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ARTICLE

Isolation and Identification of Radical Scavenging and Tyrosinase Inhibition of Polyphenols from *Tibouchina semidecandra* L.

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Phytochemical and bioactivity studies of the leaves and stem barks of *Tibouchina semidecandra* L. have been carried out. The ethyl acetate extract of the leaves yielded four flavonoid compounds, identified as quercetin, quercetin 3-*O*-α-L-(2″-*O*-acetyl) arabinofuranoside, avicularin, and quercitrin, while the stem barks gave one ellagitannin, identified as 3,3′-*O*-dimethyl ellagic acid 4-*O*-α-L-rhamnopyranoside. Evaluation of the antioxidative activity on the crude extracts and pure compounds by electron spin resonance (ESR) and ultraviolet−visible (UV−vis) spectrophotometric assays showed that the pure isolated polyphenols and the EtOAc extract possessed strong antioxidative capabilities. Quercetin was found to be the most active radical scavenger in DPPH-UV and ESR methods with SC_{50} values of 0.7 μM ± 1.4 and 0.7 μM ± 0.6 μM, respectively, in the antioxidant assay. A combination of quercetin and quercitrin was tested for synergistic antioxidative capacity;, however, there was no significant improvement observed. Quercetin also exhibited strong antityrosinase activity with a percent inhibition of 95.0% equivalent to the positive control, kojic acid, in the tyrosinase inhibition assay.

KEYWORDS: *Tibouchina semidecandra* L.; Melastomataceae; polyphenols; radical scavenging activity; tyrosinase inhibition activity

INTRODUCTION

Tibouchina semidecandra L. belonging to the Melastomataceae family is a shrub which has been introduced to Malaysia from Brazil (*1*). It bears beautiful dark purple flowers throughout the year and grows well in the frost-free areas around the world. The pristine purple flower makes it a valuable ornamental plant and a potential source for the extraction of natural food colorants (*2*). This plant is also used traditionally for both medicinal and food purposes (*2*). The main flower pigment is anthocyanin, which is natural, water-soluble, nontoxic, and reported to be safe in dietary supplements (*3*).

T. semidecandra is rich in tannins, particularly in olygomeric hydrolyzable tannins. Seven new hydrolyzable tannins, named as methylvescalagin, nobotanin A, nobotanin B, nobotanin C, nobotanin D, nobotanin E, and nobotanin F, together with several flavonoid compounds such as quercetin, myricetin, leucodelphinidin, leucocyanidin, quercetin-3-*O*-(6″-*O*-galloyl) galactoside, avicularin, and tibouchinin, have been isolated from this plant (*4*−*8*).

However, to the best of our knowledge, there has been no report on the bioactivity study of this species. This led us to reinvestigate the phytochemicals and bioactivity of the leaves and stems of this plant. In this article, we report the isolation and identification of radical scavenging and tyrosinase inhibition polyphenols from the leaves and stem barks of *T. semidecandra*.

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MATERIALS AND METHODS

General Methods. Mps. (uncorrected) were determined using Leica Gallen. Ultraviolet (UV) spectra were recorded on a UV-100PC Shimadzu spectrophotometer in methanol. IR spectra were recorded on a Perkin-Elmer 1600 series spectrophotometer. Mass spectral data were obtained from Kent Mass Spectrometry Service, U.K. NMR spectra were recorded on Bruker Avance 400 Spectrometer (400/100 MHz) and Bruker Avance 300 Spectrometer (300/75 MHz). The ESR spectra were recorded on JEOL JES-FA 100 ESR Spectrometer by using manganese dioxide as an internal standard. Thin-layer chromatography (TLC; aluminum sheets precoated with silica gel 60 F_{254}, 0.20 mm thickness, Merck) was used to detect or monitor the presence of chemical components in the crude extracts or fractions. The spots were visualized by UV light (254 and 365 nm) incooperated with anisaldehyde-sulphuric acid as the spraying agent. Vacuum liquid chromatography (VLC) was carried out on silica gel 230−400 mesh and RP SiC_{18} (Merck), and column chromatography (CC) was performed with silica gel 70−230 mesh (Merck) and Sephadex LH-20 (Pharmacia Fine Chemicals). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was from Fluka (Switzerland), vitamin C was purchased from Merck (Darmstadt, Germany), and the mushroom tyrosinase was purchased from Sigma Chemical Co. (St Louis. MO).

