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Full Length Research Paper

Correlation between enzymes inhibitory effects and antioxidant activities of standardized fractions of methanolic extract obtained from *Ficus deltoidea* leaves

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Recently, there has been increasing interest in *Ficus deltoidea* (Moracea) due to its chemical composition and the potential health benefits. The leaves of the plant have been suggested to have potential antidiabetic effects. Inhibition of carbohydrate-hydrolysing enzymes, such as α-glucosidase and α-amylase is one of the therapeutic approaches to control postprandial hyperglycemia. In this study, enzymes inhibitory effect and antioxidant properties of different fractions of methanolic extract obtained from *F. deltoidea* leaves was evaluated. Further, the possible relationship between pharmacological properties and phytochemical content of fractions was investigated. The n-butanol fraction showed significant α-glucosidase and α-amylase inhibitory effects (IC_{50} values 15.1 and 39.42 µg/ml, respectively) along with the remarkable antioxidant activity when compared to the other fractions. High performance liquid chromatography (HPLC) chemical profiling of the n-butanol fraction revealed that the contents of isovitexin (24.63 mg/g) and vitexin (8.3 mg/g) were found to be significantly higher than the other fractions. These results indicate that *F. deltoidea* could be the potential source of promising anti-diabetic drug.

Key words: *Ficus deltoidea,* enzymes, phytochemical, high performance liquid chromatography (HPLC), isovitexin, vitexin.

INTRODUCTION

Diabetes mellitus is a complex metabolic disorder that disturbs the metabolism of carbohydrates, fats and proteins, which is characterized by the elevated plasma glucose levels (Eliza et al., 2009). Postprandial hyperglycemia is the main risk factor for the development of diabetes mellitus type II (Baron, 1998) and is associated with microvascular complications in diabetic individuals (Irene et al., 2000; Lebovitz, 2001). Therefore, control of postprandial blood glucose level plays key role in treatment and decrease progression of diabetes mellitus (Kim et al., 2000). One of the latest therapeutic approaches to reduce hyperglycemia with diabetes mellitus is suppression of carbohydrate absorption after food uptake.

Alpha-glucosidase is a membrane-bound enzyme located at the epithelium of small intestine, where it hydrolyzes the final step in digestion of carbohydrates, whereas, α-amylase is a salivary enzyme that catalyses the breakdown of starch into sugars (Ali et al., 2009).

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Hence inhibition of these enzymes can significantly decrease the postprandial increase of blood glucose and therefore can be an important strategy in the management of various carbohydrates metabolic disorders including type 2 diabetes mellitus (Floris et al., 2005). Several α-glucosidase and α-amylase inhibitors such as acarbose, trestatin, amylostatin and valiolamine isolated from microorganisms (Remi and Jean, 2004) have been used to control the diabetic condition. However, the use of these drugs as the inhibitors is associated with side effects including liver disorder, abdominal distention, flatulence, meteorism and diarrhea. In such circumstance, medicinal plants were suggested as alternative medicine for prevention and treatment of diabetes because of their negligible side effects (Cheng and Fantus, 2005).

Ficus deltoidea (Moraceae) is an evergreen shrub that widely occurs in several Southeast countries commonly Malaysia and Indonesia (Adam et al., 2009). Traditionally, it is widely used to treat different diseases including diabetes mellitus, high cholesterol, high blood pressure, gout, improve blood circulation, pneumonia, diarrhea and skin infections (Hakiman and Maziah, 2009). Also, *F. deltoidea* is used as aphrodisiac; the fruit is chewed to relieve headache, toothache, and to treat cold (Sulaiman et al., 2008; Adam et al., 2007). Several studies showed that the aqueous extract of whole plant possesses anti-ulcerogenic properties (Adam et al., 2010; Sulaiman et al., 2008). In addition, wound healing activity of aqueous extract of leaves of *F. deltoidea* was also documented (Mahmood et al., 2010). Its blood glucose lowering and insulin stimulating effects were reported successively (Adam et al., 2007; Aminudin et al., 2007). Recently, toxicological study on *F. deltoidea* reported that the plant contains no toxic elements (Armaghan et al., 2011).

Though *F. deltoidea* has been used widely in Malaysian folk medicine, its pharmacological properties and active principles are still not well understood. This investigation was therefore undertaken to study the active composition of fractions prepared by bioassay guided method using α-glucosidase and α-amylase inhibitory assays, and also to find out the relationship between the phytochemical contents and the pharmacological properties of fraction of methanolic extract of *F. deltoidea* leaves.

MATERIALS AND METHODS

Chemicals and reagents

HPLC grade methanol was purchased from Merck, Germany. Folin-ciocalteau reagent, 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical, catechin, gallic acid, butylated hydroxyl anisole (BHA), butylated hydroxytolune (BHT), quercetine (QTN), 2-thiobarbituric acid (TBA), trichloroacetic acid (TCA), xylenol orange, ammonium molybdate, sodium phosphate, potassium ferricyanide, ferric chloride, potassium chloride (KCl), iron(III) chloride, manganese(II) chloride, ammonium iron(II) sulfate, sulfuric acid, hydrochloric acid, hydrogen peroxide solution, porcine pancreatic α-amylase, rat-intestinal acetone powder, *p*-nitrophenyl-a-D-glucopyranoside, starch and dinitrosalicylic (DNS) acid were purchased from Sigma Aldrich, USA. All other chemicals used were either HPLC or analytical grade.

Plant material and preparation of extract and fractions

F. deltoidea (FD) leaves were purchased from HERBagus *Sdn. Bhd.* Penang, Malaysia. The specimen (voucher number: 11204) was deposited at the herbarium of School of Biology, Universiti Sains Malaysia. The extract was prepared by maceration of oven-dried (at 37°C) leaves powder successively in petroleum ether, chloroform, methanol and distilled water at 37°C water bath. The supernatant was filtered and the solvent evaporated under reduced pressure and then lyophilized. Methanolic extract was partitioned in distilled water and n-hexane to obtain the n-heaxne fraction (HF), the aqueous part was further fractioned with dichloromethane (DF) and n-butanol (BF), to obtain respective fractions and aqueous fraction (AF).

