International Food Research Journal 19(4): 1393-1400 (2012)
Journal homepage: *http://www.ifrj.upm.edu.my*

Total phenolic content and *in vitro* antioxidant activity of *Vigna sinensis*

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Article history

Received: 26 March 2012
Received in revised form: 24 April 2012
Accepted:25 April 2012

Keywords
Antioxidant activity
Vigna sinensis
different fractions
reductive power
phenolics content

Abstract

Vigna sinensis also known as long-podded cowpea or Chinese long bean (Family:*Fabaceae*) is most widely grown in Southeast Asia. They are a good source of protein, vitamin A, vitamin C, iron, phosphorus, and potassium. The antioxidant potential of crude methanol extract, chloroform, and ethyl acetate soluble fractions of *Vigna sinensiswas* screened for *in- vitro* antioxidant activity using total phenolic content, ferric reducing power, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay, ferric thiocyanate (FTC) and thiobarbituric acid (TBA) tests. It was found that ethyl acetate fraction have maximum amount of polyphenolics compounds (2.69 mg/g GAE in concentration 0.5 mg/mL); more effective than methanol and chloroform extract.This fraction also exhibited fairly good antioxidant activity with in both TBA (17.39% mg/g GAE) and FTC (12.65% mg/g GAE) methods.

Introduction

Vigna sinensis, is also known as bora, the long-podded cowpea, asparagus bean, snake bean, or Chinese long bean kacang panjang in Indonesian and Malay. This plant is of a different genus than the common bean. They are a good source of protein, vitamin A, thiamine, riboflavin, iron, phosphorus, and potassium, and a very good source for vitamin C, folate, magnesium, and manganese. As far as our literature survey could ascertain, no information was available on the *in vitro* antioxidant activities of the *V. sinensis*. Therefore, this current investigation was to evaluate the *in vitro* antioxidant capacities of the methanol, chloroform and ethyl acetate extract of *V. sinensis*. The antioxidant activities of *V. sinensis* were measured in a concentration of 1 mg/mL using different antioxidant assays. Furthermore, the total phenolic content, the reducing power and the antioxidant activity contents were also measured and their correlation with the antioxidant activities was ascertained (Kumaran *et al*., 2007).

According to Showkat *et al*. (2011), free radicals, the partially reduced metabolites of oxygen and nitrogen, are highly toxic and reactive. The most common reactive oxygen species are superoxide anion (O^{2-}), hydrogen peroxide (H_2O_2), peroxyl radical (ROO) and highly reactive hydroxyl radical (OH). Oxidation process is one of the most vital routes for producing free radicals in food, drugs and living systems. Antioxidants are the substances that when present in low concentration significantly delay or reduce the oxidation of the substrate. Antioxidants protect the body from damaging oxidation reactions by reacting with free radicals and other reactive oxygen species within the body and hindering the process of oxidation. Hence, diseases associated with free radicals can be avoided by antioxidant therapy which gained an immense importance.

Current research is now directed towards finding naturally occurring antioxidants particularly of plant origin. Currently available synthetic antioxidants like butylated hydroxyl anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinone and gallic acid esters have been suspected to cause negative health effects. Hence, strong restrictions have been placed on their application and there was a trend to substitute them with naturally occurring antioxidants. Furthermore, these synthetic antioxidants also show low solubility and moderate antioxidant activity (Pourmorad *et al*., 2006).

Pourmorad *et al*. (2006) also statedbesides well-known and traditionally used natural antioxidants from tea, wine, fruits, spices, and medicine are already exploited commercially either as antioxidant additives or a nutritional supplements. Besides that,

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many other plant species have been investigated in search of novel antioxidants but generally there was still a demand to find more information concerning the antioxidant potential of plant species. The antioxidant activity of plants might be due to their phenolic compounds. Flavonoids are a group of polyphenolic compounds with known properties like free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory actions.

