Pertanika J. Trop. Agric. Sci. 28 (2): 111- 119 (2005)

ISSN: 1511-3701
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Determination of Flavonoid Components from *Morinda citrifolia* (Mengkudu) and Their Antioxidant Activities

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Keywords: *Morinda citrifolia*, flavanoid

ABSTRAK

Lima komponen flavonoid dapat diekstrak dan dikenal pasti daripada daun Morinda citrifolia*: quercetin 3,7-O-dimethyl ether, quercetin 3-O-methyl ether, kaempferol 3,4'-O-dimethyl ether, kaempferol 5,7-O-diarabinoside dan apigenin. Pengasingan komponen flavonoid dijalankan menggunakan teknik kertas kromatografi dengan empat sistem pelarut; BAW (n-butanol:asid asetik:air; 4:1:5), 15% asid asetik (15% HoAc), BEW (n-butanol:etanol:air; 4:1:2.2) dan air. Flavonoid dikenal pasti melalui warna di bawah cahaya ultra violet (UV) dengan atau tanpa wap NH_3, nilai R_f, hidrolisis asid kepada flavonoid aglikon dan gula, perbandingan ko-kromatografi dengan penanda piawai, analisis spektral dengan menggunakan beberapa bahan reagen. Kesemua komponen flavonoid pertama kali dilaporkan dalam genus Morinda. Aktiviti antioksidan bagi komponen flavonoid ditentukan dengan menggunakan kaedah pelunturan β-karotena. Butylated hydroxyl toloul (BHT) dan quercetin digunakan sebagai bahan penanda piawai positif. Kesemua komponen flavonoid menunjukkan penurunan aktiviti antioksidan sepanjang tempoh uji kaji. Pada minit yang ke 40, quercetin 3-O-methyl ether menunjukkan aktiviti antioksidan yang tertinggi (77.67%); diikuti oleh quercetin 3,7-O-dimethyl ether (77.00%), kaempferol 3,4'-O-dimethyl ether (66.47%), apigenin (58.10%) dan kaempferol 5,7-O-diarabinoside (56.14%). Ujian statistik ANOVA satu hala dari perisian SPSS versi 11.0 menunjukkan kesemua aktiviti antioksidan komponen flavonoid adalah berbeza secara signifikan daripada aktiviti antioksidan BHT dan quercetin ($p < 0.05$).*

ABSTRACT

Five flavonoid components were isolated and identified from Morinda citrifolia's *leaves; quercetin 3,7-O-dimethyl ether, quercetin 3-O-methyl ether, kaempferol 3,4'-O-dimethyl ether, kaempferol 5,7-O-diarabinoside and apigenin. The isolation of flavonoid components was carried using paper chromatography methods with three different system solutions namely, BAW (n-butanol:acetic acid:water; 4:1:5), 15% acetic acid (15% HoAc), BEW (n-butanol:ethanol:water; 4:1:2.2) in addition to pure water. Flavonoid was identified through their colors under ultra violet (UV) with/without NH_3, R_f values, acid hydrolysis to aglycone and sugar, co-chromatographic comparison with standard markers and spectral analysis by using a series of shift reagents. All of these flavonoid components were firstly reported in the genus Morinda. The antioxidant activities of flavonoid components were evaluated by using the β-carotene bleaching method. Butylated hydroxyl toloul (BHT) and quercetin were used as positive markers. All isolated flavonoid components showed declining antioxidant activities through out the experiment. At the 40th minute, quercetin 3-O-methyl ether showed the highest antioxidant activities (77.67%); followed by quercetin 3,7-O-dimethyl ether (77.00%), kaempferol 3,4'-O-dimethyl ether (66.47%), apigenin (58.10%) and kaempferol 5,7-O-diarabinoside (56.14%). Statistical analysis was done using ANOVA with SPSS version 11.0 software. The results showed that all antioxidant activities belonging to the flavonoid components were significantly different from BHT and quercetins' antioxidant activites ($p < 0.05$).*