High performance liquid chromatography (HPLC) analysis

The HPLC analysis of different fractions with reference to isovitexin and vitexin were performed by the methodology of Fu et al. (2007) on HPLC system equipped with quaternary pump, online degasser, auto sampler, automatic injector, column heater degasser photodiode array detector (Agilent Tech, Palo Alto, CA) and chromatographic separation were performed using Eclipse C18 reverse-phase column (250 mm × 4.6 mm) with flow rate of 1 ml/min at 30°C and sample size of 10 µl. The isocratic mobile phase constituted of methanol/water/formic acid (33:66.37:0.67, v/v/v). The sample was monitored with UV detection at 330 nm at 30°C. The chromatographic peaks of the analytes were confirmed by comparing their retention time and UV spectra with corresponding reference standards. The results were obtained by comparison of peak areas (at 330 nm) of fractions with those of reference standards.

Determination of total soluble phenolics content

Following the method of Slinkard and Singleton (1977), total soluble phenolic component of *Ficus deltoidea* was determined. 2.5 ml of 0.2 N Folin-Ciocalteu reagent was mixed with 0.5 ml of sample solution (1 mg/ml) for 8 min and 2 ml of 75 g/L Na_2CO_3 was added to the mixture. The absorbance was read at 760 nm against blank after 2 h using Fisher scientific multiskan microplate reader (Oxford, MI, USA). Quantitative measurements were performed based on a standard calibration curve of 3.1 to 100 µg/ml of gallic acid in methanol. The total phenolic content was expressed as gallic acid equivalents in mg/g of dry material.

Determination of flavonoids content

Flavonoids content of samples were determined by $AlCl_3$ colorimetric method (Jamshid et al., 2009). 100 µl of each FD fractions (1 mg/ml) and concentrations (3.1 to 100 µg/ml) of QTN (standard reference) were mixed separately with 0.1 ml of 10% (w/v) $AlCl_3$ solution, 0.1 ml of 1 M potassium acetate solution, 1.5 ml of methanol and 2.8 ml of distilled deionized water. The mixture was incubated for 30 min at room temperature, and then the absorbance was read at 415 nm. For blank, 0.1ml of 10% (w/v) $AlCl_3$ solution was replaced with distilled water. Using the standard curve, the total flavonoids content in samples were calculated as milligrams of quercetin equivalents.

DPPH radicals scavenging activity

Electron scavenging of samples was measured following the method described by Yang et al. (2006). 200 µl of DPPH solution (0.004% w/v) was added to100 ml of different concentrations (3.1 to 100 µg/ml) of methanolic solution of fractions. The mixture was allowed for 30 min in the dark at room temperature and reduction of DPPH was measured at 517 nm. The percentage of scavenging activity was evaluated by comparing with the control (100 µl methanol + 200 µl DPPH). QNT and BHT were used as reference standards. The radical scavenging activity was calculated using the following formula:

$$\text{Inhibition of DPPH scavenging activity (\%)} = \frac{\text{Absorbance of blank - Absorbance of Sample}}{\text{Absorbance of Blank}} \times 100$$

Determination of total antioxidant capacity

Phosphomolybdenum method (Saliha et al., 2010) was used to evaluate the total antioxidant capacity of different concentrations of fractions (25, 50 and 100 µg/ml). The assay was based on the reduction of Mo (VI) to Mo (V) and formation of green phosphate/Mo (V) complex at acidic pH. 300 µl of sample solution was mixed with 3 ml of reagent containing 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The mixture was allowed for 90 min incubation at 95°C in boiling water bath and the absorbance was read at 695 nm after cooling in room temperature. 300 µl of methanol was used as blank and the antioxidant capacity was expressed as the number of equivalents of QTN.

Reducing power assay (iron reducing activity)

Following the method described by Oyaizu (1986), reducing power of fractions was determined by mixing 1 ml methanolic solution of different concentrations of samples (3.1 to 100 µg/ml) with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide and the mixture was incubated for 20 min at 50°C. Subsequently, 2.5 ml of 10% (w/v) trichloroacetic acid was added and centrifuged at 3000 rmp for 10 min, and 2.5 ml of the supernatant was allowed to react for 10 min with 2.5 ml distilled water and 0.5 ml of 0.1% (w/v) ferric chloride. The absorbances of the solutions were measured at 700 nm; increase in absorbance indicated the increase in reducing power capacity of samples. QNT and BHT were used as reference standards.

***In vitro* lipid per-oxidation**

Preparation of tissue homogenate

Brain and liver homogenates were prepared from 3 months old male Sprague-Dawley rats (200 to 250 g). 40% (w/v) homogenate for the ferrous ion oxidation with xylenol orange (FOX method) was prepared in HPLC-grade methanol. For the thiobarbituric acid, non-enzymatic lipid peroxidation method, 3.3% (w/v) homogenate was prepared in 50 mM phosphate buffer (pH 7.4). All solutions were centrifuged at 5000 rpm for 15 min and the supernatants were used for the experiment.

***Ferrous xylenol* orange (FOX) method**

Lipid peroxidation was carried out at 37°C. The reaction mixture contained 10 µl of sample solution (3.1 to 100 µg/ml), 10 µl of Fenton's reagent (5 µl of 50 mM hydrogen peroxide and 5 µl of 5 mM manganese chloride), and 80 µl of each homogenate separately. 900 µl of FOX reagent (49 mg of ferrous ammonium sulfate in 50 ml of 250 mM H_2SO_4, 0.397 g of BHT, and 0.038 g of xylenol orange in 950 ml of HPLC grade methanol) was added to each sample, and left to react for 30 min at room temperature. The absorbance was read at 560 nm against blank. QTN and BHT were used as standards. The blank solution was prepared in the same manner with methanol in place of the test sample (Jiang et al., 1992). The percentage of inhibition was calculated as:

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of blank- Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

Thiobarbituric acid non- enzymatic lipid peroxidation assay

Thiobarbituric acid reactive substances were determined spectrophotometrically at 535 nm using the method of Igene et al. (1985) with minor modifications. Briefly, 0.5 ml of tissue homogenate was added to 1 ml of various concentrations of the samples solution (3.1 to 100 µg/ml). Peroxidation was initiated by adding 100 µl of 0.2 mM $FeCl_3$ after incubation for 30 min at 37°C. The reaction was terminated by the addition of 2 ml TBA-TCA-HCl reagent (15% (w/v) trichloroacetic acid, 0.375% (w/v) thiobarbituric acid and 0.25N hydrochloric acid) and further heating at 90°C for 15 min in a boiling water bath. The absorbance of supernatant was measured at 535 nm after centrifugation at 3000 rpm for 10 min. 1 ml of HPLC-grade methanol in place of test sample was used as the blank. QTN and TBA were used as reference standards. The result was expressed as percentage of inhibition.

