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Full Length Research Paper

Non enzymatic and enzymatic antioxidant activities in aqueous extract of different *Ficus deltoidea* accessions

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Ficus deltoidea **was used in this study due to its reputation in reducing risk of cancer, diabetes and heart diseases. In this study 13 accessions of** ***F. deltoidea*** **were selected and they were divided into two groups, generally named as, female and male plants based on leaf sizes and the spots present. The signature of** ***F. deltoidea*** **is that this plant has black spots representing the female leaf while red spots for the male leaf. For non enzymatic antioxidants, several methods were used, they were 2,2-diphenyl-1-picrylhydrazil (DPPH) free radical scavenging assay and ferric reducing antioxidant power (FRAP) assay for total antioxidant content. In addition, total polyphenol, flavonoid, phenolic acid and vitamin C content were also analyzed. Enzymatic antioxidants of** ***F. deltoidea*** **leaf extracts were assayed; ascorbate oxidase, peroxidase, catalase and ascorbate peroxidase. For DPPH assay, F1 has 99.87% of inhibition while the lowest is in M10 (32.86 %). F13 has the highest total percentage of antioxidant for FRAP method and the lowest is in M4. Total polyphenol content showed F13 has the highest (1.30 mg/g FW) and M10 the lowest (0.49 mg/g FW). Similar trend was observed for total phenolic acid and flavonoid content. For both assays, F1 has the highest content while M10 is the lowest. For total vitamin C content, F8 and F7 have the highest and lowest content with 6.78 and 0.61 mg/g FW, respectively. Ascorbate oxidase, peroxidase, catalase and ascorbate peroxidase were calculated using respective coefficient extinction and expressed as mg/g FW protein content. This study suggested that the extracts of the female leaves are better than male leaves in most of the assays. This is the first documented report on the antioxidants of** ***F. deltoidea*****.**

Key words: *Ficus deltoidea*, antioxidant, enzymatic, non-enzymatic, aqueous extracts.

INTRODUCTION

Natural products such as herbs, fruits and vegetables become popular in recent years due to public awareness and increasing interest among consumers and scientific community (Thaipong et al., 2006). Natural products which contain antioxidant properties such as phenolics, include flavonoids and phenolic acids (Klimczak et al., 2007), carotenoids and vitamins (Rupasinghe and Clegg, 2007). Epidemiological evidence has been provided that constituents in natural products show many biological and pharmalogical activities, including antioxidative, anti imflammatory and antiviral effects (Pawlowska et al., 2008).

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Ficus deltoidea, a plant derived from Moraceae family locally known as Mas Cotek among the Malays in Malaysia, is an evergreen shrub or small tree used traditionally to treat cardiovascular diseases and diabetes. Besides that, *F. deltoidea* also can be used as aphrodisiac, specifically to increase male virility. According to Adam et al. (2007), almost all of the parts of *F. deltoidea* plant including the roots, bark, leaves and fruits are believed to have medicinal properties. Studies on *F. deltoidea* have received tremendous responses because this plant can be used by both sexes. Unlike Eurycoma longifolia or known as Tongkat Ali which is used only by the male to increase the virility or Labisia pumila or known as Kacip Fatimah which is used only by female to facilitate childbirth as well as a post partum medication. Research on *F. deltoidea* becomes a challenge due to the lack of knowledge in this species.

According to Ventakamaran (1972), the taxonomy treatment of the Moraceae family constitutes large taxa of over fifty genera and nearly 1400 species, including some important groups like Artocarpus, Morus and Ficus.

Several species belonging to the genera of Ficus were reported to contain furanocoumarins which is an important plant phototoxins (Swain and Downum, 1990). Ventakamaran (1972) also claimed that Moraceae family contains phytochemistry related to flavonoids, flavonoids with isoprenoid substituents and stilbenes. Antioxidants from figs can protect lipoproteins in plasma from oxidation and produce a significant increase in plasma antioxidant capacity (Dueñas et al., 2008). As an example, *Ficus bengalensis* or Banyan tree which grows wild in lower Himalayas and all over India is used as treatment for several diseases according to parts of the plant: leaves for ulcer, aerial roots for gonorrhea while seeds and fruits for cooling and tonic (Shukla et al., 2004). *Ficus pumila* L. which can be found in Okinawa, Japan used by the Okinawan elders as medicinal herbs to cure some diseases such as diabetes, dizziness, high blood pressure and neuralgia (Abraham et al., 2008).

Previous research by Sulaiman et al. (2008) shows that *F. deltoidea* aqueous extract possesses positive antinociceptive activities such as acetic acid-induced abdominal writhing test, formalin test, hot plate test and involvement of opioid receptors in adult male Balb C mice and adult male Sprague-Dawley rats. Research findings by Adam et al. (2007) showed that *F. deltoidea* aqueous extract at 1000 mg/kg has hypoglycaemic activity in post prandial mild diabetic rats while at other concentrations the extracts do not have hypoglycaemic activity in normal and fasting mild diabetic rats. However, Aminudin et al. (2007) reported that the aqueous extracts of both the leaves and fruits of *F. deltoidea* at 50 mg/kg dosage significantly reduced the external glucose load. Antioxidants and their derivatives are of great importance in our daily life. Antioxidants are compounds that are capable to delay or inhibit the oxidation of lipids or other biomolecules by inhibiting the initiation or propagation of oxidizing chain reactions (Wang and Ballington, 2007). There are several reports about the plant-derived antioxidants and its derivatives, however, there is no report documented on antioxidant activities in *F. deltoidea* plant extracts. Hence, the main objective of this study is to determine non enzymatic and enzymatic antioxidant activities in different accessions of *F. deltoidea*.

MATERIALS AND METHODS

Chemicals and reagents

2,2-diphenyl-1-picrylhydrazil (DPPH), 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ) and Folin-Ciocalteu phenol reagent, catechin, polyvinylpolypyrrolidone (PVPP), ascorbic acid and 2,6-dichloroindophenol (DCPIP) were purchased from Sigma Co. St. Louis, Missouri, USA. Methanol, sodium nitrite, acetic acid, sodium hydroxide, Coomasie Blue G250, albumin fraction V (from bovine), hydrogen peroxide, aluminium chloride, gallic acid, iron (lll) chloride hexahydrate and sodium carbonate were purchased from Merck, Darmstadt, Germany. All the chemicals and reagents were of analytical grade.

