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Antioxidant and cytotoxic flavonoids from the flowers of *Melastoma malabathricum* L.

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Abstract

Phytochemical and bioactivity studies of the flowers of *Melastoma malabathricum* L. (Melastomataceae) have been carried out. The ethyl acetate extract yielded three compounds, identified as naringenin, kaempferol and kaempferol-3-*O*-D-glucoside, and methanol extract gave kaempferol-3-*O*-(2″,6″-di-*O*-*p*-*trans*-coumaroyl)glucoside and kaempferol-3-*O*-D-glucoside. The crude extracts and isolated compounds were screened for their antioxidant and cytotoxic activities. The antioxidant assay was carried out by the DPPH radical-scavenging electron spin resonance (ESR) spectroscopic method. The cytotoxicity was measured by the MTT assay against a MCF7 cell line. Naringenin, kaempferol, kaempferol-3-*O*-D-glucoside, kaempferol-3-*O*-(2″,6″-di-*O*-*p*-*trans*-coumaroyl) glucoside, ethyl acetate and methanol extracts were found to be active as radical-scavengers with IC_{50} values of 0.52 mM, 81.5 μM, 1.07 mM, 35.8 μM, 7.21 μg/ml and 6.59 μg/ml, respectively. Naringenin and kaempferol-3-*O*-(2″,6″-di-*O*-*p*-*trans*-coumaroyl)glucoside were also found to be active in inhibiting cell proliferation of MCF7 with IC_{50} values of 0.28 μM and 1.3 μM, respectively.

Keywords: *Melastoma malabathricum*; Melastomataceae; Flavonoids; Antioxidant; Cytotoxic

1. Introduction

Melastoma malabathricum L. (senduduk) is a very common herb or shrub found through out the tropics, in moist areas, where it grows as small trees 12–13 ft. high, occasionally even up to 20 ft. They may be found in the forest at the edge of a stream, on landslips or in old clearings, and they are evergreen and flower throughout the year. (Burkill, 1966).

Characteristics of these species are leaves 0.25–2 in. wide, with stalks 0.25–0.5 in. long, flowers 1–3 in. wide, calyx closely set with short chaffy and silky or silvery scale. This species spread from Madagascar and India to Australia and is very common throughout Malaysia in the lowland and mountain forests, chiefly in open places (Whitmore, 1972).

Senduduk consists of three varieties, having large, medium and small size flowers with dark purple-magenta petals, light pink-magenta petals, and (the rare variety) white petals. *Melastoma malabathricum* with the white flower is reported to have miraculous healing properties (Corner, 1951). The plants have been used in traditional Malay medicine for the treatment of diarrhoea as an astringent, post-partum treatment and haemorrhoids (Burkill, 1966), but, to the best of our knowledge, there has been no report on the phytochemicals of this plant.

Several tannins have been isolated from the dry leaves of *M. malabathricum* with light pink-magenta petals. The main tannins were the hydrolysable tannin oligomers nobotanin B, malabathrins B, malabathrins C, malabathrins D, the hydrolysable tannin monomers, 1,4,6-tri-*O*-gal-

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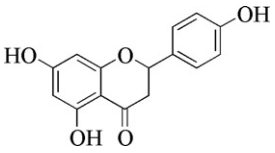
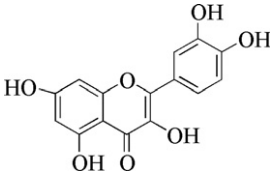
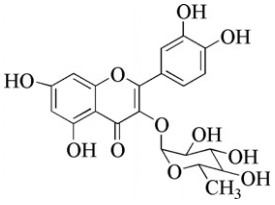
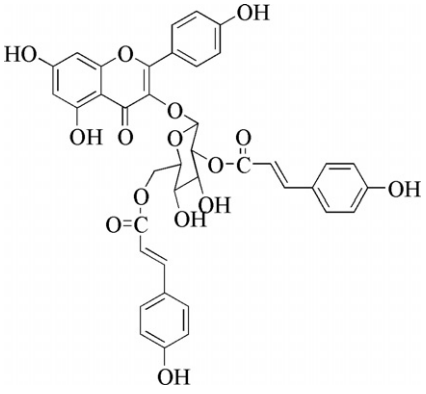
loyl-β-D-glucose, 1,2,4,6-tetra-*O*-galloyl-β-D-glucose, strictinin, casuarictin, pedunculagin, nobotanin D, pterocarinin and the hydrolysable tannin oligomers, nobotanin G, nobotanin H and nobotanin J which were recently found to exhibit potent *in vitro* antiviral activity against human immunodeficiency virus (Yoshida, Nakata, Hosotani, Nitta, & Okuda, 1992). *M. malabathricum* with light pink-magenta petals also contains β-sitosterol, α-amyrin, uvaol, sitosterol 3-*O*-β-D-glucopyranoside, quercetin, quercitrin and rutin (Nuresti, Baek, & Asari, 2003).

