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Antioxidant activity, phenolic and flavonoid contents in the leaves of different varieties of sweet potato (*Ipomoea batatas*)

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Abstract

The study of antioxidants from natural sources has gained popularity in the recent years. Six *Ipomoea batatas* leafy varieties namely Batu Kelantan, Batu Biasa, Biru Putih, Oren, Vitato and Indon were compared to assess the total phenols, flavonoids, reducing power and antioxidant activity. In this study, the Indon variety showed the highest level of total phenolic contents at 5.35 ± 0 g GAE/100 g DW. The flavonoid contents in the leaves ranged from 96 ± 47.6 µg/ g in Indon variety to 263.5 ± 43.5 µg/g in Batu Biasa variety. 1,1-diphenyl-2-picryl hydrazyl (DPPH) was used to determine the radical scavenging activity in leaves, in which the Indon and Biru Putih variety had the highest and lowest scavenging activities of 372.4 µg/ ml (IC_{50}) and 597.61 µg/ ml (IC_{50}), respectively. All varieties, except Biru Putih, showed the high radical scavenging activity compared to the ascorbic acid standard. Besides, all the leaf varieties also showed increment in their reducing power with increasing concentrations. Thus, *Ipomoea batatas* leaves can be used as a potential source of natural antioxidants.

Keywords: *Ipomoea batatas*; antioxidant assays; condensed tannins; natural antioxidants; Malaysia.
Abbreviations: DPPH- 1,1-diphenyl-2-picryl hydrazyl; GAE- Gallic acid equivalent; var-variety.

Introduction

Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are widely used in the food industry due to their abilities to prevent food deterioration and to extend the shelf life of foods (Hotta et al., 2002). However, the usage of synthetic antioxidants was found to increase the risk of cancer occurrence and liver damage in human (Ito et al., 1983; Namiki, 1990). Commonly used synthetic antioxidants such as β-carotene, vitamin C and vitamin E are widely sold in the market and have been shown to increase the risk of mortality in adult who consumed them. The exact mechanism of action is still unknown but it has been suggested that it could be due to the rigorous toxicity that they possess compared to natural antioxidants (Bjelakovic et al., 2007). Therefore, the search for alternative sources of natural antioxidant is becoming increasingly important. Examples of natural antioxidants that can be acquired through diet are chlorophylls, flavonoids, vitamin C, selenium and lycopene. The natural antioxidants in wine, fruits and vegetables have been studied widely due to their health benefits and commercial values. Besides fruits, other parts of plants such as bark, leaves, fruit peels and roots are also being exploited extensively for their antioxidant properties. For instance, antioxidant studies were conducted in green leafy vegetables such as amaranth, spinach, bak choi and kang kong as well as in leaves of guava leaves, blackberry leaves, red raspberry leaves and strawberry leaves (Wang and Lin, 2000; Yang et al., 2005). *Ipomoea batatas* or the sweetpotato plants are mainly planted for their storage roots. During the harvesting period, 95-98% of the leaves are discarded while the remaining 2-5% is used as animal food. Previous study by Islam (2006) revealed that *Ipomoea batatas* leaf extracts contained radical scavenging, antimutagenic, anticancer and antibacterial activities. Hence, these leaves can be utilised to be a potential source of natural antioxidant. On the other hand, variations in the antioxidant contents and activities of the *Ipomoea batatas* storage root were studied in different genotypes and varieties (Teow et al., 2007). The study of antioxidant capacity in *Ipomoea batatas* leaves has been limited compared to their storage roots, and up to now little research has been conducted to determine the influence of the different varieties on the antioxidant activities in the leaves of this plant. In this study, six commonly found varieties of *Ipomoea batatas* leaves in Malaysia were selected from a commercial sweet potato farm in Tanjung Sepat, Kuala Langat, Selangor, which is one of the largest sweet potato plantation in the Selangor state. The antioxidant properties in the different varieties of leaves were determined and compared using four different assays, namely Folin-Ciocalteau, Vanillin-HCl, reducing power and DPPH radical scavenging assay. The leaf variety with the highest antioxidant properties will be used as a suitable source of natural antioxidant to substitute the usage of synthetic antioxidant.

