NOESY spectra were obtained on a Bruker 500 DRX NMR Spectrometer and Varian Mercury-VX300 Fourier spectrometer. LC-MS was run on an Agilent LC-MSD system equipped with an ESI source, Bruker Daltonics 4.0, and Data analysis 4.0 software. Tyrosinase inhibitory activity was monitored on a UV-1206 Spectrophotometer (Shimadzu, Japan). Visual assessment of color development in the samples was performed with a digital camera while the relative extents of browning were measured using a Chroma meter CR-400 (Konica Minolta Sensing, Japan).

2.3 Extraction and isolation

Wood of *A. heterophyllus* (2 kg) was ground using a minigrinder (Model: DF-15, Shenzhen Laitong Company, Shenzhen, P. R. China) and packed into a paper extraction bag. Extraction was performed by maceration in 95% ethanol (3 × 10 L) at room temperature in a traditional Chinese medicine extractor (Model: YFX20T, Donghuayuan Medical Company, Beijing, P. R. China). The extract was concentrated under vacuum at 45°C on a rotary evaporator. The dried extract (62.6 g) was subjected to silica gel (200 – 300 mesh) column chromatography by successive elution with different proportions of $CHCl_3$/MeOH, 100:0, 30:1, 10:1, and 5:1. Each gradient consisted of 3 L solvent with the first gradient collected in six fractions (Fr. 1 – 6), the second in ten fractions (Fr. 7 – 16), the third in one fraction (Fr. 17), and the fourth in four fractions (Fr. 18 – 21). This gave altogether 21 fractions (Fr. 1 – 21) which were dried on a rotary evaporator, respectively. Fr. 2 (6.57 g) was further purified by silica gel column chromatography (thin-lay silica gel H) using chloroform as mobile phase to obtain artocarpin (7) (3147.9 mg) and cycloartocarpin (8) (379.3 mg). Fr. 3 and 4 were combined (3.2 g) and further purified by Sephadex LH-20 column chromatography eluted with CH_3OH to give five sub-fractions (sub-fr. 1 – 5). Sub-fr.1 was further purified by semipreparative HPLC (MeOH/H_2O 80:20) to offer brosimone I (11) (151.4 mg). Semipreparative HPLC of sub-fr. 3 (MeOH/H_2O 75:25) led to cudraflavone B (12) (11.2 mg) and that (MeOH/H_2O 80:20) of sub-fr. 5 gave isoartocarpesin (14) (2.5 mg). Combined fraction from Fr. 5 and 6 (2.25 g) was separated by MCI gel chromatography successively eluted with 50:50, 80:20, and 100:0 MeOH/H_2O to offer eight sub-fractions (sub-fr. 1 – 8). Sub-fr. 3 was purified by silica gel (200 – 300 mesh) chromatography eluted with ethyl acetate/*n*-hexane 1:2 to offer artocarpanone (5) (75.5 mg). Fr. 7 – 16 were combined (7.26 g) and separated on MCI gel column by successive elution with MeOH/H_2O of the following ratios: 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, and 100:0, each of 500 mL to offer seven sub-fractions (sub-fr. 1 – 7). Sub-fr. 3 and 4 were purified on Sephadex LH-20 column (mobile phase: MeOH/H_2O 50:50) to offer steppogenin (3) (580 mg) and norartocarpetin (4) (610.6 mg), respectively. Sub-fr. 5 was purified by semipreparative HPLC (gradient elution, 60 – 100% MeOH, 0 – 30 min) to offer artocarpesin (6) (133.1 mg), artocarpetin (10) (5.4 mg), cycloartocarpesin (9) (196.0 mg), and carpachromene (13) (16.2 mg). Fr. 17 (1.38 g) was subjected to MCI gel chromatography eluted successively

with 50:50, 70:30, 80:20, 90:10, and 100:0 MeOH/H_2O to offer five sub-fractions, respectively. Sub-fr. 1 was further separated by silica gel (thin-lay silica gel H) chromatography ($CHCl_3$/MeOH 15:1) to offer dihydromorin (2) (382 mg). Sub-fr. 2 was dried by vacuum evaporation and then re-dissolved in methanol. Precipitation was formed on standing of the solution under room conditions. After filtration, solids of compound 1 (19.5 mg) were obtained. Combined fraction from Fr. 8–21 (4.6 g) was chromatographically separated on MCI gel column eluted successively with 35:65, 50:50, 70:30, and 100:0 MeOH/H_2O to offer four sub-fractions, respectively. Sub-fr. 1 was further purified by silica gel (200–300 mesh) chromatography ($CHCl_3$/MeOH 6:1) to give cyanomaclurin (15) (1984.3 mg).

2.4 Spectral data

Dihydromorin (2): colorless needles, ^{1}H NMR (CD_3OD, 500 MHz) δ = 7.20 (1H, d, *J* = 8.9 Hz, H-6′), 6.34 (1H, dd, *J* = 8.9, 2.4 Hz, H-5′), 6.34 (1H, d, *J* = 2.2 Hz, H-3′), 5.89 (1H, d, *J* = 2.1 Hz, H-8), 5.85 (1H, d, *J* = 2.1 Hz, H-6), 5.37 (1H, d, *J* = 11.4 Hz, H-2), 4.77 (1H, d, *J* = 11.4 Hz, H-3); ^{13}C NMR (CD_3OD, 125 MHz) δ = 199.1 (s, C-4), 168.7 (s, C-5), 165.5 (s, C-9), 165.1 (s, C-7), 160.3 (s, C-4′), 158.8 (s, C-2′), 131.0 (d, C-6′), 115.7 (s, C-1′), 108.0 (d, C-5′), 103.8 (d, C-3′), 102.1 (s, C-10), 97.3 (d, C-8), 96.4 (d, C-6), 80.2 (d, C-2), 72.7 (d, C-3); ESI-MS: *m*/*z* 305 $[M + H]^+$ ($C_{15}H_{12}O_7$).

