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Evaluation of Phytochemicals and Antioxidant Properties of Leaves, Fruits and Stems of *Ficus deltoidea* Plant Extract

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ABSTRACT

The fundamental mechanism underlying a number of human neurodegenerative disorders, diabetes, inflammation, viral infections, autoimmune pathologies and digestive system disorders due to free radicals or reactive oxygen species (ROS). World is blessed with mammoth biodiversity resources, but overwhelmed with several diseases, including those with ROS as the etiological factor. In the present study, the antioxidant activity, total phenolic and flavonoids content of different parts of the plant F. deltoidea, collected from three different areas in Malaysia has been evaluated. Plant extract exhibited strong antioxidant activity. Methanolic extract of F. deltoidea possess more antioxidant principles than aqueous extracts. Among the three sources, F. deltoidea 1 (FD1) possess higher antioxidant activity than the others [FD2 and FD3]. In the present study, we discovered that fruits of F. deltoidea contain more antioxidant principles and free radical scavenging activity and the effects are significantly comparable with standard antioxidants.

Keywords: Antioxidant, Hydrogen Peroxide, DPPH, Flavonoids, Phenolics

INTRODUCTION

Major vascular risk factors, such as hypertension, diabetes, dyslipidemia smoking are coupled with a distinct boost in vascular Reactive Oxygen Species (ROS) production. There are evidences signifying that disease conditions are directly or indirectly related to oxidative damage caused by free radicals [1]. Latest epidemiological studies show that daily consumption of diet rich in antioxidant constituents such as herbal tea, fruits and vegetables significantly reduce the risk of chronic diseases such as diabetes, obesity, cardiovascular diseases, cancer and neurodegenerative diseases [2]. These defensive effects of dietary supplements have been accredited due to the presence of antioxidant compounds. Antioxidants are substances that dawdling down or prevent the oxidative chain reactions by removing free radical intermediates 3. Search for natural antioxidants, especially of plant origin, has notably increased in recent years. Natural sources, such as grains, seed oils, beans, leaf waxes, bark, roots, spices, fruits and vegetables are the richest sources of antioxidant constituents [3].

The use of Ficus species as food or pharmacological agents to progress human health has a history of about ten

thousand years. Some of the species are used in Ayurvedic and Traditional Chinese Medicine [4]. Among these Ficus species, Ficus deltoidea (Mas cotek) belong to the family of Moraceaehas gained more significance in recent days, because this meticulous herb has been widely used as a traditional system of medicine for the management of a variety of illnesses [5]. In Malaysia, *F. deltoidea* plant extract is used as herbal tea, herbal juice and herbal extract for the treatment of different illnesses such as diabetes, high blood pressure, gout, memory impairment. It is also used for treating migraine, diarrhea, skin diseases, pneumonia and cardiovascular complications [6]. It is helpful in improving blood circulation and reducing cholesterol. The herb is often used after childbirth for contracting the vagina after delivery, delaying menopause, reducing the risk of cancer and also has antinociceptive activity [7,8]. However, the antioxidant profiles of most species belonging to Ficus have remained unexamined and lack extensive documentation. Even though, extensive work has been carried out with this plant the antioxidant activity of the methanolic and aqueous extract of various parts of the plant collected from three different areas has not been revealed by any researcher. Thus, the aim of this study is to evaluate the antioxidant activity, total phenolic and flavonoids content of different parts of the plant *F. deltoidea*, collected from three different areas in Malaysia.

MATERIALS AND METHODS

Materials

Leaves, stems and fruits of *F. deltoidea* were collected from forest in Perlis (Sample 1), Kuantan (Sample 2) and Kuala Terrengganu (Sample 3) of Malaysia. The plant material was authenticated by a botanist in Botanical garden Penang, Malaysia. A Voucher specimen has been deposited in AIMST University, Malaysia. The leaves, stems and fruits of *F. deltoidea* were cut into pieces and shade dried. Then plant materials were ground into fine and coarse powder using Waring Commercial blender and sieved using Endecotts Minor sieve mesh no 10 separately. Chemicals such as 1,1-diphenyl-2-picrylhydrazyl (DPPH), Butylated hydroxy toluene (BHT), Ascorbic acid, Gallic acid, Catechin, Folin-Ciocalteu reagent and α-tocopherol were purchased from Sigma, US. All other chemicals used were of analytical grade.