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INTRODUCTION

Flavonoid is a phenolic compound and was discovered as early as the 1930s. At that time, it was known as vitamin P (Nijveldt *et al.* 2001) because of its nutritional properties. Flavonoid is abundant in plants and can be found in all plant parts; bark, leaves, fruit and stem because flavonoid is a secondary metabolite in plants. There were five major groups of flavonoid; flavone, flavanone, isoflavone, flavonol, and anthocyanin (Wang and Halliwel 2001). Based on *in vitro* experiments, flavonoid was found to have anti-inflammatory, anti-allergy, antiviral and anti-carcinogenic properties (Middleton 1998). From a biological activity perspective, flavonoids have the ability to scavenge oxygen-derived free radicals and can act as antioxidant. According to Nijveldt *et al.* (2001), flavones and catechins are the most powerful antioxidant against reactive oxygen species (ROS).

An antioxidant is a substance that can prevent the process of lipid peroxidation; a process whereby free radicals (FR) can damage membrane cells. The damaged cells can lead to many degenerative diseases (Mohd Zin *et al.* 2002). In plants, antioxidants are produced/developed to defend their structures against ROS attack during photosynthesis. By taking antioxidant supplements such as vitamins or foods high in antioxidants such as vegetables and green leaves can help counter detrimental effects of free radicals (Faridah *et al.* 2003).

Morinda citrifolia or Mengkudu is commonly used as a Malay traditional remedy and its young shoots are eaten as *ulam* or raw. *Morinda citrifolia* has been used a lot especially by post-natal women for healing menstrual problems and curing diabetes (Zainatul Suhaida 2003). The plant belongs to the *Rubiaceae* family and originated from tropical Asia especially the coast of South East Asia (Dharma 1987). Apart from its utilization in traditional medicine, the pulp is also used as hair shampoo (Jaganath *et al.* 2000). Many scientific and modern research studies have been conducted to investigate the active compounds and special abilities of this plant. From previous research that has been conducted, few pure active compounds have been revealed in the leaf of *Morinda citrifolia* like iridoids (Sang *et al.* 2003) and anthraquinone (Thomson 1987) while the biological activities that has been discovered from *Morinda citrifolia* are antioxidative (Mohd Zin *et al.* 2002), and anti-inflammatory (Li *et al.* 2003) activities.

MATERIALS AND METHODS

Plant Sources

The leaves of *Morinda citrifolia* were collected at Universiti Putra Malaysia's campus and dried in an oven at 50°C for four–five days.

Extraction of Flavonoid

Air-dried leaves of *Morinda citrifolia* were extracted using three solvents; 80% methanol, petroleum ether (60-80°C boiling point) and chloroform. 300-600 g dried leaves were pulverized and extracted using the solvents. After standing overnight for methanol and three-four days for petroleum ether (60-80°C boiling point) and chloroform, the extracts were concentrated using a rotary evaporator.

Isolation of Flavonoid

The concentrated extracts were separated by chromatography on 3 mm paper (46 x 57 cm) in BAW (4:1:5, top layer). Bands that appeared dark under UV light, changed to yellow-green or unaffected by fuming with NH_3 vapour were eluted. The concentrated eluates were separated on 3 mm paper in 15% HOAc and the same processes were repeated with BEW (*n*-butanol: ethanol:water; 4:1:2.2) and water. Flavonoid components were identified through their colors under ultra violet (UV) with/without ammonia vapor, comparison R_f values with standard markers, acid hydrolysis and spectral data (Markham 1982). The isolated compounds were also identified based on the reported compounds in the previous studies (Harborne 1967).