Steppogenin (3): white powders, ^{1}H NMR (CD_3OD, 500 MHz) δ = 7.22 (1H, d, *J* = 8.2 Hz, H-6′), 6.33 (1H, dd, *J* = 8.2, 2.4 Hz, H-5′), 6.31 (1H, d, *J* = 2.1 Hz, H-3′), 5.90 (1H, d, *J* = 2.2 Hz, H-8), 5.86 (1H, d, *J* = 2.2 Hz, H-6), 5.59 (1H, dd, *J* = 13.2, 2.9 Hz, H-2), 3.06 (1H, dd, *J* = 17.2, 13.1 Hz, H-3_a), 2.69 (1H, dd, *J* = 17.2, 2.9 Hz, H-3_b); ^{13}C NMR (DMSO-d_6, 75 MHz) δ = 196.9 (s, C-4), 166.6 (s, C-7), 163.6 (s, C-5), 163.5 (s, C-9), 158.7 (s, C-2′), 155.8 (s, C-4′), 128.4 (d, C-6′), 115. 4 (s, C-1′), 106.5 (d, C-5′), 102.4 (d, C-3′), 101.7 (s, C-10), 95.7 (d, C-6), 94.9 (d, C-8), 73.9 (d, C-2), 41.1 (t, C-3); ESI-MS: *m*/*z* $[M + H]^+$ 289 ($C_{15}H_{12}O_6$).

Norartocarpetin (4): yellow powders, ^{1}H NMR (DMSO-d_6, 300 MHz) δ = 6.15 (1H, d, *J* = 1.8 Hz, H-6), 6.42 (1H, dd, *J* = 3.0, 8.7 Hz, H-5′), 6.48 (1H, d, *J* = 1.8 Hz, H-8), 6.43 (1H, d, *J* = 3.0 Hz, H-3′), 7.00 (1H, s, H-3), 7.75 (1H, d, *J* = 8.7 Hz, H-6′); ^{13}C NMR (DMSO-d_6, 75 MHz) δ = 181.9 (s, C-4), 164.2 (s, C-7), 161.8 (s, C-4′), 161.8 (s, C-2), 161.4 (s, C-9), 159.0 (s, C-5), 157.3 (s, C-2′), 129.8 (d, C-6′), 108.5 (s, C-1′), 108.0 (d, C-3), 106.7 (d, C-5′), 103.6 (s, C-10), 103.2 (d, C-3′), 98.6 (d, C-6), 93.8 (d, C-8); ESI-MS: *m*/*z* 287 $[M + H]^+$ ($C_{15}H_{10}O_6$).

Artocarpanone (5): white powders, ^{1}H NMR (CD_3OD, 500 MHz) δ = 7.22 (1H, d, *J* = 8.0 Hz, H-6′), 6.33 (1H, dd, *J* = 8.0, 2.3 Hz, H-5′), 6.32 (1H, d, *J* = 2.3 Hz, H-3′), 6.04 (1H, d, *J* = 2.3 Hz, H-8), 6.02 (1H, d, *J* = 2.3 Hz, H-6), 5.62 (1H, dd, *J* = 13.2, 3.0 Hz, H-2), 3.80 (3H, s, OMe), 3.09 (1H, dd, *J* = 17.2, 13.2 Hz, H-3_a), 2.72 (1H, dd, *J* = 17.2, 3.0 Hz, H-3_b); ^{13}C-NMR (CD_3OD, 125 MHz) δ = 199.1 (s, C-4), 169.6 (s, C-7), 165.4 (s, C-4), 165.4 (s, C-9), 160.0 (s, C-5), 157.0 (s, C-2′), 129.1 (d, C-6′), 117. 9 (s, C-1′), 107.9 (d, C-5′), 104.2 (s, C-10), 103.6 (d, C-3′), 95.8 (d, C-8), 95.0 (d, C-6), 76.3 (d, C-2), 56.4 (q, OMe), 43.3 (t, C-3); ESI-MS: *m*/*z* 303 $[M + H]^+$ ($C_{16}H_{14}O_6$).

Artocarpesin (6): yellow powders, ^{1}H NMR (DMSO-d_6, 300 MHz) δ = 1.62 (3H, s, H-4″), 1.72 (3H, s, H-5″), 3.18 (2H, br d, *J* = 6.6 Hz, H-1″), 5.17 (1H, br t, *J* = 6.6 Hz, H-2″), 6.43 (1H, dd, *J* = 8.7, 2.1 Hz, H-5′), 6.48 (1H, d, *J* = 1.8 Hz, H-3′), 6.48 (1H, s, H-8), 6.98 (1H, s, H-3), 7.73 (1H, d, *J* = 8.7 Hz, H-6′), 10.75 (s, OH), 10.17 (s, OH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ = 181.9 (s, C-4), 161.7 (s, C-7), 161.7 (s, C-2), 161.5 (s, C-9), 158.6 (s, C-4′), 158.3 (s, C-2′), 155.1 (s, C-5), 130.7 (s, C-3″), 129.8 (d, C-6′), 122.3 (d, C-2″), 110.6 (s, C-1′), 108.7 (s, C-6), 108.1 (d, C-5′), 106.8 (d, C-3′),103.3 (s, C-10), 103.2 (d, C-3), 93.1 (d, C-8), 25.6 (q, C-4″), 21.0 (t, C-1″), 17.8 (q, C-5″); ESI-MS: *m*/*z* 355 $[M + H]^+$ ($C_{20}H_{18}O_6$).