Plant materials

Thirteen accessions of *F. deltoidea* were purchased from the Department of Agriculture Serdang, Malaysia and maintained in the glass house. Fresh leaves of 13 accessions of *F. deltoidea* were collected and washed under running tap water to clean the debris and contaminant. The leaves were grouped into two main groups, male and female plants. The male plants have small and fine leaves while the female plants have big and thick leaves. Another characteristic that need to be considered is the colour of the spot present at the back of the leaves, male plants have red dot at the back of the leaf while black colour represents for the female leaves. Alphabet M was assigned to male plants (M4, M6 and M10) while F for female plants (F1, F2, F3, F5, F7, F8, F9, F11, F12 and F13). In Malaysia, there are a lot of female accessions but only a few male accessions can be naturally found. All of *F. deltoidea* accessions used in this experiment will be deposited at the herbarium Biodiversity Unit, Institute of BioScience (IBS), Universiti Putra Malaysia, Selangor, Malaysia.

Extraction of antioxidant compounds

Extraction of antioxidant compounds was conducted employing the method modified from Wong et al. (2005). A total of 0.5 g leaves of each of the 13 accessions was cut into small pieces and placed in 150 ml conical flask. A total volume of 25 ml of distilled water was added and the flask was covered with aluminium foil. The conical flasks containing the samples were placed on an orbital shaker at room temperature for 1 h in the dark. After 1 h the samples were filtered using Whatman No. 1 filter paper and the extracts were stored at -80°C freezer.

DPPH free radical scavenging assay

DPPH free radical scavenging assay was measured using DPPH free radical test, employing method of Wong et al. (2005). The initial absorbance of DPPH in methanol was measured using spectrophotometer at 515 nm until the absorbance remain constant. A total of 40 µl of extract was added to 3 ml of 0.1 mM methalonic DPPH solution. The mixture was incubated in room temperature for 30 min before the change in absorbance at 515 nm was measured. The percent of inhibition was calculated using the formula, percent of inhibition (%) = [(A515 of control-A515 of sample)/A515 of control] x 100.

Ferric reducing antioxidant power assay (FRAP)

The FRAP assay was conducted using method of Wong et al. (2005). Two hundred microlitre of extract were added with 3 ml of FRAP reagent that was prepared with mixture of 300 mM sodium acetate buffer at pH 3.6, 10 mM 2, 4, 6-tri (2-pyridyl)-s-triazine (TPTZ) solution and 20 mM FeCl.6H2O at the ratio of 10:1:1. The reaction mixture was incubated in a water bath at 37°C for 30 min. The increase in absorbance was measured using spectrophotometer at 593 nm. The antioxidant capacity based on the ability to reduce ferric ions of the extracts was calculated as percent of antioxidant. The percent of antioxidant was calculated using the formula, percent of antioxidant (%) = [(A593 of sample-A593 of control)/A593 of sample] x 100.

Total polyphenol content

A total of 100 µl of extract were added with 2.5 ml of Folin-Ciocalteu reagent which was diluted 10 times. After 5 min of reaction, 2.5 ml of 7% of sodium carbonate was added. The mixture was incubated in room temperature for 1 h before the absorbance at 725 nm was measured. The total polyphenol content of the extract was expressed as mg gallic acid equivalents per gram of plant material on fresh basis.

Extraction of total phenolic acids and total flavonoids

Extraction of total phenolic acid and total flavonoid assay were conducted using a modified method of Marinova et al. (2005). *F. deltoidea* leaves weighing 0.5 g was grind using pestle and mortar. These samples were homogenized with 50 ml distilled water and transferred into covered flasks. Then the mixture was centrifuged for 5 min at 14000 rpm. The supernatant was collected and used in the experiment.

Total phenolic acid assay

The total phenolic acid assay was conducted as described by Marinova et al. (2005). Total phenolic acid content assay was carried out using Folin-Ciocalteu agent. One mililitre extract was added into a flask containing 9 ml of distilled water. Then 1 ml of Folin-Ciocalteu's phenol reagent was added and the mixture was thoroughly mixed. After 5 min, 10 ml of 7 % Na2CO3 were added. Then the mixture was diluted to 25 ml with the addition of 4 ml of distilled water. Then the mixture was incubated at room temperature for 90 min. Finally, the absorbance was measured using spectrophotometer at 750 nm. The total phenolic acid content was expressed as mg gallic acid equivalents (GAE)/g samples.

Total flavonoid assay

The total flavonoid assay was conducted according to Marinova et al. (2005). Total flavonoids assay was conducted using Aluminium Chloride Colormetric method. One mililitre of extract was added with 4 ml of distilled water in a flask. After that, 0.3 ml 5% NaNO2 was added. After 5 min, 0.3 ml of 10% AlCl3 was added. After the sixth minute, 2 ml of 1 M NaOH was added. Then the mixture was diluted to 10 ml by adding 2.4 ml distilled water. The mixture was mixed and the absorbance was measured at 510 nm. Total flavornoids content was expressed as mg catechin equivalents (CE)/g samples.

Total vitamin C content

Total vitamin C content was measured using modified method of Davis and Masten (1991). Each leaf samples were extracted using 1% of phosphate citrate buffer, pH 3.5 using chilled mortar and pestle. Then the homogenates was centrifuged at 10000 rpm at 4℃ for 10 min. Lastly, the supernatant was collected and used for further analysis. The supernatant was added with 1.72 mM 2,6-dichloroindophenol (2,6-DCPIP) in 3 ml cuvette and was measured at 518 nm immediately after mixing.

Preparations of enzymes extracts

For determination of antioxidant enzymes activities, enzyme extraction procedure was prepared according to Nayyar and Gupta (2006) with some modifications. Each leaf (0.5 g) was ground with 8 ml solution containing 50 mM potassium phosphate buffer (pH 7.0) and 1% polyvinylpolypyrrolidone. The homogenate was centrifuged at 15000 x g for 30 min and supernatant was collected for enzymes assays.

Ascorbate oxidase (EC 1.10.3.3) activity

Ascorbate oxidase activity was measured according to Diallinas et al. (1997). In 1.0 ml of reaction mixture contained 20 mM potassium phosphate buffer (pH 7.0) and 2.5 mM ascorbic acid. The reaction was initiated with the addition of 10 µl enzyme extract. The decreased in absorbance was observed for 3 min at 265 nm due to ascorbate oxidation and calculated using extinction coefficient, 14 mM-1cm-1.

Peroxidase (EC 1.11.1.7) activity

Peroxidase activity was determined using the guaicol oxidation method by Chance and Machly (1955). The 3 ml reaction mixture contains 10 mM potassium phosphate buffer (pH 7.0), 8 mM guaicol and 100 µl enzyme extract. The reaction was initiated by adding 2.75 mM H2O2. The increase in absorbance was recorded within 30 s at 470 nm due to the formation of tetraguaicol. A unit of peroxidase activity was expressed as the change in absorbance per min and specific activity as enzymes units per mg soluble protein (extinction coefficient 6.39 mM-1cm-1).

Catalase (EC 1.11.1.6) activity

Catalase activity was determined according to Aebi (1984) by monitoring the decomposition of H2O2. In 1 ml of reaction mixture contain potassium phosphate buffer (pH 7.0), 250 µl of enzyme extract and 60 mM H2O2 to initiate the reaction. The reaction was measured at 240 nm for 3 min and H2O2 consumption was calculated using extinction coefficient, 39.4 mM-1cm-1.

