J. Trop. Agric. and Fd. Sc. 39(1)(2011): 000–000

Free radical scavenging activity and phenolic content of *Ficus deltoidea* accessions MFD4 and MFD6 leaves

(Aktiviti pemusnahan sisa radikal bebas dan kandungan fenol daun *Ficus deltoidea* aksesi MFD4 dan MFD6)

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Keywords: *Ficus deltoidea* accessions MFD4 & MFD6, DPPH, antioxidant activity, phenolic content, flavonoid content

Abstract

Methanol extracts of leaves from *Ficus deltoidea* accessions, MFD4 & MFD6, were examined for phenolic constituents and free radical scavenging activity, to determine their potential as a source of natural antioxidants. Total phenolic and flavonoid contents were evaluated according to the Folin-Ciocalteau procedure and a colorimetric method respectively. The results showed that total phenolic compounds and flavonoid content were higher in MFD6 than MFD4. Antiradical activity determined in terms of per cent inhibition by the DPPH radical scavenging method was higher in MFD4 than MFD6.

Introduction

There is consensus of opinion that free radicals induce oxidative damage to biomolecules. This damage causes atherosclerosis, aging, cancer and several other diseases (Aruoma 1998). Moreover, free radicals are known to take part in lipid peroxidation in foods, which is responsible for rancid odours and flavours and decrease in nutritional quality. Therefore, synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and *tert*-butyhydroquinone (TBHQ) are widely used in the food industry as potential inhibitors of lipid peroxidation. However, previous studies have demonstrated that BHA and BHT accumulate in the body and result in liver damage and carcinogenesis (Ito et al. 1986; Whysner et al. 1994).

Interest in natural sources of antioxidant molecules for use in food, beverage and cosmetic industries has resulted in an extensive body of research in recent years. It is well known that natural antioxidants extracted from herbs and spices have high antioxidant activity and are used in many food applications. Of these substances, the phenolic compounds, which are widely distributed, have the ability to scavenge free radicals by single-electron transfer (Hirano et al. 2001). Several studies have reported the antioxidant activity of plant extracts and their relationship with the phenolic compound content (Aaby et al. 2004; Silva et al. 2005; Sun and Ho 2005; Yuan et al. 2005; Singh et al. 2007). Stratil et al. (2006) found high correlation between the content of phenolic substances and the total antioxidant activity of sets of samples.

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Several methods have been proposed to measure the antioxidant activity of pure compounds and plant extracts, such as FRAP (Ferric Reducing Antioxidant Power), ORAC (Oxygen Radical Absorbance Capacity), ESR (Electron Spin Resonance), ABTS (2, 2-azinobis (3-ethyl-benzothiazoline-6-sulphonate) and DPPH (2, 2-diphenyl-1-picrylhydrazyl). The DPPH method is used worldwide in the quantification of free radical scavenging activity. The reaction is based on the colour decrease occurring when the odd electron of the nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidant compounds. DPPH is known as a stable free radical, but is sensitive to light, oxygen, pH and the type of solvent used (Ozcelik et al. 2003).

Herbs and medicinal plants are becoming popular these days as more and more people are inclined to use herbal remedies in their daily life. These herbal preparations are either used for treatment of certain diseases or just for normal health and vitality, and some are used as aphrodisiac. The use of *tongkat ali,* noni fruit*, kacip fatimah,* ginseng, avocado, aloe vera*,* ginkgo biloba, *gamat* and so on has increased tremendously and the demand has led to a parallel increase of these types of products in the market. It is believed that as much as 80% of the world outside the industrialized countries relies on herbs for health (Zaidah et al. 2006). In fact, many commercially produced pharmaceutical products are derived from herbs.

Besides the above mentioned herbs, another medicinal plant that is gaining popularity is *Ficus deltoidea*. This traditional herb, known locally as *mas cotek*, is a plant from the family Moraceae and has been used for its medicinal properties. The leaves of *F. deltoidea* are boiled and the decoction is taken by women after birth. It is believed that it helps to contract the uterine and vaginal muscles, improve blood circulation and regain body strength as well as for treating disorders related to the menstrual cycle. It is also taken as medicinal tea for general health and in treating pneumonia, diabetes, hypertension, diarrhoea and gout (Musa et al. 2004).

The Rice and Industrial Crops Research Centre, MARDI, has managed to gather 35 accessions of this herb and screen their adaptability to both the open field planting and containerized planting in polybags. Results obtained showed that there are four accessions that are well adapted to both types of planting systems, i.e. MFD4, MFD6, MFD7 and MFD2.

