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Full Length Research Paper

Polyphenolic content and their antioxidant activity in leaf extract of sweet potato *(Ipomoea batatas*)

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Nowadays, phytochemicals and antioxidants in plants are raising interest in consumers for their roles in the maintenance of human health. Phenolics and flavonoids are known for their health-promoting properties due to protective effects against cardiovascular disease, cancers and other disease. In this study, total phenolic (TP), total flavonoids (TF) and antioxidant activities of the six different varieties of sweet potato (*Ipomoea batatas*) leaf extracts were determined. TP content varied from 4.47±1.88 to 8.11±2.11 mg/g DW in the leaf extracts and high content of TP was observed in Vardaman variety. TF content in the extracts ranged from 1.87±0.84 and 3.95±0.91 mg/g DW and Bush Porto Rico variety showed highest content. The antioxidant activity determined by the 1,1-diphenyl-2-picryl hydrazyl (DPPH) and ferric reduction antioxidant power (FRAP) showed high activities (IC_{50} value of 184.3 µg/mL) in the leaves of Vardaman variety while the Centennial variety showed the lowest activity (IC_{50}=450.46 µg/mL). Antioxidant activity was highly correlated with TP content (R^2=0.827, P<0.001) while, no significant correlation was observed between TF content and antioxidant activity (R^2=0.0448). Therefore, the total phenolic content could be served as a useful indicator for the antioxidant activities of sweet potatoes. This study validated the medicinal potential of the sweet potatoes leaf.

Key words: *Ipomoea batatas*, total phenolic (TP), total flavonoids (TF), 1,1-diphenyl-2-picryl hydrazyl (DPPH) assays.

INTRODUCTION

Plants are a potential source of natural antioxidants. Natural antioxidants or phytochemical antioxidants are secondary metabolites of plants (Ghasemzadeh et al., 2010a). Carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols, tocotrienols, etc. are among the antioxidants produced by plants for their own sustenance. The ability of antioxidants to prevent food deterioration and extend the shelf life of the food causes it to be used widely in the food industry (Ghasemzadeh et al., 2012;Fresco et al., 2006). However, these synthetic antioxidants are suspected to increase the risk of cancer in human and liver damage (Mavundza et al., 2010).

Antioxidants are substances that when present in low concentrations, compared to those of an oxidisable substrate significantly delay or prevent oxidation of that substance. Diet with high consumption of antioxidant rich fruits and vegetables significantly reduces the risk of many cancer diseases suggesting that, confident antioxidants could be effective agents for the inhibition of cancer spread. These agents present in the diet are a group of compounds with low toxicity, safe and generally

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Abbreviations: TP, total phenolic; **TF,** total flavonoids; **DPPH,** 1,1-diphenyl-2-picryl hydrazyl; **FRAP,** ferric reduction antioxidant power.

accepted (Fresco et al., 2006). Therefore, the search for alternative sources of natural antioxidant is becoming increasingly important. Flavonoids are also a kind of natural antioxidant substances capable of scavenging free superoxide radicals, thus displaying anti-aging properties and reducing the risk of cancer. The study of flavonoids has been intensive due to their antioxidant property that contributes to good health in human. In recent years, research about carcinogenic potential of flavonoids like as quercetin has exhibited its promise as an anticancer agent. Likewise, in vitro and in vivo studies showed that quercetin was able to inhibit viability of leukemic cells, colon and ovarian carcinoma cell and especially human breast cancer cells (Davis et al., 2000;Gibellini et al., 2010). *Ipomoea batatas* or the sweet potato is planted mainly for their storage roots. During the harvesting period, 95 to 98% of the leaves are discarded while the remaining 2 to 5% is used as animal feeds. The discarded leaves can be a potential source of natural antioxidant. In recent years, several reports have indicated that the phytochemicals in sweet potatoes displayed antioxidative or radical-scavenging activity and exerted several health-promoting functions in humans (Konczak-Islam et al., 2003; Rabah et al., 2004; Suda et al., 2003). A red-fleshed sweet potato cultivar grown in the Andean region has been reported to have higher antioxidant activity and phenolic content than a cultivar of blueberry, a fruit with high levels of antioxidants (Cevallos and Cisneros, 2003). This could suggest the potential that the *I. batatas* leaves possessed as a source of cheap natural antioxidant. The study of antioxidant capacity in *I. batatas* leaves has been limited compared to their storage roots and so far no research has been conducted to determine the levels of antioxidant activity in the different varieties of leaves. The objective of the present study was to determine medicinal value of the six different varieties of *I. batatas* leaf extracts based on their phenolic acids and flavonoids content.