Plant Materials. *Tibouchina semidecandra* L. was collected from Genting Highland, Pahang, Malaysia. A voucher specimen was deposited

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in the herbarium of the Forest Research Institute of Malaysia, Kepong, Selangor.

Extraction and Isolation. The dried leaves of *T. semidecandra* (1.2 kg) were ground and successively extracted by Soxhlet extraction for 18 h with *n*-hexane (9.0 L), EtOAc (9.0 L), and MeOH (9.0 L). The solvents from the extraction were removed under reduced pressure to yield dark green sticky oil, *n*-hexane (40.3 g, 3.3%), EtOAc (25.1 g, 2.1%), and MeOH (23.6 g, 2.0%).

The EtOAc extract (25.1 g) was fractionated by VLC on silica gel 60 (230−400 mesh) and eluted with petroleum ether/EtOAc (9:1, 8:2, 7:3, 6:4, 5.5:4.5, 5:5, 4:6, 3:7, and 2:8) and finally EtOAc to afford 52 fractions (EaCr1-EaCr52). Fractions EaCr20 to EaCr22 (1.5 g) were recombined and separated by CC on silica gel 70−230 mesh and eluted with petroleum ether/EtOAc (98:2, 95:5, 90:10, 85:15, 80:20, and 70:30) to give 156 fractions. Fractions 26−68 were combined and recrystallized using acetone and CH_2Cl_2 to yield **1** (0.025 g, 0.10%) (*9*). Fractions EaCr30−EaCr34 were combined and purified by CC on silica gel 70−230 mesh using petroleum ether/EtOAc (90:10, 85:15, 80:20, 75:25, and 70:30) to give 220 fractions. Fractions 190−200 were recombined and washed using EtOAc to yield **2** (0.033 g, 0.13%) (*10*). Fractions EaCr47 to EaCr50 were combined and subjected to CC, eluted with the gradient solvent system of CH_2Cl_2−MeOH to yield 250 fractions. Fractions 150−170 were washed with EtOAc to afford **3** (0.045 g, 0.18%) (*11*), while fractions 200−208 from the same CC were washed with acetone to yield **4** (0.039 g, 0.15%) (*12*).

The air-dried stem barks of *T. semidecandra* (1.0 kg) were ground and extracted with MeOH (4.0 L) by Soxhlet extraction for 18 h. The extract was concentrated in vacuo to give a dark green sticky liquid of the MeOH (100.0 g, 10.0%) extract. The MeOH extract (100.0 g) was fractionated by RP-VLC on RP-18 silica gel (230−400 mesh) using water, water/MeOH (3:1, 3:2, 1:1, 2:3, 1:4, and 1:9), MeOH, MeOH/CH_2Cl_2 (3:1, 1:1), and CH_2Cl_2 to afford four fractions (STA, STB, STC, and STD). Fraction STD (23.6 g) was further purified by CC on MCI gel (240.0 g) using a solvent system, water, water/MeOH, and MeOH, in the order of decreasing polarity to give 3,3′-*O*-dimethyl ellagic acid 4-*O*-α-L-rhamnopyranoside **5** (19.3 mg) as white amorphous solids; R_f 0.3 (MeOH/CH_2Cl_2 = 1:9) (*13*).

Antioxidant Activity. The UV spectrophotometric assay was conducted according to the method described by Tagashira et al. (*14*). Each sample stock solution (1.0 mg/mL) was diluted to final concentrations of 500, 250, 125, 62.5, 31.3, and 7.8 μg/mL in methanol. A total of 3.8 mL of 25 mM DPPH methanolic solution was added to 0.2 mL of sample solution of different concentrations and allowed to react at room temperature. After 30 min, the absorbance of the reaction mixture was measured at 517 nm. The DPPH solution plus methanol was used as the blank, while vitamin C was measured as the reference antioxidant. The absorbance of the blank was measured immediately at 0 min. The percentage of antioxidant activity was calculated using the following formula:

$$\%SC = \left(\frac{Ab(blank) - Ab(DPPH + sample)}{Ab(blank)}\right) \times 100$$

The SC_{50} value was determined as the concentration of each sample to give 50% of the absorbance shown by the blank. All test and analyses were run in triplicate and averaged.