Preparation of plant extract

For methanolic extract, plant materials were packed in a soxhlet extraction apparatus and extracted with ¾ volume of methanol for 72 h at 55°C. The extracts were filtered using muslin cloths and then with whatman filter paper and the solvent were removed at 30°C by using a rotary evaporator and then the samples were lyophilized. The percentage yield was calculated.

Aqueous extracts were prepared by simple maceration technique. The plant materials were placed with 4/5 of the menstruum in a closed vessel for a period of three days and the flask was allowed to shake continuously by Erla ES203D Bench top orbital shaker. The solvent extracts were filtered using muslin cloths and then with whatman filter paper and lyophilized. The percentage yield was calculated.

Preliminary phytochemical analysis

Preliminary phytochemical properties of the extract were tested for alkaloids with Mayers and Dragendroffs reagents, saponins glycosides with the ability to produce suds, cardiac glycosides with FeCl2 and H2SO4, flavonoids with the use of Mg and HCl, anthraquinones with Borntragers reaction, terpenoids with Liebermann-Burchard method and use of H2SO4, tannins with 1% gelatin and 10% NaCl solutions and all other phytochemical analyses weredone according to the standard procedure [9].

Total phenolic content

The total phenolic content was determined by Folin-Ciocalteau (FC) calorimetry method [10] with some modifications. 0.5 mL of extract (1 mg/ml) was added to 0.5 mL of Folin-Ciocalteu reagent and mixed. After 4 minutes, 1 ml of sodium carbonate solution (7%w/v) and 6 mL of distilled water was added. After 90 minutes of incubation at room temperature, the absorbance of the reaction mixture was measured at 765 nm using Tecan Infinite M1000 PRO multimode microplate reader. Gallic acid was used as reference standard and the results were expressed as milligram gallic acid equivalent (mg GAE)/g of extract. Distilled water was used as blank (without sample and reagents). The phenolic content in the plant extracts was calculated using the formula: C=A/B, where C is expressed as GAE/dry weight of the extract; A is the equivalent concentration of gallic acid established from calibration curve (mg); and B is the dry weight of the extract (g).

Total flavonoid content

The total flavonoid content was measured by aluminiumchloride colorimetric assay [11]. 1 ml of extract (1 mg/ml) was added to test tube containing 4 ml of distilled water, and then 0.3 ml of 5% w/v sodium nitrite was added. After 5 minutes, 0.3 ml of 10% w/v aluminium chloride was added. After 6 minutes of incubation, 2 ml of 1 M sodium hydroxide was added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm using Tecan Infinite

M1000 PRO multimode microplate reader. The amount of 10% w/v aluminium chloride was substituted by the same amount of distilled water in blank. Samples were analyzed in triplicate. Catechin was used as standard. Total flavonoid content of extract was expressed in mg of catechin/g of extract and calculated according to the equation:C=c x V/m, Where c is the equivalent concentration of catechin established from calibration curve (mg), V is the volume of extract (1 ml) and m is the mass of extract (1 mg).

DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging assay

The free radical scavenging activity of the extract was determined by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) as a reagent [12]. Different concentrations (0.01 to 0.06 mg) of extracts from *F. deltoidea* in 1 ml of solution were added to 3 ml of 0.004%w/v methanol solution of DPPH. The samples were incubated for 30 min in the dark at room temperature. Scavenging capacity was analyzed by spectrophotometer at 515 nm against blank (methanol). Ascorbic acid was used as a positive control. The negative control contained 3 mL of 0.004%w/v methanol solution of DPPH and 1 ml of methanol without sample extract or standard. Each sample was measured in triplicate and expressed in mean average. The free radical scavenging activity was calculated according to the following equation:DPPH scavenging activity (%)=[(Acontrol – Asample)/Acontrol] x 100, Where Acontrol is absorbance reading of the negative control (DPPH) and Asample is absorbance of the sample or standard.