Artocarpin (7): yellow powders, ^{1}H NMR (CD_3OD, 500 MHz) δ = 1.06 (3H, d, *J* = 6.8 Hz, H-5‴), 1.06 (3H, d, *J* = 6.8 Hz, H-4‴), 1.38 (3H, s, H-4″), 1.56 (3H, s, H-5″), 2.38 (1H, m, H-3‴), 3.07 (2H, d, *J* = 7.0 Hz, H-1″), 3.83 (3H, s, OCH_3), 5.08 (1H, br t, *J* = 7.1 Hz, H-2″), 6.38 (1H, dd, *J* = 1.0, 8.3 Hz, H-5′), 6.40 (1H, s, H-8), 6.51 (1H, d, *J* = 1.0 Hz, H-3′), 6.49 (1H, d, *J* = 16.2 Hz, H-1‴), 6.59 (1H, dd, *J* = 16.2, 7.1 Hz, H-2‴), 7.06 (1H, d, *J* = 8.3 Hz, H-6′); ^{13}C NMR (CD_3OD, 125 MHz) δ = 183.9 (s, C-4), 163.6 (s, C-7), 161.9 (s, C-4′), 159.0 (s, C-5), 159.7 (s, C-9), 158.0 (s, C-2), 157.9 (s, C-2′), 142.9 (d, C-2‴), 132.8 (s, C-3″), 132.6 (d, C-6′), 122.9 (d, C-2″), 122.3 (s, C-3), 117.3 (d, C-1″), 113.4 (s, C-1′), 110.5 (s, C-6), 108.1 (d, C-5′), 106.0 (s, C-10), 103.8 (d, C-3′), 90.8 (d, C-8), 34.5 (d, C-3‴), 26.0 (q, C-4″), 25.1 (t, C-1″), 23.4 (q, C-4‴), 23.4 (q, C-5‴), 17.8 (q, C-5″); ESI-MS: *m*/*z* 437 $[M + H]^+$ ($C_{26}H_{28}O_6$).

Cycloartocarpin (8): yellow powders, ^{1}H NMR (CD_3OD, 500 MHz) δ = 1.08 (3H, d, *J* = 6.8 Hz, H-5‴), 1.08 (3H, d, *J* = 6.8 Hz, H-4‴), 1.70 (3H, d, *J* = 1.0 Hz, H-4″), 1.93 (3H, d, *J* = 1.1 Hz, H-5″), 2.40 (1H, m, H-3‴), 3.89 (3H, s, OCH_3), 5.40 (1H, d, *J* = 9.4 Hz, H-2″), 6.11 (1H, d, *J* = 9.4 Hz, H-1″), 6.30 (1H, d, *J* = 2.2 Hz, H-3′), 6.47 (1H, d, *J* = 16.2 Hz, H-1‴), 6.50 (1H, dd, *J* = 2.2, 8.6 Hz, H-5′), 6.54 (1H, s, H-8), 6.61 (1H, dd, *J* = 7.0, 16.2 Hz, H-2‴), 7.59 (1H, d, *J* = 8.6 Hz, H-6′); ^{13}C NMR (CD_3OD, 125 MHz) δ = 179.7 (s, C-4), 164.8 (s, C-4′), 164.1 (s, C-7), 159.9 (s, C-5), 159.6 (s, C-2′), 157.2 (s, C-2), 156.8 (s, C-9), 143.1 (d, C-2‴), 139.9 (s, C-3″), 126.5 (d, C-6′), 122.7 (d, C-2‴), 117.2 (d, C-1‴), 111.2 (d, C-5′), 111.0 (s, C-1′), 110.6 (s, C-6), 108.6 (s, C-3), 106.4 (s, C-10), 105.2 (d, C-3′), 91.2 (d, C-8), 70.9 (d, C-1″), 34.5 (d, C-3‴), 26.2 (q, C-4″), 23.4 (q, C-4‴), 23.4 (q, C-5‴), 18.9 (q, C-5″); ESI-MS: *m*/*z* 435 $[M + H]^+$ ($C_{26}H_{26}O_6$).

Cycloartocarpesin (9): yellow powders, ^{1}H NMR (DMSO-d_6, 300 MHz) δ = 1.51 (6H, s, H-4″, 5″), 5.75 (1H,

d, J = 9.9 Hz, H-2″), 6.43 (1H, dd, J = 8.7, 2.7 Hz, H-5′), 6.48 (1H, d, J = 2.7 Hz, H-3′), 6.51 (1H, s, H-8), 6.57 (1H, d, J = 9.9 Hz, H-1″), 7.03 (1H, s, H-3), 7.76 (1H, d, J = 8.7 Hz, H-6′), 10.82 (OH, s), 10.23 (OH, s); ^{13}C NMR (DMSO-d_6, 75 MHz) δ = 182.1 (s, C-4), 162.0 (s, C-7), 162.0 (s, C-5), 159.0 (s, C-2′), 158.5 (s, C-2), 156.4 (s, C-4′), 155.5 (s, C-9), 129.8 (d, C-6′), 128.8 (d, C-2″), 114.6 (d, C-1″), 108.4 (s, C-1′), 108.1 (d, C-5′), 106.8 (d, C-3), 103.2 (d, C-3′), 104.5 (s, C-6, 10), 103.2 (d, C-3′), 94.8 (d, C-8), 77.9 (d, C-3″), 27.8 (q, C-4″), 27.8 (q, C-5‴); ESI-MS: m/z 353 $[M + H]^+$ ($C_{20}H_{16}O_6$).

Artocarpetin (10): yellow powders, ^{1}H NMR (DMSO-d_6, 500 MHz) δ = 3.85 (3H, s, OCH_3), 6.34 (1H, d, J = 2.2 Hz, H-6), 6.43 (1H, dd, J = 8.8, 2.3 Hz, H-5′), 6.48 (1H, d, J = 2.3 Hz, H-3′), 6.72 (1H, d, J = 2.2 Hz, H-8), 7.04 (1H, s, H-3), 7.81 (1H, d, J = 8.8 Hz, H-6′); ^{13}C NMR (DMSO-d_6, 75 MHz) δ = 182.1 (s, C-4), 166.6 (s, C-7), 165.0 (s, C-4′), 162.0 (s, C-2), 161.1 (s, C-9), 159.2 (s, C-5), 157.3 (s, C-2′), 129.9 (d, C-6′), 108.4 (s, C-1′), 108.1 (d, C-3), 106.9 (d, C-5′), 104.5 (s, C-10), 103.3 (d, C-3′), 97.7 (d, C-6), 92.5 (d, C-8); ESI-MS: m/z 301 $[M + H]^+$ ($C_{16}H_{12}O_6$).

