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Antioxidative and Radical Scavenging Properties of the Constituents Isolated from *Cosmos caudatus* Kunth

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Abstract – A phytochemical investigation on the methanolic extract of *Cosmos caudatus* has led to the isolation of quercetin 3-*O*-β-D-arabinofuranoside (1), quercetin 3-*O*-β-D- rhamnoside (2), quercetin 3-*O*-α-D-glucoside (3) and quercetin (4). These compounds were shown to be the antioxidative constituents of the plant when evaluated using the ferric thiocyanate (FTC) and thiobarbituric acid (TBA), and radical scavengers based on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assays.
Keywords – *Cosmos caudatus* Kunth., antioxidant activity, radical scavenger, flavonoids and quercetin glycosides.

Introduction

Vegetables and greens are believed to be a rich source of antioxidants and their dietary consumption can help counter the detrimental effects of oxygen free radicals. *Cosmos caudatus* or locally named 'ulam raja', a small herb of the family Compositae, is a common and popular traditional vegetable, often consumed raw as a salad. Medicinally, this plant is believed to promote the formation of healthy bones and said to be useful in 'cleansing the blood' (Burkill, 1966, Ismail, 2000). *C. caudatus* have previously been reported to show moderate antioxidant activity when tested using the xanthine-xanthine oxidase enzymatic assay (Norhanom *et al.*, 1999). A phytochemical investigation on the species was thus initiated in order to identify the antioxidative constituents of the plant. Previous studies on the plant have reported the presence of phenylpropane derivatives in the roots with antifungal activity (Fuzzati, *et al.*, 1993).

Experimental

General experimental – Melting points were determined on Kofler hot-stage apparatus and were uncorrected. UV and IR (in mini KBr form) spectra were recorded on a Shimadzu UV-Vis 160 and a Perkin Elmer 1650 FT-IR spectrometers, respectively. ^{1}H and ^{13}C NMR spectra were recorded on a Varian INOVA500 spectrometer at 500 MHz (^{1}H) and 125 MHz (^{13}C), respectively. ESIMS was obtained on Finnigan LCQ-Deca using 1% HOAc as buffer. Column chromatography and analytical TLC utilized Merck 9385 and Merck DC-Plastikfolien 60_{F254}, respectively.

Plant material – *Cosmos caudatus* was purchased from a wet market in Kuala Lumpur area in July 2000. A voucher specimen (K 148/02) was deposited at the herbarium of the Laboratory of Phytomedicines, Institute of Bioscience, University Putra Malaysia.

Extraction and isolation – The ground air-dried sample of *Cosmos caudatus* (800 g) was extracted three times at room temperature with methanol (MeOH). The combined MeOH extracts were evaporated under reduced pressure to give a brown gum (120 g). Twenty grams of the gum was shaken with 300 ml water and successively partitioned into petroleum ether, methylene chloride (CH_2Cl_2), ethyl acetate (EtOAc) and n-butanol (BuOH) fractions. Removal of the solvent from EtOAc fraction under reduced pressure gave a brownish gum (4 g). The EtOAc fraction (2 g) was then subjected to column chromatography (3 cm×30 cm) packed with 60 g silica gel and successively eluted with EtOAc followed by EtOAc enriched with increasing percentages of acetone (Me_2CO) and methanol (MeOH). The eluent was collected by 100 ml each and combined according to their TLC profiles to produce 6 pooled fractions. Fraction A (80 mg) was rechromatographed on silica gel using hexane-EtOAc, solvent systems, yielding quercetin (**4**, 8 mg). Fraction B (75 mg) was recrystallised using Me_2CO: $CHCl_3$ to afford quercetin 3-*O*-β-D-arabino-

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furanoside (**1**, 45 mg). Fraction C (300 mg) also was recrystallised using Me_2CO: $CHCl_3$ to afford quercetin 3-*O*-α-D-rhamnoside (**2**, 250 mg). Fraction D (350 mg) was further rechromatographed on sephadex LH-20, eluted with 100% methanol to give quercetin 3-*O*-β-D-glucoside (**3**, 15 mg).

The isolated compounds were identified based on the analyses of the spectral data and their comparison with those reported in the literature (Markham *et al.*, 1978; Lin, *et al.*, 1999; Cuendet, *et al.*, 2001; Fraisse, *et al.*, 2000; Wenkert and Gottlieb, 1997).