Ascorbate peroxidase (EC 1.11.1.11) activity

The reaction mixture for ascorbate peroxidase activity includes 100 mM tris-acetate buffer at pH 7.0, 2 mM ascorbic acid, enzyme extracts and 2 mM of H2O2 to initiate the reaction. The decrease in absorbance at 290 nm was measured and monitored for 100 s. The reaction was calculated using extinction coefficient, 2.8 mM-1cm-1 (Ali et al., 2005).

Statistical analysis

The observations were replicated thrice for each parameter, mean values were pooled and standard error (S.E.) was calculated. Statistical analysis was carried out using analysis of variance (ANOVA).

RESULTS

DPPH free radical scavenging assay

From the Figure 1, F3 leaf extract showed the highest percent of inhibition compared to other accessions leaf extracts. 99.87% of inhibition was calculated from F3 leaf extract and followed by F11 leaf extract which had 99.72% of inhibition. Other accessions which showed

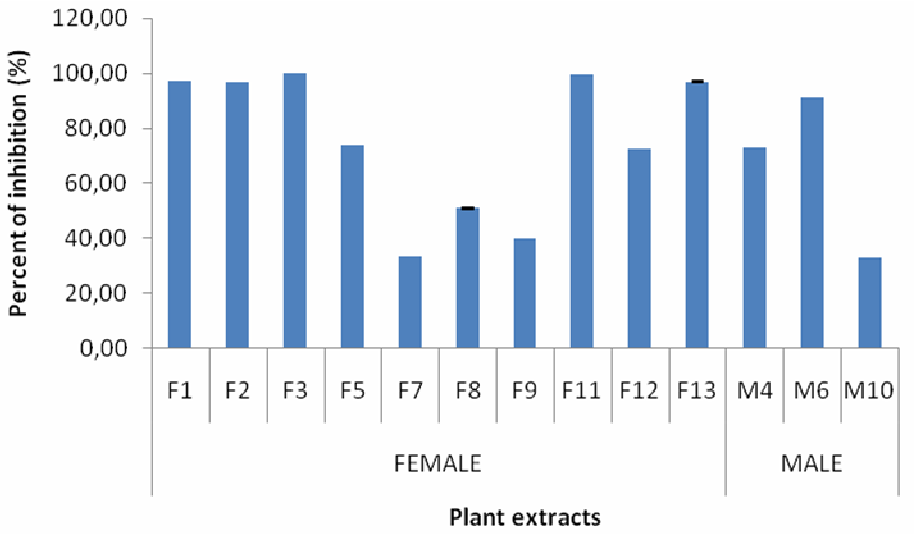

Figure 1. Total antioxidant content of female and male plant extracts expressed as percent of inhibition using DPPH method in fresh leaves of *F. deltoidea.* Values are means ± S.E. (n = 3).

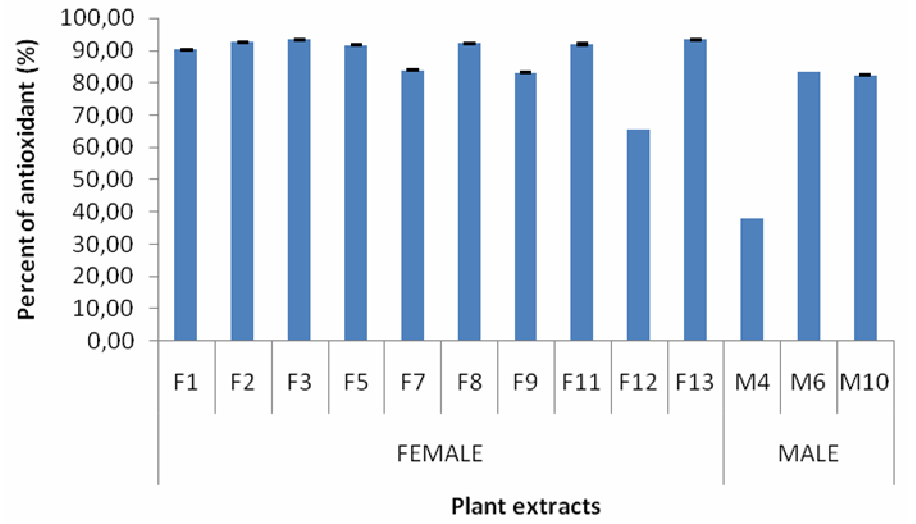

Figure 2. Total antioxidant content of female and male plant extracts expressed as percent of antioxidant using FRAP method in fresh leaves of *F. deltoidea.* Values are means ± S.E. (n = 3).

high percent of inhibition were F1, F13 and F2 leaf extracts with 97.12%, 96.97 and 96.94% of inhibition, respectively. The lowest percent of inhibition for female leaf extract was detected in F7 leaf extract with 33.41%. On the other hand, most of the male leaf extracts had antioxidant properties but lower in their activities compared to the leaf extracts of the opposite sex. The highest percent of inhibition for the male leaf extract was detected in M6 leaf extract with 91.14% of inhibition while the lowest antioxidant content was measured from M10 leaf extract with 32.86% of inhibition. This showed that female leaf extracts of *F. deltoidea* scavenge more free radicals compared to the male leaf extracts.

FRAP assay

Figure 2 showed that female leaf extracts had higher total antioxidant compared to male leaf extracts. For the female leaf extracts, percentage of antioxidant ranged from 65.46 to 93.31%. F13 leaf extract showed the highest antioxidant activities with 93.31% of antioxidant among all of the leaf extracts. Among the female leaf extracts F3, F2, F8, F11, F5 and F1 showed high percent

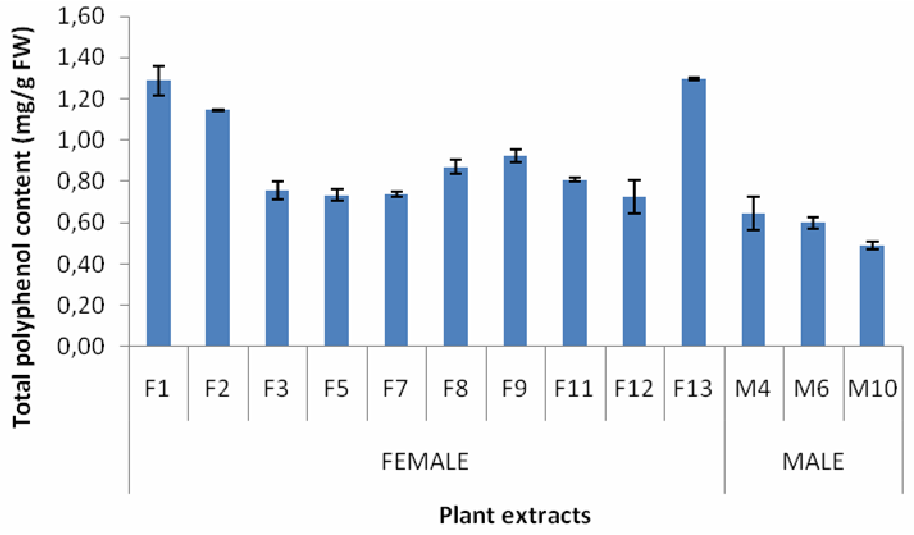

Figure 3. Total polyphenol content of female and male plant extracts in fresh leaves of *F. deltoidea*. Values are means ± S.E. (n = 3).

