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Full Length Research Paper

Total antioxidant, polyphenol, phenolic acid, and flavonoid content in *Ficus deltoidea* varieties

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This research aimed to study the antioxidant activities in hot and cold aqueous extracts extracted from fresh leaf of *Ficus deltoidea* using 2,2-diphenyl-1-picrylhydrazil (DPPH) free radical scavenging activity and ferric reducing antioxidant power (FRAP) assays. Phenolic compounds were measured using total polyphenol, phenolic acid, and flavonoid content. Different extraction conditions significantly affected the total antioxidant activities, polyphenol, phenolic acid, and flavonoid content of the extracts. The decreasing order of antioxidant activities using DPPH method in hot aqueous extracts were as follows: var. *kunstleri* > var. *trengganuensis* > var. *angustifolia*, while cold aqueous extracts: var. *kunstleri* > var. *angustifolia* > var. *trengganuensis*. The total antioxidant content using FRAP method showed the highest activity in hot aqueous extracts of F2 with 2.13 mg Trolox equivalent per gram fresh weight (TE/g FW). Hot aqueous extract for F2 and F11 contained the highest total polyphenol content with 0.88 mg gallic acid equivalent (GAE)/g FW, while the lowest in cold aqueous of M6 contained total polyphenol content with 0.47 mg GAE/g FW. Total phenolic acid ranged from 0.54 to 2.19 mg GAE/g FW in hot aqueous extracts, while 0.59 to 1.96 mg GAE/g FW for cold aqueous extract. Total flavonoid content ranged from 0.17 to 0.66 and 0.18 to 0.51 mg catechin equivalents (CE)/g FW in hot and cold aqueous extracts, respectively. All of the antioxidant compounds correlated positively with total polyphenol, phenolic acid, and flavonoid.

Key words: *Ficus deltoidea*, antioxidant, polyphenol, phenolic acid, flavonoid.

INTRODUCTION

Taxonomy investigation of genus *Ficus* and other genus that belong to Moraceae family was studied by Ventakamaran (1972) who claimed that Moraceae family constitutes large taxa of over 50 genus and nearly 1400 species, including some important genus like *Artocarpus*, *Morus*, and *Ficus.* The common name for *Ficus* is fig and it was cultivated and became an important crop world-wide for its synconium or better known as fruit which has hollow receptacle with a small opening at the apex partly closed by small scales (Dueñas et al., 2008). *Ficus* is a genus which contains about 800 species of woody trees, shrubs, and vines in Moraceae family (Abdel-Hameed, 2008). The importance of *Ficus carica* L., commonly used by Okinawan folks in Japan as beverage or herbal medicine. Four flavonoid glycosides were detected with rutin exhibited the highest antioxidant activity, while the bark, fruits, and leaves of *Ficus microcarpa* L. extracted with methanol contained high antioxidant activities, total phenolics, and had antibacterial properties towards Gram-positive and Gram-negative bacteria (Abraham et al., 2008; Ao et al., 2008). *Ficus bengalensis* (banyan tree) and *Ficus racemosa* (Indian fig) parts such as bark, fruits, and roots are used as alternative medicine for diabetes. *Ficus deltoidea* or better known as Mas Cotek among the Malay community in Malaysia is a medicinal plant derived from genus *Ficus*. Mas Cotek is a name given by local people, because of different characteristics of *F. deltoidea*: Mas stands for gold and Cotek stands for dot which gives the general idea of this plant that has

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gold dots at the upper surface of the leaf. It is an evergreen shrub or small tree used traditionally to treat cardiovascular diseases and diabetes (Hakiman and Maziah, 2009).

During daily activities, numerous reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide ion (O_2^-), peroxyl (ROO^-), peroxinitrite ($^-ONOO^-$), nitric oxide (NO^-), and hydroxide radical (OH^-) are produce through oxidative stress in living organism, biologically materials such as lipid, food and oil, and industrial products (Abdel-Hameed, 2008). ROS compounds can damage cellular proteins and lipids or form DNA adducts that lead to carcinogenic activity when they are present in high levels in living cells (Seifried et al., 2007). The redox properties of phenolic compounds allow them to act as reducing agent, hydrogen donors, quenchers of singlet oxygen, and may possess metal chelation properties (Gülçin et al., 2010). Flavonoids represent low molecular phenolics and are divided into several classes such as flavones, flavanones, isoflavones, isoflavans, pterocarpans, coumestans, anthocyanins, flavanols, and flavonols (Sultana and Anwar, 2008). Due to the medicinal properties of *F. deltoidea*, the purpose of this study was to obtain additional information on antioxidant activities (2,2-diphenyl-1-picrylhydrazil (DPPH) and ferric reducing antioxidant power (FRAP) assay) and total polyphenol, phenolic acid, and flavonoid content of different *F. deltoidea* varieties. Both hot and cold aqueous extractions were used.