Half maximal inhibitory concentration (IC50) is the amount of sample extracted in 1 ml solution necessary to decrease by 50% the initial DPPH concentration and was derived from percentage of radical scavenging activity (% inhibition) versus sample concentration plot. The results are also expressed as ascorbic acid equivalent antioxidant capacity (AEAC) in mg ascorbic acid/100 g [13] as: AEAC (mg AA/100 g)=IC50 (ascorbic acid)/IC50 (sample) × 105

Hydrogen peroxide scavenging activity

The hydrogen peroxide (H2O2) scavenging activity of *F. Deltoidea* extracts were determined by UV-Spectrophotometermethod [14]. 1 ml of extract/standard of different concentration (0.05, 0.1, 0.15, 0.2, 0.25, 0.3 mg/ml), 3.4 ml of 0.1 M phosphate buffer (pH 7.4) and 0.6 ml of 43 mM H2O2 were mixed. After incubation at room temperature for 10 minutes, absorbance was measured at 230 nm. Blank used was phosphate buffer and BHT was used as standard. The percentage scavenging activity was calculated by using the following equation: Percentage scavenging activity (%)=[(Acontrol – Asample)/Acontrol] x 100, where, Acontrol is the absorbance of the control (without extract) and Asample is the absorbance in the presence of the extract or standard.

Statistical analysis

The data are expressed as the mean ± SD. All statistical analyses were performed using Graph pad prism, version 5.0

RESULTS AND DISCUSSION

Extraction and yield

Among the two different extracts, methanolic extract produced high yield than the aqueous extract. The percentage yield of stem is low when compared with the leaves and fruits in all the three plants collected from different areas shown in Table 1. A high percentage yield (22 %) was obtained for *F. deltoidea* 3(FD3), and this variety of *F. deltoidea* can be used as a major source for the preparation of extracts.

Sample	M	A
	Percentage Yield (%)	
FD1L	12	3.8
FD1S	8	7.84
FD1F	16	15.2
FD2L	17	7.8
FD2S	8	6.3
FD3L	18	16.3
FD3S	12	13.04
FD3F	22	17.6

Table 1: Percentage yield of different parts of the plant collected from different areas. FD1: Plant collected from Perlis, FD2: Plant collected from Kuantan and FD3: Plant collected from Kuala Terrengganu and L: Leaves, S: Stem and F: Fruits. M: Methanolic extract and A: Alcoholic extract.

Phytochemical studies

Preliminary phytochemical screening on *F. deltoidea* revealed the presence of phenols, flavonoids, tannins, saponins is shown in Table 2. Chemical constituents were present in all the plant parts such as leaves, stems and fruits. Even though the plants are collected from different areas but all the plants contains same phytochemicals.

		FD1						FD2				FD3					
		L		S		F		L		S		L		S		F	
S. N	**Chemical test**	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A
1	Alkaloids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	Glycosides	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	Carbohydrates	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	Phenols	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	Reducing Sugar	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	Tannins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	Saponins	+	-	+	+	-	-	-	+	+	+	-	+	+	-	+	+
9	Volatile oil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	Anthraquinones	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	Steroids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 2: Results of Phytochemical Analyses. FD1: Plant collected from Perlis, FD2: Plant collected from Kuantan and FD3: Plant collected from Kuala Terrengganu and L: Leaves, S: Stem and F: Fruits. M: Methanolic extract and A: Alcoholic extract.