Quercetin 3,7 O-dimethyl ether

The $^{a}_{max}$ values for this component are 349, 300 (shoulder) and 265; + NaOAc 393, 305 (sh) 269; +NaOH 408, 325 (sh), 273; + Al_2Cl_3 348, 300 (sh), 397 (iv), 273 nm. The absence of a NaOAc shift indicates that the 7-position is blocked. The component gave a dark colour under UV light but changed to yellow-green when fumed with ammonia vapor (NH_3). On partial acid hydrolysis, it produced quercetin, quercetin 3-*O*-methyl ether and quercetin 7-*O*-methyl ether. Quercetin showed yellow to yellow reaction in UV + NH_3 and showed identical spectral and R_f properties to the standard marker. Quercetin 3-*O*-methyl ether showed dark to yellow color reaction in UV + NH_3. The location of the methyl group at position 3 followed from the UV spectral analysis: $^{a}_{max}$ in MeOH at 359, 266, 256 (sh), in MeOH + NaOH 385, 359 (sh), 270. Quercetin 7-*O*-methyl ether was identified because of the R_f value, colour reaction under UV + NH_3 and Co-chromatogram comparision standard marker.

Quercetin 3-O-methyl Ether

Quercetin 3-O-methyl ether was dark when viewed under UV light on paper and yellow with NH_3. The $^{a}_{max}$ values for this component are 359, 299 (sh), 255, 268 (sh). The sodium hydroxide UV spectral data established the presence of a hydroxyl group on C_3 was changed (320 nm as band III). On acid hydrolysis no sugar was detected and tentatively this component was identified as Quercetin 3-*O*-methyl ether.

Kaempferol 3,4'-O-dimethyl Ether

The color of the component was dark under UV light and unchanged after fuming. Spectral analysis; in MeOH 350, 295sh; 266, in MeOH + NaOH 406, 325 (iii), 274 nm (decompose), indicated that there was free C_3 and C_4. After hydrolysis with acid, three derivatives were isolated: kaempferol that showed unchanged yellow under UV/UV+NH_3, (366, 317sh; 266 nm), kaempferol 3-*O*-methyl ether with dark to yellow reaction under UV/UV+NH_3 and kaempferol 4'-*O*-methyl ether also known as kaempferide,'which showed unchanged dark reaction under UV/UV+NH_3 as well as the decompose reaction after NaOH was added to neutralise the sample for spectral data indicates free C_4. Identification *kaempferol, kaempferol 3-O-methyl ether* and *kaempferide* was confirmed from their spectral data, R_f values, color reaction under UV/UV+NH_3. Co-chromatography comparison with standard marker and bibliography data by Harborne (1967). This component was identified as *kaempferol 3,4'-O-dimethyl ether* because the sugar test produced negative results.

Apigenin

This component was isolated from petroleum ether (60-80°C boiling point) extract and identified using standard procedures. It produced a dark color under UV and changed to yellow after fuming with NH_3 vapor.

Kaempferol 5,7-O-diarabinoside

Kaempferol 5,7-*O*-diarabinoside was isolated from chloroform and its color under UV light was dark and turned yellow after fuming with NH_3. Its neutral spectral in MeOH was 360, 283sh; 268, 257sh. No shift between neutral spectral and spectral after addition of NaOAc reagent on band II (268 nm) indicates that the hydroxyl group at C_7 was not free. The hydroxyl group at C_5 was not free because there was no shift between the neutral spectral and the spectral after addition of Al_2Cl_3 reagent on band I (361 nm). After hydrolisis with acid, three derivatives were isolated. The first derivative was identified as kaempferol because the yellow color under UV did not change after NH_3 fuming. Its R_f and spectral data were identical to the purchased kaempferol. The second derivative was recognized as kaempferol 7-*O*-arabinose based on the spectral characteristics, R_f value, and color reaction under UV/UV+NH_3. The sugar test detected arabinose and tentatively this component is known as kaempferol 5,7-*O*-diarabinoside.