Assay for α-glucosidase inhibitory activity

In vitro α-glucosidase inhibition assay was performed according to the method described by Kwon et al. (2006) with slight modifications. Crude α-glucosidase solution was prepated using100 mg of rat-intestinal acetone powder. The powder was suspended in 1 ml of 0.9% saline, and the suspension was sonicated for 30 s. The supernatant containing the crude α-glucosidase solution was separated after centrifugation (5000 g, 30 min, 4°C). 100 µl of rat-intestinal α-glucosidase solution was pre-incubated with 50 µl of sample solution (3.1 to 100 µg/ml) at 37°C for 10 min. After pre-incubation, 50 µl of 5 mM *p*-nitrophenyl-a-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added. The reaction mixture was incubated at 37°C for 15 min. The reaction was stopped by addition of 100 µl of Tris –HCl buffer (pH 7) and the absorbance readings were recorded at 405 nm. The α-glucosidase inhibitory activity of fractions was expressed as percentage of inhibition.

Assay for porcine pancreatic α-amylase inhibitory activity

Porcine pancreatic α-amylase inhibition assay was conducted as

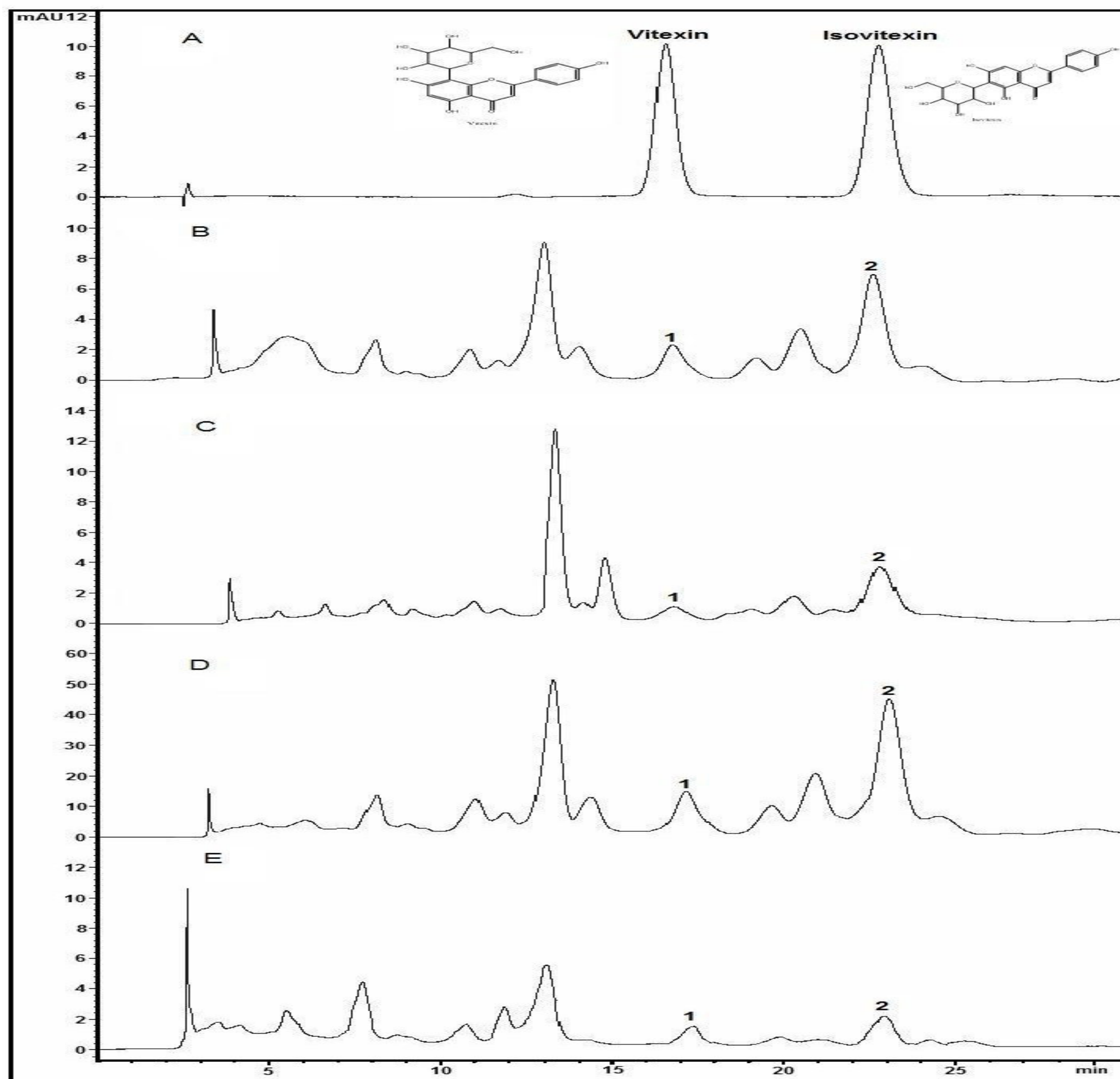

Figure 1. HPLC chromatogram of the four fractions of metanolic extract of *F. deltoidea* leaves with detector response at 330 nm. A, Standard; B, n-hexane;C, dichloromethane; D, n-butanol; E, aqueous fraction.

part of the protocol described by Kwon et al. (2006). 500 µl of sample solution (3.1 to 100 µg/ml) and 500 µl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) containing α-amylase solution (0.5 mg/ml) were incubated at 37°C for 10 min. After pre-incubation, 500 µl of 1% starch solution in 0.02 M sodium phosphate buffer was added. The reaction mixture was then incubated at 37°C for 15 min and the reaction was stopped with 1.0 ml of DNS acid; color reagent. The reaction mixture was then incubated in a boiling water bath for 5 min, cooled to room temperature, then diluted with 10 ml distilled water and absorbance was measured at 540 nm.

Statistical analysis

All assays were performed in three independent experiments and expressed as mean ± standard deviation (SD).

RESULTS

HPLC analysis of fractions of methanolic extract of *F. deltoidea* leaves

HPLC was used to identify and quantify isovitexin and vitexin in different fractions of methanolic extract of *F. deltoidea* leaves. Individual constituents were identified by comparing their peaks, UV spectra and retention times, with corresponding reference standards (Figure 1). Concentrations in the samples were estimated based on

Table 1. Phytochemical contents of fractions obtained from *F. deltoidea* leaves extract.