MATERIALS AND METHODS

Plant

A total of six accessions of *F. deltoidea* were grown and maintained in the glass house with natural environmental conditions with temperature (35/25°C) and photoperiod (16 h light/8 h dark). The leaves were grouped into their respective variety by studying the leaf shape and morphology according to classification by Corner (1969). Two accessions per variety were chosen for this study; var. *kunstleri* (labeled as F1 and F11), var. *trengganuensis* (labeled as F2 and F12), and var. *angustifolia* (labeled as M4 and M6). Six-month-old fully expended leaves were freshly harvested and used for the experiments.

Chemicals and reagents

DPPH, 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ), Trolox, Folin-Ciocalteu's phenol reagent, and catechin were purchased from Sigma Co. St. Louis, Missouri, USA. Methanol, sodium nitrite, acetic acid, sodium hydroxide, 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.

Preparation of extracts for total antioxidants and total polyphenol content

Extraction of antioxidant compounds was conducted by employing the method modified from Wong et al. (2006). A total of 0.5 g leaf of each of the six accessions was homogenized with 25 ml of boiling distilled water (100°C) in 150 ml flask covered with aluminium foil. The treatment was maintained at 100°C in the water bath for 30 min before being placed on an orbital shaker at room temperature for 1 h in the dark. For cold aqueous extraction, pre-cold distilled water at 4°C was used to homogenize the leaf sample. The temperature of the extraction was maintained at 4°C in the water bath for 30 min before being placed on an orbital shaker at room temperature for 1 h in the dark. After which the samples were filtered using Whatman No. 1 filter paper and the extracts were stored at -80°C freezer.

Preparation of the extracts for total phenolic acids and total flavonoid content

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 ground using pestle and mortar in 50 ml of boiling distilled water to obtain hot aqueous extract, while cold distilled water was used to obtain cold aqueous extract. Both extracts were maintained at desired temperature for 1 h before the mixture was centrifuged for 5 min at 14000 rpm. The supernatant was collected and kept in -80°C freezer before use.

Determination of total antioxidant content

DPPH free radical scavenging assay was measured using DPPH free radical scavenging test, employing the method described by Wong et al. (2006). The initial absorbance of DPPH in methanolic solution was measured at 515 nm using spectrophotometer (UV-2602, Labomed, Inc. USA) for control treatment. A total of 40 µl of extract was added to 3 ml of 0.1 mM methanolic DPPH solution. The mixture was incubated at room temperature for 30 min before the change in absorbance value at 515 nm was measured. The percentage of inhibition was calculated using the formula, inhibition (%) = [(A_{515} of control - A_{515} of sample) / A_{515} of control] × 100, where A_{515} is absorbance at 515 nm and Trolox was used to express total antioxidant as mg Trolox equivalent per gram fresh weight (TE/g FW) of leaf sample.

FRAP

The FRAP assay was conducted using the method of Benzie and Strain (1996). 200 µl of the extract was added with 3 ml of FRAP reagent that was prepared with a mixture of 300 mM sodium acetate buffer at pH 3.6, 10 mM TPTZ solution, and 20 mM $FeCl.6H_2O$ 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 percentage of antioxidant. The percentage of antioxidant was calculated using the formula, Antioxidant (%) = [(A_{593} of sample - A_{593} of control) / A_{593} of sample] × 100, where A_{593} is absorbance at 593 nm and control is the experiment without sample extract. Trolox was used to express total antioxidant as mg Trolox equivalent per gram fresh weight (TE/g FW) of leaf sample.

Determination of phenolic compounds

Total polyphenol content

A total of 100 µl of the 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 at room temperature for an hr 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 (mg GAE/g FW).

Total phenolic acid assay

The total phenolic acid assay was conducted as described by Singleton and Rossi (1965) using Folin-Ciocalteu method. One milliliter 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% Na_2CO_3 were added. The mixture was further 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 colorimetric method. One milliliter of the extract was added with 4 ml of distilled water in a flask. After that, 0.3 ml 5% $NaNO_2$ was added. After 5 min, 0.3 ml of 10% $AlCl_3$ was added. After the 6 min, 2 ml of 1 M NaOH was added. Then, the mixture was made to 10 ml by adding 2.4 ml distilled water. The mixture was mixed and the absorbance was measured at 510 nm. The total flavonoids content was expressed as mg catechin equivalents (CE)/g samples.