of antioxidant with 93.25, 92.47, 92.12, 91.85, 91.60 and 90.20% of antioxidant, respectively calculated from these leaf extracts. The lowest percent of antioxidant for female leaf extracts can be detected in F12 leaf extract with 65.46% of antioxidant. For the male leaf extracts, percent of antioxidant ranged from 38.15 to 83.45% of antioxidant. M4 had the lowest antioxidant content with 38.15% of antioxidant among all of the leaf extracts while M6 had the highest percent of antioxidant with 83.45% among the male leaf extracts. Although the percentage of antioxidant in male leaf extracts especially M6 and M10 were high, both of these leaf extracts cannot compete with most of the female leaf extracts.

Total polyphenol, phenolic acid, flavonoid and vitamin C content

Total polyphenol content

Figure 3 showed that F13 leaf extract had the highest total polyphenol content of 1.30 mg/g FW among all of the leaf extracts. F1 and F2 were two leaf extracts which had high polyphenol content of 1.29 and 1.15 mg/g FW, respectively. Besides that, F9, F8 and F11 leaf extracts showed moderate amount for polyphenol content of 0.92, 0.87 and 0.81 mg/g FW, respectively. In this assay, all of the female leaf extracts showed higher polyphenol content compared to that of male leaf extracts. The highest content for male leaves extract was detected in M4 leaf extract with 0.64 mg/g FW. The result from male leaf extracts was quite low compared to the lowest polyphenol content for the female leaf extracts, F5 and F12, both had 0.73 mg/g FW. The other two male leaf extracts were M6 and M10 with 0.60 and 0.49 mg/g FW, respectively.

Total phenolic acid content

According to the Figure 4, the range for total phenolic acid content is from 1.60 to 4.27 mg/g FW. F1 leaf extract showed highest total phenolic acid content with 4.27 mg/g FW followed by F7 and F2 leaf extracts with 3.49 and 3.40 mg/g FW, respectively. In addition, F9, F5 and F3 leaf extracts also exhibit high phenolic acid compared to that of the male leaf extracts with 3.33, 3.14 and 2.94 mg/g FW, respectively. Although most of the female leaf extracts will have high total antioxidant properties but sometimes male leaf extracts can have higher performance compared to several accessions of female *F. deltoidea* leaf extracts. For an instance, total phenolic content in M4 leaf extract is 2.73 mg/g FW which is higher phenolic acid content compared to F8, F11, F12 and F13 from the female leaf extracts with 2.20, 2.61, 2.14 and 2.29 mg/g FW, respectively. The lowest total phenolic acid content for female leaf extracts can be detected in F12 leaf extract with 2.14 mg/g FW while M10 leaf extract had the lowest total phenolic acid content among all of the leaf extracts.

Total flavonoid content

Figure 5 showed total flavonoid content using catechin equivalent. F1 leaf extract showed the highest total flavonoid content with 27.36 mg/g FW while M10 leaf extract showed the lowest total flavonoid content among all of the leaf extracts with 2.32 mg/g FW. Other accessions that had high total flavonoid content were F2, F5, F7 and F9 leaf extracts with 10.78, 15.53, 14.36 and 10.36 mg/g FW, respectively. F12 leaf extract had the same total flavonoid content as M6 leaf extract with 2.44 mg/g FW. M4 leaf extract exhibited 5.36 mg/g FW which

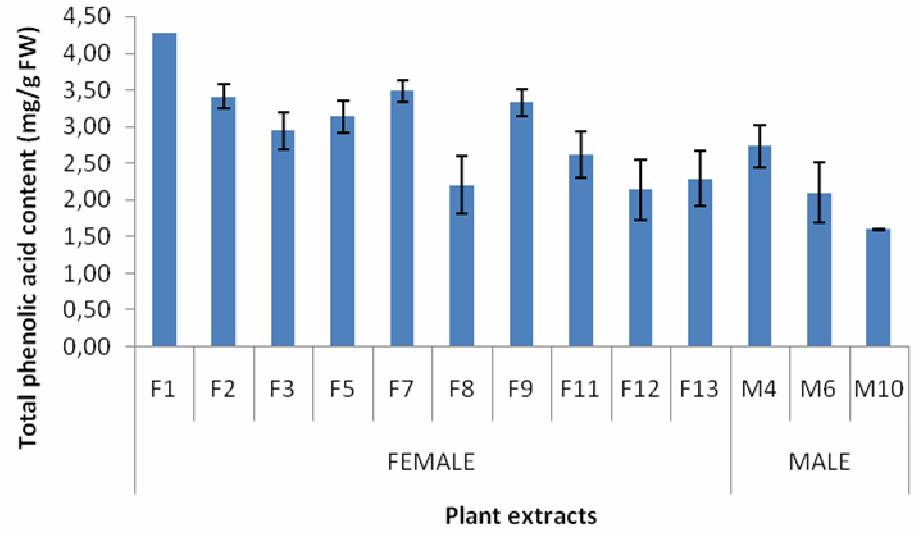

Figure 4. Total phenolic acid content of female and male plant extracts using Folin-Ciocalteu method in fresh leaves of *F. deltoidea*. Values are means ± S.E. (n = 3).