Materials and Methods

Extraction of crude extract

Fifty grams of long beans, vegetable is washed under running tap water and dried with a soft cloth. Fresh flesh was then cut into small pieces, approximately 1.5 cm×1.5 cm×1.5cm wide in size. Sample was then macerated in hexane, DMS and methanol for one hour with occasional shaking. All the procedures are carried out in a dark environment and the samples were left in dark conditions for 4 days. Universal bottles are weighed and the weights are recorded. Universal bottles are put into oven at 45°C for 30 mins. The extraction solvents are filtered with Whatman No. 41 filter paper (pore size 20-25µm). The filtered solvent is put into the rotavapor flask and concentrated under reduced pressure at 40°C using rotary vacuum evaporator (Buchi Rotavor R-200, Switzerland). The solvents are concentrated and dried in flask before stopping the evaporator procedure.

Five mL of the used solvents is added into the rotavapor flask. All the concentrated crude extract is transferred into the previous universal bottles. The bottles are labelled with the name of sample, type of solvents and the date of experiment. Universal bottles with the concentrated extract are transferred in the oven at 40°C. The concentrated extracts are dried until the solvent was evaporated completely. The universal bottles are weighed and recorded. The crude extracts are stored at 40°C before being used for the next antioxidant analysis.

Total phenolics content

The amount of total phenolics in extract was determined with Folin–Ciocalteu reagent. Standard for TPC test were using gallic acid and α-tocopherol. 4 mg of samples were weighed and dissolved in 4 ml of 70% ethanol. 0.75 mL of Folin-Ciocalteu's reagent (previously diluted 10 fold with distilled water) was added into test tube containing samples and stand at room temperature for 5 mins. 0.75 mL of sodium carbonate (60g/L) were added and left at room temperature around 90 minutes. The mixture was read at 725 nm using UV-Vis spectrophotometer.

Total antioxidant status (FRAP)

The FRAP Substrate Working Solution was then prepared by adding 25 µL of 3% Hydrogen Peroxide Solution to 10 mL of FRAP Substrate Solution. It was used within 20-30 mins. Then, assays were prepared in the 96 well plates. In wells for the Trolox standard curve, 10 µL of a Trolox Standard (from tube 1-6) and 20 µL of Myoglobin Working Solution are added. Meanwhile, in wells for the Test samples, 10 µL of a Test sample and 20 µL of Myoglobin Working Solution are added. After that, 150 µL of FRAP Substrate Working Solution was added to each well. Then, they were incubated for 5 mins at room temperature (27°C). The 70 µL of stop solution was added to each well. The Stop Solution was warmed to room temperature (27oC) and mixed until homogenous prior used. The endpoint absorbance was read at 405 nm using a plate reader.

ABTS assay

Trolox standard well

Trolox standard well is prepared by adding 10 µL of Trolox standard, 10 µL of Metmyoglobin and 150 µL of Chromogen per well in the designated wells on the plate.

Sample well

Sample well is prepared by adding 10 µL of Trolox standard, 10 µL of Metmyoglobin and 150 µL of Chromogen to wells. The reactions is initiated by adding 40 µL of hydrogen peroxide working solution to all the wells beig used, as quickly as possible. Then, the plate is covered with plate cover, followed by incubate on a shaker for 3-5 min at room temperature. The cover is removed and the absorbance is read at 750 nm or 405 nm using plate reader. The antioxidant concentration was then calculated.

Reductive potential

The standards used in this assay were gallic acid, α-tocopherol and L-ascorbic acid. 6 mg of samples extract (ethyl acetate, chloroform, and methanol of long beans extraction) were weighed and dissolved in 6 mL of distilled water. Then, the sample solutions were added in each test tube/ facon tube followed the concentration as in table 1 below. 2.5 mL, 0.2 M, pH 6.6 of phosphate buffer and 2.5 mL of 1% potassium ferricyanide solution were added. The mixture was incubated 50°C for 20 mins. 2.5 mL of 10% trichloroacetic acid were added. Then, centrifugation was carried out for 10 minutes at 1000 g. 2.5 mL supernatant was taken and mixed with 2 mL dH_2O and

0.5 mL of 0.1% $FeCl_3$. The absorbance was measured at 700 nm using a UV-Vis spectrophotometer.