Brosimone I (11): yellow powders, ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 0.84 (3H, d, J = 6.9 Hz, H-5‴), 0.87 (3H, d, J = 6.9 Hz, H-4‴), 1.44 (3H, s, H-4″), 1.71 (3H, s, H-5″), 2.22 (1H, m, H-3″), 5.18 (1H, d, J = 9.3 Hz, H-2″), 5.93 (1H, d, J = 9.3 Hz, H-1″), 6.16 (1H, d, J = 1.8 Hz, H-3′), 6.31 (1H, d, J = 16.5 Hz, H-1‴), 6.25 (1H, dd, J = 8.7, 1.8 Hz, H-5′), 6.26 (1H, s, H-8), 6.48 (1H, dd, J = 16.2, 6.9 Hz, H-2‴), 7.34 (1H, d, J = 8.7 Hz, H-6′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 178.0 (s, C-4), 162.7 (s, C-7), 161.0 (s, C-9), 159.1 (s, C-4′), 157.5 (s, C-2), 157.4 (s, C-2′), 154.3 (s, C-5), 141.1 (d, C-2‴), 137.9 (d, C-3″), 124.7 (d, C-6′), 121.0 (d, C-2″), 115.8 (d, C-1‴), 109.7 (d, C-5′), 108.3 (s, C-6), 107.0 (s, C-3),104.0 (s, C-10), 103.9 (d, C-3′), 93.4 (d, C-8), 69.1 (d, C-1″), 32.6 (d, C-3‴), 25.5 (q, C-4″), 22.4 (q, C-4‴), 22.4 (q, C-5‴), 18.2 (q, C-5″); ESI-MS: m/z 421 $[M + H]^+$ ($C_{25}H_{24}O_6$).

Cudraflavone B (12): yellow powders, ^{1}H NMR (DMSO-d_6, 300 MHz) δ = 1.36 (3H, s, H-5″), 1.41 (6H, s, C-4‴, 5‴), 1.54 (3H, s, H-4″), 2.97 (2H, d, J = 6.9 Hz, H-1″), 5.01 (1H, br t, J = 6.9 Hz, H-2″), 5.77 (1H, d, J = 9.9 Hz, H-2‴), 6.34 (1H, dd, J = 8.4, 2.1 Hz, H-5′), 6.37 (1H, s, H-8), 6.43 (1H, d, J = 2.1 Hz, H-3′), 6.61 (1H, d, J = 9.9 Hz, H-1‴), 7.07 (1H, d, J = 8.4 Hz, H-6′), 9.82 (OH, s), 9.92 (OH, s); ^{13}C NMR (DMSO-d_6, 75 MHz) δ = 181.7 (s, C-4), 162.2 (s, C-7), 160.5 (s, C-2′), 158.6 (s, C-2), 156.9 (s, C-5), 156.4 (s, C-4′), 131.3 (d, C-6′), 131.1 (s, C-3″), 128.9 (d, C-2‴), 121.4 (d, C-2″), 120.1 (s, C-3), 114.3 (d, C-1″), 110.8 (s, C-1′), 106.8 (d, C-5′), 104.4 (s, C-10), 102.6 (d, C-3′), 90.8 (d, C-8), 34.5 (d, C-3‴), 26.0 (q, C-4″), 25.1 (t, C-1″), 23.4 (q, C-4‴), 23.4 (q, C-5‴), 17.8 (q, C-5″); ESI-MS: m/z 437 $[M + H]^+$ ($C_{26}H_{28}O_6$).

Carpachromene (13): yellow powders, ^{1}H NMR (DMSO, 300 MHz) δ = 1.48 (6H, s, H-4″, 5″), 5.75 (1H, d, J = 9.9 Hz, H-2″), 6.53 (1H, s, H-8), 6.56 (1H, d, J = 10.5 Hz, H-1″), 6.80 (1H, s, H-3), 6.92 (2H, d, J = 8.7 Hz, H-3′, 5′), 7.90 (2H, d, J = 8.7 Hz, H-2′, 6′), 10.45 (OH, s); ^{13}C NMR (DMSO, 75 MHz) δ = 182.0 (s, C-4), 164.0 (s, C-7), 161.4 (s, C-5), 158.6 (s, C-2), 156.4 (s, C-4′), 155.5 (s, C-9), 128.9 (d, C-2″), 128.5 (d, C-2′, 6′), 121.0 (s, C-1′), 116.0 (d, C-3′, 5′), 114.6 (d, C-1″), 104.8 (s, C-6), 104.6 (s, C-10), 102.9 (d, C-3), 95.0 (d, C-8), 77.9 (d, C-3″), 27.8 (q, C-4″), 27.8 (q, C-5‴); ESI-MS: m/z 337 $[M + H]^+$ ($C_{20}H_{16}O_5$).