Statistical analysis

The observations were replicated thrice for each experiment and all data are presented as mean ± standard deviation (SD) of three replicates using Statistical Analysis System (SAS) software. Least significant differences (LSD) test was used to evaluate the difference between treatment means at 95% confidence interval.

RESULTS AND DISCUSSION

Determination of total antioxidant activity

F. deltoidea extract is commonly used for the treatment of many ailments, such as to facilitate childbirth, post partum medication, and to contract cervic muscles, provide extra energy, regulate blood system, headache, toothache, cold, wound, sores, and rheumatism in Malaysia (Sulaiman et al., 2008). Usually, different parts of *F. deltoidea* plant were prepared by mixing with water for oral use and this aqueous extraction was reported to contain antioxidative activities (Hakiman and Maziah, 2009).

Antioxidant activities in different plants have been extensively studied due to the ability of antioxidants to donate hydrogen to a free radical to make it stable and become unreactive species (Wang et al., 2008). The effects of different method of extractions can be a vital key to obtain high antioxidant activities. The best way to select a method for extraction is by considering factors such as extraction time, solvent consumption, extraction yield, and the quality of the extracts (Nantitanon et al., 2010).

DPPH free radical scavenging activities and FRAP of hot and cold aqueous extracts of *F. deltoidea* are shown in Table 1. The results for total antioxidant content in Table 1 were expressed as percentage of inhibition and percentage of antioxidants which were calculated from formula given earlier, respectively. Hot and cold aqueous extracts of *F. deltoidea* showed that all of the plant extracts contained antioxidant activities, but varied in values. The type of extraction conditions significantly affected the antioxidant activities. Total antioxidant content using DPPH assay expressed as percentage of inhibition of hot aqueous extracts varied from 35.26 to 43.96% and that of cold aqueous extracts varied from 36.04 to 46.77%. Percentage of antioxidant using FRAP method showed that total antioxidant content in hot aqueous extracts ranged from 64.94 to 94.78% as compared to that of cold aqueous extracts which ranged from 67.65 to 93.69% of antioxidant. The total antioxidant activity of both methods was found highest in F2 accession of hot aqueous extract, while F1 accession for cold aqueous extract. The percentage of inhibition (DPPH) for F2 extract is 43.96 and 94.78% of antioxidant using FRAP assay, while F1 cold aqueous extracts contained 46.77 and 93.69% for percentage of inhibition and antioxidant, respectively. The total antioxidant content using DPPH and FRAP methods were further evaluated using Trolox as standard curve and expressed as mg Trolox equivalent (TE)/g fresh weight (FW) as shown in Figures 1 and 2. The standard curves of Trolox were generated using different concentrations of Trolox for both DPPH and FRAP methods with the R^2 value of 0.997 and 0.996, respectively.

In DPPH method, plant extracts will act as radical scavengers and function to quench peroxide radical to terminate peroxidation chain reaction that is caused by lipid peroxidation process, thus improving the quality of food product (Liu and Yao, 2007). In the presence of radical scavengers as hydrogen donor, DPPH will be transformed into non-radical form and the reduction of DPPH radicals can be measured at 515 nm (Prior et al., 2005). The highest antioxidant activities using DPPH method was found in cold aqueous extract of F1 accession and the lowest activity of antioxidant was found in cold aqueous extracts of F12 accession with 4.22 and 2.80 mg/g FW, respectively (Figure 1). In hot aqueous extraction, F1 accession from var. *kunstleri* and F2 accession from var. *trengganuensis* contained the highest total antioxidant activity with 3.85 and 3.92 mg/g TE/g FW, respectively. Meanwhile, the lowest activity of total antioxidant can be found in F12 (var. *trengganuensis*) and M6 (var. *angustifolia*) with 2.99 and 3.03 mg TE/g FW, respectively for hot aqueous extract. Although, the highest antioxidant for hot aqueous extract can be found in var. *trengganuensis*, the lowest activity of

Table 1. Total antioxidant content in DPPH and FRAP assays and expressed as percentage of inhibition and percent of antioxidant, respectively.