MATERIALS AND METHODS

Plant material

Six different varieties of *I. batatas* leaves were used in this experiment namely *Beauregard*, *Bush Porto Rico*, *Centennial*, *Georgia Jet*, *Jewell* and *Vardaman*. The *I. batatas* leaves were collected during the harvesting period (April, 2011) from the Tropical Pertanian farm (TPU) in University Putra Malaysia. The average light intensity in farm was 1165 µmol/m^2/s, day and night temperatures were recorded at 30±1.0 and 20±1.5°C, respectively, and relative humidity at about 70 to 80%. Matured leaves were harvested and thoroughly washed with water to remove dirt. After drying under shade, leaves were dried by freeze drier and were ground into fine powder using a mortar and kept at -80°C for future analysis.

Extract preparation

Leaves were freeze dried to constant weights prior to being used in the extraction. One gram of the powder was extracted continuously with methanol (80%, 50 ml). The solution was then swirled for 1 h at room temperature using an orbital shaker. Extracts were then filtered under suction and stored at -20°C for further use.

Total flavonoids determination

The TF were measured following a previously reportedspectro-photometric method (Ghasemzadeh et al., 2010a). Briefly, extracts of each plant material (1 ml) were diluted with 4 mL of H_2O in a 10 ml volumetric flask. Initially, 5% $NaNO_2$ solution (0.3 ml) was added to each volumetric flask; after 5 min, 10% $AlCl_3$ (w/w) was added; and at 6 min, 1.0 M NaOH (2 ml) was added. Absorbance of the reaction mixture was read at 430 nm. Quercetin used as a standard and total flavonoid contents was expressed as mg quercetin/g DW.

Total phenolics determination

The total phenolic content was determined following the method of Ghasemzadeh et al. (2010a). Briefly, 1 ml of extract was added to deionized water (10 ml) and Folin–Ciocalteu phenol reagents (1.0 mL). After 5 min, 20% sodium carbonate (2.0 ml) was added to the mixture. The solution was kept in total darkness, and the absorbance was measured at 750 nm using a spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan). Gallic acid used as standard and total phenolic contents was expressed as mg gallic acid/g DW.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma–Aldrich. Butylated hydroxytoluene (BHT) and α-tocopherol were purchased from Merck (India). In order to determine the radical scavenging ability, the method reported by Mensor et al. (2001), was used. Briefly, an alcohol solution of DPPH (1 ml, 3mg/ml) was added to 2.5 ml samples containing different concentrations of extracts originating from leaves of the *I. batatas* varieties. The samples were first kept in a dark place at room temperature and their absorbance was read at 518 nm after 30 min. The antiradical activity (AA) was determined using the following formula:

AA% = (Abs: sample - Abs: empty sample)/Abs: control) × 100

Blank samples contained 1 ml ethanol + 2.5 ml of various concentrations of plant extract; control sample contained 1 mL of 0.3 mM DPPH + 2.5 mL ethanol. The concentration of the samples, the control, and the empty samples were measured in comparison with ethanol. The synthetic antioxidants BHT (butylated hydroxytoluene) and α-tocopherol were used as positive controls.

Ferric reducing antioxidant potential (FRAP Assay)

The stock solutions included 300 mM acetate buffer, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3$ solution. Acetate buffer (25 ml) and TPTZ (2.5 ml) were mixed, and 2.5 ml $FeCl_3$ added. Plant extracts (150 µl) were added to 2850 µl of the FRAP solution and kept for 30 min in the dark. The absorbance was measured at 593 nm using a spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan) (Benzie and Strain, 1996).

Statistical analysis

The experimental results were expressed as mean ± standard

Table 1. Total phenolic and flavonoid contents of the leaf extracts of *Ipomoea batatas.*

Varieties	TP (mg gallic acid/g DW)	TF (mg quercetin/g DW)
Georgia Jet	6.21±1.52[c]	2.47±1.17[b]
Vardaman	8.11±2.11[a]	1.87±0.84[c]
Beauregard	6.89±1.62[c]	3.25±1.3[a]
Bush Porto Rico	4.79±2.41[d]	3.95±0.91[a]
Centennial	4.47±1.88[d]	2.31±0.78[b]
Jewell	7.32±2.51[b]	2.98±0.92[b]

All analyses are the mean of triplicate measurements ± standard deviation. Means not sharing a common letter were significantly different at $P \leq 0.05$.

deviation of three replicates. Where applicable, the data were subjected to one-way analysis of variance (ANOVA) and the differences among samples were determined by Duncan's multiple range test using the statistical analysis system (SAS, 1999) and MSTATC programs. P-value of < 0.05 was regarded as significant.