Antioxidant Assay (β-carotene Bleaching Method)

Fresh leaves were used to determine the antioxidant activities. The leaves were crushed by using mortar and extracted using 80% methanol for 2 hours by stirring with a magnetic stirrer. The antioxidant activity of the crude extract and pure flavonoid compounds were evaluated by the β-carotene bleaching method (Veliglou 1998) with some modification to suit the equipment in the laboratory. A solution of β-carotene was prepared by dissolving 1 mg of powder β-carotene in 5 ml of chloroform. 60 μl of 95% linoleic acid, between 20 and 600 μl of the sample or control (80% methanol) or standard marker were pipetted into a 250 ml round bottom flask and covered with aluminium foil. Three ml of β-carotene prepared previously was added into the same flask. After chloroform was removed by vacuum using a rotary evaporator, 150 ml of distilled water was added to the flask and shaked vigorously for 60 seconds. Aliquots (5 ml) of this emulsion were transferred into test tubes to make triplicates. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm using a spectrophotometer. The tubes were placed at 50°C in dri-block. Absorbance measurements were recorded every 20 minutes for 2 hours. Butylated hydroxyl toloul (BHT) and quercetin were used as positive standard markers for comparative purposes. The antioxidant activities of the methanolic extract and flavonoid compounds were determined by using the following formula. The antioxidant activity was calculated in terms of percent inhibition relative to the control after 40 minutes incubation.

Antioxidant activity (%) formula:

1. $$dr = \frac{(\ln a/b)}{t}$$

ln = natural log
dr = degradation rate
a = absorbance value at 0 minute
b = absorbance value at observation time (0, 20, 40, 60, 80, 100, 120 minute)
t = observation time (0, 20, 40, 60, 80, 100, 120 minute)

2. Antioxidant activity (%)

$$= \frac{\text{dr control} - \text{dr sample}}{\text{dr control}} \times 100$$

The principle of β-CB determines the oxidative degradation of β-carotene in an emulsion containing linoleic acid. To measure these products, spectrophotometry has been used (Cruz *et al.* 1999). β-carotene in the model system undergoes rapid discoloration in the absence of an antioxidant. This is because of the coupled oxidation of β-carotene and linoleic acid, which generates free radicals. The linoleic acid free radical formed upon the abstraction of a hydrogen atom from one of its methylene groups attacks the highly unsaturated β-carotene molecules. As β-carotene molecules loose their double bonds, the system loses its characteristic orange colour, which can be monitored using spectrophotometry. The presence of a flavonoid as an antioxidant can hinder the extent of β-carotene destruction by neutralizing the linoleate free radical and other free radicals formed in the system.

RESULT AND DISCUSSION

Flavonoid Identification

In this study, five flavonoid components were isolated and identified from the leaves of *Morinda citrifolia* viz; quercetin 3,7-*O*-dimethyl ether, quercetin 3-*O*-methyl ether, kaempferol 3,4'-*O*-dimethyl ether, kaempferol 5,7-*O*-diarabinoside and apigenin. Quercetin 3,7-*O*-dimethyl ether, quercetin 3-*O*-methyl ether and kaempferol 3,4'-*O*-dimethyl ether were isolated from methanolic extract. Kaempferol 5,7-*O*-diarabinose and apigenin were isolated from chloroform and petroleum ether extracts respectively. The color reactions under UV/

TABLE 1

R_f value, color and spectral data of flavonoid component from leaves of *Morinda citrifolia*