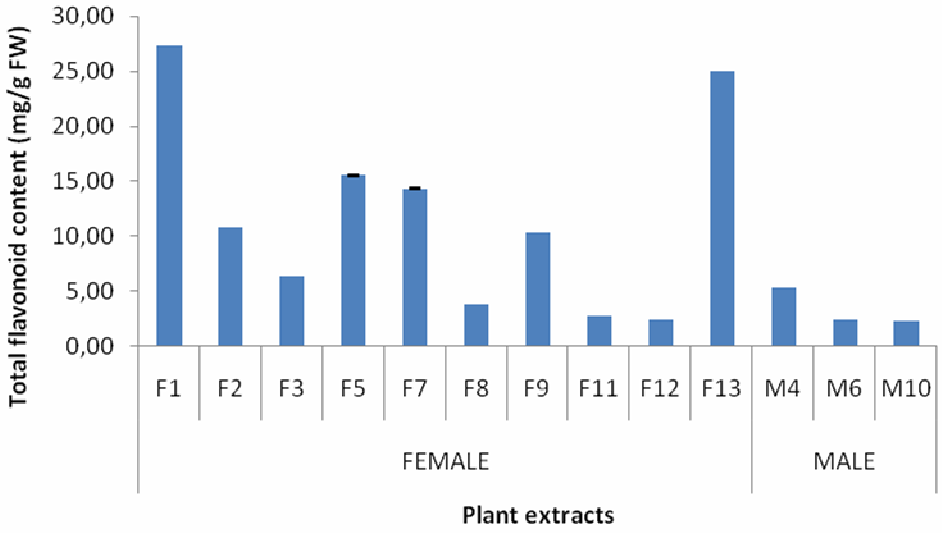

Figure 5. Total flavonoid content of female and male plant extracts using aluminium chloride colormetric method in fresh leaves of *F. deltoidea*. Values are means ± S.E. (n = 3).

exceeded the total flavonoid content of F8, F11 and F12 leaf extracts with 3.86, 2.73 and 2.44 mg/g FW, respectively. M4 leaf extract also the highest total flavonoid content among the male leaf extracts. Lastly, the lowest total flavonoid content among all of the leaf extracts was found in M10 leaf extract with 2.32 mg/g FW.

Total vitamin C

According to Figure 6, the highest total vitamin C content was found in F8 leaf extract with 6.78 mg/g FW but the second highest total vitamin C content was found in M10 leaf extract with 6.36 mg/g FW among all of the leaf extracts. Several accessions like F2, F3, F5 and F12 leaf extracts showed high total vitamin C content with 4.17, 2.63, 4.01 and 3.26 mg/g FW, respectively. Other accessions for the female leaf extracts did not perform as good as the female leaf extracts in assays mentioned earlier. The lowest total vitamin C content of all leaf extracts was detected in F7 leaf extract with 0.61 mg/g FW. It was found that M6 and M4 leaf extracts with 3.63 and 1.29 mg/g FW, respectively had higher total vitamin C compared to several female leaf extracts such as F1, F7, F9 and F11 with 0.83, 0.61, 0.83 and 0.92 mg/g FW, respectively. In this assay M4 leaf extract showed lowest total vitamin C content among male leaf extracts with

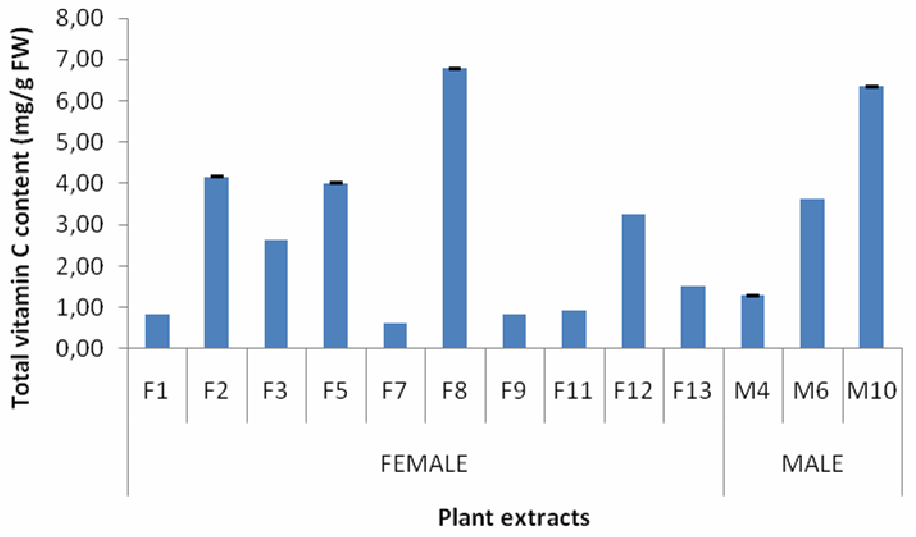

Figure 6. Total vitamin C of female and male plant extracts in fresh leaves of *F. deltoidea*. Values are means ± S.E. (n = 3).

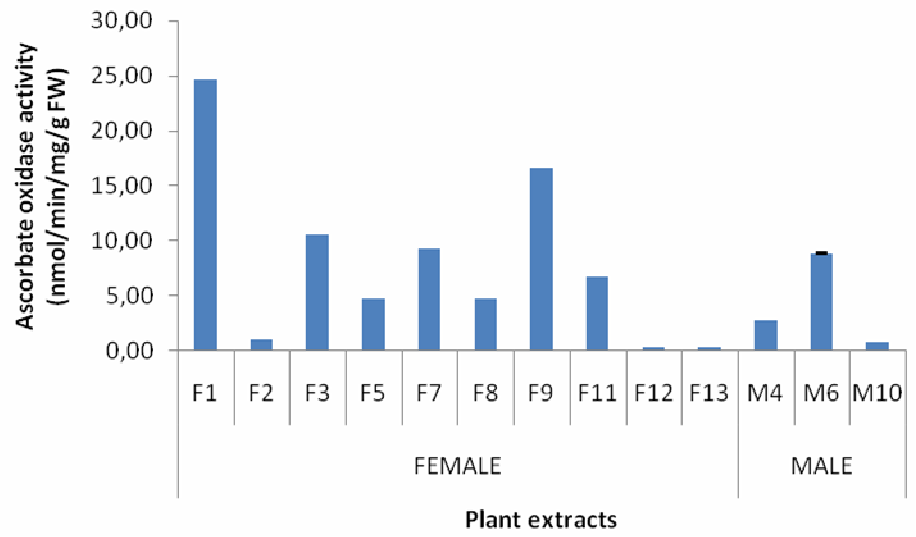

Figure 7. Ascorbate oxidase activity of female and male plant extracts of fresh leaves of *F. deltoidea*. Values are means ± S.E. (n = 3).

1.29 mg/g FW.

Ascorbate oxidase, peroxidase, catalase and ascorbate peroxidase activities

Ascorbate oxidase activity

According to Figure 7, F1 leaf extract showed the highest ascorbate oxidase activity with 24.64 nmol/min/mg/g FW followed by F9, F3 and F7 leaf extracts with 16.62, 10.57 and 9.28 nmol/min/mg/g FW, respectively. Besides that, M6 leaf extract had the highest ascorbate oxidase activity for the male leaf extracts with 8.88 nmol/min/mg/g FW while the lowest ascorbate oxidase content for male leaf extract was found in M10 leaf extract with 0.84 nmol / min /mg/g. M6 leaf extract also had greater activity than several leaf extracts from the female leaf extracts such as F11, F8 and F5 leaf extracts with 6.77, 4.78 and 4.73 nmol/min/mg/g FW, respectively. Among all of the leaf extracts, F13 leaf extract showed the lowest ascorbate oxidase activity with 0.36 nmol/min/mg/g FW.