Ferric thiocyanate (FTC) method

Four mg of sample extract is weighed and the sample is mixed with 4.0 mL absolute ethanol, 4.1 mL of 2.52% linolenic in absolute ethanol, 4.0 mL of 0.05 M phosphate buffer (pH 7) and 3.9 mL distilled water. The mixture solution is mixed in the tube with a screw cap and then placed in the dark oven at 40°C for about 10 mins. 0.1 mL of the solution is taken and is added with 9.7 mL of 75% ethanol and 0.1 mL of 30% ammonium thiocyanate. 0.1 mL of 0.02 M ferrous chloride is added in 3.5 % HCl to the reaction mixture and the absorbance is read at 532 nm precisely 3 mins after the addition. Gallic acid, L-ascorbic acid and α-tocopherol were used as standards.

Thiobarbituric acid (TBA) method

One mL of sample solution from FTC method was added with 2.0 mL of 20% trichloroacetic acid (TCA) + 2.0 mL 0.67% thiobarbituric acid (TBA) in the test tube. The mixture was placed in water bath (95oC) for 10 mins. After cooling, it was then centrifuged at 3000 rpm for 20 mins. The absorbance of the supernatant was read at 532 nm using UV-Vis spectrophotometer.

Results and Discussion

Determination of total phenolic compounds

Plant phenolics are the widest spread secondary metabolite in plant kingdom. These compounds have received much attention as potential natural antioxidant in terms of their ability to act as both efficient radical scavengers and metal chelator. Therefore, it is worthwhile to determine the total amount of phenolic content in the plant chosen for the study. The Folin-Ciocalteu reagent is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric assay of phenolic and polyphenolic antioxidants. However, this reagent does not measure the total phenols only; it also will react with any reducing substance. Perhaps there were other components that can react with the reagent such as ascorbic acid (Shahidi and Naczk, 1995). Besides, various phenolic compounds have different response to this assay (Singleton and Rossi, 1965). It works by measuring the amount of the substance being tested needed to inhibit the oxidation of the reagent. However, the measurement of colour changes after two hours storage could be used to determine the existence of phenol in samples. This may due to the antioxidant properties of plant extract that react as reductant agent which known as redox action.

Javanmardi *et al*. (2003) stated that antioxidant activity of plant extracts is not limited to phenolics. Activity may also come from the presence of other antioxidant secondary metabolites, such as volatile oils, carotenoids, and vitamins, among others. The total phenolic content is expressed in gallic acid equivalence (GAE) withα-tocopherolas positive control. Higher phenolic content was observed in higher extract concentration. At concentration 0.1 mg/mL all extracts shows lowest phenolic content, whereby the highest phenolic content is observed in ethyl acetate extract, 1.59 mg/g and the lowest is in methanol extract, 0.01 mg/g. As the concentration of the extract increase, the phenolic content also increase. The highest phenolic content is recorded in 0.5 mg/mL concentration with α-tocopherol extract shows highest reading (4.28 mg/g), followed by ethyl acetate (2.69 mg/g), chloroform (2.25 mg/g) and the lowest is methanol extract (2.05 mg/g). The total phenolic contents of V. sinensis are shown in Table 1.

Based on the analysis, the phenolic content are increased by concentration of the solution. At concentration 0.5 mg/mL, all extract shows maximum amount of phenolic content, including α-tocopherol. This indicates that at higher concentration, more phenols needed to inhibit the oxidation of Folin-Ciocalteu reagent. Total phenolic content of ethyl acetate extract (control) shows best value compared to chloroform and methanol extract. Methanol shows the least total phenolic content when using this Folin-Ciocalteu method. The total phenolic content increases with the concentration. Using this method, the *V. sinensis* extract inethyl acetate displayed highest phenolic content; 2.693 mg/g, followed by chloroform extract and methanol extract.