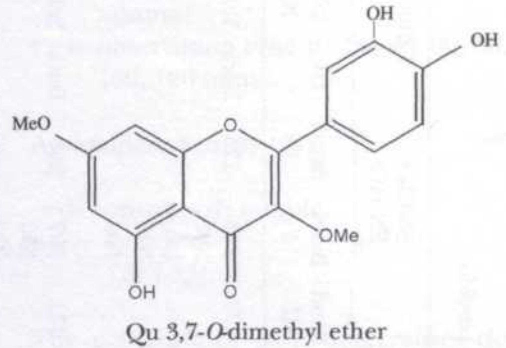
	R_fs value				Spectrum in 80% MeOH		+NaOAc		+NaOAc $+H_3BO_3$		+NaOH		$+AlCl_3$		$+AlCl_3$ + 2N HCl		Color	
	BAW	BEW	15% HOAc	H_2O	Band I	Band II	Band I	Band II	Band I	Band II	Band I	Band II	Band I	Band II	Band I	Band II	UV	UV + NH_3
Qu 3,7-*O*-diMethyl ether	47	64	69	54	349 297 (sh)	265	393 305 (sh)	269	354 299 (sh)	265	408 325 (sh)	273	348 300 (sh) 397 (iv)	273	343 300 (sh) 396 (iv)	273	Dark	Yellow
Qu 3-*O*-Methyl ether	58	49	54	33	359 299 (sh)	255 268 (sh)	379 319 (sh)	268 389	376	260	406 320 (sh)	274	401 341 (sh) 299 (sh)	271	397 350 (sh) 299 (sh)	269	Dark	Yellow
Km 3,4'-*O*-dimethyl ether	67	51	60	39	350 295 (sh)	266	386 308 (sh)	273	352 297 (sh)	266	406 325 (sh) 386 (iv)	274	348 300 (sh) 395 (iv)	272	346 300 (sh)	273	Dark	Yellow
Km 5,7-*O*-diarabi-noside	75	83	57	46	342 328 (sh) 297 (sh)	265	341 303 (sh)	269	346 296 (sh)	266	383 321 (sh)	272	340 381 (sh) 303 (sh)	273	322.6	269 242 (sh)	Dark	Dark
Apigenin	75	67	65	22	347 282 (sh)	266	381 304 (sh) 360 (sh)	272	357 299 (sh)	268	399 328 (sh)	275	340 381 (sh) 303 (sh)	273	383 300 (sh)	274	Dark	Yellow

UV+ NH_3 vapor, R_f values and spectral properties of the identified flavonoid components are shown in Table 1.

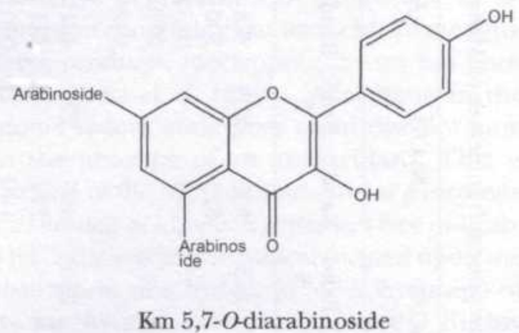
Flavonoids that have been revealed in *Rubiaceae* family in previous study were rutin and kaempferol 3-*O*-rutinoside from the leaves of *Randia formosa* (Sahpaz *et al.* 2000). Cimanga *et al.* (1995), identified quercetin, quercetin 7,4'-*O*-dimethyl ether, luteolin 7-*O*-glucoside, apigenin 7-*O*-glucoside, quercetin 3-*O*-rhamnoside, kaempferol 3-*O*-rhamnoside, quercetin 3-*O*-rutinoside, kaempferol 3-*O*-rutinoside and chrysoeriol neohesperidoside from the leaves of *Morinda* morindoides. From a similar study, Siti Juhaida (2000) found quercetin 3-*O*-rhamnoside, kaempferol 3-*O*-rutinoside, myricetin 3-*O*-galactoside from the leaves of *Morinda citrifolia.* The difference between the results of previous studies and this study is the flavonoid's rate of syhthesis and acumulation. There were many factors that contribute to both processes such as light, pH and soil's salinity (Onyilagha *et al.* 2003). The condition of the plants played a major role too, as plants infected with any kind of disease will tend to synthesize flavonoids in higher concentration as a mechanism of protection. (Manthey *et al.* 2000). According to Manach *et al.* (1996) the concentration of flavonoids varies for different plant organs.