Assay	Hot aqueous extracts*	Cold aqueous extract*
DPPH assay (Percentage of inhibition, %)		
var. *kunstleri*		
F1	43.32 ± 0.54	46.77 ± 0.48
F11	37.90 ± 0.60	40.62 ± 0.43
var. *trengganuensis*		
F2	43.96 ± 0.77	39.77 ± 0.63
F12	35.26 ± 0.32	40.62 ± 0.56
var. *angustifolia*		
M4	38.62 ± 0.45	37.64 ± 0.32
M6	35.67 ± 0.31	36.04 ± 0.43
FRAP assay (Percentage of antioxidant, %)		
var. *kunstleri*		
F1	93.15 ± 0.98	93.69 ± 1.03
F11	90.98 ± 2.04	83.85 ± 1.18
var. *trengganuensis*		
F2	94.78 ± 0.84	90.73 ± 0.95
F12	80.14 ± 0.79	85.24 ± 0.78
var. *angustifolia*		
M4	87.69 ± 0.99	82.03 ± 0.75
M6	68.11 ± 0.69	70.39 ± 0.67

*****Each data values represents the mean of three replicates ± standard deviation.

antioxidant also was found in the same variety. This finding suggests that different accessions in the same variety may differ greatly in the activity of antioxidant. The average of total antioxidant content for hot aqueous in every variety is 3.56, 3.46, and 3.19 mg TE/g FW for var. *kunstleri*, *trengganuensis*, and *angustifolia*, respectively, while 3.89, 3.13, and 3.16 mg TE/g FW for cold aqueous extract, respectively. The activity of antioxidant in different variety can be ordered in decreasing value as follow: var. *kunstleri* > var. *trengganuensis* > var. *angustifolia* for hot aqueous extracts and var. *kunstleri* > var. *angustifolia* > var. *trengganuensis* for cold aqueous extracts. Generally, the hot aqueous extract of *F. deltoidea* showed higher level of antioxidant activity for most accessions when compared with that of cold aqueous extract. A similar finding was reported in coconut oil extracted under hot and cold conditions. The coconut extracted in hot condition was superior for all concentrations tested (Seneviratne et al., 2009). In another study, cold aqueous extract of green tea was found to be more effective in scavenging ability of DPPH as compared to that of hot aqueous extract with 31.7 to 36.3% and 29.1 to 34.0%, respectively (Lin et al., 2008). The value of percentage of inhibition is in agreement with the result in Table 1 which showed low antioxidant activity in both aqueous extract using DPPH assay probably due to low affinity of aqueous extract to react with DPPH radicals. On the other hand, hot aqueous extract of green tea effective for reducing power which suggested that cold brewing method of green tea would be a new alternative way to brew tea (Lin et al., 2008).

The antioxidant activities of hot and cold aqueous extracts were evaluated by comparing their ability to form ferrous tripyridyltriazine complex which can be detected spectrophotometrically at 593 nm. Total antioxidant content using FRAP method showed that the highest activity was found in hot water extract of F2 belonging to the var. *trengganuensis* with 2.13 mg TE/g FW, while the lowest in M6 (var. *angustifolia*) extract with 0.58 mg TE/g FW (Figure 2). Total antioxidant content for hot aqueous extract ranged from 0.58 to 2.13 mg TE/g FW, while cold aqueous extract ranged from 0.60 to 1.81 mg TE/g FW. The highest antioxidant content for cold aqueous extract was found in F1 extracts, while the lowest is in M6 with 1.81 and 0.6 mg TE/g FW, respectively. *F. deltoidea* var. *angustifolia* contained the lowest antioxidant activity in

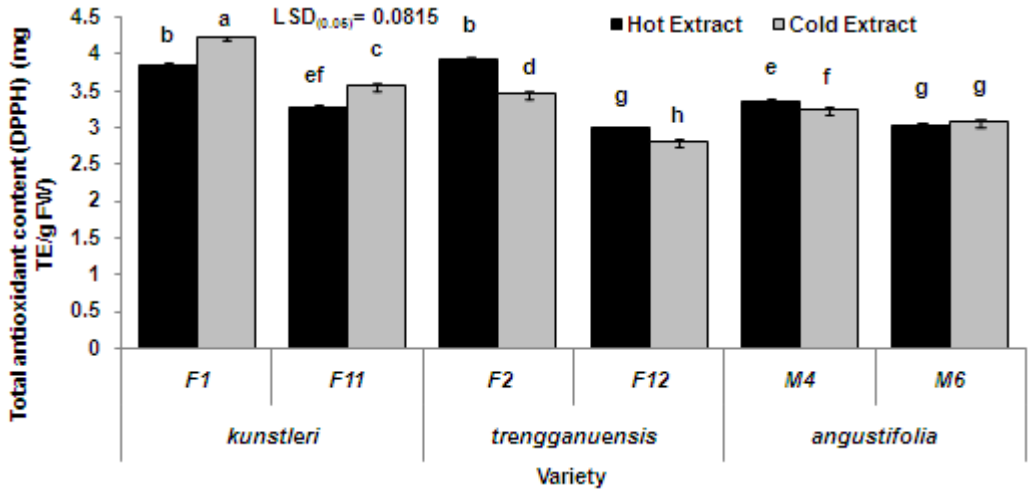

Figure 1. Total antioxidant content of six accessions of *F. deltoidea* using DPPH method expressed as Trolox equivalent in hot and cold aqueous extractions. Values are means ± SD (n = 3) followed by different letters to indicate the significant differences ($p < 0.05$) between the values.