Total phenolic content

Table 3 shows that the aqueous extract samples contain phenolic content ranging from 83.30-204.21 mg GAE/g and methanol extract samples contain phenolic content ranging from 101.23-533.77 mg GAE/g. The results of aqueous and methanolic extract showed that the leaves and fruits have higher total phenolic content compared to the stem. Phenolic content is highest in fruits: FD1F (533.7 mg GAE/g); FD3F (331.5 mg GAE/g) when compared to leaves and stem. Phenolic content is lowest in stem; FD1S (111.9 mg GAE/g); FD2S (101.2 mg GAE/g); FD3S (148.3 mg GAE/g). Both leaves and fruits are the suitable plant parts to be used for extract preparation and activity assay because it contains high phenolic content. Besides all,methanolic extract of samples showed higher phenolic content compared to the aqueous extract. This may indicate that phenols can dissolve better in methanol and it is better to do the extraction of plants using methanol as extracting solvent if phenols are required as the active phytochemical.

	(mg GAE/g)	(mg GAE/g)
Samples	**A**	**M**
FD1L	96.89 ± 2	238.52 ± 1
FD1S	84.41 ± 1	111.92 ± 1
FD1F	405.72 ± 3	533.77 ± 3
FD2L	122.96 ± 1	263.36 ± 2
FD2S	83.30 ± 1	101.23 ± 4
FD3L	171.29 ± 2	292.09 ± 3
FD3S	128.77 ± 1	148.39 ± 2
FD3F	204.21 ± 3	331.59 ± 5

Table 3: Total Phenolic Content. FD1: Plant collected from Perlis, FD2: Plant collected from Kuantan and FD3: Plant collected from Kuala Terrengganu and L: Leaves, S: Stem and F: Fruits. M: Methanolic extract and A: Alcoholic extract.

Total flavonoid content

The total flavonoid content values are summarized in Table 4. The results were obtained from aluminium chloride colorimetric assay with correction for the presence of catechin. All the methanolic extract of *F. deltoidea* showed higher value of catechin equivalent than that of the aqueous extract. This indicated that methanolic extracts contain

higher amount of flavonoid compared to the aqueous extracts. Among the eight extracts studied, the fruit extracts of *F. deltoidea* have the highest total flavonoid content values; FD1F (419.7 mg of catechin/g) and FD3F (208.3 mg of catechin/g), which is higher than that of leaves and stem extracts.

TOTAL FLAVONOID CONTENT		
Samples	(mg of catechin/g)	(mg of catechin/g)
	A	M
FD1L	63.71±1	78.58±2
FD1S	42.41±1	52.49± 1
FD1F	410.86±2	419.73±3
FD2L	101.48±2	108.36±3
FD2S	37.66±1	58.88±1
FD3L	166.14±1	176.38±4
FD3S	67.51±1	74.66±0.2
FD3F	105.70±4	208.36±2

Table 4: Total Flavonoid Content. FD1: Plant collected from Perlis, FD2: Plant collected from Kuantan and FD3L: Plant collected from Kuala Terrengganu and L: Leaves, S: Stem and F: Fruits. M: Methanolic extract and A: Alcoholic extract.

Antioxidant assay

DPPH radical scavenging activity: In DPPH assay, methanolic extract of the plant wasestimated, as the methanolic extract showed higher value in total phenolic and flavonoid content test. The DPPH scavenging effects of *F. deltoidea* are shown in Figure 1. The DPPH radical scavenging activity of ascorbic acid and methanolic extracts of *F. deltoidea* increases in a dose-dependent manner. Ascorbic acid exhibited higher DPPH scavenging activity (91.83%) than all the plant extracts when tested at concentration of 0.01 to 0.06 mg/ml. This represent the antioxidant activity of the plant extracts is weaker than that of ascorbic acid. At the highest concentration of 0.06 mg/ml, the fruit extracts showed high percentage of DPPH radical scavenging activity (88.06% for FD1F, 77.38% for FD3F) followed by leaves extracts (69.79% for FD3L, 67.65% for FD2L and 65.92% for FD1L) and stem extracts (58.79% for FD3S and 57.06% for FD2S 54.77%). The IC_{50} for DPPH radical scavenging activity was reported in Table 5**.** In DPPH assay, the lower the IC_{50} the better is the ability to scavenge the radicals, particularly peroxy radicals which are the propagators of the autoxidation of lipid molecules and break the free radical chain reaction [15]. It was observed that the fruits of *F. deltoidea* have low IC_{50} [FD1F (0.015 mg/ml) and FD3F (0.02 mg/ml)] and are very potent radical scavengers, but stems of *F. deltoidea* [FD1S (0.037 mg/ml), FD2S (0.028 mg/ml), FD3S (0.035 mg/ml)] possess low IC_{50} values and exhibited low scavenging property. The results were also expressed as ascorbic acid equivalent antioxidant capacity (AEAC) in mg ascorbic acid/100 g in Table 6. Fruit extracts showed higher AEAC than the leaves and stem extracts.