Fraction[a]	Total phenolics (mg/g)	Total flavonoids (mg/g)	Isovitexin (mg/g)	Vitexin (mg/g)
HF	211.05 ± 0.004	0.44 ± 0.02	3.81 ± 0.05	4.05 ± 0.08
DF	273.82 ± 0.002	1.29 ± 0.05	2.46 ± 0.03	3.33 ± 0.04
BF	416.155 ± 0.008	1.6 ± 0.03	24.63 ± 0.04	8.3 ± 0.03
AF	72.36 ± 0.002	0.16 ± 0.01	1.91 ± 0.06	4.27 ± 0.04

[a]HF, n-Hexane fraction; DF, dichloromethane fraction; BF, n-butanol fraction; AF, aqueous fractions. The results are expressed as mean ± S.D.

Table 2. IC_{50} values (µg/ml) of fractions of methanolic extract of *F. deltoidea* leaves obtained from different antioxidant assays.

Test sample[a]		HF	DF	BF	AF	QTN	BHT	Acarbose
Assays								
DPPH		51.07	35.43	9.74	62.3	3.2	14.11	ND[b]
Reducing power		116.37	71.24	38.55	109.09	8.6	ND[b]	ND[b]
Total antioxidant capacity		152.5	120.2	58.94	245.5	32.62	ND[b]	ND[b]
Hydroxyperoxide FOX method	Liver	88.45	56.38	25.47	120.51	11.18	27.68	ND[b]
	Brain	73.99	53.58	24.56	91.38	7.02	25.24	ND[b]
Hydroxyperoxide TBARS method	Liver	105.3	81.5	35.3	130.8	11.9	35.5	ND[b]
	Brain	87.01	51.03	18.9	117.9	8.56	28.54	ND[b]
α- glucosidase inhibitors		42.61	37.12	15.1	74.1	ND[b]	ND[b]	5.27
α- amylase inhibitors		94.50	68.9	39.42	150.15	ND [b]	ND[b]	18.39

[a]HF, n-Hexane fraction; DF, dichloromethane fraction; BF, n-butanol fraction and AF, aqueous fraction; QTN, Quercetine; BHT, butylated hydroxytoluene; [b]ND, not determined.

the calibration curves of isovitexin and vitexin in the range of 5 to 200 µg/ml, and the quantitative percentage of dry weight of bioactive markers (isovitexin and vitexin) was calculated from the following formulas: Y=23.90X – 65.442 (R^2 =0.9992, n=6), Y=28.305X-28.245 (R^2 =9982, n=6), respectively, where Y is the peak area of the analyte and X was the concentration of the analyte (µg/ml) (Table 1).

According to HPLC chromatogram, it could be noticed that the n-butanol fractions contained highest amount of isovitexin and vitexin than compared to the other fractions. The chromatographic profile indicated that BF contained other chemical constituents beside isovitexin and vitexin.

Phytochemical screening

The results of the phenolics and flavonoids content of the fractions of methanol extract from *F. deltoidea* leaves are shown in Table 1. The results reveal that the phenolics content of the n-butanol (416.15 mg/g) was statistically higher than that of the other fractions. Also, the flavonoids content of the fractions depicted in Table 1 indicate that n-butanol fraction (1.6 ± 0.03 mg/g) had higher flavonoid content than other tested samples. The order of total flavonoids and phenolics content in the samples was given as; n-butanol> dichloromethane> n-hexane > aqueous fractions.

Antioxidant activities fractions of metanolic extract of *F. deltoidea* leaves

The antioxidant efficacy of fractions of metanolic extract of *F. deltoidea* leaves were evaluated by *in vitro* lipid peroxidation, DPPH radical scavenging, reducing power and total antioxidant capacity assays. The degree of lipid peroxidation in tissue homogenates was assessed by two different methods. In Ferrous xylenol orange (FOX) method, Fenton's reagent (H_2O_2 $^+$ Mn) was used as peroxidation inducer, and the resulting ferrous xylenol orange, under acidic conditions that reveals the presence of lipid hydroperoxide was measured spectrophoto-metrically at 560 nm. In the other method; lipid peroxidation was determined by measuring the level of TBARS using the TBA-TCA-HCl reagent at 532 nm. QTN was found to be more effective than the fractions in curbing the generation of lipid peroxides in both method, whereas, the n-butanol fraction of *F. deltoidea* exhibited