Isoartocarpesin (14): yellow powders, ^{1}H NMR (CD_3OD, 500 MHz) δ = 1.10 (6H, d, J = 6.7 Hz, H-4″, 5″), 2.42 (1H, m, H-3″), 6.42 (1H, dd, J = 8.7, 2.3 Hz, H-5′), 6.44 (1H, d, J =2.3 Hz, H-3′), 6.46 (1H, s, H-8), 6.55 (1H, dd, J = 16.2, 1.0 Hz, H-1″), 6.69 (1H, dd, J = 16.2, 7.2 Hz, H-2″), 7.14 (1H, s, H-3), 7.75 (1H, d, J = 8.7 Hz, H-6′); ^{13}C NMR (CD_3OD, 125 MHz) δ = 184.7 (s, C-4), 164.0 (s, C-2), 163.5 (s, C-7), 163.5 (s, C-5), 160.5 (s, C-2′), 157.3 (s, C-9), 142.7 (d, C-2″), 131.0 (d, C-6′), 117.6 (d, C-1″), 110.9 (s, C-1′), 110.3 (s, C-6), 109.2 (d, C-5′), 108.4 (d, C-3), 105.1 (d, C-10),104.3 (s, C-3′), 94.4 (d, C-8), 34.5 (d, C-3″), 23.4 (q, C-4″, 5″); ESI-MS: m/z 355 $[M + H]^+$ ($C_{20}H_{18}O_6$).

Cyanomaclurin (15): colorless powders, ^{1}H NMR (CD_3OD, 500 MHz) δ = 7.10 (1H, d, J = 8.4 Hz, H-11), 6.34 (1H, dd, J = 8.4, 2.4 Hz, H-10), 6.18 (1H, d, J = 2.3 Hz, H-8), 5.89 (1H, d, J = 2.1 Hz, H-2), 5.74 (1H, d, J = 2.1 Hz, H-4), 5.23 (1H, dd, J = 3.4, 1.9 Hz, H-13), 4.99 (1H, t, J = 2.2 Hz, H-14), 4.18 (1H, t, J = 3.1 Hz, H-15); ^{13}C NMR (CD_3OD, 125 MHz) δ = 64.3 (d, C-14), 67.0 (d, C-15), 73.1 (d, C-13), 95.5 (d, C-4), 96.5 (d, C-2), 100.5 (s, C-10), 103.8 (d, C-8), 109.8 (d, C-10), 114.1 (s, C-6), 133.2 (d, C-11), 155.9 (s, C-5, 7), 159.9 (s, C-9), 160.5 (s, C-1), 160.7 (s, C-3). ESI-MS: m/z 289 $[M + H]^+$.

2.5 Mushroom tyrosinase inhibition assay

Tyrosinase inhibitory activity of *A. heterophyllus* extract and isolated compounds was determined by spectrophotometric method. The procedure followed that described by Vanni *et al*. [17]. All compounds were first dissolved in DMSO at 1.0 mg/mL and then diluted to different concentrations using DMSO. The above sample solution (30 μL) was diluted with 970 μL 0.05 mM sodium phosphate buffer (pH 6.8) in a test tube. This was followed by addition of 1 mL L-tyrosine solution (0.1 mg/mL) and finally 1.0 mL mushroom tyrosinase solution (200 U/mL) was added. After thorough mixing by vortex, initial absorbance at 490 nm was measured. The set of test solutions were then incubated for 20 min at 37°C before checking for change in their absorbance at the same wavelength (490 nm). Percent inhibition of tyrosinase activity was calculated as follows:

$$\% \text{ inhibition} = (A - B)/A \times 100$$

where A is the absorbance at 490 nm without test sample and B is the absorbance at 490 nm with test sample.