Results and Discussion

Flavonoid contents in the Ipomoea batatas leaf extracts

Phytochemicals in plants have long been studied in the prevention of certain chronic diseases besides the maintaining freshness in fruits and prolonging food storage. The antioxidant properties in plants are contributed by the presence of phytochemicals such as phenolics, anthocyanins and other flavonoid contents (Cao et al., 1997). Some of the naturally found antioxidant in plants includes vitamins, phenolics, flavonoids, dietary glutathione and endogenous metabolites (Larson, 1988). The antioxidant capacity in plants was found to be influenced by cultivars, maturity and other environmental factors such as sunlight exposure. A study conducted by Kacharava et al. (2009) showed that irradiation can affect the antioxidant level in cabbage and beetroot leaves while pre-treatment before the extraction process and stage of leaf maturity affect the antioxidant activity in the guava's leaves (Nantitanon et al., 2010). Besides, the antioxidant activities in the *Ipomoea batatas* roots were found to be different among various cultivars (Prior and Cao, 2000). Previous study was also illustrated that the leaves of the *Ipomoea batatas* contained higher antioxidants and phytochemicals compared to their storage roots. In this study, leaves of six different varieties of the *Ipomoea batatas*, commonly found in Malaysia, were studied for their antioxidant properties as well as their potential as a suitable source for alternative source of natural antioxidant. The study of flavonoids has been intensive due to their antioxidant properties that contribute to good health of human kind. The action of flavonoids can be divided into two different mechanisms: scavenging or the chelating process (Cook and Samman, 1996). Condensed tannins or proanthocyanidins are flavonoids that consist of two or more flavan-3-ol such as catechin, epicatechin or gallocatechin. Catechin is a flavonoid which contains two benzene rings in its structure and was found to be the most powerful scavenger compared to its counterparts categorised under the different classes of flavonoids. Therefore, catechin is often used as standard to measure the content of flavonoids in the leaf samples. The principle used in this assay is that vanillin is protonated in acidic solution, thereby giving a weak carbocation that reacts with the flavonoids rings. The intermediate compound is dehydrated and gives a red compound (Nakamura et al., 2003) which can be measured spectrophoto-metrically. The structure of flavonoids has known to contribute to the oxidative properties of the extract. Green leafy vegetables are known to contain high level of antioxidants activity which is partially contributed by the presence of flavonoids in these vegetables. The flavonoids contents (µg/ml) in the different varieties of the *Ipomoea batatas* leaf extracts were calculated using the standard curve for catechin with the equation $y = 0.0037x - 0.0125$, $r^2 = 0.996$.

It was observed that the flavonoids contents ranged between 96 ± 47.6 µg/g and 263.5 ± 43.5 µg/g (Fig 1). The Batu Biasa variety has the highest flavonoids content at 263.5 ± 43.5 µg/g followed by the Batu Kelantan variety, whereas the Biru Putih and Oren varieties had almost similar total flavonoids contents. However, the Indon variety contained the lowest flavonoids content compared to the others at 96 ± 47.6 µg/g. In this study, the differences in the total flavonoid contents were statistically significant between the different varieties. Comparatively, a study conducted by Koo and Mohamed, (2001) concluded that highest content of total flavonoids is found in leaves of onion (1497.5 mg/kg quercetin, 391.0 mg/kg luteolin and 832.0 mg/kg kaempferol) followed by black tea (1491.0 mg/kg) and papaya shoots (1264.0 mg/kg). On the other hand, commonly consumed vegetables such as soybean sprout (78.5 mg/kg), red spinach (29.5 mg/kg) and kailan (14.5 mg/kg) showed lower flavonoids contents compared to all the *Ipomoea batatas* leaf extracts used in this study. Hence, this result can conclude that leaves of all *I. batatas* varieties in this study have the potential to be a suitable source of cheap flavonoids.