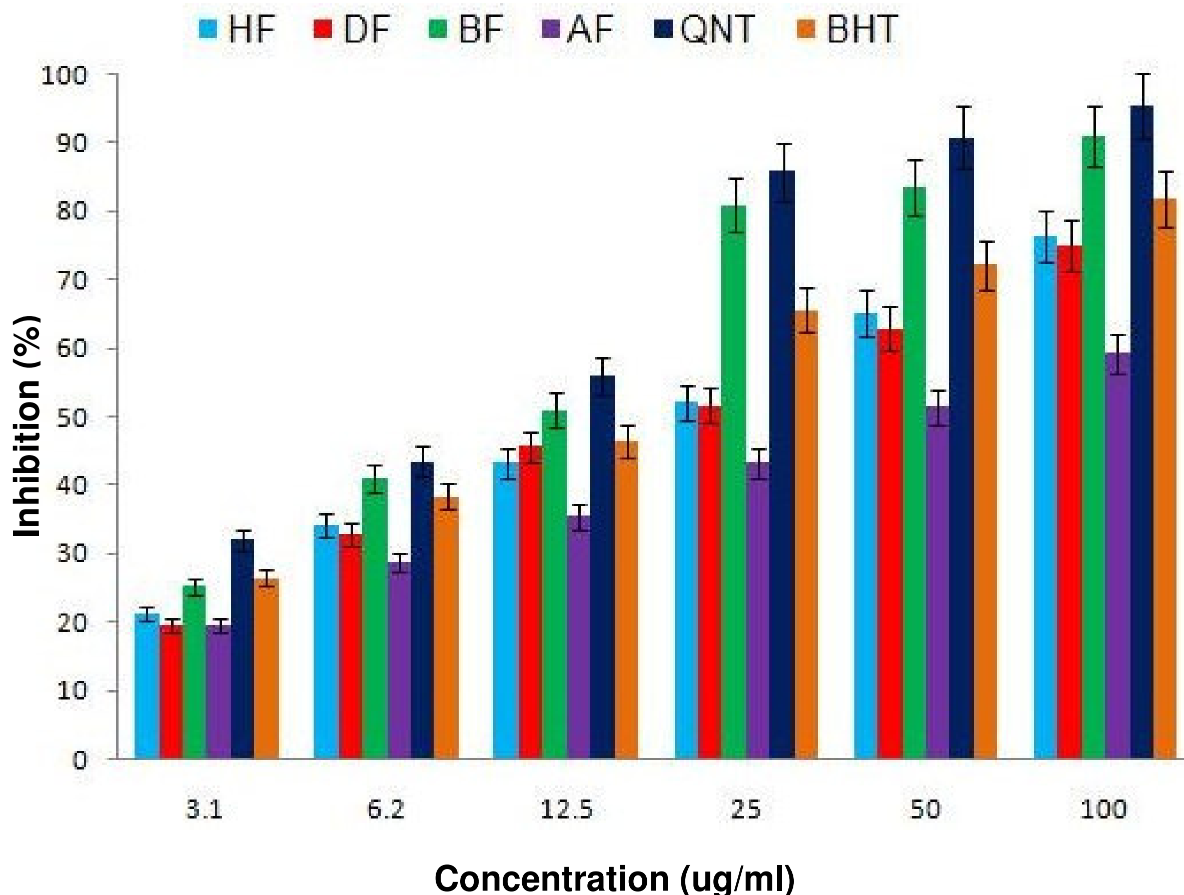

Figure 2. DPPH radical scavenging activity of fractions of metanolic extract of *F. deltoidea* leaves. Values are expressed as percentage of inhibition per µg/ml of test sample. HF, n-Hexane fraction; DF, dichloromethane fraction; BF, n-butanol fraction; AF, aqueous fractions; QTN, Quercetine; BHT, butylated hydroxytolune.

significant anti-lipidperoxidation activity in a dose dependent manner (Table 2, Figures 5 and 6).

The activity of fractions to reduce DPPH radical to diphenylpicylhydrazine was detemined by the ability of the fractions in quenching the DPPH free radical which inturn declines the absorbance at 517 nm. The results given in Table 2 illustrate that n-butanol fraction exhibited a significant dose-dependent DPPH scavenging activity, as the free radical was drastically quenched by the fraction with IC_{50} 9.74 µg/ml. Dichlromethyl fraction also showed significant activity with IC_{50} 35.43 µg/ml when compared to the control. On the other hand, the n-hexane and aqueous fractions demonstrated moderate DPPH quenching activity with IC_{50} 51.07 µg/ml and 62.3 µg/ml, respectively (Figure 2). The results of reducing power and total anti-oxidant capacity assays are depicted in Figures 3 and 4, respectively. Consistently, n-butanol fraction of *F. deltoidea* exhibited significant activity in both assays, whereas other fractions showed less activity (Table 2). Moreover, the n-butanol fraction demonstrated the activity on a par with the standard reference BHT in almost all assays.

The inhibitory activity of *F. deltoidea* fractions on α-amylase

Table 2 shows the inhibitory effects of *F. deltoidea* fractions on α -amylase activity. As shown in Figure 7, the highest percentage of inhibition was observed in n-butanol fraction with IC_{50} 39.42 µg/ml. Likewise, the α-amylase inhibitory effect of acarbose (positive control) and the inhibitory effect of *F. deltoidea* fraction on α -amylase increased with increasing concentrations. On the basis of increasing order of IC_{50}, the sequence of enzyme inhibitory activity were found to be: n-butanol> dichloromethane >n-hexane >aqueous fractions.

The inhibitory activity of *F. deltoidea* fractions on α-glucosidase

Spectrophotometrically standard procedure was used to assess the inhibitory potency of four different fractions of *F. deltoidea* leaves against α-glucosidase by measuring hydrolysis of *p*-nitrophenyl glycoside. (Figure 8) As

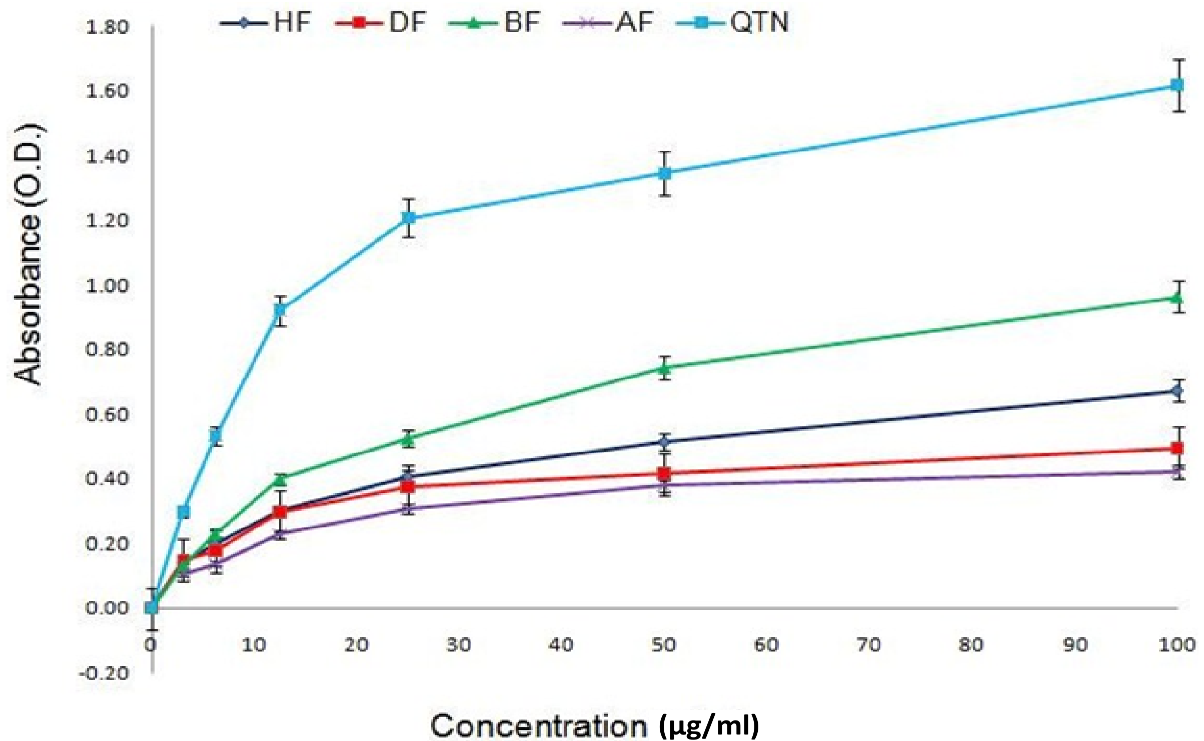

Figure 3. Reducing power of fractions of metanolic extract of *F. deltoidea* leaves as compared to QTN. Values were the average of triplicate experiments. HF, n-Hexane fraction; DF, dichloromethane fraction; BF, n-butanol fraction; AF, aqueous fractions; QTN, quercetine; BHT, butylated hydroxytoluene.