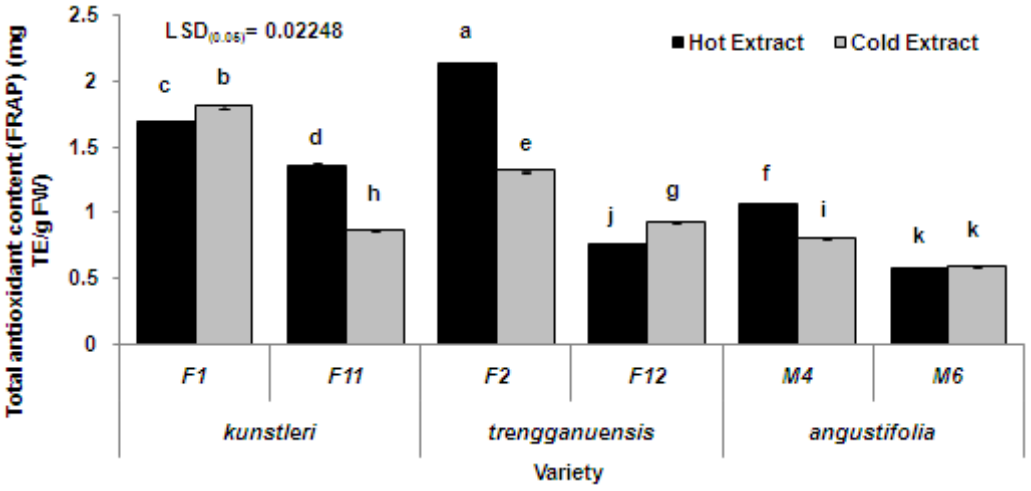

Figure 2. Total antioxidant content of six accessions of *F. deltoidea* using FRAP method expressed as Trolox equivalent in hot and cold aqueous extractions. Values are means ± SD (n = 3) followed by different letters to indicate the significant differences ($p < 0.05$) between the values.

both aqueous extract. The same patterns of result exhibited by var. *trengganuensis* in DPPH and FRAP assays where one accession contained high antioxidant activity (F2), while F12 contained low antioxidant activity in both extracts. The decreasing order for total antioxidant content in both extracts is as follow: var. *kunstleri* > var. *trengganuensis* > var. *angustifolia* with 1.52, 1.45, and 0.82 mg TE/g FW and 1.34, 1.13, and 0.71 mg TE/g FW for hot and cold aqueous extracts, respectively. The antioxidant activity of hot aqueous extract was superior as compared to that of cold aqueous extract with an average of 1.26 and 1.06 mg TE/g FW for hot and cold aqueous extracts, respectively which compliments with the finding of total antioxidant found in DPPH method. Previous study using FRAP method in 45 plant species found high differences between the lowest and highest FRAP value of the plant extracts studied up until 369 fold which is contrary to this study of with only three and two fold in hot and cold aqueous extracts, respectively (Li et al., 2008).