Total phenolic contents in the Ipomoea batatas leaf extracts

The *Ipomoea batatas* leaves were found to contain radical scavenging, antimutagenic, anticancer and antibacterial activities in previous study (Islam, 2006). The presence of the phenolics might contribute to the protective properties in the *Ipomoea batatas* leaves. In this study, the total phenolic contents were expressed as leave's dry weight for comparison with the results from previous data. The water content in the leaves was found to reach up to 82% of the fresh weight in the *I. batatas* var Biru Putih leaves, while approximately contained 81% for the Batu Kelantan, Oren and Indon leaves varieties. Both the Vitato and Batu Biasa varieties were recorded lower water content values at 76% and 79%, respectively. The Folin-Ciocalteau method was commonly used to determine the total phenolics in the substrate and usually incorporates the usage of gallic acids as the standard (Waterhouse, 2001). The colour of Folin-Ciocalteau reagent changes from yellow to blue upon the detection of phenolics in the extracts which is normally due to the chemical reduction of tungsten and molybdenum oxides mixture in the reagent. In this study, methanol was used to dilute the gallic acid standard because gallic acid showed higher solubility in methanol compared to water and other solvents (eg. ethanol) (Daneshfar et al., 2008).

The total phenolic contents among the different varieties were expressed in terms of gallic acid equivalents using the standard curve equation $y = 0.0012x + 0.0007$, $r^2 = 0.998$ (Fig 2). The total phenolic contents in the different varieties were between 2.78 ± 0.11 and 5.35 GAE g/100 g DW and were significantly different from each other. *Ipomoea batatas* var. Indon showed the highest amount of total phenolics content at 5.35 g/100 g DW, whereas the Biru Putih variety had the lowest total phenolics content (2.78 ± 0.11 g DW) among the varieties studied. Previous study on the total phenolics content in some other varieties of *I. batatas* leaves exhibited a range from 1.42 to 17.1 g/100g dry weight (Islam et al., 2002a). The deviation in the total phenolic contents might be attributed to the geographical factors as well as the different cultivation methods. Previous study conducted by Hajihmahmoodi et al. (2008) on the usage of olive pulp extract as a potential source of natural antioxidants showed that the highest level of total polyphenol in the Iranian olive cultivar was found in the Mishen cultivar which contained approximately 2.997±0.361 g GAE/100g. The *Ipomoea batatas* leaves used in this study have higher total phenolic contents compared to olive pulp and thus could be served as a potential source of natural antioxidant. Phenolics are important mainly because of their function to scavenge the free radicals in the human body and to help maintaining healthy body by scavenging or removing the reactive oxygen species (ROS).

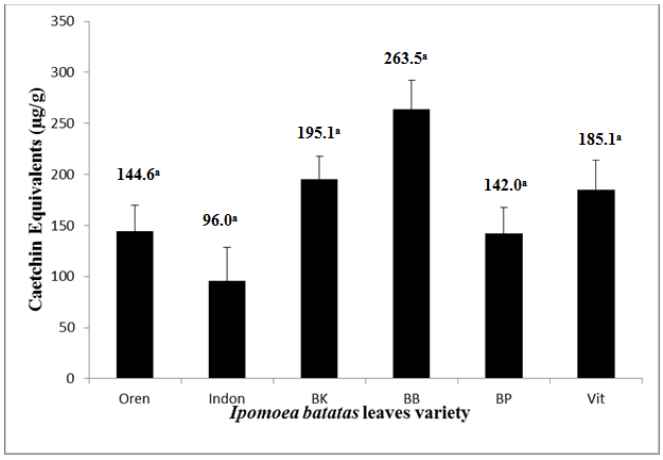

Fig 1. Flavonoid contents in six different varieties of *Ipomoea batatas* leaf extract expressed as catechin equivalent (µg/g). Means followed by different letters are significantly different ($p \leq 0.05$) (n=6).