The electron spin resonance (ESR) assay was conducted according to the method described by Ohtani et al. (*15*). Each sample stock solution (1.0 mg/mL) was diluted to final concentrations of 500, 250, 125, 62.5, 31.3, and 7.8 μg/mL in absolute ethanol. A total of 200 μL of 25 mM DPPH ethanolic solution was added to 200 μL of sample solution of different concentrations in an Eppendorf tubes. The reaction mixture was shaken for 10 s and then was transferred to a flat cell and fitted into cavity of the ESR spectrometer. The spin adduct was measured on an ESR spectrometer exactly 30 s later. The ethanolic DPPH solution was used as the blank, while vitamin C was used as the reference antioxidant.

Measurement conditions: magnetic field, 336.0 mT; modulation frequency, 100 kHz; microwave frequency, 9.42 GHz; microwave power, 0.998 m;, sweep time, 30 s; temperature, 298 K. The percentage of scavenging capacity (% SC) of the sample was calculated as follows:

$$\%SC = \left(\frac{\text{peak height(blank)} - \text{peak height(DPPH + sample)}}{\text{peak height(blank)}}\right) \times 100$$

Antityrosinase Activity. The tyrosinase enzyme assay was conducted according to the method described by Isao Kubo et al. (*16*) with slight modifications. Each sample (5 mg) was dissolved in the different solvents (50 μL) on the basis of their solubility to give a final concentration at 0.03% (w/v). Two millimolar L-DOPA (300 μL) solution was mixed with phosphate (K_2HPO_4) buffer (pH 6.5, 670 μL). Then, each sample solution (30 μL) and tyrosinase (100 units/mL, 10 μL) in aqueous solution (added last) were added in this order to the mixture. This solution was immediately monitored for the formation of dopachrome by measuring the linear increase in optical density at 492 nm for 1 min. The tyrosinase plus L-DOPA solution was used as the blank, while kojic acid was measured as the reference antityrosinase. The percentage of antityrosinase activity was calculated using the following formula.

$$\%IC = \left(\frac{\text{absorbance(blank)} - \text{absorbance(tyrosinase + sample)}}{\text{absorbance(blank)}}\right) \times 100$$

Statistical analysis. Statistical analyses were performed using Sigma Plot 8.0. Data were presented as the means of the standard error of triplicate samples.

RESULTS AND DISCUSSION

Phytochemical Investigation. The EtOAc extract of the leaves was subjected to the VLC technique for fractionation. Repeated gravity column chromatography either on silica gel or Sephadex LH-20 on these fractions gave four flavonoids **1**−**4**. Fraction STD (23.6 g) resulted from the fractionation of the MeOH extract of the stem barks was purified by CC on MCI gel (240.0 g) using the solvent system water, water/MeOH, and MeOH in the order of decreasing polarity to give an ellagitannin, **5**.

The structures of these pure compounds were elucidated spectroscopically using ^{1}H NMR, ^{13}C, and 2D NMR, IR, UV, and MS as well as by comparison with literature data, as quercetin (**1**) (*17*), quercetin 3-*O*-α-L-(2″-*O*-acetyl)-arabinofuranoside (**2**) (*10*), avicularin (**3**) (*11*, *17*), quercitrin (**4**) (*16*), and 3,3′-*O*-dimethyl ellagic acid 4-*O*-α-L-rhamnopyranoside (**5**) (*13*, *18*) (**Figure 1**). This is the first report of the isolation of quercetin 3-*O*-α-L-(2″-*O*-acetyl)-arabinofuranoside (**2**), quercitrin (**4**), and 3,3′-*O*-dimethyl ellagic acid 4-*O*-α-L-rhamnopyranoside (**5**) from this plant.

Quercetin (**1**) was obtained as a colorless solid; mp 308−310 °C. EIMS *m*/*z* 302 $[M]^+$, $C_{15}H_{10}O_7$. ^{1}H NMR (400 MHz, CD_3OD): δ12.70 (1H, s, O−H), 7.74 (1H, d, *J* = 2.1 Hz, H-2′), 7.64 (1H, dd, *J* = 2.1 Hz, 7.4 Hz, H-6′), 6.90 (1H, d, *J* = 7.4 Hz, H-5′), 6.54 (1H, d, *J* = 2.1 Hz, H-8), 6.27 (1H, d, *J* = 2.1 Hz, H-6). ^{13}C NMR (100 MHz, CD_3OD): δ 175.9 (C-4), 164.1 (C-7), 161.0 (C-5), 156.8 (C-9), 147.3 (C-3′), 146.6 (C-2), 144.8 (C-4′), 135.2 (C-3), 122.7 (C-1′), 120.2 (C-6′) 114.8 (C-2′), 114.6 (C-5′), 103.1 (C-10), 97.8 (C-6), 93.0 (C-8) were consistent with previously published data (*9*).