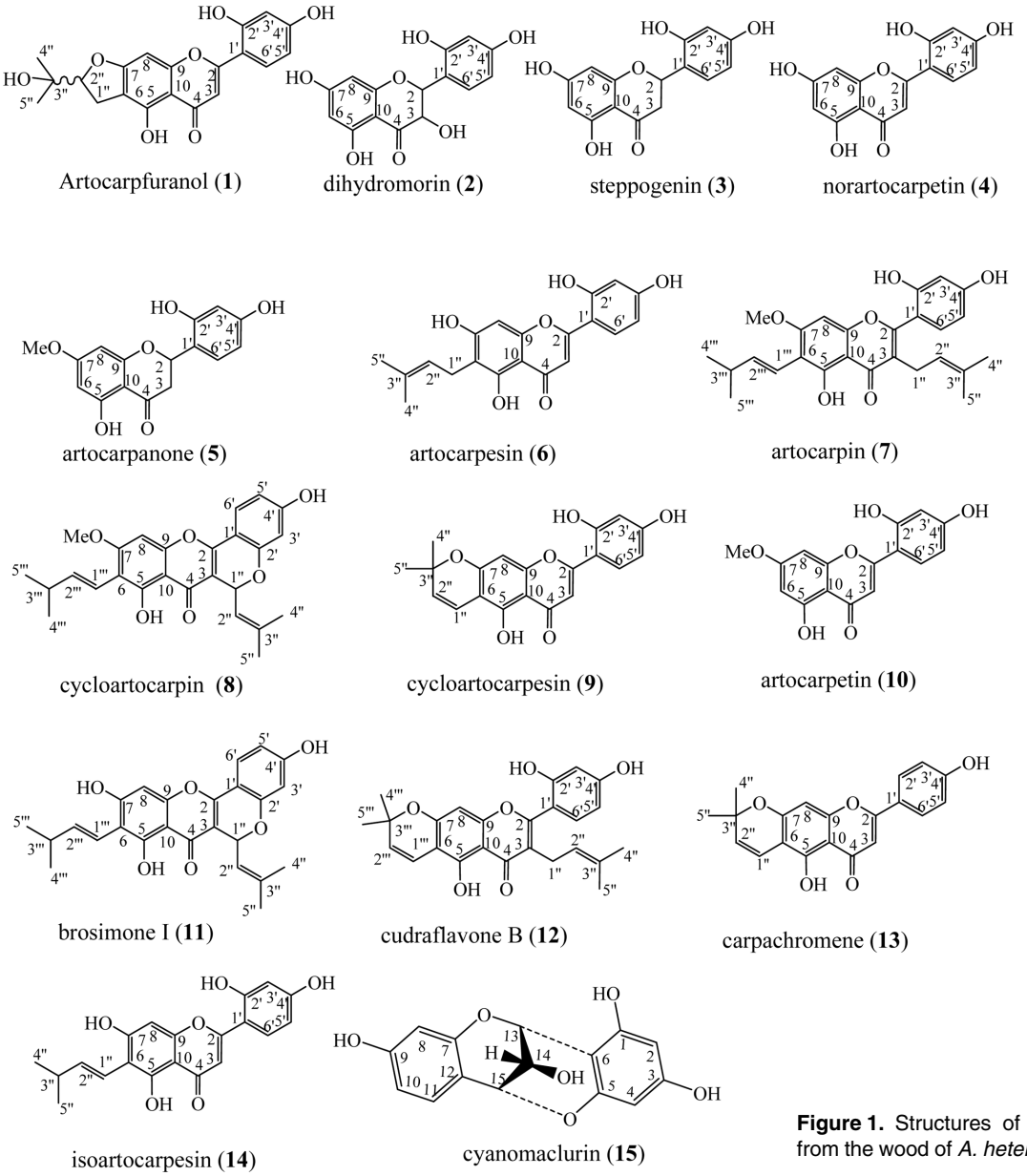

Figure 1. Structures of compounds isolated from the wood of *A. heterophyllus*.

Triplicate experiments were performed and IC_{50} value (concentration of test sample which results in 50% reduction in tyrosinase activity relative to the control) was determined for each compound.

2.6 Antibrowning effects of *A. heterophyllus* extract on fresh-cut apple slices

Apples of comparable size were cleaned and cut into 4 mm-thick slices. The slices were treated by dipping in 400 mL of the corresponding test solution or distilled water (control) for 5 min and drained. Samples were then placed in plastic Petri dishes, and stored at room temperature for 24 h. Hexplicate samples were prepared for each treatment and the experiment was repeated three times. Test solutions used for the above samples included aqueous solutions of ascorbic acid (0.5%), 4-hexylresorcinol (0.01%), *A. heterophyllus* extract (0.05%), *A. heterophyllus* extract (0.03%), ascorbic acid (0.5%) + 4-hexylresorcinol (0.01%), ascorbic acid (0.5%) + *A. heterophyllus* extract (0.05%), and ascorbic acid (0.5%) + *A. heterophyllus* extract (0.03%). Visual assessment of color development in the samples was performed with a digital camera while the relative extents of browning were measured with a tristimulus reflectance colorimeter (Minolta CR-400 Chroma Meter). Center of the apple slices was in touch with the lens of the Minolta CR-400 Chroma meter when taking the readings (L^*, a^*, and b^* values). Measurements were made immediately following each treatment and at timed intervals thereafter.

Table 1. NMR Data and HMBC correlations of Compound **1** in DMSO (δ in ppm, *J* in Hz)

Position	δ_H	δ_C	HMBC correlations
2		161.7 (s)	
3	7.00 (1H, s)	106.7 (d)	C-2, C-4
4		182.0 (s)	
5		155.6 (s)	
6		108.7 (s)	
7		166.0 (s)	
8	6.54 (1 H, s)	88.6 (d)	C-7, C-9, C-4
9		157.1 (s)	
10		104.4 (s)	
1′		108.5 (s)	
2′		161.7 (s)	
3′	6.48 (1H, d, *J* = 2.2)	103.2 (d)	C-4′, C-2′
4′		158.7 (s)	
5′	6.42 (1H, dd, *J* = 8.8, 2.2)	108.0 (d)	C-3′, C-6″
6′	7.85 (1 H, d, *J* = 8.8)	129.7 (d)	C-2′, C-4′
1″	3.04 (2H, d, *J* = 8.6)	25.9 (t)	C-7, C-5, C-6, C-10, C-2″, C-3″
2′	4.71 (1H, dd, *J* = 9.5, 8.6)	91.3 (d)	C-7, C-6, C-2″, C-3″, C-4″, 5″
3″		70.0 (s)	
4″, 5′	1.14, 1.12 (6H, s)	24.8, 25.7 (q)	C-3″, C-2″
5-OH	13.27 (1H, OH)		C-5, C-6, C-10
4′-OH	10.74 (1H, OH)		C-4′
2′-OH	10.16 (1H, OH)		C-2′

2.7 Statistical analysis

The analysis of variance (ANOVA) and Tukey's multiple range test for comparison of means and least significant differences were performed with the obtained data using the SAS system (SAS Institute, Cary, NC). $p < 0.05$ was selected as the level decision for significant differences.

3 Results and discussion

Separation of the major compounds from the wood of *A. heterophyllus* was achieved by classic column chromatography on silica gel, Sephadex LH-20, MCI GEL, and with the aid of semipreparative RP HPLC. Structures for 14 known compounds were identified by comparison with the literature data as dihydromorin [18], steppogenin [19], norartocarpetin [8], artocarpanone [9], artocarpesin [20], artocarpin [21], cycloartocarpin [22], cycloartocarpesin [23], artocarpetin [9], brosimone I [24], cudraflavone B [25], carpachromene [26], isoartocarpesin [27], and cyanomaclurin [9]. Their structures are shown in Fig. 1.