Quercetin 3,7-*O*-dimethyl ether has been found before in the leaves and trichome of *Nicotiana attenuata* (wild tabacco) (Roda *et al.* 2003). Quercetin 3-*O*-methyl ether has been found in the fruits of *Rhamnus disperma* (Marzouk *et al.* 1999), stem and fruit of *Optunia ficus-indica* var. saboten (Go *et al.* 2003). To the best fo our knowledge, all the five flavonoid components isolated from the leaves *of M. citrifolia* in this study are pioneer findings for the genus *Morinda.*

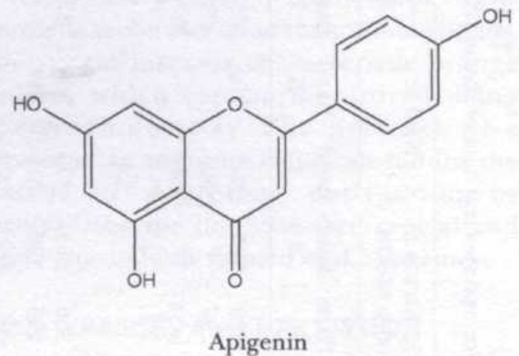
Structure of Isolated Flavonoid from Morinda citrifolia



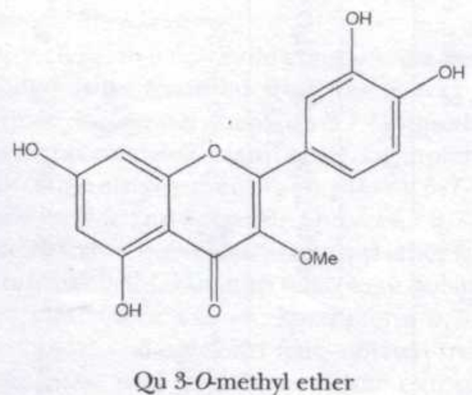
Qu 3,7-*O*-dimethyl ether



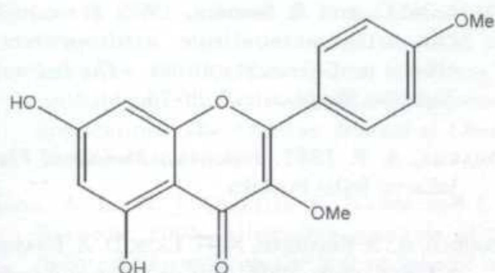
Km 5,7-*O*-diarabinoside



Apigenin



Qu 3-*O*-methyl ether



Km 3,4'-*O*-dimethyl ether

emulsion containing linoleic acid and flavonoid methylated antioxidant properties with time. The correlations indicated decreasing antioxidant activity during the reaction for all flavonoid components isolated from *Morinda citrifolia* leaves (*Fig. 1*). For the purpose of comparison, the 40th minute was selected and all antioxidant activities of the samples and standards for that time were compared (*Fig. 2*). Unfortunately, antioxidant