Determination of total polyphenol, phenolic acid, and flavonoid content

Statistical analysis showed that different extraction

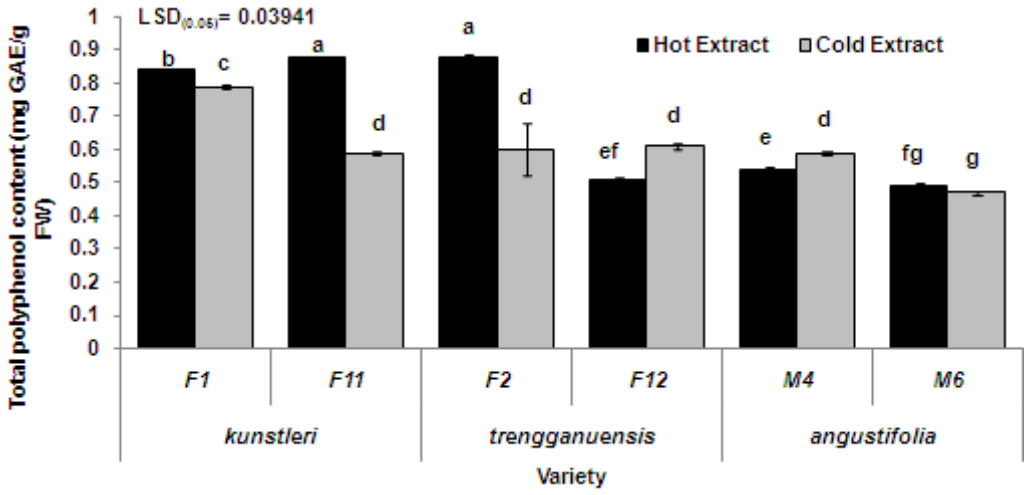

Figure 3. Total polyphenol content of six accessions of *F. deltoidea* in hot and cold aqueous extractions expressed as gallic acid equivalent. Values are means ± SD (n = 3) followed by different letters to indicate the significant differences ($p < 0.05$) between the values.

conditions significantly affected the activities of total polyphenol, phenolic acid, and flavonoid contents at $p < 0.05$. Figure 3 shows the results obtained from total polyphenol content quantified using Folin-Ciocalteu method and expressed as mg gallic acid equivalent (GAE)/g FW of hot and cold aqueous extracts. Total polyphenol content of hot and cold aqueous extracts of *F. deltoidea* accessions ranged from 0.49 to 0.88 mg GAE/g FW and 0.47 to 0.79mg GAE/g FW, respectively. The highest total polyphenol content can be observed in hot aqueous extract of F11 and F2 with 0.88 mg GAE/g FW, while the lowest activity can be found in cold aqueous extract of M6 with 0.47 mg GAE/g FW. The highest activity of total polyphenol in cold aqueous extract can be found in F1 extract. F1 and F11 extracts (var. *kunstleri*) and F2 (var. *trengganuensis*) contained high total polyphenol content especially in hot aqueous extract. The other three accession extracts; F12 (var. *trengganuensis*), M4 and M6 (var. *angustifolia*) contained low total polyphenol content. Total polyphenol capacity of *F. deltoidea* in different varieties can be arranged in decreasing order; var. *kunstleri* > var. *trengganuensis* > var. *angustifolia* for both hot and cold aqueous extracts. The results collected in Figure 3 showed that the temperature of extractions plays an important role in polyphenol content of *F. deltoidea* extracts. Most of the plant extracts showed high total polyphenol content in hot aqueous extracts which suggested that hot aqueous leads to superior extraction as compared to that of cold aqueous extraction for *F. deltoidea*. This is in agreement with the study in beverages made from *Hibiscus sabdariffa* flowers named *karkadè* which have high total polyphenol content in hot treatment as compared to cold treatment which conclude that the antioxidant ability of polyphenol compounds do not destroyed during heating process (Prenesti et al., 2007).

The results for total phenolic acid are as shown in Figure 4 and expressed as mg gallic acid equivalent (GAE)/g FW. Total phenolic acid of different accessions of *F. deltoidea* extracts ranged from 0.36 to 2.19 mg GAE/g FW for hot aqueous extract, while 0.59 to 1.96 mg GAE/g FW for cold aqueous extract. Although, hot aqueous extract of F11 accession in total polyphenol contained the highest activity, F11 extract exhibited the lowest total phenolic acid content with 0.36 mg GAE/g FW. The highest total phenolic acid content can be detected in hot aqueous extract of F2 followed by F1 with 2.19 and 1.95 mg GAE/g FW, respectively. In cold aqueous extract, F2 extract again exhibited the highest total phenolic acid content with 1.96 mg GAE/g FW followed by F1 and F11 with 1.12 and 0.91 mg GAE/g FW, respectively. The lowest total phenolic acid content can be found in M6 extract with 0.59 mg GAE/g FW. In average, total phenolic acid content was superior in var. t*rengganuensis* for both extracts followed by var. *kunstleri* and lastly var. *angustifolia* with 1.37, 1.16, and 0.72 mg GAE/g FW for hot aqueous extract, while 1.39, 1.02, and 0.7 mg GAE/g FW for cold aqueous extract. Total phenolic content was studied using hot and cold infusion of different type of teas, and result was that most of the teas studied showed high total phenolic content in hot infusion as compared to that of cold infusion. The highest phenolic content of hot infusion was found in Lyons black tea, but the highest activity of total phenolic content of all infusions was found in white tea, although white tea infusion is the only tea infusion that has low total phenolic activity in hot infusion (Venditti et al., 2010). Furthermore, total phenolic content in hot aqueous extract was found to be the highest as compared to ethanolic and ethyl acetate extracts that suggested that aqueous extract is