Compound 1: Quercetin 3-*O*-β-D-arabinofuranoside. Yellow amorphous solid; m.p. 247-248°C (lit. 246-247°C); $FeCl_3$, Mg/HCl: positive; UV λ_{max} (MeOH) 258, 357 nm; (+NaOH) 271, 330, 403 nm; (+$AlCl_3$) 273, 403 nm; (+$AlCl_3$/HCl) 270, 359, 400 nm. IR V_{max} (KBr) cm^{-1}: 3430, 1652, 1608, 1504, 1068; ^1H NMR and ^{13}C NMR are consistent with literature (Markham *et al.*, 1978; Fraisse, *et al.*, 2000; Wenkert and Gottlieb, 1997); LC-ESIMS m/z 435 $(M+H)^+$, 303 $[(M+H)$-arabinofuranose$]^+$.

Compound 2: Quercetin 3-*O*-β-D-rhamnoside. Yellow amorphous solid; m.p. 182-184°C (lit. 182-185°C); $FeCl_3$, Mg/HCl: positive; UV λ_{max} (MeOH) 258, 353 nm; (+NaOH) 271, 330, 395 nm; (+$AlCl_3$) 273, 395 nm; (+$AlCl_3$/HCl) 269, 355, 397 nm. IR, ^1H NMR and ^{13}C NMR are consistent with literature (Markham *et al.*, 1978; Lin, *et al.*, 1999; Wenkert and Gottlieb, 1997); LC-ESIMS m/z 449 $(M+H)^+$, 303 $[(M+H)$-rhamnose$]^+$.

Compound 3: Quercetin 3-*O*-β-D-glucoside. Yellow amorphous solid; m.p. 215-216°C (lit. 214-216°C); FeCl, Mg/HCl positive; UV λ_{max} (MeOH) 290, 360 nm; (+NaOH) 326, 408 nm; (+ $AlCl_3$) 303, 317, 419nm; (+$AlCl_3$/HCl) 303, 357, 405 nm. IR, ^1H NMR and ^{13}C NMR are consistent with the literature (Markham *et al.*, 1978; Lin, *et al.*, 1999; Cuendet, *et al.*, 2001; Wenkert and Gottlieb, 1997); LC-ESIMS m/z 465 $(M+H)^+$, 303 $[(M+H)$-glucose$]^+$.

Compound 4: Quercetin. Yellow crystal; m.p 312-314°C (lit. 313-314°C); FeCl, Mg/HCl positive; ^1H NMR and ^{13}C consistent with the literature (Markham *et al.*, 1978; Wenkert and Gottlieb, 1997); LC-ESIMS, m/z 303 $(M+H)^+$.

Antioxidant assay

Ferric thiocyanate (FTC) method – This assay was carried out as described in the modified method of Kikuzaki and Nakatani (1993). A mixture of 2 mg of the test sample in 4 ml of 99.5% ethanol, 4.1 ml of 2.51% linoleic acid in 99.5% ethanol, 8.0 ml of 0.02 M phosphate buffer (pH 7.0) and 3.9 ml of distilled water contained in screw-cap vial (Φ38×75 mm) was placed in an oven at 40°C in the dark. To measure the extent of antioxidant activity, 0.1ml of the reaction mixture was transferred to a test tube (Φ13×150 mm) and to it, 9.7 ml of 75% (v/v) aqueous ethanol followed by 0.1 ml of 30% aqueous ammonium thiocyanate and 0.1 ml of 0.02 M ferrous chloride in 3.5% hydrochloric acid. Three min after the addition of ferrous chloride to the reaction mixture, the absorbance was measured at 500 nm. The measurement was taken every 24 h until one day after absorbance of the control reached its maximum value.

Thiobarbituric acid method – The test was conducted according to method of Kikuzaki and Nakatani (1993). Test samples were prepared in a similar manner as in the FTC method. 1 ml of 20% aqueous solution of trichloroacetic acid and 2 ml 0.67% aqueous solution of thiobarbituric acid solution were added to 2 ml of the test sample solution. The mixture was placed in a boiling water bath for 10 min. After cooling, it was centrifuged at 3000 rpm for 20 min. Absorbance of the supernatant was measured at 532 nm. Antioxidant activity was recorded based on absorbance on the last day.