In this report, we wish to report the isolation and structural elucidation of four flavonoids (**1–4**) from the flowers of *M. malabathricum*, together with their bioactivities.









2. Materials and methods

2.1. General methods

Mps. (uncorr.) were determined using the Leica Gallen III apparatus. UV absorbance was recorded on a Shimadzu UV-160 spectrophotometer in methanol. IR spectra were recorded on a Perkin–Elmer 1650 FTIR spectrophotometer. NMR spectra were recorded on a JEOL JNH A500 Spectrometer and measured at 500 MHz and 125 MHz, respectively. Vacuum liquid chromatography (VLC) and column chromatography (CC) were carried out using silica gel 230–400 mesh, Merck 9385 and 70–230 mesh, Merck 7734. Sephadex LH-20 was purchased from Pharmacia (Uppsala, Sweden). ESR spectra were obtained on a JEOL JES-FA100 instrument using manganese oxide (MnO) as an internal standard. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was from Fluka (Switzerland), dimethylsulfoxide and MTT were from Sigma–Aldrich (St. Louis, MO, USA), DMEM/F-12 was purchased from Gibco-BRL Life Technologies (Auckland, NZ), vitamin E and vitamin C were purchased from Merck (Darmstadt, Germany) and tamoxifen was purchased from Calbiochem, affiliate of Merck (Darmstadt, Germany).

2.2. Plant material

Melastoma malabathricum was collected from Johor, Malaysia in 2002 and identified by Dr. Rusdi Tamin of Universitas Andalas, Padang, Indonesia. A voucher specimen (YANTI-2061) was deposited at the Herbarium of Universitas Andalas (ANDA) Padang, Indonesia.

2.3. Extraction and isolation

The air-dried and powdered flowers of *M.malabathricum* (87.9 g) were extracted by Soxhlet extractor for 18 h with *n*-hexane, EtOAc and MeOH, successively. Evaporation of the respective solvent gave the *n*-hexane (1.3 g, 1.5%), EtOAc (1.5 g, 1.7%) and MeOH (2.3 g, 2.6%) extracts.

The EtOAc extract (1.3 g) was fractionated by VLC on silica gel 60 (230–400 mesh), using *n*-hexane and *n*-hexane:EtOAc (9:1, 8:2, 5:5), EtOAc, and EtOAc:MeOH (9:1, 8:2, 5:5) as eluents to afford nine fractions (EF1–EF9). Fraction EF4 (0.7 g) was purified by column chromatography on silica gel 70–230 mesh and eluted with *n*-hexane–EtOAc (9:1, 8:2, 7:3, 5:5), EtOAc, EtOAc–MeOH (9:1, 8;2, 7:3) and MeOH afforded four fractions (1–5, 6–15, 16–25, 25–48). The combined fractions 25–48 were purified on Sephadex LH-20 with MeOH to afford **1** (0.005 g, 0.006%) and **2** (0.027 g, 0.03%).

Naringenin (**1**) was obtained as brownish needles with mp. 250–252 °C, lit. (Kuo, Lee, & Lai, 2000) 249–252 °C; R_f 0.6 in EtOAc; UV λ_{max}^{MeOH} nm (log ε): 320 (4.3), 290 (4.4); +NaOMe, 315, 242; +NaOAc, 322, 291 (sh), 207; +NaOAc/H_3BO_3, 320, 291; +$AlCl_3$ 368, 306; +$AlCl_3$/HCl, 368, 306; IR υ_{max}^{KBr} cm^{-1}: 3453, 3282, 1685, 1601, 1518, 1249, 1156, 1083, 828; ^1H NMR (acetone-d_6): δ 12.19 (s, OH), 7.41 (2H, dd, $J = 8.8$, 2.3 Hz, H-2′, H-6′), 6.90 (2H, dd, $J = 8.8$, 2.3 Hz, H-3′, H-5′), 5.97 (2H, d, $J = 1.5$ Hz, H-6, H-8), 5.47 (1H, dd, $J = 12.9$, 3.0 Hz, H-2), 3.19 (1H, dd, $J = 12.9$, 17.1 Hz, H-3β), 2.74 (1H, dd, $J = 17.1$, 3.0 Hz, H-3α); ^{13}C NMR (acetone-d_6): δ 178.2 (C-4), 166.5 (C-7), 164.5 (C-5), 163.5 (C-4′), 157.8 (C-9),

127.9 (C-2′, C-6′), 115.4 (C-3′, C-5′), 102.5 (C-10), 96.0 (C-6), 95.0 (C-8), 79.0 (C-2), 42.7 (C-3); CIMS: *m/z* 273 $[M+H]^+$, (Found: C, 66.18%; H, 4.41%; $C_{15}H_{12}O_5$).