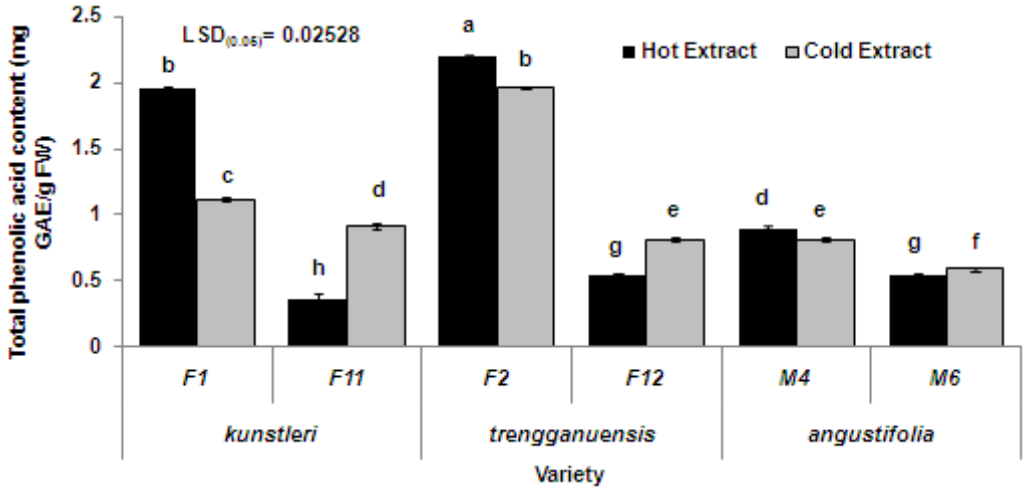

Figure 4. Total phenolic acid content of six accessions of *F. deltoidea* in hot and cold aqueous extractions expressed as gallic acid equivalent. Values are means ± SD (n = 3) followed by different letters to indicate the significant differences ($p < 0.05$) between the values.

superior to extract phenolic compounds due to its polarity (Nantitanon et al., 2010). In the study of Leguminosae family, extracts from the leaf of *Caesalpinia pulcherrima* was found to contain the highest total phenolic content with 5030 mg GAE/100 g FW, followed by *Bauhinia kockiana* and *Cassia surattensis* with 4220 and 3330 mg GAE/100 g FW, respectively (Chew et al., 2009). The activity of total phenolic was higher as compared to our findings in *F. deltoidea* accessions extracts which may be due to different extraction preparation, initial weight of sample used, and different species of plant extracts. High content of total phenolic content was expected in *Salvia cedronella* which is known to have rich amount of phenolics compound with 116 µg pyrocatechol/mg extract (Yesilyurt et al., 2008).

The total flavonoid content in hot and cold aqueous extract of *F. deltoidea* accessions are summarized in Figure 5 and expressed as mg catechin equivalent (CE)/g FW. Total flavonoid content in hot aqueous extract varied from 0.17 to 0.66 mg CE/g FW, while cold aqueous extract ranged from 0.18 to 0.51 mg CE/g FW. The highest total flavonoid content in hot aqueous extract was found in F1 extract with 0.66 mg CE/g FW and followed by F2 extract with 0.63 mg CE/g FW, while the lowest total flavonoid content for hot aqueous extract can be found in F11 extract with 0.17 mg CE/g FW. In cold aqueous extract, the highest activity can be found in F2 extract with 0.51 mg CE/g FW, followed by F1 and F11 with 0.34 mg CE/g FW. The lowest total flavonoid content for cold aqueous extract was found in F12 extract with 0.18 mg CE/g FW. The order of average total flavonoid activity by variety in decreasing order is as follows: var. *kunstleri* = var. *trengganuensis* (0.42 mg CE/g FW) > var. *angustifolia* (0.23 mg CE/g FW) for hot aqueous extract, and var. *trengganuensis* (0.35 mg CE/g FW) > var. *kunstleri* (0.34 mg CE/g FW) > var. *angustifolia* (0.23 mg CE/g FW) for cold aqueous extract. Another study of hot aqueous extract in *Coprimus comatus* showed that the activity of total flavonoid as well as phenolic was low as compared to ethanolic extract, but no cold aqueous extract comparison was made (Li et al., 2010). Although, *S. cedronella* was reported to have high phenolic compounds, low total flavonoid content was found in its extract with 24.44 µg quercetin/mg FW which suggest that total flavonoids and its derivatives are not the main contributor to the high phenolics activity (Yesilyurt et al., 2008).