Two accessions, MFD4 and MFD6, were chosen in this study with the main objectives of evaluating the total phenolic and flavonoid contents as well as the free radical scavenging activity of methanol extracts of these accessions. These investigations are important for gaining more information on the potential natural antioxidants from *F. deltoidea* accessions for further application of the natural antioxidants in food product development.

Materials and methods

Preparation of plant extracts

Leaves from two accessions of *Ficus deltoidea*, MFD4 and MFD6, were obtained from MARDI station, Telong, Kelantan. On arrival at the laboratory in Serdang, the leaves were immediately washed under running tap water and then oven-dried for 48 h at 50 °C. The dried leaves were ground using a blender and then mixed with 100% methanol (500 ml) at ambient temperature overnight and shaken during the extraction time to ensure complete extraction. The extracts were filtered through Whatman No. 4 paper. The residue was re-extracted by repeating the above steps under the same conditions until the extraction solvents became coluorless. The filtrate was collected, placed in the rotary evaporator and the methanol was evaporated from the supernatants at 50 mm Hg pressure and 50 °C. The thick and viscous extracts

were then kept in air-tight amber bottles and stored in the freezer at 4 °C to prevent oxidative damage until further use.

Determination of plant extract yield
The yield of the evaporated extracts was calculated based on dry weight basis using the equation (1) shown below:

$$\text{Yield } (\%) = (W_1 \times 100)/W_2 \quad (1)$$

where W_1 was the weight of extract after evaporation of methanol and W_2 was the dry weight of the fresh plant sample.

Proximate analysis
Proximate composition (moisture, ash, total fat, crude protein and crude fibre) of *F. deltoidea* accessions MFD4 and MFD6 was carried out according to AOAC (1984). All analyses were carried out in triplicate.

Determination of total phenolic content
The total phenolic content was determined using the Folin-Ciocalteau reagent (Singleton and Rossi 1965) with some modifications. Each evaporated thick and viscous extract (~1.0 g ± 0.01 g) was diluted with 20 ml of 80% methanol. The mixture was shaken for 1 h and filtered through Whatman No.1 filter paper to get the extract solution. Each plant extract solution (200 µl) was transferred into a test tube containing 8.0 ml distilled water and then mixed thoroughly with 0.5 ml Folin-Ciocalteau reagent (prediluted 10-fold with distilled water). After mixing for 5 min, 1.0 ml of 20% (w/v) sodium carbonate was added. The mixtures were agitated with a vortex mixer, and then allowed to stand for a further 90 min in ambient temperature. The absorbance of plant extracts and a prepared blank were measured at 725 nm using a spectrophotometer (UV–vis model 50 Probe, Cary). The concentration of total phenolic compounds in all plant extracts was expressed as mg of gallic acid equivalents (GAE) per gram dry weight of plant, which was determined from known concentrations of gallic acid standard prepared similarly. Data were reported as a mean ± standard deviation for three replications.

Determination of total flavonoid content
The flavonoid content was measured using a colorimetric assay developed previously by Zhishen et al. (1999). One ml of the extract or standard solution of catechin was added to a 10 ml volumetric flask. Distilled water was added to make up to a volume of 5 ml. At zero time, 0.3 ml of 5% (w/v) sodium nitrite was added to the flask. After 5 min, 0.6 ml of 10% (w/v) $AlCl_3.6H_20$ was added and allowed to stand for 6 min, then 2 ml of 1 M NaOH was added to the mixture, followed by the addition of 2.1 ml distilled water. Absorbance was read at 510 nm against the blank (water) and flavonoid content was expressed as mg catechin equivalents (CAE) per 100 g of dry weight. Samples were analysed in triplicate.

Determination of free radical scavenging activity
The hydrogen atom or electron donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of purple coloured methanol solution of DPPH. This spectrophotometric assay uses stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a reagent (Cuendet et al.1997; Burits and Bucar 2000). One ml of various concentrations of the extracts in methanol was added to 3 ml of a 0.004% methanol solution of DPPH. After a 40 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. The percentage of inhibition of free radical DPPH by the extracts was calculated as follows:

$$\text{Inhibition } (\%) = (A_{blank} - A_{sample}/A_{blank}) \times 100 \quad (2)$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound.

Statistical analysis

Each of the measurements described above was conducted in triplicate and the mean data ± SD (standard deviation) reported. The data recorded on the sample were statistically analysed using the 2004 Statistical Analysis Software (SAS) package (version 9.1.2 of SAS Institute, Inc. Cary, NC). Statistically significant differences (p <0.05) in the chemical properties of the samples were determined by one way analysis of variance (ANOVA). Duncan multiple range test (DMRT) was used to determine significant differences between the means.