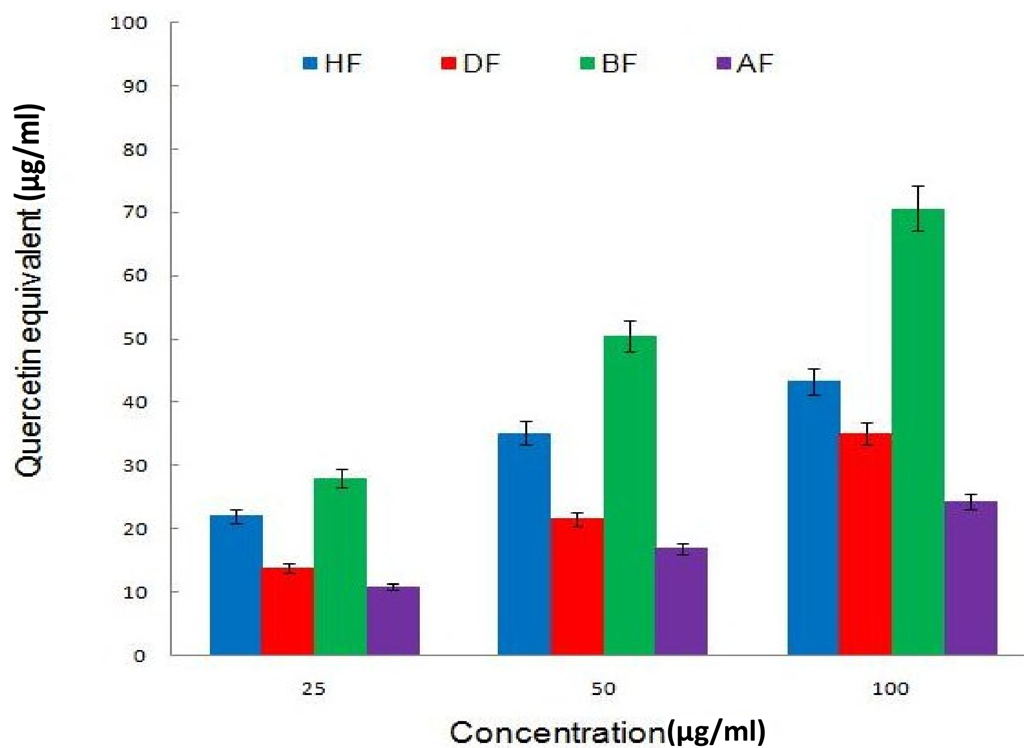

Figure 4. Total antioxidant capacity assessed by measuring formation green phosphate/Mo (V) complex of acidic pH at 695 nm in the presence of fractions of metanolic extract of *F. deltoidea* leaves. Values are as expressed as µg/ml of quercetin equivalents.

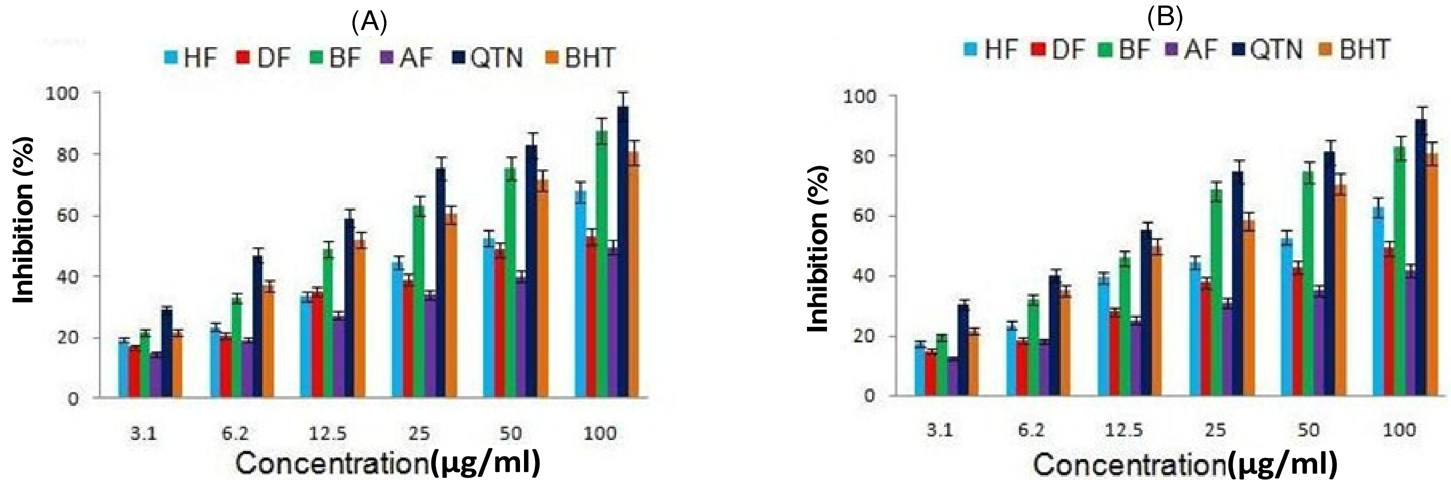

Figure 5. Effect of concentrations (3.1 to 100 µg/ml) of fractions of metanolic extract of *F. deltoidea* leaves in inhibition of lipid peroxidation generated by Fentons Reagent in brain (A) and liver (B) homogenate (40% w/v) in the Fox method during 60 min at 37°C. The values are expressed as percentage of inhibition per µg/ml of fraction. HF, n-Hexane fraction; DF, dichloromethane fraction; BF, n-butanol fraction; AF, aqueous fractions; QTN, quercetine; BHT, butylated hydroxytoluene.