Peroxidase activity

Figure 8 showed peroxidase activity which ranged from 0.07 – 0.94 nmol / min /mg/g FW. The highest peroxidase

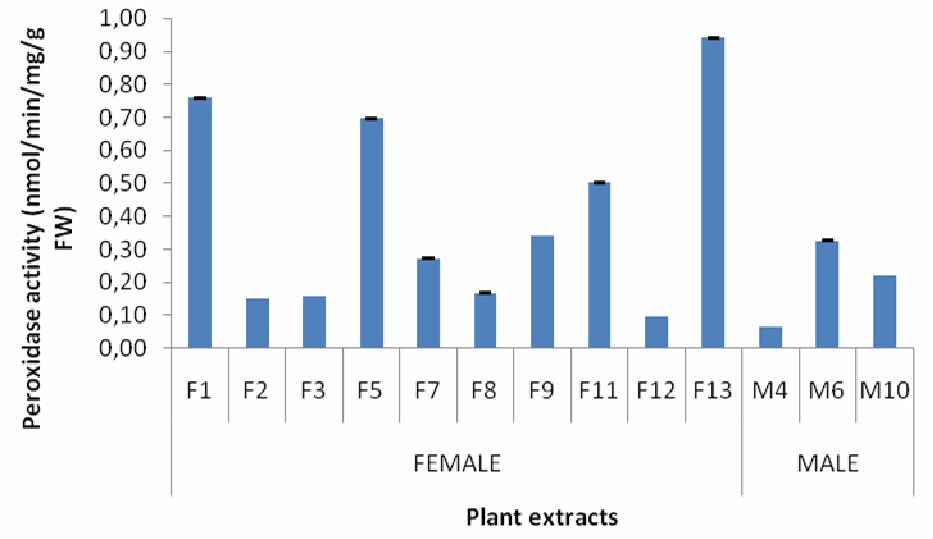

Figure 8. Peroxidase activity of female and male plant extracts of fresh leaves of *F. deltoidea*. Values are means ± S.E. (n = 3).

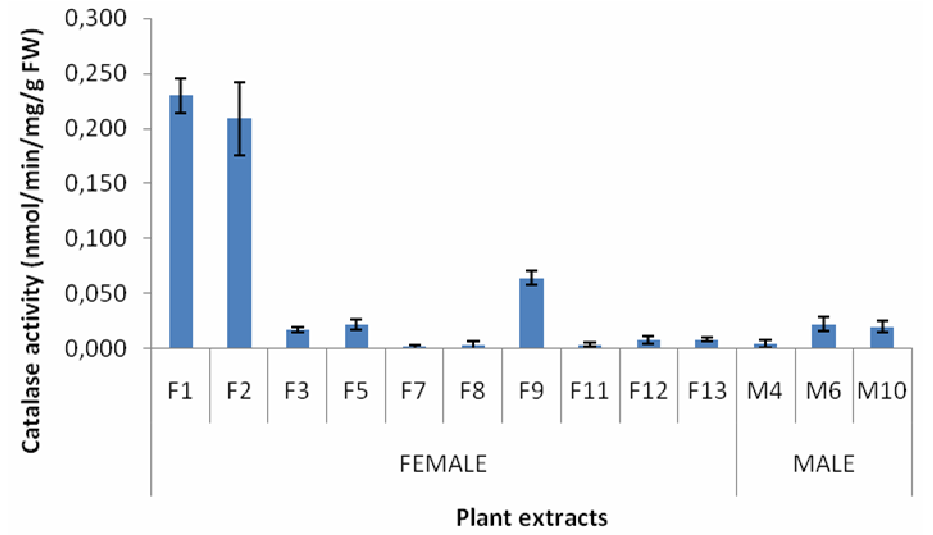

Figure 9. Catalase activity of female and male plant extracts of fresh leaves of *F. deltoidea*. Values are means ± S.E. (n = 3).

activity belongs to F13 leaf extract with 0.94 nmol / min / mg/g FW and followed by F1 and F5 leaf extracts with 0.76 and 0.70 nmol/min/mg/g FW, respectively. F11, F9 and M6 leaf extracts had moderate activity of peroxidase with 0.50, 0.34 and 0.33 nmol/min/mg/g FW, respectively. Low activity of peroxidase can be found in F3, F2 and F12 leaf extracts with 0.16, 0.15 and 0.10 nmol/min/mg/g FW, respectively. The lowest peroxidase activity among the female leaf extract can be found in F12 leaf extract with 0.10 nmol/min/mg/g FW. The highest peroxidase activity among the male leaf can be detected in M6 leaf extract with 0.33 nmol/min/mg/g FW while the lowest peroxidase activity was detected in M4 leaf extract with 0.07 nmol/min/mg/g FW.

Catalase activity

From Figure 9, only three bars which had high catalase activity can be observed. They were F1, F2 and F9 leaf extracts with 0.230, 0.203 and 0.064 nmol/min/mg/g FW, respectively. The other leaf extracts showed low activity of catalase. For example, F5 and M6 leaf extracts, both had 0.022 nmol/min/mg/g FW while F12 and F13 leaf extracts, both had 0.008 nmol/min/mg/g FW. The lowest catalase activity among all of the leaf extracts can be detected in F7 leaf extract with only 0.002 nmol/min/mg/g

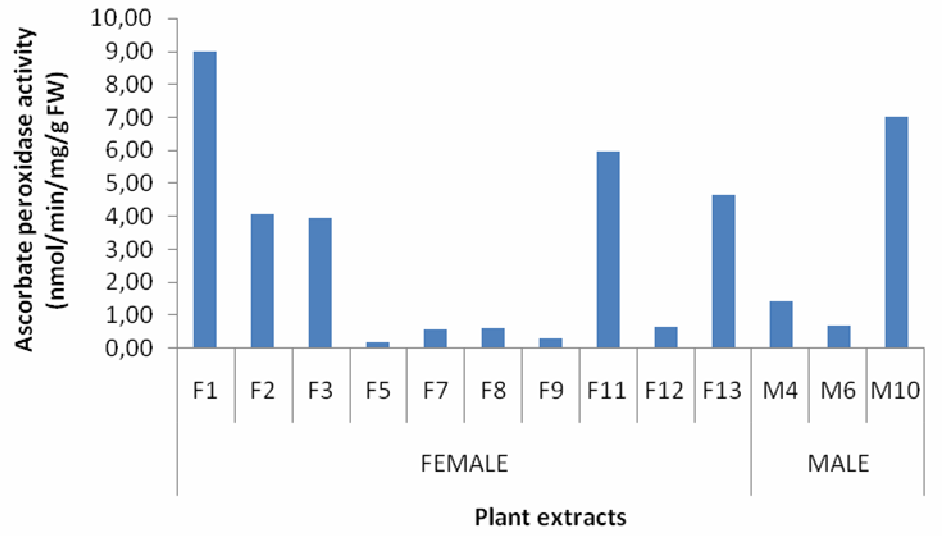

Figure 10. Ascorbate peroxidase activity of female and male plant extracts of fresh leaves of *F. deltoidea*. Values are means ± S.E. (n = 3).