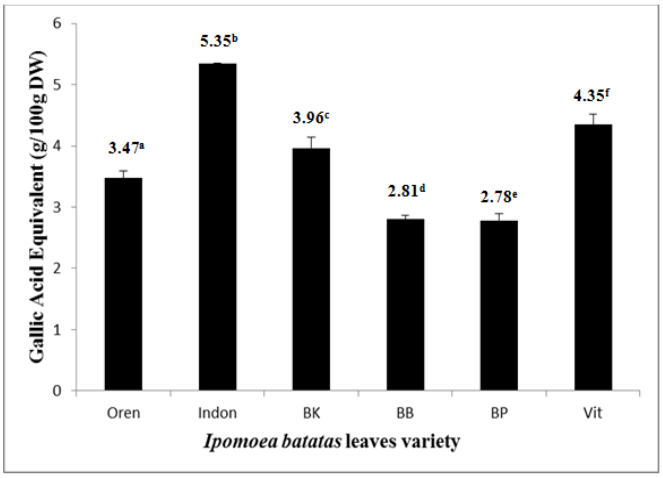

Fig 2. Total phenolic contents in six different varieties of *Ipomoea batatas* leaf extract expressed as gallic cid equivalent (g/100g DW). Means followed by different letters are significantly different ($p \leq 0.05$) (n=6).

Antioxidant activity

Free radicals and reactive oxygen species (ROS) contribute to the occurrence of many degenerative diseases such as arthritis, cirrhosis, cancer, Alzheimer and aging which can be prevented by the presence of antioxidants. There are two main types of antioxidants namely primary and secondary antioxidants which differ in their mechanisms of action (Lim et al., 2007). Primary antioxidants scavenge free radicals and donate a hydrogen atoms or electrons to make the free radicals more stable. On the other hand, secondary antioxidants act by suppressing the formation of radicals thus preventing oxidative damage. DPPH (1,1-diphenyl-2-picryl hydrazyl) is a free radical generating compound which is used to determine the radical scavenging activity of extracts. One of the known free radical scavenging activity that occurs exogenously in human body is the inhibition mechanism of lipid oxidation (Barros et al., 2007). The DPPH radical scavenging assay has been used widely to evaluate the radical scavenging activity of the different type of antioxidant substances (Cotelle et al., 1996). In this assay, the radical scavenger present in the sample extract will decolorized the purple coloured methanolic DPPH solution to yellow due to the reduction of the stable DPPH radicals to diphenyl-pricrylhydrazine in the presence of hydrogen-donating antioxidant (Shon et al., 2003). The colour changes will allow the detection of the scavenging activity at 517 nm. IC_{50} is often used to express the amount or concentration of extracts needed to scavenge 50% of the free radicals. The IC_{50} value is inversely proportional to the scavenging activity of the leaf extract. The scavenging activities among the different varieties were shown in Fig 3. The Indon variety had the highest scavenging activity with the IC_{50} value of 372.4 µg/ml while the Biru Putih variety had the lowest scavenging activity (IC_{50} = 597.61 µg/ml). The descending order of radical scavenging activity in the leaves of different *I. batatas*' varieties is as follow: Indon (IC_{50} 372.4 µg/ml) > Batu Kelantan (IC_{50} 468.31µg/ml) > Vitato (IC_{50} 475.32 µg/ml) > Batu Biasa (IC_{50} 516.98 µg/ml) > Oren (IC_{50} 545.39 µg/ml) > Biru Putih (IC_{50} 597.61 µg/ml). All the *Ipomoea batatas* leaf extracts had higher radical scavenging activity compared to ascorbic acid (vitamin C) standard (IC_{50} = 569.6 µg/ml) except for the Biru Putih variety. This showed that the *I. batatas* leaf extracts contained high amount of radical scavenging compounds with proton-donating ability. The lower radical scavenging activity observed in the Biru Putih leaves is perhaps attributed to the lower total phenolics and flavonoids contents in these leaves. In this study, the total phenolics contents and the radical scavenging activity of the leaves are likely to showed good relationship. The Indon variety with highest total phenolics contents showed higher radical scavenging activity whereas the Biru Putih variety with the lowest total phenolics contents had lower radical scavenging activity. Several studies had also reported the relationship between the high level of phenolics (eg. phenolic acid) and radical scavenging activity (Bertoncelj et al., 2007; Céspedes et al., 2008; Garcia- Alonso et al., 2004; Park et al., 2008). The high level of scavenging activity in the *Ipomoea batatas* leaves was also shown in the study by Yang et al. (2005) in which *I. batatas* leaves ranked first with the highest DPPH radical scavenging activity among 23 commonly consumed vegetables in Taiwan. The reducing power assay, on the other hand, is used to test the reducing capability of the *Ipomoea batatas* leaf extracts to convert the potassium ferricyanide (Fe^{3+}) complex to form potassium ferrocyanide (Fe^{2+}).