Antioxidant assays (FRAP)

The ferric reducing ability of plasma (FRAP) method measures the antioxidant capacity of a given substance, as compared to the standard. The principle FRAP method is based on the reduction of a ferric-

Table 1. Total phenolic content in ethyl acetate, chloroform, methanol and α-tocopherol extracts of *Vigna sinensis*

Concentration (mg/mL)	Total phenolic content (mg/g) GAE			
	Ethyl acetate	Chloroform	Methanol	α-tocopherol
0.1	1.59	0.11	0.01	0.66
0.2	1.63	0.42	0.04	1.25
0.3	2.03	1.34	0.66	2.69
0.4	2.32	1.94	1.61	3.26
0.5	2.69	2.25	2.05	4.28

Table 2. Absorbance and the antioxidant concentration of extract solutions

Extract	Absorbance, y	Antioxidant concentration, x (mM)
Chloroform	1.380	0.022
Ethyl acetate	1.371	0.006
Methanol	1.448	0.143

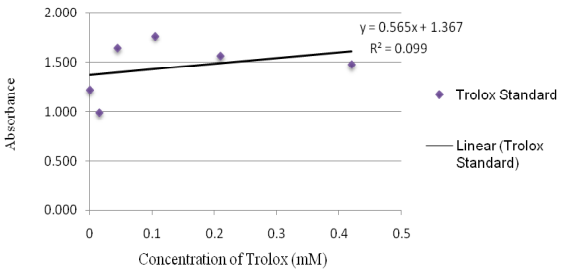

Figure 1. Antioxidant analysis by FRAP method shows the absorbance reading for different concentrations. By using equation of trolox standard, antioxidant concentration, (mM) = [(y – 1.3676) / 0.5653] × 1 mg/mL

tripyridyltriazine complex to its ferrous colored form in the presence of antioxidants. Reducing power is to measure the reductive ability of antioxidant, and it is evaluated by the transformation of Fe (III) to Fe (II) in the presence of the sample extracts (Gülçin *et al.*, 2003). From Figure 1, antioxidant concentration increased with an increased in extracts' absorbance. The data was shown that all the samples increased their antioxidant concentration when the absorbance of extracts was increased. Meanwhile, reducing power increased with an increased in extracts concentration. Therefore, the result shows that all the samples increased their reducing ability when the concentration of extracts was increased. This result was similar to the study reported by Gülçin *et al.* (2003) and Noriham *et al*. (2004), who demonstrated antioxidative activity on *Pimpinella anisum* seeds extracts and four types of Malaysian plants. From Table 2, the methanol has the highest antioxidant followed by chloroform, and ethyl acetate. This meant that the methanol has the highest ability to reduce Fe (III) followed by chloroform, and ethyl acetate. This result of methanol was similar to that reported by Suganya *et al*. (2007), who demonstrated antioxidative activity on *Psidium guajava* of Thailand plants. The ability to reduce Fe (III) may be attributed from hydrogen donation from phenolic compounds which is also related to presence of reductant agent (Shimada *et al*., 1992).

However, these results of antioxidant value were different with ABTS where the highest antioxidant was ethyl acetate followed by methanol, and chloroform. The difference appeared in the antioxidant value ranking obtained by FRAP and ABTS method was might be due to the human errors had been occurred during the experiment was carried out. For instance, the solution was pipette more or less than required by the standard method in the wells. This error could be occurred as the wells of the kits were too small, colourless, and numerous in number which easily could get confused when handling them. In addition, the number and position of hydroxyl group of phenolic compounds also rule their antioxidant activity (Rice-Evans *et al*., 1995). In addition, the myoglobin working solution was used in the FRAP procedure. Reliable and reproducible results would be obtained when the assay procedure was carried out with a complete understanding of the information contained in the package insert instructions and with adherence to good laboratory practice.

The factors that might affect the performance of the assay include proper instrument function, cleanliness of glassware, quality of deionized water, and accuracy of reagent and sample pipetting, washing technique, and incubation times/ temperatures. Besides that, 3% Hydrogen peroxide (H_2O_2) was used in FRAP and ABTS methods. It is the simplest peroxide (a compound with an oxygen-oxygen single bond). Hydrogen peroxide is a clear liquid, slightly more viscous than water that appears colourless in dilute solution. The oxidizing capacity of hydrogen peroxide is so strong that it is considered a highly reactive oxygen species. Hydrogen peroxide is naturally produced in organisms as a by-product of oxidative metabolism. Nearly all living things (specifically, all obligate and facultative aerobes) possess enzymes known as peroxidases, which harmlessly and catalytically decompose low concentrations of hydrogen peroxide to water and oxygen.