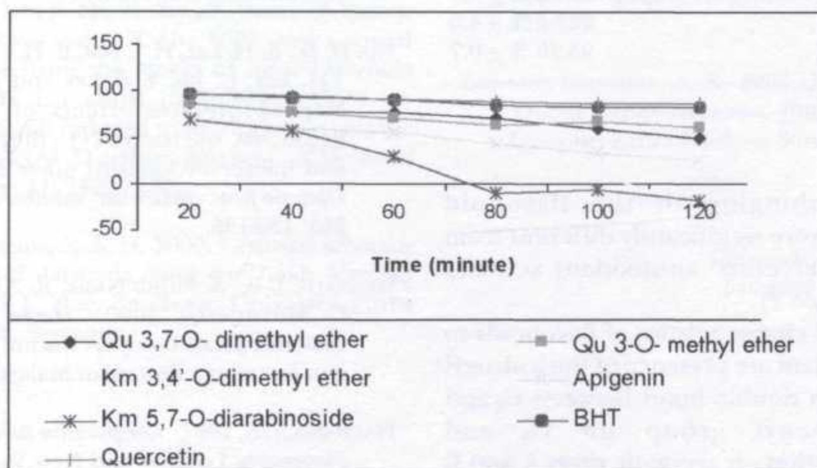


Fig. 1: Flavonoid components' antioxidant activities during 120 minutes

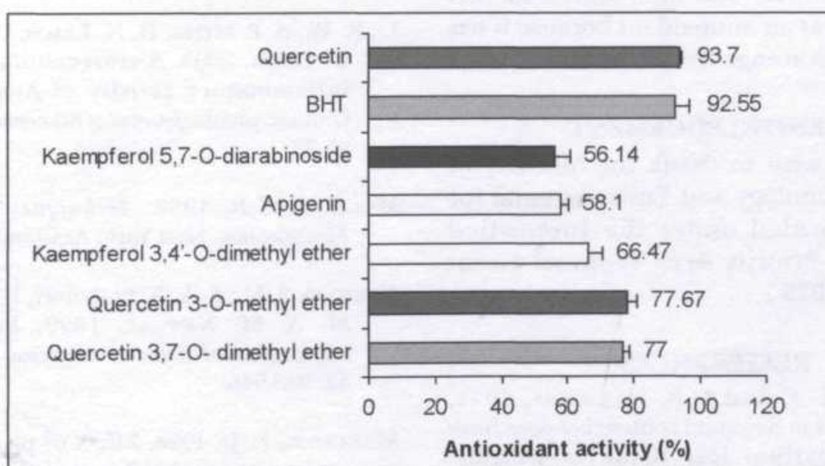


Fig. 2: Comparison of flavonoid antioxidant activities at 40 minutes

Antioxidant Activities

Based on the antioxidant activities formula, there were inverse correlations between the oxidative degradation of *β*-carotene in an properties of all the samples were lower than the standards; quercetin (93.7%) and BHT (92.55%). Based on the statistical test comparing all the mean values, all antioxidant

TABLE 2
Differences of antioxidant properties values between samples and standards

Samples	Antioxidant properties values (%)
Quercetin 3,7-*O*-dimethyl ether	77.00 % ± 1.4[a]
Quercetin 3-*O*-methyl ether	77.67 % ± 3.4[a]
Kaempferol 3,4'-*O*-dimethyl ether	66.47 % ± 4.2[a]
Kaempferol 5,7-*O*-diarabinoside	58.10 % ± 2.5[a]
Apigenin	56.14 % ± 4.6[a]
BHT	92.55 % ± 4.0
Quercetin	93.70 % ± 0.7

a: $p<0.05$

activities belonging to the flavonoid components were significantly different from BHT and quercetins' antioxidant activites ($p<0.05$) (Table 2).

The main characteristics of flavonoids to be an antioxidant are presence of the hydroxyl group at C_3, a double bond between C_2 and C_3, a carbonyl group on C_4 and polyhydroxylation on aromatic rings A and B (Cook and Samman 1996). A study by Go *et al.* (2003) showed that quercetin 3-methyl ether can act as an antioxidant because it has the ability to scavenge free radicals.

ACKNOWLEDGEMENT

The authors wish to thank the Ministry of Science, Technology and Environmental for funding provided under the Intensified Research in Priority Area Proposal Grant (IRPA No: 54075)

REFFERENCES

BATE-SMITH, E. C and J. B. HARBORNE. 1971. Differences in flavonoid content between fresh and herbarium leaf tissue in *Dillenia*. *Phytochemistry* **10**: 1055-1058.

CIMANGA, K., T. DE BRUYNE, A. LASURE, Q. LI, L. PIETERS, M. CLAEYS, D.V. BERGHE, K. KAMBU, L. TONA and A. VLIETINCK. 1995. Flavonoid *O*-glycosides from the leaves of *Morinda morindoides*. *Phytochemistry* **38**: 1301-1303.

COOK, N. C. and S. SAMMAN. 1996. Flavonoid – chemistry, metabolisme, cardioprotective effects and dietary sources. *The Journal of Nutrition Biochemistry* **7**: 66-76.

DHARMA, A. P. 1987. *Indonesia Medicinal Plant.* Jakarta: Balai Pustaka.