The potassium ferrocyanide will then react with ferric chloride to form ferrous complex which can absorb maximally at 700nm (Arulpriya et al., 2010). All the *Ipomoea batatas* varieties showed almost similar pattern of increment in their reducing power with the increase in the extracts concentrations (Fig 4). The Batu Kelantan and Indon varieties showed overall higher reducing ability compared to other varieties but the differences among the different varieties were not significant. The Biru Putih variety had steep increase compared to the other varieties from 400 to 600 µg/ml. The Oren and Batu Biasa variety on the other hand had overall lower reducing power compared to the other varieties. The reducing capacity of a plant is much related to the presence of biologically active compounds with potent donating abilities. Besides, total phenolics, flavonoids, and anthocyanins have also been reported to contribute to the high antioxidant activity in the *Ipomoea batatas* leaves (Islam et al., 2002b). In this study, the Indon variety which has

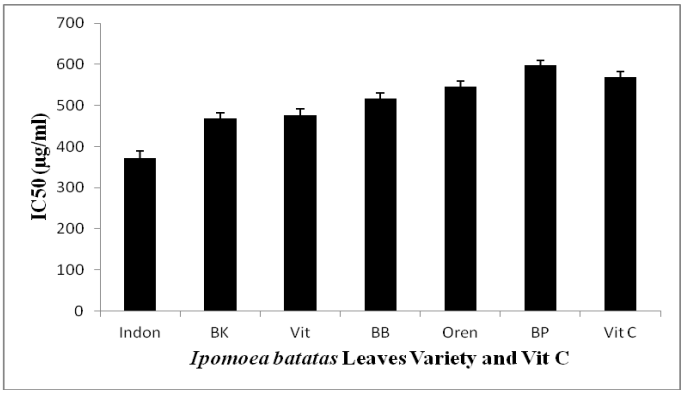

Fig 3. IC_{50} values of *Ipomoea batatas* plant extracts required to scavenge the DPPH free radicals. Lower IC_{50} value indicates higher antioxidant activity. Extracts: Indon = *Ipomoea batatas* var Indon, BK = *Ipomoea batatas* var Batu Kelantan, Vit = *Ipomoea batatas* var Vitato, BB = *Ipomoea batatas* var Batu Biasa, Oren = *Ipomoea batatas* var Oren, BP = *Ipomoea batatas* var Biru Putih and Vit C = Vitamin C (L-Ascorbic Acid) (n=6).

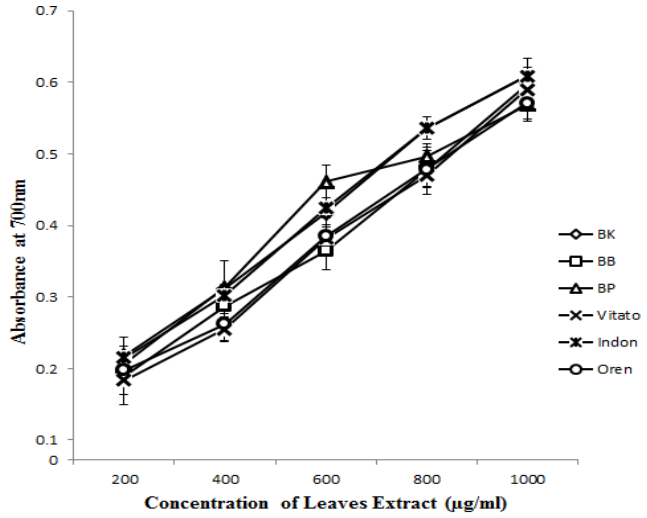

Fig 4. The reducing power of the different varieties of *Ipomoea batatas* leaf extracts at different concentrations. Extracts: Indon = *Ipomoea batatas* var Indon, BK = *Ipomoea batatas* var Batu Kelantan, Vit = *Ipomoea batatas* var Vitato, BB = *Ipomoea batatas* var Batu Biasa, Oren = *Ipomoea batatas* var Oren and BP = *Ipomoea batatas* var Biru Putih (n=6).