Antioxidant assay (ABTS)

ABTS is used as a substrate with hydrogen peroxide for a peroxidase enzyme. The formal reduction potentials for ABTS are high enough for it to act as an electron donor for the reduction of molecular oxygen and hydrogen peroxide. It is thus useful for testing food extracts and most food extracts are highly-coloured. It is also viable for both aqueous and lipophilic systems. The ABTS radical caption is reactive towards most antioxidants including phenolics, thiols and Vitamin C.The concentration of the antioxidant in the sample is inversely proportional to the absorbance of the radical caption produced by 2,2'-azino-bis-(3-ethylbenzothiozoline-6-sul-fonate) (ABTS). Trolox standard is used as standard in this

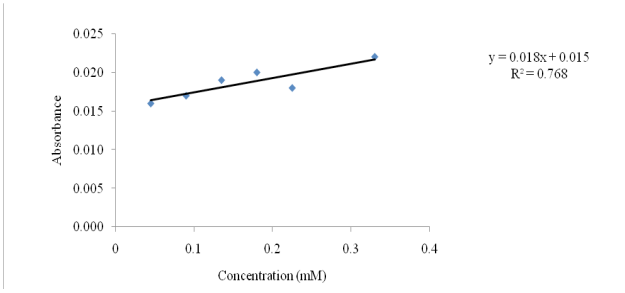

Figure 2. Trolox standard graph

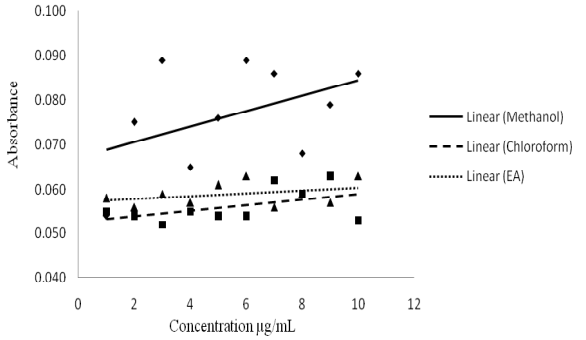

Figure 3. Reducing power of the samples extract/fractions by Fe^{3+} - Fe^{2+} transformation

ABTS assay. Trolox is Hoffman-LaRoche's trade name for 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, a water-soluble derivative of vitamin E. It is an antioxidant, like vitamin E, and is used in biological or biochemical applications to reduce oxidative stress or damage. Figure 2 showed the graph of Trolox standard that would use as the standard reference.

The value of R^2 is 0.768. The value of R^2 is to determine how closely the data conform to a linear relationship. Thus the value of R^2 of the graph is indicated that the graph have rather poor fit line. The equation got from the graph used to calculate the value of antioxidant crude extract. Hence, antioxidant concentration, (mM) = [(y – 0.015) / 0.018] × 1 mg/mL.The highest antioxidant, mM was observed for the ethyl acetate which is 312.4 mM (5.639±0.037) followed by 226.2 mM of methanol, (4.087±2.584) and 171.0 mM of chloroform, (1.093±0.076). This is due to the higher absorbance of crude extract, the higher the value of antioxidant. Its high absorbance value indicated that the mechanism of antioxidant action of this fraction was as a hydrogen donor and it could terminate the oxidation process by converting free radicals to the stable forms. Some other studies also show ethyl acetate extract displayed a higher reducing activity compared to the other fractions (Hanane *et al*., 2010).

Determination of reductive potential

The reducing power assay serves as a significant indicator of potential antioxidant activity. Although, different mechanism was proposed for the antioxidant activity such as prevention of chain initiation, binding of transition-metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Santanu *et al*., 2010). Crude extract and different fractions of *V. sinensishas* showed concentration dependant reductive effects. The highest reducing activity was again observed for the methanol fraction and gallic acid from the standard sample.