Quercetin 3-*O*-α-L-(2″-*O*-acetyl)-arabinofuranoside (**2**) was isolated as a yellow crstalline solid, mp 185−187 °C. FABMS *m*/*z* 477 $[M + H]^+$, $C_{22}H_{20}O_{12}$. ^{1}H NMR (300 MHz, CD_3OCD_3): δ 7.66 (1H, d, *J* = 2.1 Hz, H-2′), 7.57 (1H, dd, *J* = 2.1 Hz, 8.4 Hz, H-6′), 6.99 (1H, d, *J* = 8.4 Hz, H-5′), 6.49 (1H, d, *J* = 1.8 Hz, H-8), 6.27 (1H, d, *J* = 1.8 Hz, H-6), 5.80 (1H, s, H-1″), 4.91 (1H, d, *J* = 3.6 Hz, H-2″), 4.46 (1H, s, H-3″), 3.89 (1H, m, H-4″), 3.60 (2H, d, *J* = 3.6 Hz, H-5″), 2.10 (3H, s, H-7″). ^{13}C NMR (75 MHz, CD_3OCD_3): δ 178.1 (C-4), 171.2 (C-6″), 165.0 (C-7), 163.5 (C-5), 157.5 (C-2), 157.2 (C-9), 148.6 (C-4′), 145.0 (C-3′), 122.2 (C-6′), 122.2 (C-1′), 116.8 (C-2′) 114.9 (C-5′), 108.9 (C-1″), 116.0 (C-10), 98.9 (C-6), 93.7 (C-8), 86.9 (C-4″), 80.3 (C-2″), 80.5 (C-3″), 60.2 (C-5″), 20.1 (C-7″), consistent with previously published data (*10*).

Avicularin (**3**) was obtained as yellow needles; mp. 170−175 °C. FABMS *m*/*z* 435 $[M + H]^+$ $C_{20}H_{18}O_{11}$. ^{1}H (300 MHz, CD_3OD): δ 7.54 (1H, d, *J* = 2.1 Hz, H-2′), 7.52 (1H, dd, *J* = 2.1 Hz, 8.4 Hz, H-6′), 6.92 (1H, d, *J* = 8.4 Hz, H-5′), 6.41 (1H, d, *J* = 2.1 Hz, H-8), 6.22 (1H, d, *J* = 2.1 Hz, H-6), 5.48 (1H, s, H-1″), 4.35 (1H,

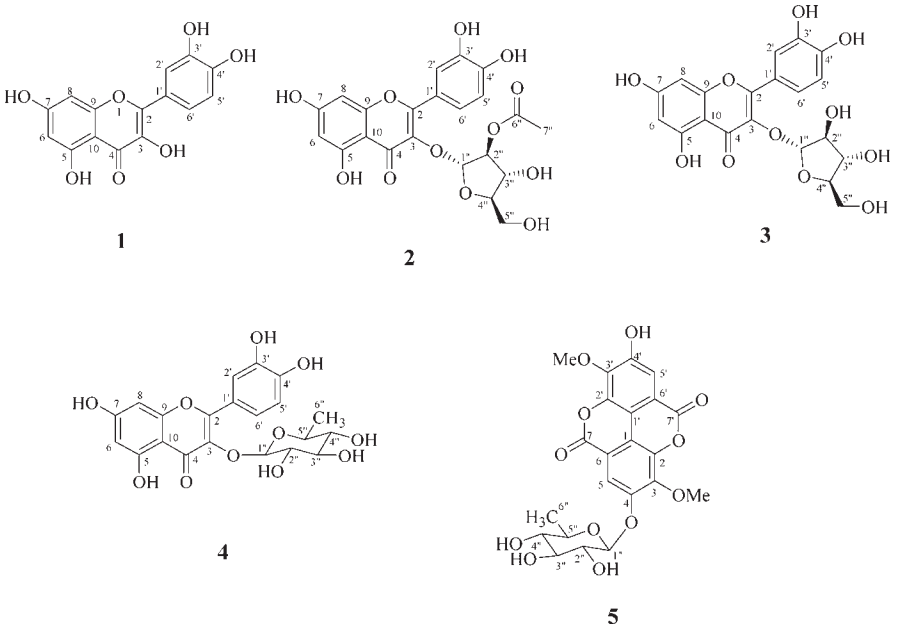

Figure 1. Structures of polyphenols from *Tibouchina semidecandra*.