RESULTS AND DISCUSSION

Flavonoids and total phenol contents of the extracts

The level of phenolic compounds in methanolic extracts of the six different varieties of *I. batatas* leaf extracts are presented in Table 1. The flavonoids values were between 1.87±0.84 and 3.95±0.91 mg/g DW. The Bush Porto Rico variety has the highest condensed flavonoids content at 3.95±0.91 mg/g DW followed by the Beauregard variety. The Centennial and Georgia Jet varieties had almost similar TF contents. However, the Vardaman variety contained the lowest TF content compared to the others at 1.87±0.84 mg/g DW. The differences in the total flavonoids content were statistically significant between the different varieties. Hence, different levels of TF were observed for the different varieties of the *Ipomoea batatas* leaves used despite all of them belonging to the same plant species.

Comparison of the TF results between *Bush Porto Rico* (2.78 to 3.95 mg/g DW) and other plants, for example, onion leaves (1.54 mg/g DW), semambu leaves (2.041 mg/g DW), black tea (1.41 mg/g DW), papaya (1.26 mg/g DW), bird chilli (1.66 mg/g DW), garlic (1.2 mg/g DW) and guava (1.12 mg/g DW) (Khoo and suhaila , 2001), shows the good potential of this component in this variety. It can be concluded that all the *I. batatas* leaves varieties used in this study could be used as a cheap source of flavonoids.

The TP contents were studied among the different varieties and results were shown in Table 1. The values of total phenol were between 4.47±1.88 to 8.11±2.11 mg/g DW. This study showed that, the TP contents of the different varieties were significantly different from each other. Vardaman variety with total phenol content of 8.11 mg/ g DW had the highest amount compared to the other varieties. This showed that the six varieties of *I. batatas* leaves used in this study did not fall into the higher tier and the deviation might takes into account the cultivar and the place of cultivation of the plant.

TP content in *I. batatas* varieties showed week potential when compared to other medicine plants, for example Calotropis (14.94 mg/g DW), Hibiscus (29.96 mg/g DW), Parthenium (8.29 mg/g DW) and Kigelia (15.04 mg/g DW) and Ginger (39.06 mg/g DW) (Ghasemzadeh et al., 2010b). Polyphenols have the function to scavenge the free radicals in human body and to help maintain healthy body by scavenging or removing the reactive oxygen species (ROS). Hence, both the polyphenols and flavonoids play important roles as antioxidant in human body (Ghasemzadeh et al., 2011). It was observed that some phenolic compounds are highly correlated with the antioxidant activity in plant such as phenolic acid (gallic acid) and polyphenol (flavonoids) (Ghasemzadeh et al., 2010a; Hasna and Afidah, 2009). In previous studies, the *I. batatas* leaf extracts were found to have radical scavenging activity, antimutagenic, anticancer and antibacterial activities (Konczak-Islam et al., 2003). Hence, these protective properties of the *I. batatas* leaf extracts might be attributed to the presence of the high levels of polyphenols and flavonoids compounds.

Antioxidant activity

Free radicals and reactive oxygen species (ROS) contribute to the occurrence of many degenerative diseases such as arthritis, cirrhosis, cancer, Alzheimer's disease and aging. Antioxidant extracted form plants are useful in preventing these degenerative diseases. At a concentration of 200 µg/ml, the scavenging activity of the methanol extract of Vardaman variety leaves reached to 62.12%, while at the same concentration DPPH activity in other varieties were 49.3% (Beauregard), 41.7% (Jewell), 39.64% (Bush Porto Rico), 36.73% (Georgia Jet) and 32.8% (Centennial), (Figure 1). IC_{50} is used to express the amount or concentration of extracts needed to scavenge 50% of the free radicals. The value of IC_{50} is inversely proportional to the scavenging activity of the