Kaempferol (**2**) was obtained as a yellow powder, m.p. 270–273 °C, lit. (Iwashina et al., 1995) 271–272 °C; R_f 0.5 in EtOAc, UV λ_{max}^{MeOH} nm (log ε): 365 (3.9), 258 (4.0); +NaOMe 415, 312 (sh); +NaOAc 413, 258; +NaOAc/H_3BO_3 414, 258; +$AlCl_3$ 412, 339, 300 (sh), 265; +$AlCl_3$/HCl 408, 366, 294 (sh), 264; IR v_{max}^{KBr} cm^{-1}: 3339, 3186, 1658, 1614, 1508, 1382, 1315, 1175, 818; CIMS: *m/z* 287 $[M+H]^+$, (Found: C, 62.94%; H, 3.50%; $C_{15}H_{10}O_6$); ^1H NMR (acetone-d_6): δ 12.19 (s, OH), 8.16 (2H, d, $J = 9.3$, 2.4 Hz, H-2′, H-6′), 7.02 (2H, d, $J = 9.3$, 2.4 Hz, H-3′, H-5′), 6.55 (1H, d, $J = 2.1$, H-8), 6.28 (1H, d, $J = 2.1$, H-6); ^{13}C NMR (acetone-d_6): δ 178.2 (C-4), 164.2 (C-7), 161.5 (C-5), 159.3 (C-4′), 129.5 (C-2′, C-6′), 122.5 (C-1′), 115.5 (C-3′, C-5′), 98.4 (C-8), 93.6 (C-6), 157.0 (C-9), 108.7 (C-10).

Fraction EF6 (0.8 g) from the EtOAc extract was purified by CC on silica gel 70–230 mesh, using *n*-hexane–EtOAc (8:2, 7:3, 5:5, 4:6), EtOAc, EtOAc–MeOH (9:1, 7:3, 5:5) and MeOH as solvents to afford five fractions (1–3, 4–9, 10–11, 12–25, 26–32). The fraction 12–25 was rechromatographed on Sephadex LH-20 with MeOH to give compound **3** (0.056 g, 0.06%).

The MeOH extract (2.0 g) was fractionated by VLC on silica gel 60 (230–400 mesh) and eluted with EtOAc, EtOAc:MeOH (9:1, 7:3, 5:5) and MeOH to afford five fractions (MF1–MF5).

Fractions MF1 and MF2 (0.7 g) were combined and purified by CC on silica gel 70–230 mesh, using EtOAc, EtOAc–MeOH (9:1, 7:3, 5:5) and MeOH as solvents to give new fractions (1–5, 6–15, 16–19, 20–25, 26–38). Fraction 26–38 was purified through Sephadex LH-20, using MeOH to afford compound **3** (0.039 g, 0.04%) and compound **4** (0.011 g, 0.01%).

Kaempferol 3-*O*-D-glucoside (**3**) was obtained as a yellow powder with m.p. 245–247 °C, lit. (Iwashina et al., 1995) 246–248 °C; R_f 0.3 in EtOAc; UV λ_{max}^{MeOH} nm (log ε): 340 (4.0) (3.9), 265 (4.1); + NaOMe 392, 322 (sh), 272; + NaOAc 360, 337 (sh), 301, 269; +NaOAc/H_3BO_3 337, 299, 268; +$AlCl_3$ 388, 338 (sh), 272; +$AlCl_3$/HCl 387, 339 (sh), 272; IR v_{max}^{KBr} cm^{-1}: 3283, 1684, 1607, 1559, 1497, 1209, 1069, 834; ^1H NMR (CD_3OD): δ 1.06 (2H, dd, $J = 6.9$, 1.8 Hz, H-2′, H-6′), 6.89 (2H, dd, $J = 6.9$, 2.1 Hz, H-3′, H-5′), 6.39 (1H, d, $J = 1.9$ Hz, H-8), 6.20 (1H, d, $J = 1.9$ Hz, H-6), 5.23 (1H, dd, $J = 7.5$, 2.4 Hz, H-1″), 3.71 (1H, dd, $J = 11.8$, 2.4 Hz, H-2″), 3.55 (1H, dd, $J = 11.8$, 5.4 Hz, H-3″), 3.47–3.43 (1H, m, H-4″), 3.34–3.31 (2H, m, H-6″), 3.25–3.22 (1H, m, H-5″); ^{13}C NMR (CD_3OD): δ 178.2 (C-4), 164.2 (C-7), 161.5 (C-5), 159.3 (C-4′), 129.3 (C-2′, C-6′), 122.5 (C-1′), 113.1 (C-3′, C-5′), 97.2 (C-6), 92.0 (C-8), 157.0 (C-9), 108.7 (C-10), 101.2 (C-1″), 75.5 (C-4″), 75.1 (C-2″), 72.8 (C-3″), 68.4 (C-5″), 59.6 (C-6″); FABMS: *m/z* 449 $[M+H]^+$, (Found: C, 56.25%; H, 4.46%; $C_{21}H_{20}O_{11}$).