Gallic acid, α-tocopherol and L-ascorbic acid were used as standard. Gallic acid acts as an antioxidant and helps to protect human cells against oxidative damage. It was found to show cytotoxicity against cancer cells, without harming healthy cells. α-tocopherol as positive control and widely used as commercial antioxidant in market. It has been claimed that the α-tocopherol form is the most important lipid-soluble antioxidant by protects membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction. The process removes the free radical intermediates and prevents the propagation reaction from continuing. Ascorbic acid as redox catalyst reduces and neutralizes reactive oxygen species such as hydrogen peroxide. Ascorbic acid is also a substrate for the enzyme ascorbate peroxidase, which act as stress resistance in plants (Santanu *et al*., 2010).

Based on the Figure 3, methanol and gallic acid had the highest reducing power respectively. The higher absorbance of the reaction mixture indicated greater reducing power. The reducing properties are generally associated with the presence of different reductants. The antioxidant action of reductants is based on the breaking of the free radical chain by donating a hydrogen atom. Reductones also react with certain precursors of peroxide, thus preventing peroxide formation. The reductive power of different fractions of long bean extract and gallic acid may be the reason for their antioxidant activity.

Correlation of antioxidant concentration by using FRAP assay and ABTS assay

As the antioxidant potential of crude methanol extract, chloroform, and ethyl acetate (EA) soluble fractions were used to determine the antioxidant concentrations in *V. sinensis*, they have different polarities respectively to show the relationship between free radical-scavenging activities or antioxidant activity by using ABTS assay and the reducing power by using FRAP assay. It was clearly seen that the higher the polarity of solvent used in the extraction process, the higher was the antioxidant

activity obtained should be. As the methanol has the higher polarity compared to chloroform and ethyl acetate, it showed the higher antioxidant activity in FRAP assay but ethyl acetate showed the higher antioxidant in ABTS assay. This is might be due to the methanol extract in ABTS assay had mixed with other substances that also had caused its CV value is higher than 5%. Thus, the result of antioxidant activity in the methanol extract of ABTS assay cannot be accepted.

Besides, the parameter for ABTS assay was quite poor (R^2 = 0.768) while the parameter for FRAP assay was good (R^2 = 0.099). This suggested that antioxidant components, existing in each fraction, possessed different predominant mechanisms of action. Moreover, the non-linear relationship for ABTS assay mechanism parameters might be due to other effects involving the nature of the active compounds existing in the foreign substances and their synergistic effects.

Ferric thiocyanate (FTC) method

Antioxidant activity of crude extracts is measured using FTC and TBA methods. The ferric thiocyanate (FTC) method measures the amount of peroxide produced during the initial stages of lipid oxidation, in which peroxide reacts with ferrous chloride and form ferric ions. The ferric ion then combines with ammonium thiocyanate and produce ferric thiocyanate. This substance is red in colour. The darker the colour, the higher will be the absorbance (Huda-Faujan *et al*., 2009).

The percentage of inhibition is calculated using the following formula:

$$\% \text{ Inhibition} = 100 - [(A_1/A_0) \times 100]$$

Whereby A_0 is the absorbance of the control reaction and A_1, is the absorbance in the presence of the sample extract. (Santanu *et al*., 2010). From the FTC analysis, it was found that the antioxidant activity of *V. sinensis*. Its shows that all samples of crude extract had been oxidized when stored for seven days at 40-45°C. *V. sinensis* crude extract showed the lowest absorbance at the first day. Blank showed the lowest absorbance values at 0.0575, followed by ethyl acetate with absorbance at 0.0590, methanol (0.6000), chloroform (0.0610), ascorbic acid (0.0620), α-tocopherol (0.0630) and lastly gallic acid (0.0635). Absorbance of each samples increase progressively by time of incubation. Lower absorbance values indicate higher antioxidant activities. Antioxidant activities are higher during the initial of the experiment than the end of the experiment.