purple storage roots showed the highest level of radical scavenging and reducing power activity compared to the other varieties with different flesh colours (orange and yellow). Correspondingly, in a separate study conducted by Teow et al. (2007), the purple fleshed *I. batatas* storage roots showed the highest antioxidant activity followed by orange coloured storage roots while the yellow and white fleshed storage roots showed the lowest antioxidant activity. In addition, the level of antioxidant in these purple roots was found to be on par with apple, avocados and grapefruit (Wu et al., 2004). Moreover, differences were also observed in the total antioxidant activity among the purple fleshed *I. batatas* roots grown in different geographical area (Teow et al., 2007). In a study conducted by Osiru et al. (2009), the yield of *I. batatas* among the different varieties was found to be affected by the plant tolerance against viruses and other fungal agent and this could suggest the differences in their total antioxidant activity despite of cultivation under similar condition (Osiru et al., 2009). Besides *I. batatas*, sorghum also showed a similar pattern in antioxidant activity with the higher colour intensity, which correlates to a higher total antioxidant activity (Awika et al., 2003). However, up to date, no study has been conducted to determine the relationship between the colour and the antioxidant activity of the *I. batatas* storage roots with the antioxidant activity in their leaves. Therefore, we hope to incorporate more varieties of *Ipomoea batatas* leaves from the different geographical areas in the future so that larger comparison can be conducted and the antioxidant components in the leaves can be utilised entirely. This study has also shown the potential of *I. batatas* leaves as a cheap and beneficial source of natural antioxidant.

Materials and methods

Chemicals

DPPH, vitamin C or L-ascorbic acid, vanillin, catechin and Folin-Ciocalteau reagent were purchased from Sigma (MO, USA). All the chemical solvents (AR grade) used in this study were purchased from Sigma (MO, USA).

Plant materials

Six different varieties of *Ipomoea batatas* leaves were used in this experiment namely *Ipomoea batatas* var. Batu Kelantan (BK), *Ipomoea batatas* var. Batu Biasa (BB), *Ipomoea batatas* var. Biru Putih (BP), *Ipomoea batatas* var. Oren (Oren), *Ipomoea batatas* var. Vitato (Vit) and *Ipomoea batatas* var. Indon (Indon). The *Ipomoea batatas* leaves were collected in two harvesting periods (January and June 2010) from a commercial sweet potato farm in Tg. Sepat, Kuala Langat, Selangor which is located about 90 kilometres from the Post Harvest Laboratory, University of Malaya. The matured leaves were harvested and used in this study (from position 9^{th} to 18^{th} on the stalk) whereas senescence and necrotic leaves were omitted from analyses. The leaves were labelled and annotated with the date of collection and deposited at the laboratory. Firstly, the leaves were thoroughly washed before any antioxidant analyses to remove the dirts. Then, the leaves were ground into fine powder with liquid nitrogen and stored at -80 ℃ freezer prior to analyses. Methanol was added to the powdered leaves sample (0.5 g of powdered leaves to 50 ml of methanol ratio) and the mixture was placed in a shaking incubator at 25 ºC and 150 rpm for 1 hour. The mixture was centrifuged and the supernatant were used for antioxidant analyses. All the leaf extracts were prepared freshly prior to antioxidant analyses. Water content in the *Ipomoea batatas* leaves was calculated by drying the leaves completely in the oven for 48 hours. The percentage of the leaves dry weight was determined after drying.

Total flavonoids determination

The vanillin-HCl assay was used to determine the amount of total flavonoids in the leaf extract (Sun et al., 1998). Diluted catechin standards (250 µl) were added with vanillin-HCl (1 ml) reagent in a 2 ml microcentrifuge tube. Then, the reaction mixture was incubated in a water bath for 20 min at 30 ºC. The absorbance was measure at 500 nm and blanked with 80% methanol. The steps above were repeated by substituting

catechin with the leaf extracts. The calculation for the total flavonoids was based on Sun et al. (2008).