Kaempferol-3-*O*-(2″,6″-di-*O*-*p*-*trans*-coumaroyl)glucoside (**4**) was obtained as a yellow powder, m.p. 172–174 °C, lit. (Skaltsa, Verykokidou, Harvala, Karabourniotis, & Manetas, 1994) 174–175 °C, $[\alpha]_D^{24}$ − 69 ($c = 0.42$ in MeOH); UV λ_{max}^{MeOH} nm (log ε): 314 (3.6), 269 (3.5); +NaOMe 363, 273; +NaOAc 315, 270, 205; +NaOAc/H_3BO_3 312, 305, 271; +$AlCl_3$ 312, 273; +$AlCl_3$/HCl 313, 275; IR v_{max}^{KBr} cm^{-1}: 3247, 1687, 1655, 1604, 1514, 1360, 1171, 831; ^1H NMR (acetone-d_6) δ 2.77(1H, s, OH), 8.14 (2H, d, $J = 8.8$ Hz, H-2′, H-6′), 7.70 (1H, d, $J = 15.9$ Hz, H-β‴″), 7.56 (2H, d, $J = 8.7$ Hz, H-2‴″, H-6‴″), 7.45 (1H, d, $J = 15.9$ Hz, H-β‴), 7.40 (2H, d, $J = 8.5$ Hz, H-2‴, H-6‴), 6.97 (2H, d, $J = 8.7$ Hz, H-3′, H-5′), 6.89 (2H, d, $J = 8.7$ Hz, H-3‴″, H-5‴″), 6.87 (2H, d, $J = 8.5$ Hz, H-3‴, H-5‴), 6.44 (1H, d, $J = 1.9$ Hz, H-6), 6.41 (1H, d, $J = 15.9$ Hz, H-α‴″), 6.20 (1H, d, $J = 1.9$ Hz, H-8), 6.16 (1H, d, $J = 15.9$ Hz, H-α‴), 5.90 (1H, d, $J = 7.6$ Hz, H-1″), 5.42 (1H, dd, $J =$ 7.6 and 9.7 Hz, H-2″), 4.28 (2H, m, H-6″), 4.04 (1H, d, $J = 3.0$ Hz, H-4″), 3.98 (1H, dd, $J = 9.7$ and 3.0 Hz, H-3″), 3.96 (1H, m, H-5″); ^{13}C NMR (Acetone-d_6) δ: 162.0 (C-2), 133.5 (C-3), 177.9 (C-4), 163.9 (C-5), 98.7 (C-6), 162.3 (C-7), 93.7 (C-8), 156.9 (C-9), 104.8 (C-10),121.9 (C-1′), 131.2 (C-2′), 115.2 (C-3′), 159.9 (C-4′), 115.2 (C-5′), 131.2 (C-6′), 166.2 (C-γ‴″), 144.7 (C-β‴″), 114.3 (C-α‴″), 126.1 (C-1‴″), 130.0 (C-2‴″), 115.8 (C-3‴″), 159.7 (C-4‴″), 115.8 (C-5‴″), 130.0 (C-6‴″), 166.3 (C-γ‴″), 144.9 (C-β‴″), 115.0 (C-α‴″), 126.3 (C-1‴″), 130.1 (C-2‴″), 115.9 (C-3‴″), 132.9 (C-4‴″), 115.9 (C-5‴″), 130.1 (C-6‴″), 99.2 (C-1″), 72.8 (C-2″), 71.8 (C-3″), 69.1 (C-4″), 73.4 (C-5″), 62.8 (C-6″); FABMS *m/z*: 763 $[M+Na]^+$, 741 $[M+H]^+$, (Found: C, 63.24%; H, 4.32%; $C_{39}H_{32}O_{15}$).