Samples	**IC_{50} (mg/ml)**
Ascorbic Acid	0.0046
FD1L	0.0296
FD1S	0.0378
FD1F	0.0153
FD2L	0.0287
FD2S	0.0387
FD3L	0.0216
FD3S	0.0356
FD3F	0.0206

Table 5: Half Maximal Inhibitory Concentration (IC_{50}). FD1: Plant collected from Perlis, FD2: Plant collected from Kuantan and FD3: Plant collected from Kuala Terrengganu and L: Leaves, S: Stem and F: Fruits.

Samples	AEAC (mg AA/100g sample)
FD1L	15684.0 ± 17
FD1S	12307.2 ± 13
FD1F	30475.4 ± 34
FD2L	16179.2 ± 57
FD2S	12028.4 ± 190
FD3L	21512.6 ± 29
FD3S	13047.2 ± 102
FD3F	22574.8 ± 132

Table 6: Ascorbic Acid Equivalent Antioxidant Capacity (mg AA/100 g sample). FD1: Plant collected from Perlis, FD2: Plant collected from Kuantan and FD3: Plant collected from Kuala Terrengganu and L: Leaves, S: Stem and F: Fruits.

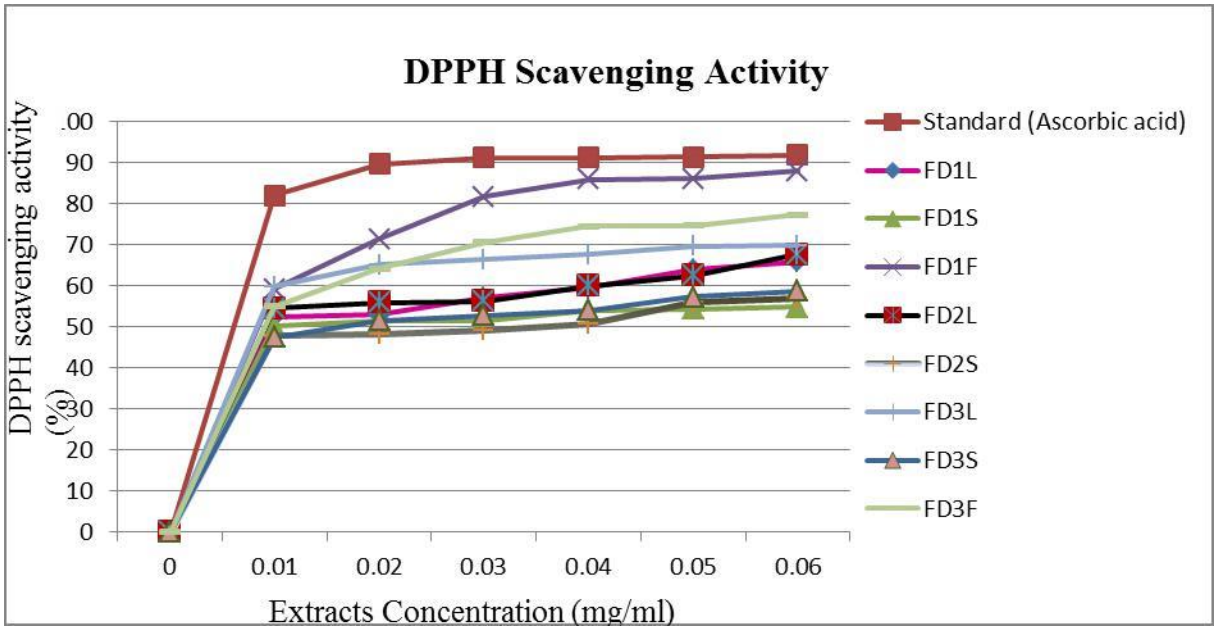

Figure 1: DPPH Scavenging Activity Assay. FD1: Plant collected from Perlis, FD2: Plant collected from Kuantan and FD3: Plant collected from Kuala Terrengganu and L: Leaves, S: Stem and F: Fruits.

Hydrogen peroxide scavenging activity

The ability of the extracts to effectively scavenge hydrogen peroxide is displayed in Figure 2 and it was compared with that of BHA, Ascorbic acid and α-tocopherol were used as standards at lowest concentration of 0.05 mg/ml. The extracts were capable of scavenging hydrogen peroxide in a concentration dependent manner. For standards, highest percentage of scavenging activity was showed by α-tocopherol (75.57%), followed