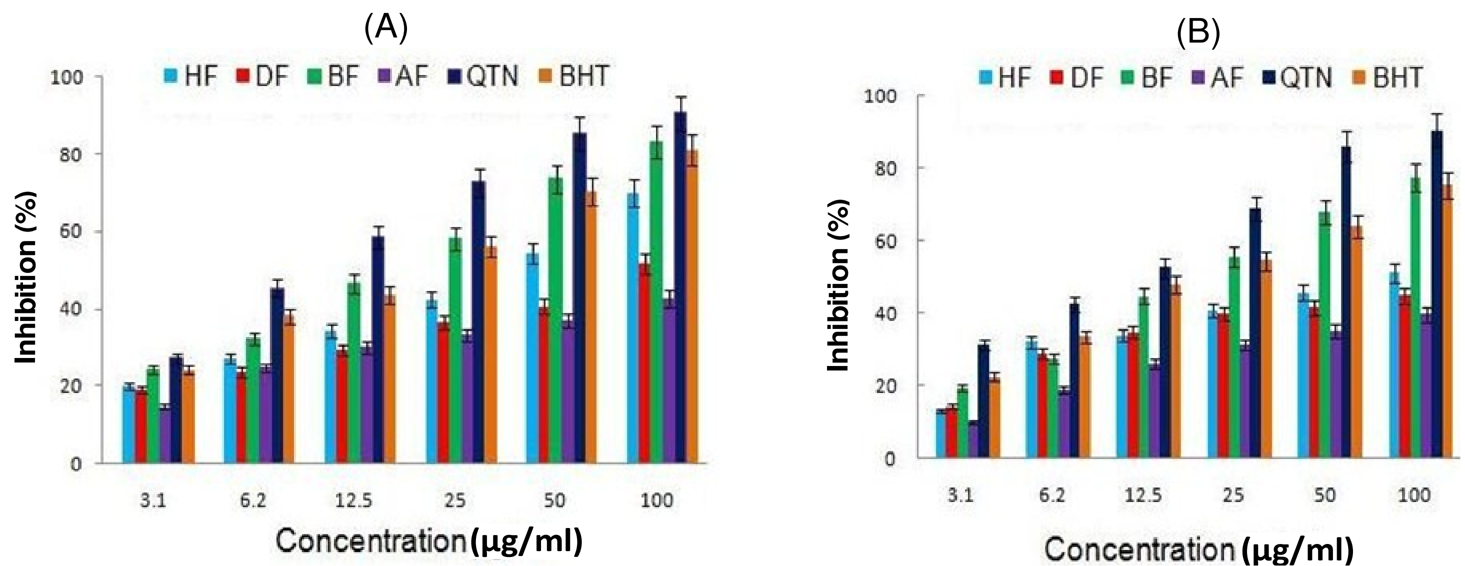

Figure 6. Effect of concentrations (3.1 to 100 µg/ml) of fractions of metanolic extract of *F. deltoidea* leaves on TBARS content generated by $FeCl_3$ in brain (A) and liver (B) homogenate (3.3% w/v) in the TBA method. The values are expressed as percentage of inhibition per µg/ml of fraction. HF, n-Hexane fraction; DF, dichloromethane fraction; BF, n-butanol fraction; AF, aqueous fractions; QTN, Quercetine; BHT, butylated hydroxytoluene.

shown in Table 2, all fractions possessed concentration-dependent inhibitory activity on mailman α-glucosidase, while the most potent tested sample was found to be n-butanol fraction with IC_{50} 15.1 µg/ml. Based on the increasing order of IC_{50} values on the enzyme inhibitory activity, the order of the fractions is given as: n-butanol> dichloromethane >n-hexane >aqueous fractions.

DISCUSSION

Inhibition of α-glucosidase and α-amylase is considered to be efficient strategy in the treatment of carbohydrate metabolic disorders including diabetes mellitus type II (Floris et al., 2005), cancer (Olden et al., 1991) and HIV(Saul et al., 1983). The various beneficial effects of these inhibitors are due to crucial role of enzymes in metabolism of carbohydrate (Remi and Jean, 2004). Delay in digestion of carbohydrate plays important role in the control of postprandial hyperglycemia, hyper in-sulinemea, as well as in decreasing risk of cardiovascular disease (Maki, 2004; Misra et al., 2010).

Assessment of enzymes inhibitory activity of the four different fractions of methanolic extract of *F. deltoidea*

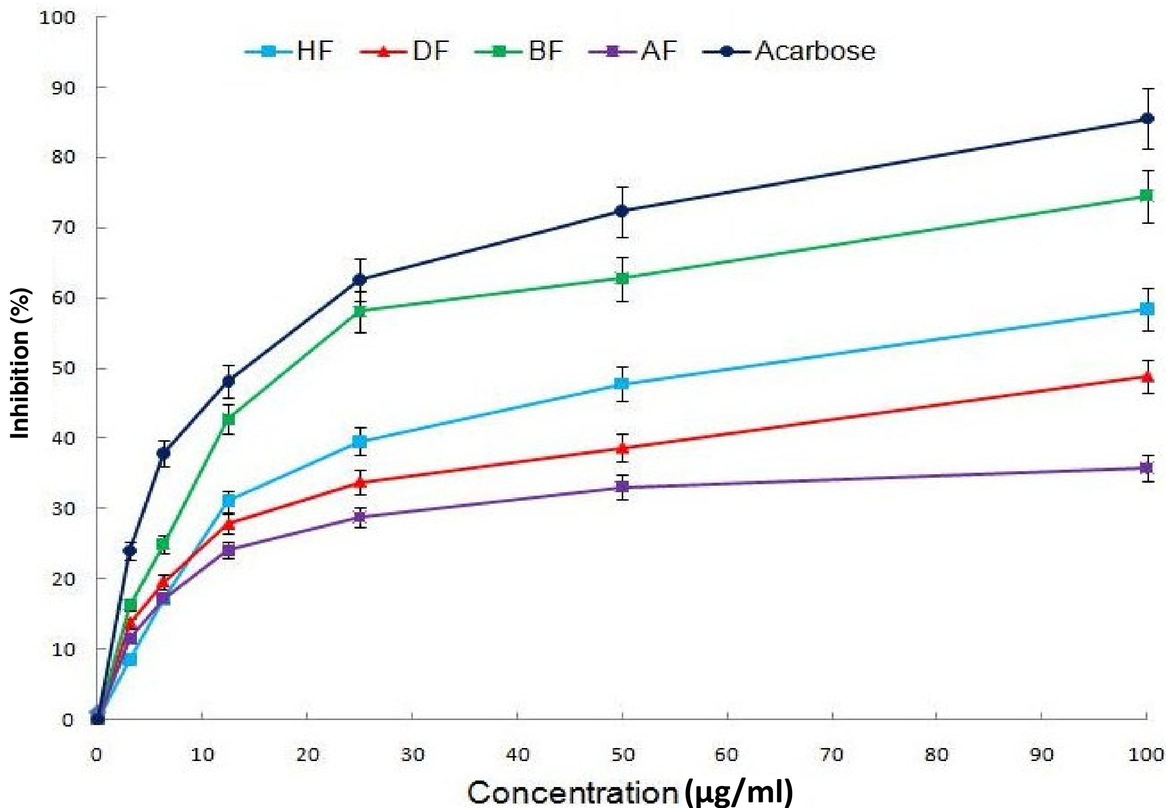

Figure 7. Porcine pancreatic α-amylase inhibitory effect of different concentrations (3.1 to 100 µg/ml) of fractions of metanolic extract of *F. deltoidea* leaves measured at 540 nm and expressed as percentage of inhibition by µg/ml of test sample. HF, n-Hexane fraction; DF, dichloromethane fraction; BF, n-butanol fraction; AF, aqueous fraction.