2.4. Antioxidant activity

DPPH radical-scavenging, using ESR (electron spin resonance), was carried out according to the method described by Ohtani et al. (2000) with slight modification. The ethanolic solution of the test sample 100 μl (1 mg/ml) was added to 100 μl of DPPH (39.43 M) in ethanol solution. After shaking vigorously for 10 s, the solution was transferred to a flat cell. The ESR spectra were recorded after 40 s. The conditions of the ESR spectrometer were set at room temperature, power: 1 mW, magnetic field: 336.000 ± 5 mT, field modulation width: 0.5 mT, sweep time: 30 s and time constant: 0.03 s. The scavenging effect of DPPH was calculated by the equation below:

$$\%\text{DPPH Scavenging} = \frac{\text{PHDPPH} - \text{PHsample}}{\text{PHDPPH}} \times 100\%$$

where: PH = peak height of the third and the fifth line signals of the DPPH radical.

Vitamin E and vitamin C were used as standard positive control.

2.5. Cytotoxic activity

Cytotoxicity assay was carried out using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay according to Mosmann (1983). MCF7 was cultured

DMEM/F-12 with 15 mM HEPES buffer, L-glutamine and pyridoxine hydrochloride supplemented with 10% foetal bovine serum and 1% penicillin–streptomycin in a 96-well plate at a density of $6x10^4$ cells/ml. After reaching confluent (2×10^5 cells/ml), the cells were treated with the sample. The sample was dissolved with dimethylsulfoxide (DMSO) and the final concentration of DMSO was 0.1% (v/v). Different concentrations of the sample were prepared with serial dilution. Dimethylsulfoxide (0.1%) was used a control. The experiment was allowed to proceed for 48 h at 37 °C in a humidified 5% CO_2 atmosphere. At the end of this period, supernatants were discarded. To minimize the interference of residue of the supernatant, the adherent cells were washed twice with phosphate buffer saline (PBS), then 20 μl of MTT stock solution (5 mg/ml) were added to each well and the plates were further incubated overnight at 37 °C. DMSO (100 μl) was added to each well to solubilize the water-insoluble purple formazan crystals. After 1 h, the absorbance was measured at 570 and 655 nm (reference) with a microplate reader. Tamoxifen was used as a commercial standard anticancer agent. The 50% reduction in cell number relative to the control or IC_{50} was estimated visually.

$$\text{Cells number after treatment} = \frac{\text{Abs. sample}}{\text{Abs. DMSO}} \times 2 \times 10^5 \text{ cells/ml}$$

2.6. Statistical analysis

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

3. Results and discussion

3.1. Phytochemical investigation

The dried and powdered flowers of *M. malabathricum* (87.9 g) were extracted (Soxhlet) for 18 h with *n*-hexane, EtOAc and MeOH, successively. The EtOAc extracts were subjected to several chromatographic techniques (VLC and CC) to yield three flavonoids **1–3**, while the MeOH extract after purification afforded **3** and **4**.

By correlating with melting points and spectral data (UV, IR, ^1H, ^{13}C NMR and MS) of literature values, compounds **1–4** were identified as naringenin (**1**) (Markham, 1982; Kuo et al., 2000; Bohm, 1998; Corticchiato et al., 1995), kaempferol (**2**) (Ohmura, Ohara, Hashida, Aoyama, & Doi, 2002; Hadizadeh, Khalili, Hosseinzadeh, & Khair-Aldine, 2003; Hamzah & Lajis, 1998), kaempferol-3-*O*-D-glucoside (**3**) (Hamzah & Lajis, 1998; Iwashina et al., 1995) and kaempferol-3-*O*-(2″,6″-di-*O*-*p*-*trans*-coumaroyl)glucoside (**4**) (Fiorini, David, Fouraste, & Vercauteren, 1998; Harborne, Mabry, & Mabry, 1975; Nasser & Singab, 1998; Skaltsa et al., 1994; Tomas-Barberan, Gil, Ferreres, & Tomas-Lorente, 1992), respectively. This is the first report of the isolation of these flavonoids from the flowers of this plant.

3.2. Bioactivity assay

3.2.1. Antioxidant activity

Oxidative stress can damage many biological molecules. Proteins and DNA are significant targets of cellular injury. Another target of free radical attack in biological systems is the lipids of the cell membranes. Lipid peroxides are potentially toxic and possess the capacity to damage mast cells. Accumulation of lipid peroxides has been reported in atherosclerotic plaques, in brain tissues damaged by trauma or oxygen deprivation, and in tissues poisoned by toxins (Middleton, Kandaswami, & Theoharides, 2000).