Results and discussion

Yield of methanol extracts

The yields of methanol extracts of MFD4 and MFD6 accessions were 19.6% and 26.1% respectively. These yields were calculated based on a dry weight basis, to eliminate the influence of the different moisture contents of the plants.

Proximate analysis

The proximate compositions of the *F. deltoidea* accessions MFD4 and MFD6 are presented in *Table 1*. Expressed on fresh weight basis (per 100 g), the moisture content of MFD4 (11.79 %) was lower than MFD6 (11.83%). The total ash mean value was 8.81 g for MFD4 and 6.47 g for MFD6. These results showed that both of the samples contained some minerals which could be useful in improving health.

The mean values for fat and protein were 3.30 g and 13.56 g/100 g sample for MFD4 and 2.36 g and 11.89 g/100 g sample for MFD6, respectively. The mean value of crude fibre is 22.07 g/100 g for MFD4 and 22.43 g/100 g for MFD6.

These results indicated that MFD4 has higher values for total ash, total fat and crude protein but lower values for moisture. There is no significant difference at p <0.05 between both accessions for crude fibre content.

Total phenolic and flavonoid contents

Phenolic compounds are very important plant constituents because they exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals (Pokorny 2001). Flavonoids are phenolic compounds, which are very effective antioxidants (Yanishlieva-Maslarova 2001). The Folin-Ciocalteau method is a rapid and widely-used assay to investigate the total phenolic content but it is known that different phenolic compounds have different responses to the Folin-Ciocalteau method (Kähkonen et al. 1999). Most determinations of flavonoids have been based on spectrophotometer (Muller et al. 2005). Aluminium chloride dissolved in methanol was often used as derivatization reagent because the reaction between aluminium chloride and flavonoid is sensitive and selective for total flavonoids determination (Meda et al. 2005; Lin and Tang 2007).

Table 1. Proximate composition of *Ficus deltoidea* accessions MFD4 and MFD6 (per 100 g sample)

	MFD4	MFD6
Moisture (%)	11.79 ± 0.194b	11.83 ± 0.423a
Total ash (g)	8.81 ± 0.0336a	6.47 ± 0.3321b
Total fat (g)	3.30 ± 0.0112a	2.36 ± 0.1265b
Crude protein (g)	13.56 ± 0.0076a	11.89 ± 0.3223b
Crude fibre (g)	22.07 ± 0.4166a	22.43 ± 0.3541a

Data was expressed as mean ± SD, each value is a mean of triplicate reading
Means with the same letter are not significantly different (p <0.05)

In this study, a spectrophotometric quantification of flavonoids with aluminum chloride was used. Chang et al. (2002) showed that the real content of total flavonoids must be determined by the aluminum chloride method which is specific only for flavones and flavonols, and by the 2,4-dinitrophenylhydrazine method that is specific for flavanones.

Therefore, in this work, the total phenolic and total flavonoid contents were calculated in units of mg gallic acid equivalent (GAE) and mg catechin equivalent (CAE) respectively. The total phenolic content differed between MFD4 and MFD6 and each accession contained a lower total flavonoid content than the total phenolic content, since other compounds besides flavonoids are phenolic substances in plants (Pietta 2000).

As illustrated in *Table 2*, MFD4 with a lower total phenolic content (5.0502 ± 0.0428 mg GAE/g dry weight), also exhibited a low total flavonoid content (1.0111 ± 0.0279 mg CAE/g dry weight) compared to MFD6, which exhibits higher total phenolic and total flavonoid contents (5.2964 ± 0.0478 mg GAE/g dry weight and 2.0250 ± 0.0362 mg CAE/g dry weight respectively).

Free radical scavenging activity of MFD4 and MFD6 leaf extracts

The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors. The DPPH method can be used for solid or liquid samples and is not specific to any particular antioxidant component, but applies to the overall antioxidant capacity of the sample. Antioxidant activity has been expressed in various ways including the percentage of the reagent used, the oxidation inhibition rate and so on. The radical scavenging activity of samples in terms of per cent inhibition of *F. deltoidea* accessions were then compared to that of ascorbic acid (Vitamin C) and α-tocopherol (Vitamin E). Results are shown in *Table 3*.

Results of DPPH radical scavenging ability showed that MFD4 exhibited higher free radical scavenging ability (60.09%) than MFD6 (48.88%) as shown in *Table 3*. However, α-tocopherol as a standard reference exhibited highest free radical scavenging ability (97.67%) followed by ascorbic acid (94.80%).