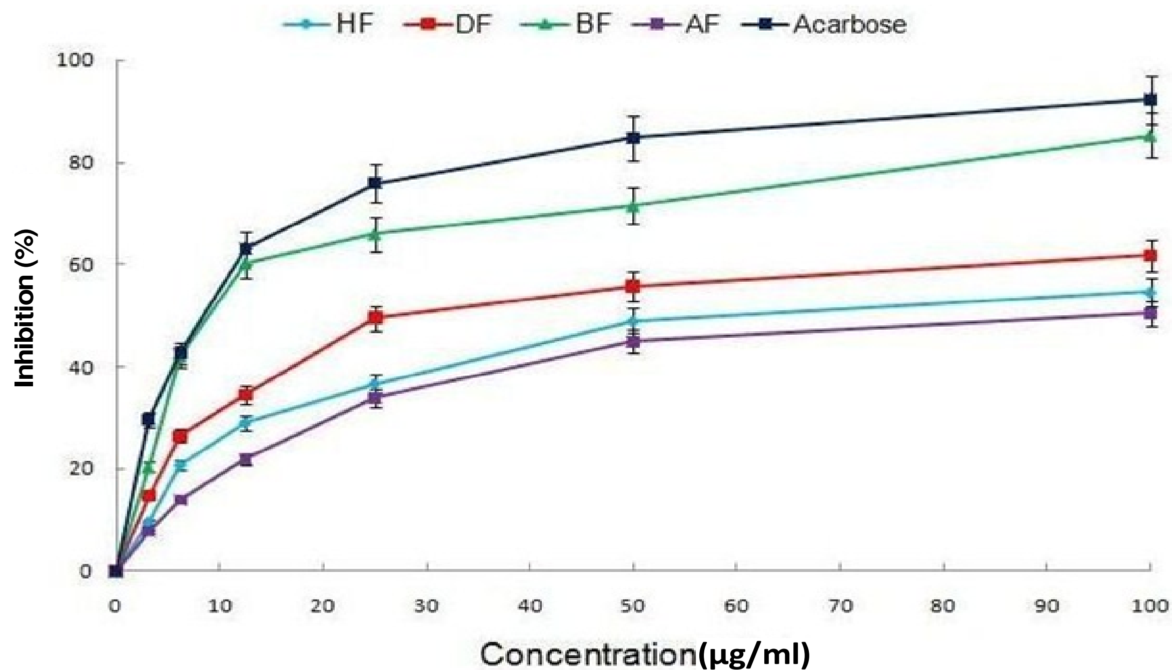

Figure 8. Mammalian α- glucosidase inhibitory activity of different concentrations (3.1 to 100 µg/ml) of fractions of metanolic extract of *F. deltoidea*. Leaves were quantified by measuringthe released *p*-nitrophenol at 400 nm and expressed as percentage of inhibition by µg/ml of test sample. HF, n-Hexane fraction; DF, dichloromethane fraction; BF, n-butanol fraction; AF, aqueous fractions.

Table 3. The R values (correlation coefficients) between antioxidant activities and α-glucosidase, α-amylase and lipid peroxidation in different systems of assessment.

Total antioxidant [a]	α-amylase	α-glucosidase	FOX-brain	Fox-Liver	TBA-Brain	TBA-Liver
HF	0.942	0.992	0.997	0.98	0.92	0.979
DF	0.977	0.997	0.953	0.973	0.975	0.998
BF	0.962	0.963	0.984	0.994	0.994	0.998
AF	0.999	0.998	0.965	0.963	0.988	0.995

[a]HF, n-Hexane fraction; DF, dichloromethane fraction; BF, n-butanol fraction; AF, aqueous fraction.

leaves showed that n-butanol fraction exhibited dose dependent α-glucosidase and α-amylase inhibitory potency which was more pronounced than the other fractions of the methanolic extract. More also, assessment of antioxidant capacity of fractions of the methanolic extract of *F. deltoidea* leaves proved that again n-butanol fraction showed higher antioxidant activity among all fractions, which was consistence with the result of phytochemical analysis that presented the higher amount of phenolics and flavonoids content in n-butanol fraction. HPLC analysis also confirmed the presence of higher phenolic compounds particularly the C-glycosylflavones, such as isovitexin and vitexin in n-butanol fraction of the methanolic extract.

Since the last decade, the antioxidant activity of phenolic and flavonoid compounds has received much attention, as it is well documented that antioxidant activity of these compounds can help in preventing some serious pathological and chronic conditions (Rakesh et al., 2001). Furthermore, they are also capable of inhibiting carbohydrate digestive enzymes due to their protein binding ability (Griffiths and Moseley, 1980; Hara and Honda, 1992). The results of antioxidant activies showed that the fraction rich in phenolics and flavonoids content had stronger antioxidant activities. Further quantitative analysis showed there was positive correlation between total antioxidant activity and phenolic content (R value: 0.993). These results therefore support the role of phenolic compounds in antioxidant activity, which is in agreement with other studies (Hiroyuki et al., 2001; Matsui, 2001). Fraction with higher phenolic and flavonoid contents, as well as higher total antioxidant activity, also showed higher enzyme inhibitory activity. The results of this study are in agreement with other research that reported high antioxidant activity and α-glucosidase inhibitory effect of phenolic rich extract (Esra et al., 2004). Furthermore, these results reveal a direct correlation between antioxidant activities and α-glucosidase and α-amylase inhibitory activities (Table 3).

Conclusion

Isovitexin and vitexin from *F. deltoidea* have been identified to be responsible for enzymes' inhibitory effect. This is the first report of α-glucosidase and α-amylase inhibitory compounds from *F. deltoidea.* Isovitexin is C-glycosyl flavones that are found to have α-glucosidase inhibitory, which may reduce postprandial hyperglycemia and diabetic complication. In addition, this fraction offers other advantages including antioxidant activity and reducing lipid peroxidation. The fraction can also reduce the harmful effects of oxidative stress in diabetics, therefore aiding in the management of diabetes.

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