One of the methods for measuring antioxidant potential is through determining the free radical inhibitory ability of different antioxidant by using very stable free radicals, such as DPPH$^{\cdot}$ in methanol solution. The reduction of DPPH$^{\cdot}$ is followed by monitoring the decrease in its absorbance at a characteristic wavelength during the reaction.

The mechanism of reaction between antioxidant and DPPH depends on the structural conformation of the antioxidant. Some compounds react very quickly with DPPH, reducing a number of DPPH molecules equal to the number of the hydroxyl groups (Bondet, Williams, & Berset, 1997).

Electron spin resonance spectrometry is considered to be the least ambiguous method for the detection of free radicals. ESR has been successfully employed to study the free radical-scavenging activity of antioxidants by using stable

Table 1
The IC_{50} values of the crude extracts and the isolated compounds (**1–4**) from the flowers of *M. malabathricum* by the ESR spectrometry method

Sample	IC_{50} (mean ± SD)	Percent inhibition 7.8 μg/ml (mean ± SD)
Kaempferol-3-*O*-(2″,6″-di-*O*-*p*-*trans*-coumaroyl)-*β*-glucopyranoside (**4**)	35.8 μM ± 0.5	31.2 ± 4.5
Naringenin (**1**)	0.52 mM ± 0.5	42.5 ± 0.7
Kaempferol (**2**)	81.5 μM ± 0.7	38.6 ± 0.09
Kaempferol-3-*O*-D-glucoside (**3**)	1.07 mM ± 0.4	24.6 ± 0.3
Ethyl acetate extract	7.21 μg/ml ± 0.5	53.2 ± 1.3
Methanol extract	6.59 μg/ml ± 0.8	59.3 ± 1.4
Vitamin E	210.2 μM ± 0.1	50.9 ± 0.07
Vitamin C	8.01 μM ± 0.5	82.2 ± 0.3

Data represent means ± SD of three independent experiments performed in triplicate.

free radicals, such as DPPH·. In addition to ESR measurement, the stable DPPH radical can be used to study the reaction kinetics of antioxidants, quantify and compared the free radical-scavenging capacities of different antioxidants (Yu, 2001).

Kaempferol, as a major aglycone in the methanol extract of the leaves of *Melilotus elegans,* which is used for treatment of haemorrhoid and lacerated wounds in Ethiopia, was formulated into creams and reported to have anti-inflammatory activity. (Mariam et al., 2005).

Kaempferol-3-*O*-(2″,6″-di-*O*-*p*-*trans*-coumaroyl)glucoside, isolated from *Quercus dentata* (Fagaceae), was reported to show strong suppression tyrosyl phosphorylation of neutrophils proteins by inhibiting of protein tyrosine kinase (Meng et al., 2001).

The methanol extract was a stronger free radical-scavenger (59.3% inhibition) than was the ethyl acetate extract (53.2% inhibition) (Table 1). The flavonoid **4**, previously isolated from the methanol extract, was a more active antioxidant than were **2** and **3**, which were isolated from the ethyl acetate extract. The pronounced radical-scavenging activity of compound **4** is due to the presence of two *p*-coumaroyl acid groups which are located at the 2″ and 6″ positions in the glucose ring. Thus, it is proposed that the presence of compound **4** contributed to the higher antioxidant activity of the methanol extract.

Compound **1** was found to be less active than were **2** and **3**, due to the lack of an unsaturated heterocyclic ring (C-ring), as well as the lack of a 3-OH group (Table 1). The unsaturation in the C ring is important, to allow electron delocalization across the molecule for stabilization of the aryloxyl radical (Evans, Miller, & Paganga, 1995).

The free radical-scavenging of flavonoids is also dependent on the presence of a free 3-OH. Flavonoids with a 3-OH and 3′,4′-cathecol are reported to be 10-fold more potent (Heim, Tagliaferro, & Bobilya, 2002). Fig. 1 shows the intensity of DPPH signals, at a concentration 7.8 μg/ml for the isolated compounds, with vitamin E and vitamin C as positive controls.

3.2.2. Cytotoxic activity

The use of antineoplastic drugs is based on their selective toxicity toward malignant cells. These drugs take advantage of the differences between malignant and normal cells, such as the state of proliferation or innate metabolism. Ideally, this goal should be attained with little or no effect on normal cells. Many therapies now combine a variety of modalities to eradicate this problem. These include surgery and radiotherapy, the use of specific metabolic inhibitors (antihormones), modifiers of nucleic acid synthesis, modifiers of DNA function, antimitotic agents, antiangiogenic agents and immune potentiators (Lewis & Lewis, 2003).