Compound 1 was found to be a new compound and its structure was elucidated by interpretation of MS and NMR spectroscopic data. Compound 1 gave a molecular ion peak $[M + H]^+$ at *m/z* 371 in positive ESI-MS, consistent with a molecular formula of $C_{20}H_{18}O_7$. The ^{1}H-NMR spectrum of compound 1, which was run in DMSO-d_6 (Table 1) showed the presence of five aromatic protons, with three of them at δ = 6.42 (1H, dd, *J* = 8.8, 2.2 Hz, H-5′), 6.48 (1H, d, *J* = 2.2 Hz, H-3′), and 7.85 (1H, d, *J* = 8.8 Hz, H-6′) assignable to a 2′,4′-dihydroxy-substituted B ring of a flavone structure, and two additional singlets at δ = 7.00 (1H, s) and 6.54 (1H, s, H-6 or H-8) assignable to the protons on C3 and C6/C8 of a flavone structure. The ^{13}C-NMR spectrum showed 20 carbon signals, with 15 of them assignable to a flavone skeleton, and the remaining five at 24.8 (q), 25.7 (q), 25.9 (t), 70.0 (s), and 91.3 (d) assignable to a modified prenyl group [28, 29]. An $-OCH-CH_2-$ spin system was evident from the ^{1}H and COSY spectra. HMBC (Fig. 2) supported the correlations between the oxygenated methine and methylene protons of this spin system and the quaternary carbons, C-6 (δ = 108.7) and C-7 (δ = 166.0), and thus the presence of a furan ring which is fused to the flavone

Table 2. Mushroom tyrosinase inhibition activity of compounds isolated from *A. heterophyllus* (*n* = 3)

Compounds	IC_{50} (μM) ± SD
Artocarpfuranol (1)	47.927 ± 0.522
Dihydromorin (2)	10.3454 ± 0.141
Steppogenin (3)	0.5733 ± 0.0080
Norartocarpetin (4)	0.4647 ± 0.0059
Artocarpanone (5)	1.5450 ± 0.0043
Artocarpesin (6)	0.5254 ± 0.0141
Artocarpin (7)	>1000
Cycloartocarpin (8)	>1000
Cycloartocarpesin (9)	>1000
Artocarpetin (10)	200.852 ± 3.165
Brosimone I (11)	60.280 ± 1.165
Cudraflavone B (12)	166.551 ± 2.481
Carpachromene (13)	94.376 ± 1.337
Isoartocarpesin (14)	0.6628 ± 0.0165
Cyanomaclurin (15)	68.733 ± 1.078
Kojic acid	71.627 ± 1.493

Table 3. *a** values of apple slices treated with different antibrowning agents in water (with 5% ethanol)[a)]

	0 h	3 h	6 h	12 h	24 h
Control (water)	−3.42 ± 0.57	−1.05 ± 1.00a	−0.73 ± 1.01a	−0.57 ± 0.95a	−0.36 ± 1.15a
0.5% Vc	−3.96 ± 0.48	−1.73 ± 1.65b	−1.64 ± 1.62b	−1.62 ± 1.51b	−1.36 ± 1.53b
0.05% extract	−3.69 ± 0.76	−3.34 ± 0.93c	−3.11 ± 0.86c	−2.41 ± 0.90c	−1.35 ± 1.00b
0.01% 4-HR	−3.47 ± 0.85	−3.53 ± 0.79c	−3.32 ± 0.84c	−2.49 ± 1.74c	−2.06 ± 1.02c
0.01% 4-HR + 0.5% Vc	−4.03 ± 0.46	−4.18 ± 0.45d	−4.16 ± 0.47d	−4.05 ± 0.48d	−3.87 ± 0.52d
0.03% extract + 0.5% Vc	−4.13 ± 0.44	−4.14 ± 0.64d	−4.02 ± 0.62d	−3.78 ± 0.68d	−3.09 ± 1.12d
0.05% extract + 0.5% Vc	−4.30 ± 0.45	−4.47 ± 0.49d	−4.35 ± 0.51d	−4.22 ± 0.54d	−3.81 ± 0.61d

a) Each value is expressed as the mean ± SD ($n = 18$). Means with different letters in the same column are significantly different ($p < 0.05$).

moiety. The presence of two isolated methyl groups (C-4″ and C-5″) belonging to a hydroxyisopropyl moiety of the furan ring located next to the methine proton was evident from the peaks at δ = 1.12 and 1.14 and carbon signals at δ = 24.8, 25.7, and 70.0 ppm [30]. HMBC correlations within the furan ring as shown in Table 1 led to the assigned structure of compound 1. Thus, the structure of the new compound 1 was elucidated as 7-(2, 4-dihydroxyphenyl)-4-hydroxy-2- (2-hydroxypropan-2-yl)-2,3-dihydrofuro(3,2-g) chromen-5-one, named as artocarpfuranol. Tyrosinase inhibitory activity of compounds 1–15 were compared using tyrosinase inhibition assay and their activities were expressed as their IC_{50} values (Table 2). IC_{50} values of 7-(2, 4-dihydroxyphenyl)-4-hydroxy-2-(2-hydroxypropan-2-yl)-2, 3-dihydrofuro (3, 2-g)chromen-5-one (artocarpfuranol), dihydromorin, steppogenin, norartocarpetin, artocarpanone, artocarpesin, and isoartocarpesin for mushroom tyrosinase activity were 47.93, 10.3, 0.57, 0.46, 1.54, 0.52, and 0.66 μM, respectively, much stronger than kojic acid (IC_{50} = 71.6 μM), a well-known tyrosinase inhibitor. Moreover, several of the above identified compounds, steppogenin, norartocarpetin, artocarpesin, and isoartocarpesin had their IC_{50} similar to that of glabridin (IC_{50} = 0.57 μM), a well-known tyrosinase inhibitor isolated from licorice roots and has been used in expensive cosmetic products. Tyrosinase inhibition test results showed that both the number and the location of hydroxyl groups affected the tyrosinase inhibitory activity of flavonoids with the later parameter playing more important roles. In particular, simultaneous occurrence of a hydroxyl group at 2′ and 4′ position of the B ring and 5 and 7 position of the A ring would greatly enhance the resultant tyrosinase inhibitory activity of the compound. Examples were compounds 2, 3, 4, 6, and 14. When some of these hydroxyl groups were associated with other group or substituted, activity of the compound would be significantly compromised. For example, substitution of the hydroxyl group at 7 position of the A ring led to much weaker tyrosinase inhibitory activity (compound 3 *vs.* compound 5).