dd, J = 0.9 Hz, 2.7 Hz, H-2″), 3.90 (1H, m, H-3″), 3.86 (1H, m, H-4″), δ 3.51 (2H, t, J = 3.0 Hz, 1.8 Hz, H-5″). ^{13}C NMR (75 MHz, CD_3OCD_3): δ 178.9 (C-4), 165.0 (C-7), 163.2 (C-5), 157.5 (C-2), 157.2 (C-9), 148.8 (C-4′), 145.0 (C-3′), 134.1 (C-3) 122.2 (C-1′), 122.0 (C-6′), 117.0 (C-2′) 116.7 (C-5′), 109.5 (C-1″), 116.0 (C-10), 98.3 (C-6), 94.0 (C-8), 88.0 (C-4″), 82.1 (C-2″), 78.3 (C-3″), 62.2 (C-5″), consistent with previously published data (*11*).

Quercitrin (**4**) was isolated as yellow needles; mp 179−182 °C. FABMS m/z 449 $[M + H]^+$ $C_{21}H_{20}O_{11}$. ^{1}H NMR (300 MHz, CD_3OD): δ 7.24 (1H, d, J = 2.4 Hz, H-2′), 7.22 (1H, dd, J = 2.4 Hz, 8.4 Hz, H-6′), 6.82 (1H, d, J = 8.4 Hz, H-5′), 6.27 (1H, d, J = 2.1 Hz, H-8), δ 6.10 (1H, d, J = 2.1 Hz, H-6), δ 5.25 (1H, d, J = 1.5 Hz, H-1″), 4.12 (1H, dd, J = 1.5 Hz, 3.3 Hz, H-2″), 3.65 (1H, dd, J = 3.3 Hz, 9.0 Hz, H-3″), 3.22 (1H, m, H-4″), 3.31 (1H, m, H-5″), 0.84 (3H, d, J = 6.0 Hz, H-6″). ^{13}C NMR (75 MHz, CD_3OD): δ 178.9 (C-4), 164.0 (C-7), 162.2 (C-5), 157.2 (C-2), 156.2 (C-9), 148.8 (C-4′), 145.0 (C-3′), 134.1 (C-3) 122.2 (C-1′), 122.0 (C-6′), 116.0 (C-2′) 115.7 (C-5′), 105.9 (C-10), 102.5 (C-1″), 98.3 (C-6), 94.0 (C-8), 72.5 (C-4″), 71.1 (C-2″), 70.3 (C-3″), 70.1 (C-5″) 18.6(C-6″), consistent with previously published data (*12*).

3,3′-*O*-Dimethyl ellagic acid 4-*O*-α-L-rhamnopyranoside (**5**) was obtained as a white amorphous solid; R_f 0.4 ($MeOH/CH_2Cl_2$ = 1:9) with mp 188 °C (lit. [12] 186 °C). FABMS m/z: 477 $[M + H]^+$, $C_{22}H_{20}O_{12}$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.74 (1H, s, H-5), 7.45 (1H, s, H-5′), 5.53 (1H, d, J = 1.6 Hz, H-1″), 4.09 (3H, s, -OMe), 4.08 (3H, s, -OMe), 3.95 (1H, dd, J = 3.3 Hz, 9.0 Hz, H-2″), 3.72 (1H, m, H-3″), 3.52 (1H, m, H-5″), 1.12 (3H, d, J = 6.0 Hz, H-6″). ^{13}C NMR (100 MHz, DMSO): δ 158.4 (C-7′), 158.2 (C-7), 152.9 (C-4′), 150.3 (C-4), 141.7 (C-2′), 141.4 (C-2), 140.9 (C-3), 140.1 (C-3′) 113.9 (C-6), 112.5 (C-6′), 111.8 (C-1′) 111.6 (C-1), 111.5 (C-5), 110.8 (C-5′), 99.8 (C-1″), 71.6 (C-4″), 70.4 (C-2″), 70.3 (C-3″), 70.1 (C-5″), 61.6 (O−CH_3), 61.0 (O−CH_3), 18.0 (C-6″)^{1}H, and ^{13}C NMR were consistent with previously published data (*13*).