DPPH free radical scavenging activity assay – The potential antioxidant activity of plant extracts was assessed on the basis of its scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (Cottelle *et al.*, 1996). Reaction mixtures containing test sample (dissolved in methanol) and 300 μM DPPH methanolic solution in 96-well microtiter plates were incubated for 30 min, and absorbance were measure at 517 nm using Spectramax Plus (Molecular Devices) UV/Vis microplate reader. The IC_{50} value was determined as the concentration of each sample required to give 50% of the optical density shown by the control. All test analyses were run in triplicates and averaged. Standard antioxidant, i.e. ascorbic acid (Sigma USA) was used as positive control.

Results and Discussion

Phytochemical work up on the ethyl acetate fraction obtained from the crude methanolic extract of *Cosmos caudatus* have yielded quercetin (**4**) and its three glycosides, quercetin 3-*O*-β-D-arabinofuranoside (**1**), quercetin 3-*O*-α-D-rhamnoside (**2**) and quercetin 3-*O*-β-D-glucoside (**3**). The four compounds gave positive cyanidin test, a general reagent for identification of flavonoids. The four compounds were structurally identified based on their spectral data (UV, IR, ^1H NMR and ^{13}C NMR, LC-ESIMS) and comparison with literature data. Their UV spectra exhibited characteristic absorbance for flavonols (Harbone *et al.*, 1975) while their IR spectra showed characteristic absorptions for aromatic ring, carbonyl and hydroxyl groups at 1608 cm^{-1}, 1652 cm^{-1} and 3430 cm^{-1} respectively. The ^1H spectra of all three glycosides has the basic quercetin as an aglycone coupled

with the respective sugar moieties, arabinofuranose, rhamnose and glucose. In each case the point of connectivity was through C-3 by comparison with literature data (Markham *et al.*, 1978). The relative large coupling constant (J=7.5 Hz) of anomeric proton indicated the β-configuration of glycoside linkages for compound **1** and **3**. The sugar moiety of compound **2** was determined to be α-rhamnose by the J values of the anomeric proton signals and the ^{13}C-NMR spectrum. The LC-MS data of each glycoside exhibited the respective $(M+H)^+$ and $[(M+H)\text{-sugar}]^+$ peaks. This confirmed the respective sugar moieties attached to the quercetin aglycone.

Several methods have been used to evaluate the antioxidant activity of plant extracts and their chemical constituents. In this paper, antioxidant activity of the isolated compounds was determined using ferric thiocyanate (FTC), thiobarbituric acid (TBA) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assays. The FTC and TBA assays are used to determine whether the antioxidant activity occurs through lipid peroxidation, where the former assay measures the amount of peroxide in the early stages of peroxidation whereas the latter measures the total decomposition products. The two assays are normally carried out in conjunction with each other since compounds showing positive results in the FTC assay are expected to also show positive results in the TBA assay. On the other hand, the DPPH assay is to determine whether the compounds are also acting as free radical scavengers. In this assay, antioxidants present as free radical scavengers should pair up with the stable DPPH free radical forming 1,1-diphenyl-2-picrylhydrazine. The antioxidant activities of the isolated compounds were also compared with the standard antioxidants, ascorbic acid and α-tocopherol.

From the FTC and TBA assays, all four compounds showed strong antioxidant activity (Fig. 1 and 2). The activity was in the order of **4** > **1** > **2** > **3** > α-tocopherol. Table 1 showed the DPPH radical scavenging activity of compounds **1** to **4**. The scavenging activity was in the order of ascorbic acid > **4** > **1** > **2** > **3**.