Knowing that quite a number of compounds isolated from *A. heterophyllus* had potent inhibitory activity against mushroom tyrosinase, and most of these compounds are polyphenols with strong antioxidant activities, it was proposed that its extract may have high potential as antibrowning agents for practical application in food products. Δ*E*, *h**, *L**, *a**, and *b** have been the most frequently used parameters for measuring the extent of browning reactions in fruits and vegetables. A decrease in *L** and an increase in *a** or D*E* mean the occurrence of browning. *a** value was reported to be more sensitive to browning and has been used to effectively monitor browning in fresh-cut pear [31, 32] and apple [33]. A recent research study also found that *a** and Δ*E* (total color difference) were the best parameters to monitor browning on fresh-cut Fuji apple surfaces [34]. The current study chose *a** as a key marker for estimating the antibrowning activity of *A. heterophyllus* extract, in addition to visual observation. As in some of earlier studies, the change of *b** was also used for monitoring browning [35], in current study, the change of *b** was also evaluated. In our previous study on the antibrowning effect of oxyresveratrol and *Morus alba* twig extract on apple slices, we discovered that mulberry twig extract, and oxyresveratrol needed to be used in combination at least with ascorbic acid to exhibit their antibrowning effects and in combination with 0.5% ascorbic acid in the test solution, *M. alba* twig extracts (0.1%), oxyresveratrol (0.01%), and 4-hexylresorcinol (0.01%) were all found to be effective in maintaining the appearance of the apple slices (kept at room temperature with open access to air) without significant color changes for at least 24 h. As the major tyrosinase inhibitors we identified in the wood extract of *A. heterophyllus* have similar functional moieties as oxyresveratrol and 4-hexylresorcinol, they possibly also need to be used in combination with ascorbic acid to achieve antibrowning effects. Our experimental results supported this hypothesis. Different concentrations (0.03, 0.05, and 0.1%) of *A. heterophyllus* extract with/without 0.5% ascorbic acid in the dipping solutions were tested. 4-Hexylresorcinol (0.01%) with 0.5% ascorbic acid in the dipping solution was used as positive control. However as 0.1% of *A. heterophyllus* extract had poor solubility in the dipping solution, this level of application was not included in further tests. Based on the change of *a** value, *A. heterophyllus* extract (0.03 or 0.05%) and 4-hexylresorcinol, each in combination with 0.5% ascorbic acid

Table 4. *L** values of apple slices treated with different antibrowning agents in water (with 5% ethanol)[a)]

	0 h	3 h	6 h	12 h	24 h
Control (water)	76.08 ± 1.91	72.31 ± 2.23a	71.76 ± 2.45a	71.58 ± 2.48a	72.19 ± 2.77a
0.5% Vc	76.05 ± 2.52	72.37 ± 3.26a	72.31 ± 3.51a	72.71 ± 3.57a	73.58 ± 3.57ac
0.05% Extract	76.72 ± 1.09	75.40 ± 2.38b	75.79 ± 1.80b	75.20 ± 1.93b	74.79 ± 2.07bc
0.01% 4-HR	76.50 ± 1.76	76.38 ± 1.93bc	75.95 ± 1.91b	75.99 ± 2.03bc	76.34 ± 2.19bd
0.01% 4-HR + 0.5% Vc	77.22 ± 1.57	76.75 ± 2.41bc	76.38 ± 2.48b	76.61 ± 2.50bc	77.34 ± 2.68d
0.03% Extract + 0.5% Vc	77.47 ± 2.10	77.32 ± 2.41b	76.94 ± 2.82b	76.81 ± 3.11c	77.32 ± 3.29d
0.05% Extract + 0.5% Vc	77.60 ± 1.55	77.05 ± 1.64c	77.09 ± 1.77b	76.87 ± 1.76bc	77.99 ± 1.62d

a) Each value is expressed as the mean ± SD ($n = 18$). Means with different letters in the same column are significantly different ($p < 0.05$).

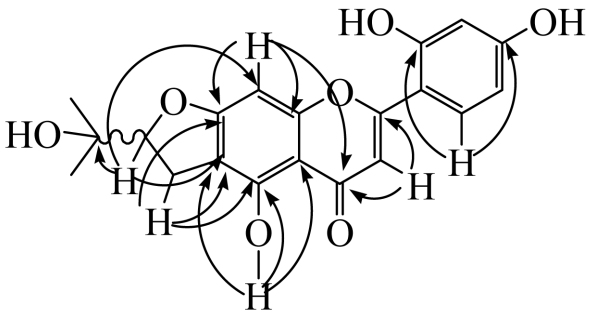

Figure 2. The key HMBC correlations observed for compound 1.

showed better activity than *A. heterophyllus* extract (0.03 or 0.05%), 4-hexylresorcinol, or ascorbic acid alone with significant statistical difference ($p < 0.05$) (Table 3). The effect was prominent even after 3 h storage of the samples at room temperature. Similar pattern was observed when *L** was used as the parameter for monitoring browning (Table 4). In our study, we found with the presence of 0.5% ascorbic acid in the dipping solution, both 0.03 and 0.05% extract showed significant antibrowning effects on apple slices in the 24 h testing period, and the effects were comparable to treatment with 0.01% 4-hexylresorcinol in combination with 0.5% ascorbic acid, suggesting the potential commercial value of *A. heterophyllus* as antibrowning agent for fresh-cut fruit products. However as no textural and sensory property data were collected in this study, it is unknown whether with the addition of *A. heterophyllus*, the nutritional values and organoleptic properties of the apple slices were changed or not. Further investigation in these areas would be required.

4 Concluding remarks

In conclusion, we systematically studied the phytochemicals in the wood of *A. heterophyllus* and elucidated the structures of 15 compounds obtained from it. Among them, one is a novel compound and several showed significant tyrosinase inhibitory activity. Natural extracts rich in these inhibitors may therefore have good potential as antibrowning agents to be applied in real food systems.

The authors have declared no conflict of interest.

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