Phenolic substances have been shown to be responsible for the antioxidant activity of plant materials (Rice-Evans et al. 1996). Although most antioxidant activities from plant sources are derived from phenolic-type compounds (Bravo 1998; Cai et al. 2004), antioxidant activity does not always correlate with the presence of large quantities of these phenolic compounds,

Table 2. Total phenolic and flavonoid contents of *Ficus. deltoidea* accessions MFD4 and MFD6(mg GAE/g dry weight)

	Total phenolic	Total flavonoid phenolic
MFD4	5.0502 ± 0.0428b	1.0111 ± 0.0279b
MFD6	5.2964 ± 0.0478a	2.0250 ± 0.0362a

Data are expressed as the average of three determinations ± SD
Values in the same column that are followed by a different letter are significantly different at $p < 0.05$

Table 3. Percentage inhibition of DPPH radical scavenging activity of *Ficus deltoidea* accessions MFD4 and MFD6

	Inhibition of DPPH radical scavenging activity (%)
α-tocopherol	97.67 ± 0.05a
Vitamin C	94.80 ± 0.64b
MFD4	60.09 ± 0.64c
MFD6	48.88 ± 0.68d

Each result is expressed as mean ± standard deviation of triplicate values
Means with the same letter are not significantly different with at $p < 0.05$

hence both data need to be examined together.

Interestingly, MFD4 had lower total phenolic and flavonoid contents, whereas its antioxidant capacity was higher (*Tables 2–3*) than MFD6 which had higher total phenolic and flavonoid contents. Therefore, no association could be found in this study between total phenols and DPPH reduction as well as between total flavonoids and DPPH for both extracts of *F. deltoidea* accessions MFD4 and MFD6. Although some studies have demonstrated a correlation between phenolic content and antioxidant capacity (Yang et al. 2002), the results in this work are in agreement with many other findings. Ismail et al. (2004) reported no correlation between total phenolic contents and antioxidant capacities of extracts from five types of vegetables. The relationship between antioxidant capacities and phenolic composition was also not found in fruit berry, fruit wines (Heinonen et al. 1998) or in plant extracts (Kähkonen et al. 1999).

No correlation between total phenolic contents and antioxidant activities was observed owing to the presence of the following factors. The antioxidant activity observed was not solely from the phenolic contents of *F. deltoidea* accessions, MFD4 and MFD6, but could possibly be due to the presence of some other phytochemicals as well as the synergistic effects among them, which also contribute to the total antioxidant activity. On the other hand, total phenolic contents determined according to the Folin-Ciocalteau method is not an absolute measurement of the amount of phenolic materials. Different types of phenolic compounds have different antioxidant activities, which is dependent on their structure.

Conclusion

The different accessions of *F. deltoidea* had different amounts of total phenolic and flavonoid contents. Both compounds were higher in MFD6 than MFD4. However, antiradical activity determined in terms of per cent inhibition by the DPPH radical scavenging method was highest in MFD4 than MFD6. These results indicated that different accessions of *F. deltoidea* possibly contain different types of phenolic compounds, which have different antioxidant activities. Thus, the antioxidant activity of an extract cannot be predicted on the basis of its total phenolic content. The potency of these accessions could provide a scientific basis for the health benefit claimed for *F. deltoidea* in folk medicine. Further work is required to elucidate these results *in vivo* and to identify the phenolic compounds, which are responsible for the antioxidant activities measured.

Acknowledgement

The authors wish to acknowledge MARDI (JP-RF-0096) for giving financial support for this study. Thanks are also due to Ms Nazarifah Ibrahim, Ms Rosnah Othman and Mr Mohd. Fazli Ali at Food Technology Research Centre, MARDI for their technical assistance.

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Abstrak

Kandungan jumlah fenol serta aktiviti pemusnahan sisa radikal bebas pada ekstrak metanol *Ficus deltoidea* aksesi MFD4 dan MFD6 telah dikaji bagi menentukan potensi herba ini sebagai salah satu sumber antioksida semula jadi. Kandungan jumlah fenol dan *flavonoid* masing-masing dinilai menggunakan prosedur *Folin-Ciocalteau* dan kaedah kolorimetrik. Keputusan yang diperoleh menunjukkan kandungan jumlah fenol dan flavonoid lebih tinggi di dalam MFD6 berbanding dengan MFD4. Bagi penentuan aktiviti pemusnahan sisa radikal bebas dalam bentuk peratusan perencatan oleh *reagen* DPPH pula, MFD4 menunjukkan peratusan perencatan yang lebih tinggi berbanding dengan MFD6.

Accepted for publication on 11 April 2011