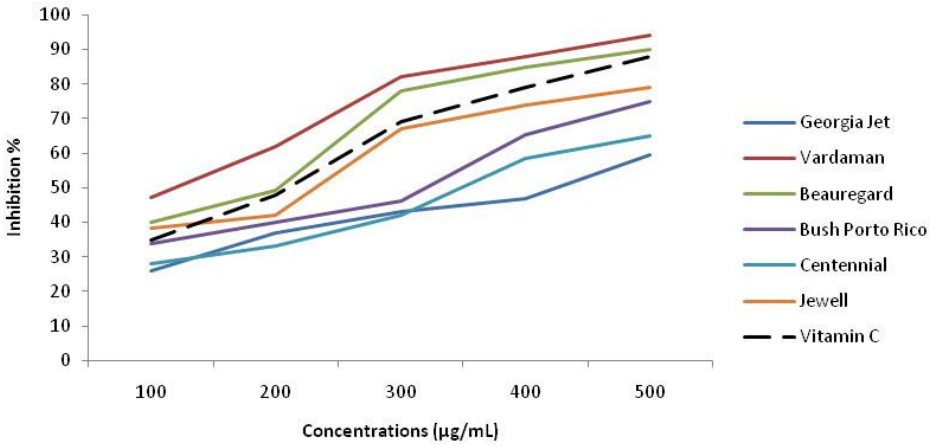

Figure 1. DPPH radical scavenging activity of the leaf extract of *Ipomoea batatas* varieties.

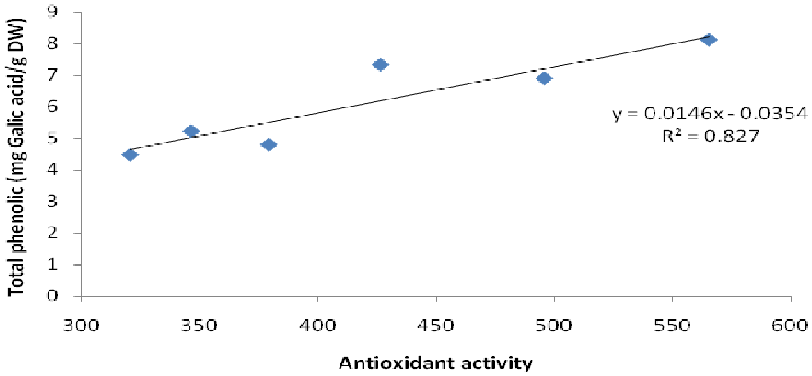

Figure 2. Correlation between antioxidant activity and total phenolic contents.

leaf extract and was shown in Figure 1. The Vardaman variety has the highest scavenging activity with the IC_{50} value of 184.3 µg/ml while the Centennial variety has the lowest activity (IC_{50}= 450.46 µg/ml). The order of the radical scavenging activity in descending order for the different varieties of leaf extracts is as follows: Vardaman (IC_{50} 184.3 µg/ml) > Beauregard (IC_{50} 226.92 µg/ml) > Jewell (IC_{50} 257.4 µg/ml) > Bush Porto Rico (IC_{50} 326.6 µg/mL) > Georgia Jet (IC_{50} 381.2 µg/mL) > Centennial (IC_{50} 450.46 µg/mL). Between these varieties, Vardman and Beauregard showed higher radical scavenging activity compared to the ascorbic acid (vitamin C) standard (IC_{50} = 238.6 µg/mL). This showed that the leaf extracts have high amount of radical scavenging compounds with proton-donating ability. The lower radical scavenging activity observed in the *Centennial* leaves variety was perhaps contributed by the lower total polyphenols and flavonoids level. This is further confirmed by the higher radical scavenging activity observed in the Vardaman leaves variety which contains higher level of total polyphenol.

In this study, the leaf extracts which found to have higher TP contents showed higher radical scavenging activity. Figure 2 and 3, shows that the antioxidant activity was highly correlated with TP content (R^2=0.827, P<0.001) while, no significant correlation was observed between TF content and antioxidant activity (R^2=0.0448). These findings further supported the idea of a positive relationship between TP and antioxidant activities of plants. However, antioxidant activity is found to be linearly proportional with phenolic compounds. Oktay et al. (2003) and Ghasemzadeh et al. (2011) reported strong positive relationships between total flavonoids contents and antioxidant activity, which appears to be the trend in many plant species.