Several studies on the relationship between flavonoid

Table 1. Percent inhibition of DPPH radical scavenging effects and IC_{50} values of compounds **1** to **4** and ascorbic acid as positive control

Samples	% Inhibition (125 μg/ml)	IC_{50} (μM)
1	83.22	19.4
2	80.93	26.3
3	81.69	46.2
4	81.82	18
Ascorbic acid	90.67	3.4

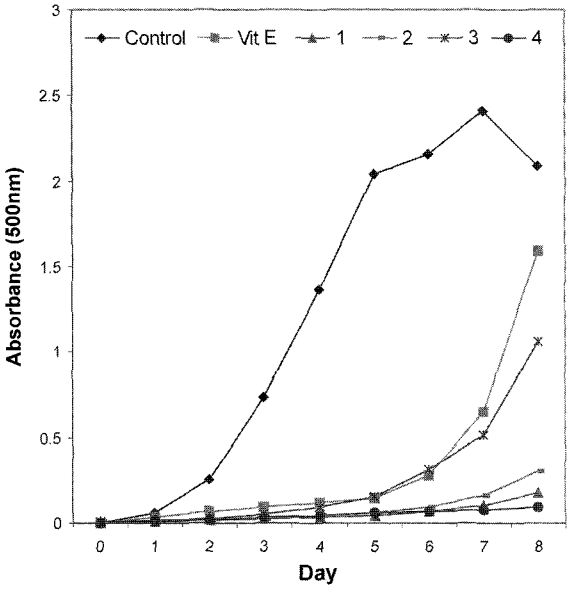


Fig. 1. Antioxidant activity of compounds **1-4** using FTC method (each sample, final concentration 0.01% w/v).

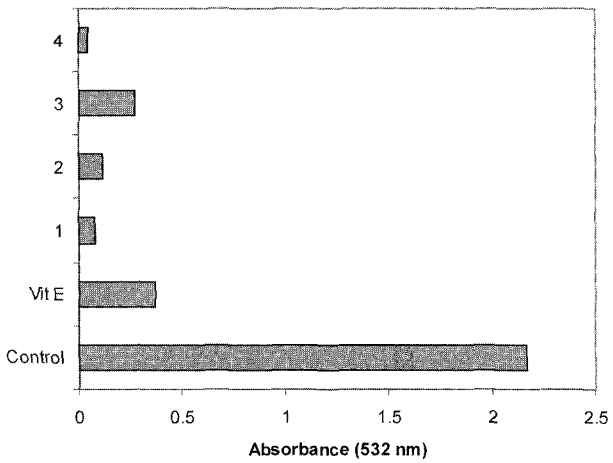
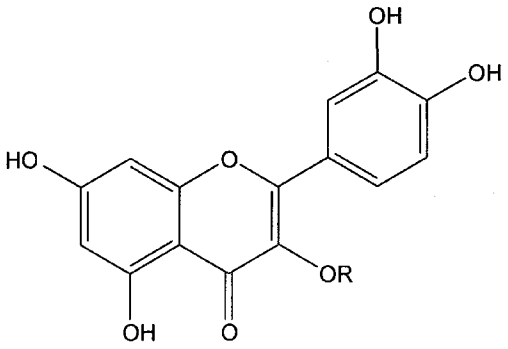


Fig. 2. Antioxidant activity of compounds **1-4** using TBA method (each sample, final concentration 0.01% w/v).

structure and their radical scavenging activity have been reported (Pietta, 2000; Mathiesen, *et al.*, 1997). The presence of a hydroxy group at C-3 is said to have great influence on the antioxidant effect of a flavonoid as exemplified by the greater radical-scavenging capacity exhibited by compound **4**. Glycosylation on this hydroxyl group however reduces the activities as shown by compounds **1**, **2** and **3**. Bors *et al.*, (1990) have also proposed that the presences of both the 3- and 5-OH groups are one of the three important structural requirements for a compound to exhibit high radical scavenging activity. The other two are the presence of *ortho* dihydroxyl (catechol) groups and an α,β-unsaturated ketone in ring B.

In conclusion, the results of our experiments demonstrated



Quercetin 3-*O*- arabinofuranoside (R= arabinofuranose)(**1**)

Quercetin 3-*O*- rhamnoside (R= rhamnose) (**2**)

Quercetin 3-*O*- glucoside (R= glucose) (**3**)

Quercetin (R= H) (**4**)

Fig. 3. The flavonoid and flavonoid glycosides isolated from *C. caudatus*.

that all the flavonoids tested posses antioxidant activity, consistent with other reports (Peng, *et al.*, 2003). The results also indicated that glycosylation at C-3 with different sugar moieties reduced the antioxidant activity. Among the three quercetin 3-*O*-glycosides, the glucoside appeared to be the least active in both assays while the arabinofuranoside was the most active. Similar activity pattern was also observed in a previous study (Braca, *et al.*, 2002).