Correlation, *r*, between total antioxidant content and phenolic compounds

From Table 2, it can be concluded that all antioxidant and phenolic compounds studied correlate each other positively. The highest correlation can be found in interaction between total flavonoid content and total phenolic acid content for hot aqueous extracts with r = 0.99. Other high correlation can be found in interaction between total antioxidant content (DPPH) with total phenolic acid and flavonoid in hot aqueous extract and interaction between total phenolic acid content and total flavonoid content of cold aqueous extract with r = 0.95. Most of the correlations were higher in total antioxidant content using FRAP method as compared to that of DPPH method in both aqueous extracts studied. Correlation between total antioxidant and total phenolic content in Mexican maize showed r = 0.65, while in this study r = 0.95 and 0.36 for hot and cold aqueous extracts, respectively (Lopez-Martinez et al., 2009). This correlation study suggests that hot aqueous extract was

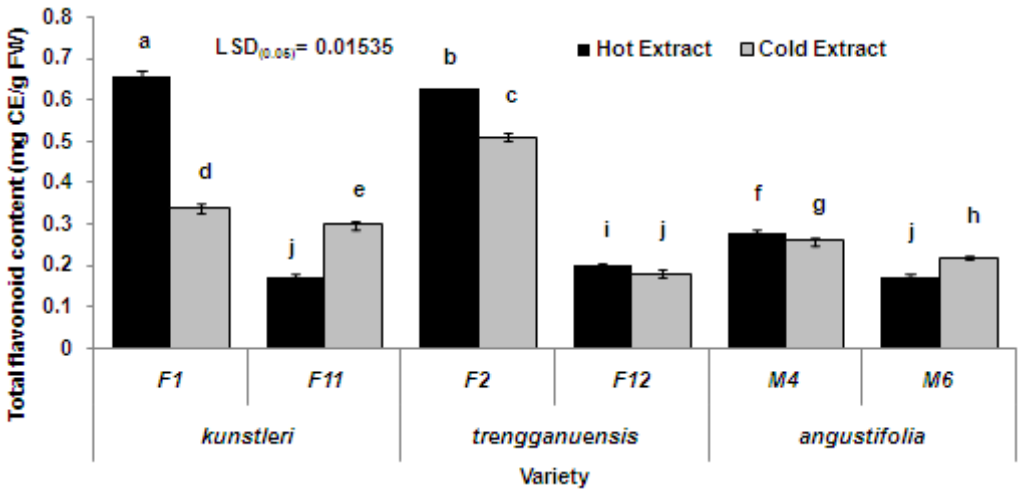

Figure 5. Total flavonoid content of six accessions of *F. deltoidea* in hot and cold aqueous extractions expressed as catechin equivalent. Values are means ± SD (n = 3) followed by different letters to indicate the significant differences ($p < 0.05$) between the values.

Table 2. Correlation between total antioxidant content (DPPH and FRAP) versus phenolic compounds (total polyphenol, total phenolic acid, and total flavonoid content) in hot and cold aqueous extracts of *F. deltoidea*. All of the correlation values are significant at $p < 0.05$.

Extract	FRAP	Polyphenol	Phenolic acid	Flavonoid
Hot aqueous				
DPPH	0.94	0.76	0.95	0.95
FRAP	-	0.89	0.84	0.85
Polyphenol	-	-	0.57	0.62
Phenolic acid	-	-	-	0.99
Cold aqueous				
DPPH	0.81	0.77	0.36	0.53
FRAP	-	0.91	0.58	0.58
Polyphenol	-	-	0.30	0.29
Phenolic acid	-	-	-	0.95

better than that of cold aqueous extract. Positive correlation was found between total antioxidant using DPPH method and total phenolic content in selected herbs of Leguminosae family with $r = 0.975$ (Chew et al., 2009) and it is comparable to our finding with $r = 0.95$.

Conclusion

This research indicates that all the extracts from *F. deltoidea* accessions contained satisfactory content of total antioxidant activities using DPPH and FRAP methods. Similarly, the total polyphenol, phenolic acid, and flavonoid contents were considerably significant. From the data obtained from these two methods, FRAP method gave higher correlation values when compared with that of DPPH method. This finding suggests that FRAP method was better than DPPH method due to better correlation value obtained for both extracts. From a health point of view, it can be concluded that extracts of *F. deltoidea* accessions contained potent antioxidant activities contributed by total polyphenol, phenolic acid, and flavonoid compounds studied in both hot and cold aqueous extracts.

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