The high scavenging activity of the *I. batatas* leaves was further confirmed by the study conducted by Yang et al. (2005) in which sweet potato leaves ranked in the first place with highest DPPH radical scavenging activity among 23 commonly consumed vegetables in Taiwan. Several methods are known to measure the total antioxidant capacity of herbs, including FRAP assay, which we have adopted in this study. The FRAP assay depends upon the reduction of ferric tripyridyltriazine

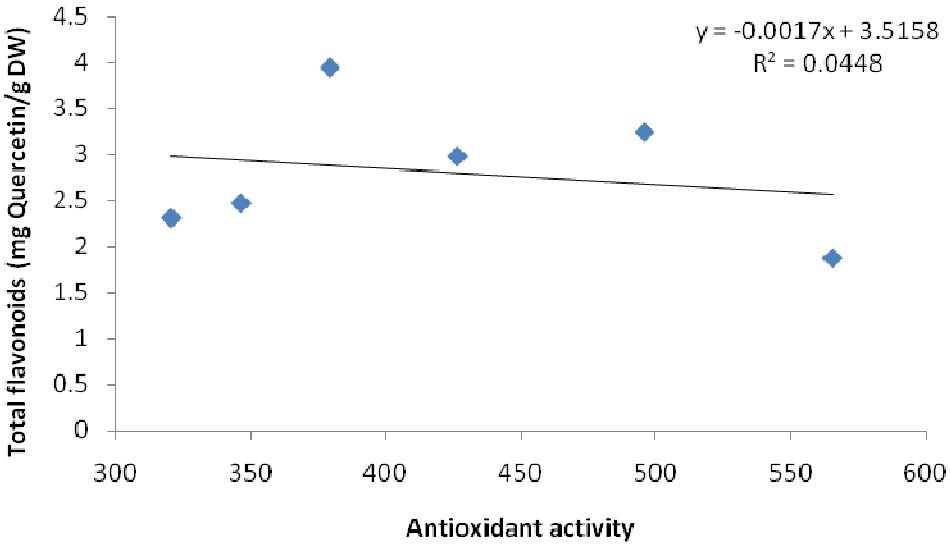

Figure 3. Correlation between antioxidant activity and total flavonoid contents.

Table 2. FRAP assay activity and IC_{50} value of the leaf extract of *Ipomoea batatas* varieties.

Varieties	FRAP (µmol Fe (II)/g DW)	IC_{50} (µg/ml)
Georgia Jet	346.3±4.24^{c}	381.2
Vardaman	565.3±8.42^{a}	184.3
Beauregard	495.7±6.73^{b}	226.92
Bush Porto Rico	379.2±5.21^{c}	326.6
Centennial	320.5±4.17^{c}	450.46
Jewell	426.4±6.42^{b}	257.4
Vitamin C	746.2±10.52	238.6

All analyses are the mean of triplicate measurements ± standard deviation. Means not sharing a common letter were significantly different at *P≤0.05*.

(Fe(III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe(II)-TPTZ) at low pH.

All the *I. batatas* varieties showed almost similar pattern of increment in their reducing power when the concentrations of the leaf extracts increase (Table 2). The Vardaman and Beauregard varieties showed overall higher reducing ability respectively 565.3±8.42 and 495.7±6.73 µmol Fe (II)/g DW compared to other varieties. Lower reducing power (320.5±4.17 µmol Fe (II)/g DW) was observed in Centennial variety. The reducing capacity of the plant is much related to the presence of biologically active compounds with potent donating abilities. Beside total phenol and total flavonoids, the phytochemical anthocyanins also contributed to the high antioxidant activity of the *I. batatas* leaves (Islam et al., 2003).

In this study, the Vardaman leaves variety which has purple storage roots showed the highest level of radical scavenging and reducing power activity compared to the other varieties with different flesh colour.

Conclusion

The study clearly indicates that, it is important to measure the antioxidant activity using various radicals and oxidation system and to take both phenolic content and antioxidant activity into account while evaluating the antioxidant potential of plant leaf extracts. The leaf extracts of all six *I. batatas* varieties showed the presence of antioxidants and the ability to scavenge free radicals. Antioxidant activities varied widely among the sweet potato varieties. There were good correlations among the TP content and antioxidant activity, and this correlations suggest that the antioxidant activities of the medicinal plants can be mainly ascribed to their phenol compounds. The present study suggested that the crude extract as well as the phenolics from *I. batatas* could be

used for future development of naturally-occurring antioxidants. A greater research effort should be devoted to confirm antioxidant activities of the phenolics from *I. batatas* which may be applied in food, medicinal and agriculture industry as a source of antioxidative agents. Thus, it seems that leaf of sweet potato especially vardaman variety could be used for traditional medicine and food flavoring.

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