by BHT (70.22%) and Ascorbic acid (51.84%) [α-tocopherol> BHT> Ascorbic acid]. For extracts, FD1F shows the highest scavenging activity (71.89%) and it scavenges better than BHT and Ascorbic acid. All the stem extracts showed lower scavenging activity [FD1S (11.61%), FD2S (8.23%), FD3S (12.37%)] compared to fruit and leaves extract. From this observation, the fruit extract of *F. deltoidea* showing significant antioxidant activity when compared to leaves and stem extract. A concentration dependent increase in scavenging activity was observed for all the extracts. At higher concentration the percentage scavenging activity was comparable with the standard (Table 7).

Percentage activity	Samples	Concentration µg/ml					
		0.05	0.10	0.15	0.20	0.25	0.30
	α-tocopherol	75.57	77.44	81.43	83.96	86.66	89.73
	Ascorbic Acid	51.84	59.08	65.87	72.73	79.78	86.44
	BHT	70.22	73.17	77.23	80.19	84.90	86.50
	FD1L	22.96	33.90	45.06	55.12	67.18	78.92
	FD1S	11.61	20.47	37.65	46.91	61.27	74.69
	FD1F	71.89	74.89	78.65	80.12	85.04	86.12
	FD2L	28.14	39.68	55.19	64.60	78.29	92.55
	FD2S	8.23	19.66	33.37	49.07	68.93	83.02
	FD3L	25.68	36.92	48.15	57.99	67.61	79.93
	FD3S	12.37	23.21	39.71	58.49	76.49	88.89
	FD3F	59.75	64.43	70.71	74.05	79.52	84.32

Table 7: Hydrogen Peroxide Free Radical Scavenging Activity of plant extract. FD1: Plant collected from Perlis, FD2: Plant collected from Kuantan and FD3: Plant collected from Kuala Terrengganu and L: Leaves, S: Stem and F-Fruits.