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References

Burkill, I. H. *A Dictionary of the Economic Products of the Malay Peninsular. Vols. 1 and 2*. Ministry of Agriculture Cooperative, Kuala Lumpur, Malaysia (1966).

Bors, W., Heller, W., and C. Michel., Flavonoid as antioxidants: Determination of radical scavenging efficiencies. *Methods Enzymol.* **186**, 343-355 (1990).

Braca, A., Chandra, S., Matteo, P., Ivano, M., and Jeannette, M., Antioxidant activity of flavonoids from *Licania licaniaeflora*. *J. Ethnopharmacol.* **79**, 379-381 (2002).

Cotelle, N., Bernier, J. L., L. Catteau, J. P., Pommery, P., Wallet, J. C. and E. M. Gaydou., Antioxidant properties of hydroxyl-flavones. *Free Radical Biology and Medicine.* **20**, 35-43 (1996).

Cuendet, M., Potterat, O., and K. Hostettmann., Flavonoids and Phenylpropanoid derivatives from *Campanula barbata*. *Phytochem.* **56**, 631-636 (2001).

Fraisse, D., Hetz, A., Carnat, A., Carnat, A. P., and Lamaison, J. L., Quercetin 3-arabinopyranoside, a major flavonoid compound from *Alchemilla xanthochlora*. *Fitoterapia* **71**, 463-464 (2001).

Fuzzati, N., Sutarjadi, Dyatmiko, W., Abdul Rahman and Hostettmann, K., Phenylpropane derivatives from roots of *Cosmos caudatus*. *Phytochem.* **39**, 409-412 (1995).

Harbone, J. B., Mabry, T. J., and Mabry, H., *The Flavonoids*. London: Chapman and Hall, 1975, pp. 64.

Ismail, S., *Sayuran Tradisional Ulam dan Penyedap rasa*. Universiti Kebangsaan Malaysia, Bangi, 2000.

Kikuzaki, H., and Nakatani, N., Antioxidant effects of some ginger constituents. *J. Food Sci.* **58**, 1407-1410 (1993).

Lin, J. H., and Lin, Y. T., Flavonoids from the leaves of *Loranthus kaoi* (Choa) Kiu. *J. Food and Drug Anal.*. **7**, 185-190 (1999).

Markham, K. R., Ternai, B., Stanley R., Geiger, H., and Mabry, T. J., Carbon-13 NMR studies of flavonoids-III. *Tetrahedron* **34**, 1389-1397 (1978).

Mathiesen, L., Malterud, K. E. and Sund, R. B., Hydrogen bond formation as basis for radical scavenging activity: A Structure-activity study of *C*-methylated dihydrochalcones from *Myrica gale* and structurally related acetophenones. *Free Rad. Biol. and Med.* **22**, 307-311 (1997).

Miller, B., *Antioxidants Made Simple*. Bruce Miller Ent. Inc., Petaling Jaya, 1995, pp. 131

Norhanom, A. W., Ashril, Y., Mustafa, A. M., Anti-tumour Activity in Local Ulam. In *Phytochemicals and Biopharmaceutins from the Malaysian Rain Forest*, A. M. Ali *et al.* (ed.), 1999, pp. 135-141.

Peng, Z. F., Dieter, S., Alfred, B., Ramanathan, S., Goh, N. K., Chia, T. F., Tan, S. N., and Chia, L. S., Antioxidant flavonoids from leaves of *Polygonum hydropiper* L. *Phytochem.* **62**, 219-228 (2003).

Pietta, P. G., Flavonoids as antioxidants. *J. Nat. Prod.* **63**, 1035-1042 (2000).

Pietta, P. G., Simonetti, P., and Mauri, P., Antioxidant activity of selected medicinal plants. *J. Agric. Food Chem.* **46**, 4487-4490 (1998).

Wenkert, E., and Gottlieb, H. E., Carbon-13 nuclear magnetic resonance spectroscopy of flavonoid and isoflavonoid compounds. *Phytochem.* **16**, 1811-1816 (1997).

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