Total phenolics determination

Total phenolics contents of the leaf extract were determined using Folin-Ciocalteau assay (Waterhouse, 2001) method with modifications. Diluted gallic acid (50 µl) was added to the 250 µl Folin-Ciocalteau reagent and the mixture was incubated for 5 min at room temperature. Sodium carbonate solution (750 µl) was then added, mixed and incubated for 2 hours at room temperature. The absorbance was recorded at 765 nm using the UV-200 spectrophotometer (MRC). A standard curve was drawn using gallic acid of different concentrations (100 mg/l, 200 mg/l, 300 mg/l, 400 mg/l and 500 mg/l). The steps above were repeated by substituting gallic acid with leaf extracts. The concentration of the leaf extracts used was modified (100 mg/ml) to ensure the total phenolic value within the excepted standards' range. The concentration of total phenolics in the leaf extract was calculated based on the equation from the standard curve and expressed in terms of gallic acid equivalent (GAE g/100 g dry weight).

DPPH radical scavenging assay

1,1-diphenyl-2-picryl hydrazyl (DPPH) was used to determine the free-radical scavenging activity of the *Ipomoea batatas* leaf extract. This assay was performed using the protocols by Oyaizu (1986) and Bae and Suh (2007). A 0.1 mM DPPH solution (Sigma, USA) was prepared in 80% methanol, and then 1 ml of this solution was added to 500 µL of the different concentrations of vitamin C (L-ascorbic acid) as standard. The mixture was vortexed for 15 seconds and incubated in the water bath at 37 ºC for 30 min. The absorbance was read at 517 nm. The steps above were repeated by replacing vitamin C with the different concentrations of leaf extract in methanol. The initial leaf extract (in 1 mg/ml) was diluted with the specific volume of methanol to prepare the required concentration of leaf extract. The IC_{50} values were calculated from the graph which represents the concentration of the sample required to scavenge 50% of the DPPH free radicals.

Reducing power assay

The method was based on Oyaizu's (1986) procedures with modifications. The different concentration of leaf extracts were prepared by dilution with methanol. For instance, 400 µl of the 1 mg/ml leaf extract was diluted with 100 µl of methanol to prepare the extract with the required concentration of 800 µg/ml. The leaf extracts (50 µl) of the different concentrations were added with 200 µl of 0.2 M phosphate buffer (pH 6.6) and 200 µl of 1% potassium ferricyanide. The mixture was incubated in the water bath for 20 min at 50 ºC. Trichloroacetic acid (250 µl) was added to the mixture and was centrifuged at 1000 rpm for 10 min at room temperature. The supernatant (500 µl) was added with 500 µl of deionised water and 100 µL of 0.1 % ferric chloride. The mixture was incubated in the oven at 37 ºC for 10 min. Absorbance was recorded at 700 nm.

Statistical Analysis

All the analyses were conducted in triplicates for each harvesting season. The antioxidant values for the leaf extracts were evaluated with the one-way ANOVA and Tukey's Multiple Range Test using SPSS software (SPSS 19, IBM). P values less than 0.05 were considered to be statistically significant. Values were expressed in means ± SD.

Conclusion

In conclusion, the leaf extracts of all six *Ipomoea batatas* varieties have shown the presence of total flavonoids, total phenolics, reducing activity and the ability to scavenge free radicals. *Ipomoea batatas* var Indon used in this study contained highest level of total phenolics and scavenging activity with the IC_{50} value of 372.4 µg/ml and thus a potential source for antioxidants. Besides, all the different varieties of *Ipomoea batatas* leaves are found to be stronger scavenger compared to vitamin C standard except for the Biru Putih variety. The reducing power of all the *Ipomoea batatas* leaf extracts increased with concentration with the Batu Kelantan and Indon varieties having overall higher level of reducing power. Despite differences in their storage roots, all the *Ipomoea batatas* leaves varieties contained antioxidants that are beneficial to the human body. Hence, the *Ipomoea batatas* var Indon used in this study can be suggested as a suitable source of natural antioxidants.

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