Humans ingest about 1 g of flavonoids daily in their diet, and they are increasingly associated with cytoprotective antitumor properties. The mechanisms responsible

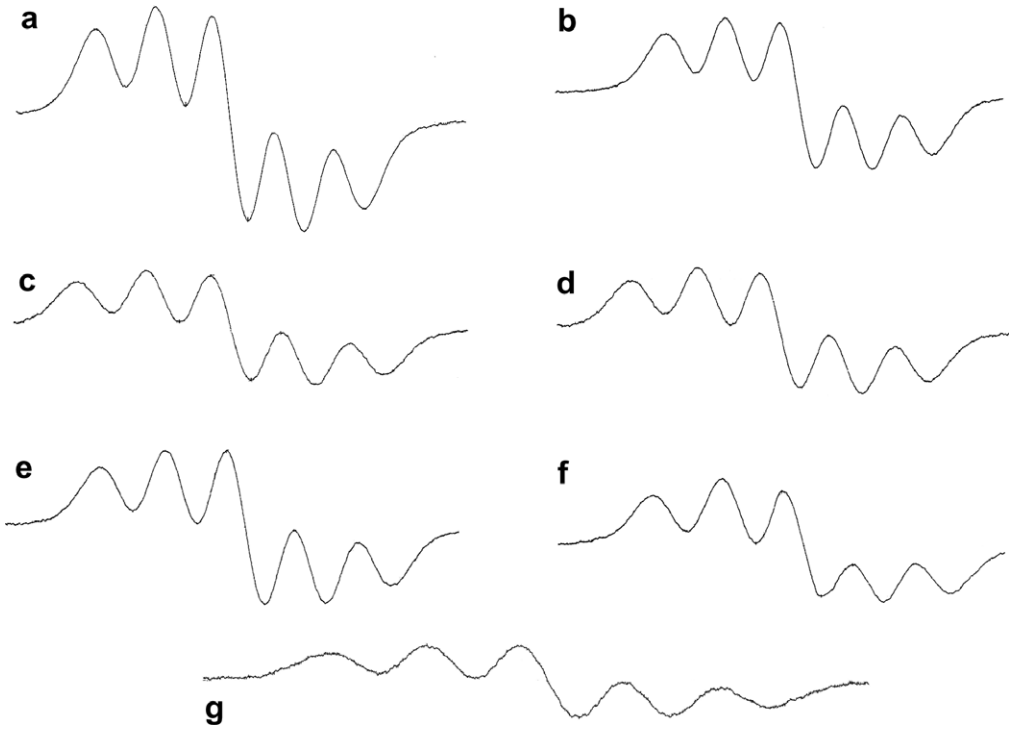


Fig. 1. Scavenging activity of, (b) Compound **4** + 25.3 mM DPPH (percent inhibition: 31.2%), (c) Compound **1** + 25.3 mM DPPH (percent inhibition: 42.5%), (d) Compound **2** + 25.3 mM DPPH (percent inhibition: 38.6%), (e) Compound **3** + 25.3 mM DPPH (percent inhibition: 24.6%), (f) Vitamin E + 25.3 mM DPPH (percent inhibition: 51.0%), (g) Vitamin C + 25.3 mM DPPH (percent inhibition 82.2%) against (a) DPPH (25.3 mM) free radical by ESR spectrometry.