FARIDAH, A., S. KHOZIRAH, N. H. LAJIS, D. A. ISRAF and Y. UMI KALSOM. 2003. Antioxidative and radical scavenging properties of the constituents isolated from *Cosmos caudatus* Kunth. *Natural Product Sciences* **9**: 245-248

GO. H. D., K. H. LEE, H. J. KIM, E. H. LEE, Y. S. SONG, Y.H. LEE, C. JIN, Y. S. LEE and J. CHO. 2003. Neuroprotective effects of antioxidant flavonoids, quercetin, (+) - dihydroquercetin and quercetin-3-methyl ather isolated from *Optunia ficus - indica* var. *saboten. Brain Research* **965**: 130-136.

JAGANATH, I. B., S. MOHD NASIR, R. A. RAHMAN and C. MUTHUVELU. 2000. *Herba Berpotensi di Malaysia.* Kuala Lumpur: Institut Penyelidikan dan Kemajuan Pertanian Malaysia.

HARBORNE, J. B. 1967. *Comparative Biochemistry of the Flavonoids.* London and New York: Academic Press.

LI, R. W., S. P. MYERS, D. N. LEACH, G. D. LIN and G. LEACH. 2003. A cross-cultural study: anti-inflammatory activity of Australian and Chinese plants. *Journal of Ethnopharmacology* **85**: 25-32.

MARKHAM, K.R. 1982. *Techniques of Flavonoid Identification.* New York: Academic Press.

MARZOUK, S. M., S. A. A. EL-TOUMY, I. MERFORT and M. A. M. NAWWAR. 1999. Polyphenolic metabolites of *Rhamnus disperma. Phytochemistry* **52**: 943-946.

MIDDLETON, E. Jr. 1998. Effect of plant flavonoids on immune and inflammatory cell function. *Adv. Exp. Med. Biol.* **439:** 175–182.

MOHD ZIN, Z., A. ABDUL-HAMID and A. OSMAN. 2001. Antioxidative activity of extracts from Mengkudu (*Morinda citrifolia* L.) root, fruit and leaf. *Food Chemistry* **78:** 227-231.

NIJVELDT R. J., E. VAN NOOD, D. F. VAN HOORN, P. G. BOELENS, K. VAN NORREN and P. A. M VAN LEEUWEN. 2001. Flavonoids: a review of probable mechanisms of action and potential applications *The American Journal of Clinical Nutrition* **74:** 418-425.

RODA, A. L., N. J. OLDHAM, A. SVATOS and I. T. BALDWIN. 2003. Allometric analysis of the induced flavonols on the leaf surface of wild tabacco (*Nicotiana attenuata*). *Phytochemistry* **62**: 527-536.

SANG, S., G. LIU, K. HE, N. ZHU, Z. DONG, Q. ZHENG, R. T. ROSEN and C. T. HO. 2003. New unusual iridoids from the leaves of noni (*Morinda citrifolia* L.) show inhibitory effect on ultraviolet B-induced transcriptional activator protein-1 (AP-1) activity. *Bioorganic & Medicinal Chemistry* **11**: 2499-2502.

SITI NOR JUHAIDA, S. A. H. 2000. Ekstraksi sebatian flavonoid daripada daun dan buah *Morinda citrifolia* L. Bac. Sc. Tesis, Universiti Putra Malaysia, Selangor.

THOMSON, R. H. 1987. *Naturally Occurring Quinones III. Recent Advances.* London: Chapman & Hall.

VELIGLOU, Y. S., G. MAZZA., L. GAO and B. D. OOMAH. 1998. Antioxidant activity and total phenolics in selected fruits, vegetable and grain products. *Journal Agricultural Food Chemistry* **46**: 4113-4117.

WANG, H. and K. HELLIWELL. 2001. Determination of flavonols in green and black tea leaves and green tea infusions by high-performance liquid chromatography. *Food Research International* **34:** 223-227.

ZAINATUL SHUHAIDA, A. R. 2003. *Herba dan Ubatan Tradisi Zaman-berzaman.* **Jilid I:** 64-65. Kuala Lumpur: Zebra Editions Sdn. Bhd.

(Received: 25 February 2006)
(Accepted: 13 October 2006)