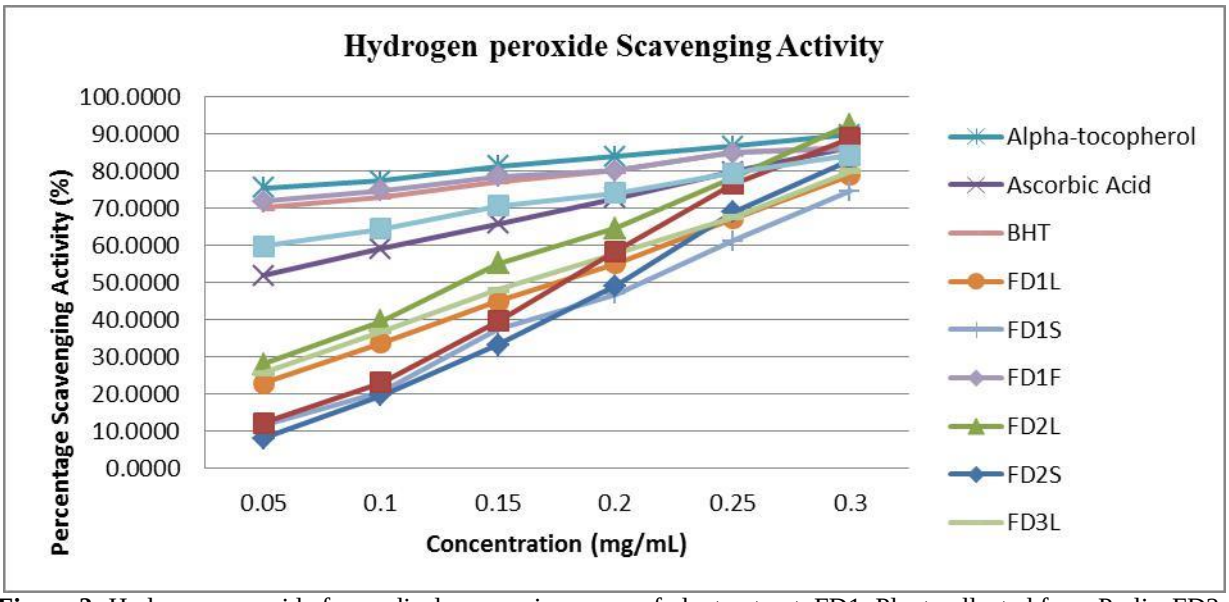

Figure 2: Hydrogen peroxide free radical scavenging assay of plant extract. FD1: Plant collected from Perlis, FD2: Plant collected from Kuantan and FD3: Plant collected from Kuala Terrengganu and L: Leaves, S: Stem and F: Fruits.

DISCUSSION

Many studies reported that medicinal plant with antioxidant compounds play an important role in stress management, anti-aging, aphrodisiac effect and prevention of various chronic illnesses such as diabetes, hypertension, cancer, inflammation and neurodegenerative disorders [4-6]. Ficusdeltoidea is a widely used medicinal herb in Malaysia as herbal tea; herbal juice and herbal extracts for management of various illnesses. These herbal preparations are believed to have anti-aging, memory enhancer, anti-stress, and aphrodisiac effect [4]. In the present study, the preliminary phytochemical screening revealed the presence of antioxidant principles such as flavonoids and phenols in all the three plants of *F. deltoidea* collected from different areas. The total flavonoids and phenolic content was higher in fruits than in leaves and stem. Further, the presence of antioxidant principles was confirmed by in-vitro antioxidant assay. In DPPH and H2O2 scavenging assay, *F. deltoidea* showed good antioxidant activity. Methanolic extract of *F. deltoidea* possess more antioxidant principles than aqueous extracts. Among the three sources, *F. deltoidea* 1 (FD1) possess higher antioxidant activity than the others [FD2 and FD3]. From the observation, we confirmed the antioxidant activity of *F. deltoidea*. Even though FD 3 has given higher

yield than FD1 and 2, but FD1 can be the best resource for the pharmacological activity and formulation of herbal preparation as it contains high antioxidant principles. The results of this study will give the scientific evidence for the usage of this plant as an herbal medicine.

CONCLUSION

In conclusion, *F. deltoidea* possess good antioxidant activity. The presence of antioxidant principles could be the basis for anti-aging, memory enhancing, anti-stress and aphrodisiac effect. *F. deltoidea* 1 can be the best source for the formulation of herbal preparation since it contains high quantity of antioxidant principles and significantly high antioxidant activity than other varieties. In Malaysia, *F. deltoidea* leaves are the most commonly used plant part for herbal preparation. In the present study, we discovered that the fruits of *F. deltoidea* contain more antioxidant principles and free radical scavenging activity and those effects are significantly comparable with standard antioxidants. The future scope includes the isolation of antioxidant principles with special emphasis on in-vitro and in-vivoscreening for various pharmacological activities.

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