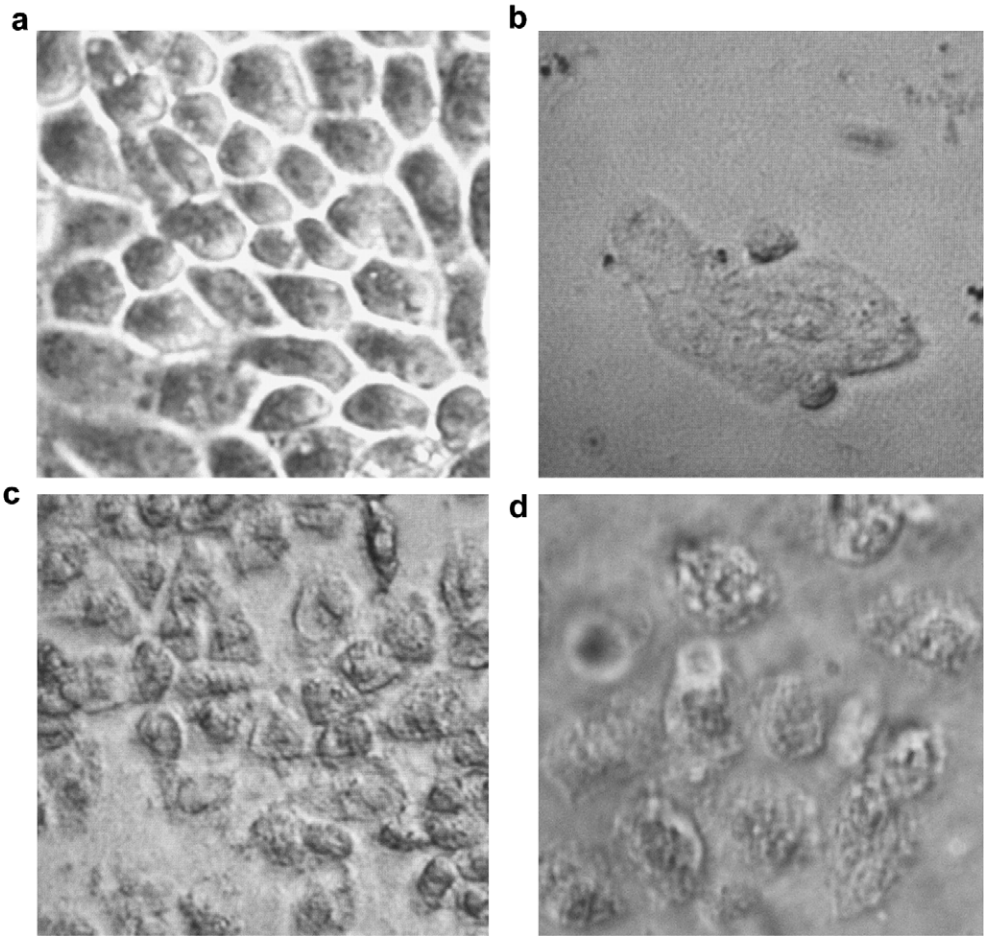


Fig. 2. Morphology of Human Breast Cancer (MCF 7). (a) Confluent cells (untreated), (b) after 48 h of treatment with compound **4**, (c) after 48 h of treatment with **1** and (d) after 48 h of treatment with tamoxifen.

for these effect have not yet been elucidated but may involve interaction with xenobiotic metabolizing enzymes to alter the metabolic activation of potential carcinogens (Rodgers & Grant, 1998). Some flavonoids cause apoptosis in human tumor cells and act as antiproliferative agents in cancer cells (Middleton et al., 2000).

One of the mechanism by which flavonoids might exert their cytoprotective anticancer effect is through interaction with the cytochrome P-450 mixed function oxidase system, either by inhibition or induction of the isoform of this enzyme. This may lead to decreased metabolic activation of potential carcinogens (Rodgers & Grant, 1998).

The ethyl acetate extract showed a change in the cell morphology of MCF7 cells line at a concentration 500 μg/ml while, at the same concentration, the methanol extract did not display any activity. Compounds **1** and **4** showed a significant anticancer effect against MCF7 by affecting cell proliferation and changing the cell morphology (Fig. 2). Compounds **1** and **4** were found to be active in a dose-dependent manner with IC_{50} values of 1.3 μM (SD ± 0.002) and 0.28 μM (SD ± 0.004), respectively. Thus, it is proposed that compound **1** contributed to the anticancer activity of the ethyl acetate extract. As for the methanol extract, it is suggested that the antagonist effects of the compounds present in the extract play an important role in not affecting the cell proliferation.

The IC_{50} value of **4** was lower than that of the positive control, tamoxifen, which had the IC_{50} 0.76 μM (SD ± 0.005). Based on the cell morphology, it was proposed that compound **4** was active against human breast cell cancer by inhibiting cell proliferation. Meanwhile, compound **1** inhibited cell proliferation, followed by cell lyses.

Compound **4** has a free hydroxyl group at position 3 and a *para*-hydroxyl group in ring B, which probably increase the activity of this compound. Thus, this compound could be a useful anticancer agent.

In conclusion, kaempferol-3-*O*-(2″,6″-di-*O*-*p*-*trans*-coumaroyl)glucoside, **4,** exhibits antiradical effects in the ESR method with an IC_{50} value of 35.8 μM. Kaempferol-3-*O*-(2″, 6″-di-*O*-*p*-*trans*-coumaroyl)glucoside, **4,** and naringenin, **1,** showed significant antiproliferation effects against MCF7 by affecting cell proliferation and changing the cell morphology. Their effects were active in a dose-dependent manner with IC_{50} values of 0.28 μM and 1.3 μM, respectively.

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