FW. Among the male leaf extracts, M6 leaf extract showed highest catalase activity with 0.022 nmol/min/mg/g FW while the lowest can be detected in M4 leaf extract with 0.004 nmol/min/mg/g FW.

Ascorbate peroxidase activity

Figure 10 showed that F1 leaf extract had the highest ascorbate peroxidase activity while F5 leaf extract had the lowest activity for ascorbate peroxidase among all of the leaf extracts with 9.01 and 0.20 nmol/min/mg/g FW, respectively. Second highest ascorbate peroxidase activity was observed in M10 leaf extract with 7.01 nmol / min / mg/g FW followed by F11, F13, F2 and F3 leaf extracts with 5.98, 4.66, 4.07 and 3.96 nmol/min/mg/g FW, respectively. F7, F8 and F9 leaf extracts had very low activity of ascorbate peroxidase with 0.58, 0.61 and 0.31 nmol /min/mg/g FW, respectively. The lowest ascorbate peroxidase activity among the male leaf extracts can be detected in M6 leaf extract with 0.70 nmol/min/mg/g FW.

DISCUSSION

DPPH free radical scavenging assay and FRAP assay

Results obtained from this study revealed that both methods, DPPH free radical scavenging assay and FRAP assay are reliable, rapid, easy and accurate assays, it can be applied for monitoring the activity of numerous samples over a limited period of time (Maisuthisakul et al., 2008; Klimczak et al., 2007). DPPH is a stable nitrogen-centered free-radical, and their color change from violet to yellow when is reduced by either the process of hydrogen- or electron- donation (Liu et al., 2008). DPPH method is recommended by many authors because this method is repeatable and provides an accurate assay for measuring the antioxidant activity (Klimczak et al., 2007). Behbahani et al. (2007) also suggested the model of scavenging the stable DPPH radical is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods.

According to Thaipong et al. (2006), FRAP assay also could be used to determine antioxidant activity as well as DPPH assay because both assays showed high reproducibility. When the samples reacted with FRAP solution, dark blue color of solution will appear which refers to the ferrous tripyridyltriazine complex. This complex was detected at 593 nm. The extracts which exhibit high antioxidant such as in some of the female accessions produced more ferrous tripridyltriazine complexes than the other accessions. Ferrous tripridyltriazine complexes were produced as product from the reaction in which the samples had the ability to reduce Fe3+ to Fe2+. The greater Fe3+ reduced to Fe2+ the higher total antioxidant content will be observed.

Since there is no available scientific data on antioxidant activity in *F. deltoidea*, no comparative studies can be made. Thaipong et al. (2006) claimed that DPPH free radical scavenging assay and FRAP assay showed no differences among determinations. Based on the results obtained for total antioxidant properties, it confirmed the claims by Thaipong et al. (2006) where the DPPH free radical scavenging assay and FRAP assay have no significant different among determinations. Studies by Ao et al. (2008) of antioxidant activities in Ficus microcarpa showed that leaves extracts using DPPH assay produced higher antioxidant activities compared to 2,2-azinobis (3-ethyl-benzothiazoline-6-fulfonic acid) diammonium salt (ABTS) assay and phenazine methosulfate-nicotinamide adenine dinucleotide – reduced (PMS – NADH) system

superoxide-radical scavenging assay. Previous reports (Patthamakanokporn et al., 2008) on antioxidant activities in different fruits extracts using oxygen radical absorbance capacity (ORAC) assay and FRAP assay showed that different fruits extracts showed different antioxidant activities. Both of these assays expressed high antioxidant activities but some fruits extracts have higher antioxidant activities in ORAC assay and some fruits extracts have higher activity of antioxidant using FRAP assay. DPPH assay and FRAP assay are suitable methods due to the rapidness and accuracy of the results obtained. Wong et al. (2006) reported that DPPH assay and FRAP assay have strong correlation between the mean values in all samples tested. Wong et al. (2006) concluded that the strong correlation indicated that the compounds present in the aqueous extracts capable of reducing DPPH radicals were also able to reduce ferric ions.

The trend for DPPH free radical scavenging assay of 13 accessions of *F. deltoidea* leaf extracts did not vary from ferric reducing antioxidant potential (FRAP) assay. When Figure 1 and Figure 2 were compared, the trend for some leaf extracts of *F. deltoidea* accessions was almost the same. F2, F3 and F13 leaf extracts in both of the Figures showed high antioxidant activities. The percentage for DPPH free radical scavenging activity in Figure 1 was higher than percentage from FRAP method from Figure 2. The probable reason for this trend to be happened is due to the compounds that are more reactive towards DPPH free radical compared to ferric ions. In this study, the highest percent of inhibition using DPPH method is 99.87% while only 93.31% of antioxidant can be detected using FRAP method as shown in Figure 2. To date, there is no report on antioxidative properties in *F. deltoidea*. Various reseachers reported high antioxidant content in different species of Ficus using different methods to analyse. Ao et al. (2008) reported high antioxidant activity from leaf compared to bark and fruit extracts of *Ficus microcarpa*. This study concludes that most plant of genus Ficus may have high antioxidant activity especially in the leaf of the plant.

Total polyphenol, phenolic acid, flavonoid and vitamin C content

Flavones, flavonols and proanthocyanidins are well known specific compounds associated with antioxidant activity in plants (Skerget et al., 2005). Phenolics can be classified into two groups, polyphenols and simple phenols which contain phenolic acids (Marinova et al., 2005). Most of the antioxidant properties in plants are also due to polyphenol, phenolic acid, flavonoid and vitamin C.

According to Li et al. (2006), polyphenols is one of the most numerous groups of substances in plant kingdom ranging from simple molecules, such as phenolic acids, to complex compounds, such as tannins. In addition, polyphenols function in trapping and scavenging free radicals due to their antioxidant properties. Other functions of polyphenols are regulating nitric oxide, decreasing leukocyte immobilization, inducing apoptosis, inhibiting cell proliferation and angiogenesis, and exhibit phytoestrogenic activity (Arts and Hollman, 2005). Phenolic acids are hydroxylated derivatives of benzoic and cinnamic acids. The most common hydroxycinnamic acid derivatives are p-coumaric, caffeic and ferulic acids which frequently occur in food as simple esters with quinic acid or glucose (Mattila and Kumpulainen, 2002). Flavonoids constitute a group of natural compounds that occur in fruits, vegetables, wine, tea, chocolate and other cocoa products. Daily dietary intake of flavonoids and similar polyphenols exceeds that of antioxidative vitamins and provitamins (Sies et al., 2005). According to Hernandez et al. (2006), vitamin C is the most important vitamin for human nutrition. L-ascorbic acid (AA) is the main biologically active form of vitamin C. L-ascorbic acid is then reversibly oxidized to form L-dehydroascorbic acid (DHA).