At the end of the experiment, all samples showed higher absorbance values than the day 1. Ethyl acetate extract showed the lowest absorbance value with 0.107, followed by chloroform (0.110), methanol (0.111), and blank (0.117). Compared to standards, gallic acid displays the highest absorbance (0.123), followed by ascorbic acid (0.094), and α-tocopherol (0.092). Higher absorbance values showed lower antioxidant activities. Progressing days causes the antioxidant to reduce and this might be due to exposure to light. As antioxidant is easily damaged by light, it is crucial to ensure every procedure being carried out in a dark area. The percentage of inhibition in ascending order is ethyl acetate, followed by methanol and lastly chloroform. A higher percentage of inhibition, the higher antioxidant activity was recorded. After three days of storage, crude extract exhibited good effect in inhibiting linoleic acid oxidation from previous days. The percentage of inhibition increases from day 1 to day 3 and the subsequent measurements are in a scattered pattern with some higher and some lower. This might be probably due to handling error as incubation only proceeds up to third days.

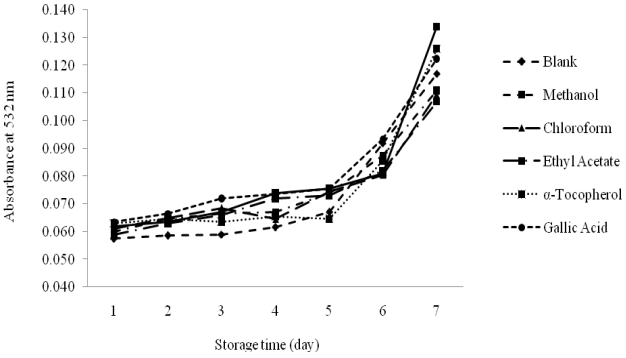

Figure 4. Antioxidant properties of crude extract determined with the FTC method

Figure 4 also depicts that the antioxidant activities also increased with the increasing concentration of *V. sinensis* crude extract. It is clearly shown that there is a positive correlation between total phenolic content and the antioxidant activities. The phenolic compounds may donate hydrogen and terminate the free radical reaction chain by changing it to the stabile compounds (Amarowicz *et al*., 2000). None of the crude extract of *V. sinensis* showed absorbance values greater than the blank, the one without crude extracts at the initial and end point of FTC method. This clearly indicates the presence of antioxidant activity in the blank. Ethyl acetate shows the strongest antioxidant activity compared to methanol and chloroform.

Thiobarbituric acid (TBA) method

FTC is used to measure the production of peroxide compound at the initial stage of oxidation while TBA test is used to measure the secondary product of

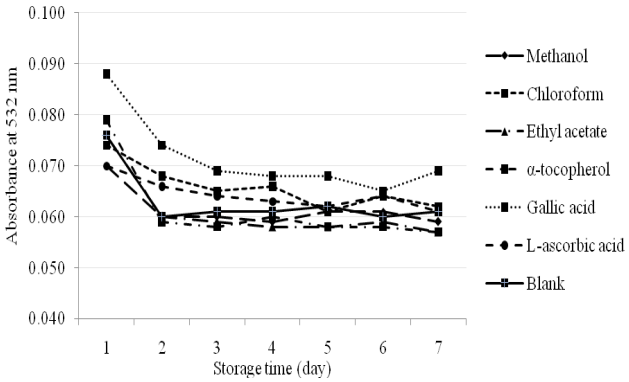

Figure 5. Absorbance of antioxidant activities as measured by the TBA method

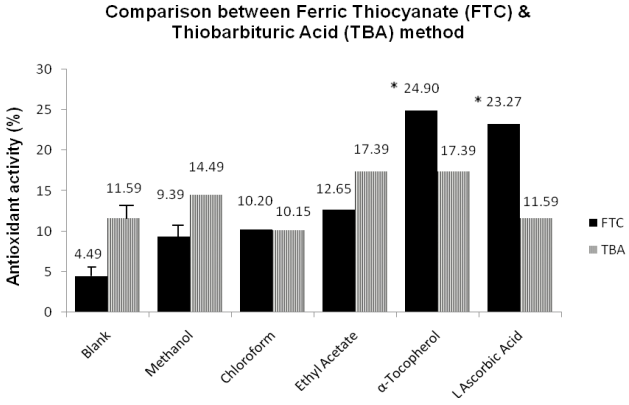

Figure 6. A comparison between antioxidant activities of five extracts using ferric thiocyanate (FTC) & thiobarbituric acid (TBA) methods with α-tocopherol and ascorbic acid as standards. Asterisk (*) indicates significant difference at $p<0.05$