Bioaassay Studies. *Antioxidant Activity*. Antioxidants can be divided into two main categories, oxidant scavengers and antioxidant enzymes. Organic antioxidant scavengers are small molecular compounds that readily reduce free radicals, peroxides, and oxidized molecules, thereby neutralizing their effect and protecting functionally sensitive proteins, lipids, and nucleic acids from oxidative damage (*19*). One free radical can begin a destructive process of removing electrons from stable compounds forming many reactive oxygen species (ROS), transforming stable compounds into a variety of free radicals such as superoxide, superoxide conjugate acid, singlet oxygen, hydroxyl radical, and organic and peroxy free radical (*20*).

One of the methods for measuring antioxidant potential is through determining the free radical inhibitory ability of the different antioxidants by using very stable free radicals such as DPPH in methanol solution. The reduction of DPPH was monitored by the decrease in its absorbance at a characteristic wavelength during the reaction.

The antioxidant activities of crude and pure samples were determined by the free radical scavenging method, i.e., the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) method using a UV spectrophotometer and an ESR spectrometer.

Table 1 shows the scavenging concentration to obtain 50% of the maximum scavenging capacity of DPPH by the crude extracts and isolated compounds at the minimum sample concentration of 7.8 μg/mL. The final solution sample always turns light yellow in color after completing the reaction; therefore, its absorption inhibition cannot reach 100% compared to that in the colorless methanol solution. We found that the EtOAc extract of the leaves possesses the most active radical scavenger in this method compared to other crude extracts with SC_{50} of 10.3 μg/mL. The methanol extract of the leaves also exhibits good results as a free radical scavenger followed by the methanol extract of the stem barks with SC_{50} of 24.1 μg/mL and 55.8 μg/mL, respectively. Methanolic extracts were active to scavenge radicals due to the presence of polyphenol compounds in these crude extracts (*21*,*22*). Quercetin **1** showed the highest ability to act as free radical scavengers with SC_{50} values of 0.7 μM followed by quercitrin **4** with SC_{50} of 74.1 μM. Quercetin 3-*O*-α-L-(2″-*O*-acetyl)-arabinofuranoside **2**, avicularin **3**, and 3,3′-*O*-dimethyl ellagic acid 4-*O*-α-L-rhamnopyranoside **5** exhibited moderate scavenging capacity with SC_{50} of 169.0 μM, 236.4 μM, and 292.2 μM, respectively.

It was of interest to examine whether there was the operation of a synergistic effect that contributes to the enhancement of antioxidative activity of a flavonoid in the presence of other flavonoids. A combination of two flavonoid compounds, quercetin **1**

Table 1. Scavenging Capacity of the Isolated Polyphenols and Crude Extracts from *T. semidecandra* L. by the UV Spectroscopy Method As Measured by SC_{50} Values

samples	SC_{50} (mean ± SD)	percent inhibition 7.8 μg/mL (mean ± SD)
EtOAc extract of leaves	10.3 ± 1.1 μg/mL	40.0 ± 0.3
methanol extract of leaves	24.1 ± 1.8 μg/mL	32.8 ± 1.6
methanol extract of stem barks	55.8 ± 1.2 μg/mL	13.7 ± 1.1
quercetin (**1**)	0.7 ± 1.4 μM	57.6 ± 0.5
quercitrin (**4**)	74.1 ± 0.4 μM	15.4 ± 1.8
quercetin 3-*O*-α-L-(5″-*O*-acetyl)-arabinofuranoside (**2**)	169.0 ± 1.8 μM	12.2 ± 2.2
avicularin (**3**)	236.4 ± 1.2 μM	11.8 ± 1.5
3,3′-*O*-dimethyl ellagic acid 4-*O*-α-L-rhamnopyranoside (**5**)	292.2 ± 1.3 μM	8.4 ± 1.7
vitamin C	8.3 ± 1.2 μM	30.8 ± 0.7