There are claims that phenolics compounds and its derivatives are strongly correlated with antioxidant activities (Maisuthisakul et al., 2007). In this study, total polyphenol, phenolic acid and flavonoid content are well correlated with total antioxidant activities. Some of the accessions such as F1 and F3 leaf extracts constantly show high activities whether antioxidant activity or phenolics compounds. This is in an agreement with research done by Wong et al. (2006) on antioxidant activities in aqueous extracts of selected plants. Wong et al. (2006) reported that aqueous extracts of *Mentha arvensis* L., *Polygonium hydropiper*, *Manihot esculenta* L., *Eugenia polyantha*, *Ipomea babatas*, *Sauropus androgynus* and *Piper betel* had high antioxidant activities and also had high total polyphenol content. Similarly observed from Figure 3, F1, F2 and F13 leaf extracts had high total polyphenol content and also had high total antioxidant content in both DPPH free radical scavenging assay and FRAP method. The trend for Figure 3 can be observed in Figures 4 and 5 since polyphenol, phenolic acid and flavonoids contains phenolic groups. From Figures 3 - 5, there is no correlation and similarities between phenolics compounds and vitamin C content as shown in Figure 6. According to Figure 6, total vitamin C content differs from results obtained for total antioxidant content in Figures 1 and 2. As an example, F1 and F13 leaf extracts that continuously showed high total antioxidant content in Figures 1 and 2 did not show the same pattern in Figure 6 where, F1 and F13 leaf extracts showed low total vitamin C content. This study agreed with previous report by Gil et al. (2002) which claimed that vitamin C may not necessarily have the same pattern with antioxidant activities and not the main antioxidant in some samples. Previous studies on antioxidant activities in fruits indicate that vitamin C is not the main antioxidant but phenolics compounds are responsible for the observed activities (Kalt et al., 1999).

Report by Makris et al. (2007) showed that total polyphenols and total flavonoids can be found in different

types of grapes and other agri-food solid wastes food such as olive tree leaves, apple peels, onion peels, potato peels and carobs. It was found that grape seeds contain the greatest total polyphenols content while total flavonoid content had lower amount as the total flavonoid content was over 99% of total polyphenol. Maisuthisakul et al. (2008) revealed that plants with high antioxidant activities also have high total phenolic and flavonoid content. These are in agreement with our study that plants extracts which have high antioxidant activities possess high antioxidative compounds such as polyphenol, phenolic acid and flavonoid. Plants such as Eugenia siamensis Craib., Cratoxylum formosum Dyer., Erythrina crista Galli. and Careya sphaerica Roxb. possessed strong antioxidant activity, total phenolic and flavonoid contents (Maisuthisakul et al., 2008). Likewise, the same pattern was found in cocoa which have strong antioxidant activities and also have high total phenolic and flavonoid content (Lee et al., 2003).

Ascorbate oxidase, peroxidase, catalase and ascorbate peroxidase activities

Reactive oxygen species (ROS) get special attention lately due to many factors such as drought, cold, heat, herbicides and heavy metals. All of these factors lead to increasing number and accumulation of ROS in plant cells (Noctor and Foyer, 1998). Scientific research shows that ROS are harmful to the cell because they can raise the oxidative level through loss of cellular structure and function (Lee at al., 2007). ROS detoxification agents in cells include antioxidative enzymes such as ascorbate oxidase, peroxidase, catalase and ascorbate peroxidase. Johnson et al. (2003), said that ROS detoxification agents also includes non enzymatic antioxidants such as flavones, anthocyanins, carotenoids and ascorbic acid.

According to Murata et al. (2008), ascorbate oxidase (EC 1.10.3.3) is a member of the multicopper oxidase family and can be obtained from higher plants such as green zucchini squash and cucumber and fungal species. Ascorbate oxidase catalyzes the one-electron oxidation of ascorbate with the concomitant four-electron reduction of dioxygen to water (Solomon et al., 1996). Peroxidases (EC 1.11.1.7) are referring to heme containing enzymes which are able to oxidise organic and inorganic compounds using hydrogen peroxide as co-substrate. The non-specificity of peroxidase makes the enzyme suitable to a broad range of electron donor substrates (Halliwell, 2006). Catalase (EC 1.11.1.6) is a tetrahedrical protein, constituted by four heme groups which catalyze the dismutation of hydrogen peroxide in water and oxygen (Scandalios, 1987). Ascorbate peroxidase (EC 1.11.1.11) functions as hydrogen peroxide detoxification and gluthathione regeneration via ascorbate-gluthathione pathway. Ascorbate peroxidase is able to scavenge hydrogen peroxide produced by superoxide dismutase using ascorbate as an electron donor (Noctor and Foyer, 1998).

From the results obtained for enzymatic antioxidants, the enzymes for antioxidative study were not stable. It was shown on all of the results obtained for enzymatic antioxidant that the plant extracts did not have similar pattern between the other antioxidative enzymes studied. Only F1 leaf extract showed high activity and it is consistent from assay to assay. Report on ascobate oxidase activities in Phalaenopsis plantlet by Ali et al. (2005) showed that ascorbate oxidase activity increase significantly after exposed to different light intensities which indicated the oxidation by molecular oxygen of ascorbic acid to dehydroascorbate, with the formation of water. Dionisio-Sese and Tobita (1998) reported that peroxidase activity increase in most of the rice seedling in response to salinity stress and may cause retarded growth due to inhibition of cell elongation. Catalase activities in C3 plants such as wheat are higher than C4 plants (maize) (Nayyar and Gupta, 2006). It is because C4 plants have high in ascorbate peroxidase, glutathione reductase and dehydroascorbate reductase activities compared to C3 plants. Carias et al. (2008) found out that, 3.8 folds of ascorbate peroxidase activities occurred when treated with Phragmites australis for the first time as an adaptative response regarding the plant's necessity to detoxify the hydrogen peroxide formed. Ascorbate peroxidase activities were found to be higher in the leaves extracts than roots and stems extracts which suggested that ascorbate peroxidase is mainly located in the chloroplast (Carias et al., 2008).

With all the data obtained on non enzymatic and enzymatic antioxidant activities in aqueous extract of different *F. deltoidea* it is suggested to further the study on total antioxidant properties using different methods and parameters to find optimum and best condition to extract and assay the samples. Furthermore, enzymatic antioxidant also need further study of other enzymes that may regulate in the plants due to their importance as plant first response in defense againts free radicals.

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