oxidation. During the oxidation process, peroxides are gradually decomposed to lower molecular weight compounds. One such compound is malonaldehyde, which is measured by the TBA method (Mohd Zin *et al*., 2002). The TBA analyses of *V. sinensis* extract in seven days are shown in Figure 5. Mostly the absorbance readings are decrease by day until day seven, except for chloroform and gallic acid. Chloroform absorbance reduced from day one until day five, but increase dramatically at day six before undergo reduction at day seven. In contrast, gallic acid shows increasing absorbance from the first day until day five, before slightly reduced at day six. At day seven, all extract shows reduction in absorbance reading. The value is in range 0.057-0.065. Low absorbance correlate to high antioxidant activity.

There are differences of the antioxidant activities trend between TCA and TBA method. TCA method shows increasing value of absorbance, while TBA shows increasing value on day two and falls prior to day seven. The total antioxidant activity of the FTC method is lower than the TBA method on day seven. Using FTC method, the ethyl acetate extract from the vegetable of Vigna sinensis displayed highest antioxidant activity followed by methanol extract and chloroform extract. However, using TBA ethyl acetate comes with the highest antioxidant activity as in FTC, followed by methanol and chloroform extract as least antioxidant activity. The results are inversely correlated between FTC and TBA.

From Figure 6, antioxidant activities of samples' extract from TBA method are higher than that of FTC method. In contrast, the antioxidant activities of standards (α-tocopherol and ascorbic acid) seen to be higher from FTC compared to TBA method. This may indicate that the amount of peroxide in the initial stage of lipid peroxidation is greater than the amount of peroxide in the secondary stage as reported by Rahmat *et al*., (2003). However, the result negatively correlated to samples' extract. This also shows that the antioxidant activity detected with the FTC method was lower than that detected with the TBA method for samples' extract.It is highly possible that several compounds of different polarity may contribute to the antio*xidative activity of V.sinensis extract (*Mohd Zin *et al*., 2002). In addition, antioxidative activities observed in these plants could be the synergistic effect of more than two compounds that may present in the plant. It has been reported that most natural antioxidative compounds often work synergistically with each other to produce a broad spectrum of antioxidative activities that creates an effective defence system against free radical attack (Lu and Foo, 1995).

Conclusion

The results expressed in this study are the first information on the antioxidant activities of *Vigna* sinensis. Total phenolic contents are influenced by the concentration of extract. Among all the fractions, the ethyl acetate fractions exhibited the highest total phenolic content comparing to methanol and chloroform fraction. The antioxidant capacity of *V. sinensis* measured by FRAP and ABTS method revealed different results. Using FRAP method, methanol displayed the highest antioxidant activities, and followed by chloroform and ethyl acetate. In contrast, ABTS method shows ethyl acetate extract to be the highest antioxidant activities compared to methanol and chloroform. Using FTC and TBA method, the ethyl acetate extract from the vegetable of Vigna sinensis displayed highest antioxidant activity followed by methanol extract and chloroform extract. From this study, it is suggested that *Vigna sinensis* content high in phenolic compound and antioxidant capacity in ethyl acetate extracts as compared to methanol and chloroform.

Acknowledgements

The authors would like to thank Universiti Sultan Zainal Abidin (UniSZA) for the financial aid and Faculty of Food Technology for providing the facilities. The authors also would like to acknowledge Mr. Roslan Arshad and Mr. Hazlan Harun for their assist from Faculty of Food Technology, UniSZA, Mr. Syed Ahmad Tajudin B. Tuan Johari and all staff at Chemistry Laboratory, Faculty of Agriculture and Biotechnology, UniSZA.

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