Table 2. Scavenging Capacity of the Isolated Polyphenols from *T. semidecandra* L. by the ESR Spectrometry Method As Measured by SC_{50} Values

samples	SC_{50} (mean ± SD)	percent inhibition 7.8 μg/mL (mean ± SD)
quercetin (**1**)	0.7 ± 0.6 μM	72.3 ± 0.7
quercitrin (**4**)	42.6 ± 1.5 μM	27.2 ± 0.3
Quercetin 3-*O*-α-L-(5″-*O*-acetyl)-arabinofuranoside (**2**)	144.7 ± 1.8 μM	30.1 ± 1.8
avicularin (**3**)	255.3 ± 1.2 μM	22.3 ± 2.0
3,3′-*O*-dimethyl ellagic acid 4-*O*-α-L-rhamnopyranoside (**5**)	312.2 ± 1.7 μM	13.8 ± 1.5
EtOAc extract of leaves	9.9 ± 1.1 μg/mL	42.0 ± 0.9
methanol extract of leaves	20.4 ± 1.7 μg/mL	35.4 ± 1.4
methanol extract of stem barks	112.8 ± 1.1 μg/mL	13.7 ± 1.1
vitamin C	8.0 ± 0.5 μM	80.2 ± 0.3

and quercitrin **4**, was tested for synergistic antioxidative capacity; however, no significant improvement was observed. This suggests that the capability of the scavenging DPPH radical remained about the same in terms of the antioxidative potential per mole of antioxidant, although the total antioxidative capability was enhanced with the addition of another flavonoid.

The ESR spectrometric assay quantitatively measured the free radical scavenging activity of the antioxidant against the DPPH radical. The 25 mM DPPH radicals give a typical ESR spectrum as shown in **Figure 2**. These peaks will be reduced on the basis of the strength of the antioxidative capability when an antioxidant was added to the ethanolic DPPH solution.

The scavenging concentrations to obtain 50% of the maximum scavenging capacity of DPPH by the tested compounds are listed in **Table 2**. The radical scavenging activity of these compounds was found to be concentration dependent. Once again, quercetin **1** was found to possess the strongest ability to act as a free radical scavenger with SC_{50} values of 0.7 μM, followed by quercitrin **4** with SC_{50} of 42.6 μM. The EtOAc extract of the leaves possess the strongest radical scavenging activities in this method compared to the other crude extracts with SC_{50} 9.9 μg/mL. This was due to the presence of several flavonoid compounds which were very active as a radical scavenger in this crude extract such as quercetin **1**, quercetin 3-*O*-α-L-(2″-*O*-acetyl)-arabinofuranoside **2**, avicularin **3**, and quercitrin **4**. These results correlated well and matched with the results obtained from the UV spectrophotometric assay.

Flavonoids are a group of compounds characterized by a C_6-C_3-C_6 configuration and can participate in hydrogen donating. The position and the number of hydroxyl groups on the ring dictate the antioxidant activity of flavonoids (*23*, *24*). Quercetin **1** exhibited the strongest radical scavenger in both methods due to both reasons. Hydroxyl groups at the 3′ and 4′ position (cathecol) in the ring B arrangement of quercetin that form ortho-dihydroxylation are the main feature of the most potent scavengers of the DPPH radicals (*25*). This was supported by the structure of this compound which has a heterocyclic C-ring consisting of a 4-keto group, a 2,3-double bond, and a 3-hydroxyl group, which allows the formation of a huge π-bond that links the A and B

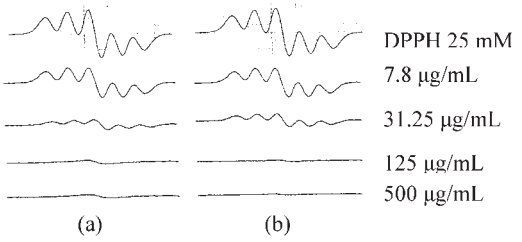

Figure 2. ESR spectra of the scavenging effects of (**a**) vitamin C and (**b**) quercetin on the 25 mM DPPH radical at various concentrations.

rings for electron delocalization. This helps to stabilize the aryloxyl radical after hydrogen donation in the process of scavenging the free radicals (*17*). However, glycoside substitutes at the C-3 position such as rhamnoside in quercitrin **4** and arabinoside in avicularin **3** will reduce the potency of the aglycone as a radical scavenger due to fewer intermediate structures that can be formed.

Tyrosinase Inhibition Assay. The mushroom tyrosinase used for the bioassay was purchased from Sigma Chemical Co. (St Louis. MO). Although mushroom tyrosinase differs somewhat from other sources, this fungal source was used for the experiment because it is readily available. Since the mode of inhibition depends on the structure of both the substrate and inhibitor, L-DOPA was used as the substrate in this experiment unless otherwise specified. Therefore, inhibitors discussed in this assay are inhibitors of the diphenolase activity of mushroom tyrosinase, and their effect on the enzyme was determined by spectrophotometry (dopachrome formation at 492 nm).

Table 3 shows the percentage of inhibition of all sample tested in this assay. Quercetin **1** exhibited strong antityrosinase activity with a percent inhibition of 95.0% compared to kojic acid as a reference inhibition with 93.8%. This was followed by the hexane extract and EtOAc extract of the leaves with percent inhibition of 75.7% and 33.8%, repectively. In contrast to quercetin **1**, its 3-*O*-glycoside analogues, quercetin 3-*O*-α-L-(2″-*O*-acetyl)-arabinofuranoside **2**, avicularin **3**, and quercitrin **4** did not exhibit any inhibitory activity for the L-DOPA oxidation. The results obtained

Table 3. Percentage Inhibition of the Tyrosinase Inhibition Assay

solvent	sample	inhibition (%)
aqueous	kojic acid (**6**)	93.8
acetone	quercetin 3-*O*-α-L-(2″-*O*-acetyl)-arabinofuranoside (**2**)	
DMSO	3,3′-*O*-dimethyl ellagic acid 4-*O*-α-L-rhamnopyranoside (**5**)	5.3
EtOAc	EtOAc extract of the leaves	33.8
hexane	hexane extract of the leaves	75.8
methanol	quercetin (**1**)	95.0
	avicularin (**3**)	
	quercitrin (**4**)	
	methanol extract of the stem barks	
	methanol extract of the leaves	

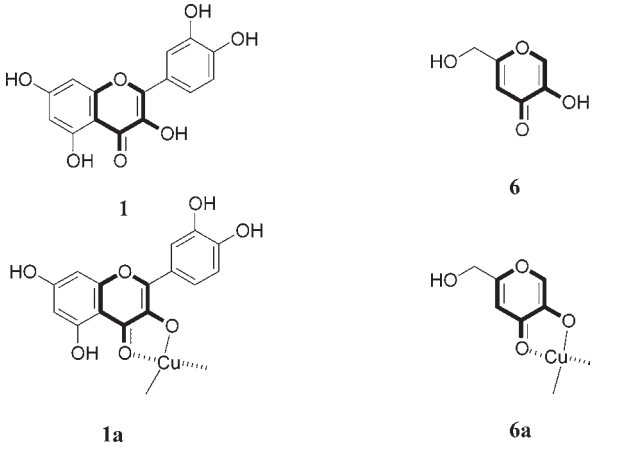

Figure 3. Chemical structures of quercetin (**1**) and kojic acid (**6**) showing the 3-hydroxy-4-keto moiety and copper chelation of quercetin (**1a**) and kojic acid (**6a**).

indicate that the free hydroxyl group at the C-3 position plays an important role in eliciting tyrosinase inhibitory activity. It appears that, as shown with a bold line in **Figure 3**, a portion of the structure **1a** (3-hydroxy-4-keto moiety) in quercetin (**1**) was clearly superimpossable with kojic acid, **6**. The inhibition exerted by kojic acid is well established as coming from its ability to chelate copper in the enzyme (*26*, *27*). The structural similarity of **1a** and **6a** suggests that quercetin (**1**) can also chelate the copper in the tyrosinase enzyme.

In conclusion, quercetin 3-*O*-α-L-(2″-*O*-acetyl)-arabinofuranoside (**2**), quercitrin (**4**), and 3,3′-*O*-dimethyl ellagic acid 4-*O*-α-L-rhamnopyranoside (**5**) were isolated for the first time from *T. semidecandra*, whereas quercetin 3-*O*-α-L-(2′-*O*-acetyl)-arabinofuranoside (**2**), and 3,3′-*O*-dimethyl ellagic acid 4-*O*-α-L-rhamnopyranoside (**5**) was found for the first time in the genus *Tibouchina*. Quercitrin (**4**) has also been obtained previously from *T. pulchra* (*28*), and *T. grandifolia* (*29*). The result also revealed that polyphenol compounds, especially quercetin (**1**), were potential free radical scavengers. Quercetin (**1**) was also found to be the most promising natural tyrosinase inhibitor.

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