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Antioxidative activity of water extract of sweet potato leaves in Taiwan

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ABSTRACT

This study reports the preparation of four varieties of water extract from sweet potato leaves from Taiwan, including TNG10, TNG57, TNG66 and YSP, and evaluates their antioxidative activity. The EC_{50} values (scavenging DPPH radicals) of TNG10, TNG57, TNG66 and YSP were 0.27 ± 0.01, 0.19 ± 0.01, 0.41 ± 0.02, and 0.31 ± 0.02 mg/ml, respectively, on a freeze-dry weight basis. The total phenolic contents of these water extracts were in the order: TNG57 > TNG10 > TNG66 > YSP. The TNG10 and TNG57 extracts exhibited better reducing power and scavenging effects of superoxide radicals than did TNG66 and YSP. At a concentration of 1 mg/ml, TNG10 and TNG57 significantly protected HaCaT cells from H_2O_2-induced cytotoxicity. The water extracts of YSP had more flavonoids than had those of TNG66 which may have contributed to their higher activity in many antioxidative assays. These results suggest that the water extracts of all four varieties of sweet potato leaves, and especially TNG10 and TNG57, display potent antioxidative effects.

1. Introduction

The modern dietary habit, a low vegetable but high-calorie, high-fat and high-sugar intake, is usually associated with chronic diseases such as atherosclerosis, neurodegeneration, aging, cancer, diabetes mellitus, and inflammation (Brunetti et al., 2009; Duračková, 2010; Khandrika, Kumar, Koul, Maroni, & Koul, 2009). This is likely due to endogenous and exogenous factors that create an imbalance between free radicals and antioxidative defence systems, provoking oxidative stress (Duračková, 2010). Therefore, sufficient intake of antioxidants may be necessary to repress oxidative stress and prevent diseases and aging (Gaziano, 1994; Lenaz, 1998). Antioxidative substances, such as phenolic compounds, flavonoids, tocopherol, and ascorbic acid, appear in many fruits and vegetables (Suzuki et al., 2004). A diet of natural foods with antioxidant compounds can protect the human body from oxidative stress and associated chronic diseases induced by endogenous and exogenous factors (Morganti, 2009).

Sweet potato is the fifth most important food crop in terms of fresh weight (CGIAR, 2005). In Taiwan, sweet potato was the most abundant coarse crop from 2004–2008 (COA, 2008). Sweet potato can attenuate oxidative damage, inflammation, aging and hypertension with its many antioxidant compounds, including polyphenolic compounds, flavonoids, and proteins (Huang, Chen, Hou, Lin, & Lin, 2004; Rumbaoa, Cornago, & Geronimo, 2009; Zhang et al., 2009). The methanol extract of sweet potato leaves has the highest phenolic acid content, followed by the peel, whole root, and flesh tissue (Truong, McFeeters, Thompson, Dean, & Shofran, 2007).

The antioxidative activity of the phytochemicals in sweet potato leaves can be measured by their total phenol contents, reducing power, metal ion chelating activity, and scavenging activity against free radicals, such as superoxide anion, and hydrogen peroxide (Huang, Chu, Juang, & Wang, 2010; Singh & Rajini, 2004). This study investigates the antioxidant compounds and antioxidative activity of water extracts of different varieties of sweet potato leaves in Taiwan, and also, the effect of water extract of sweet potato leaves on hydrogen peroxide-induced cytotoxicity in HaCaT cells.

2. Materials and methods

2.1. Materials

1,1-Diphenyl-2-picrylhydrazyl (DPPH·), $FeCl_2{\cdot}4H_2O$, trichloroacetic acid, phenazine methosulfate (PMS), nicotinamide adenine dinucleotide (NADH), nitroblue tetrazolium (NBT), ferrozine, ferric chloride ($FeCl_3$), and potassium ferricyanide ($K_3Fe(CN)_6$), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma chemical Co. (St. Louis, MO). Dulbecco's Modified Eagle's Medium (DMEM), foetal bovine serum (FBS), and penicillin–streptomycin were purchased from Gibco BRL (Life Technology, Paisley, Scotland).

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2.2. Samples

Samples Tainung 10 (TNG10), Tainung 57 (TNG57), Tainung 66 (TNG66), and Yellow leaf (YSP) were provided by Chiayi Agricultural Experiment Station, Agriculture Research Institute (AGI), Taiwan. The leaf of TNG10 is heart-shaped with a green colour .The stem colour is purple red and the root is spindle-shaped with white skin and white flesh. The leaf of TNG57 is palm-shaped with green colour, and the root is spindle-shaped with yellow skin and yellow flesh. The leaf of TNG66 is heart-shaped with green colour, and the roots are spindle-shaped with red skin and red flesh. Yellow leaf is a local sweet potato line. The top part of the vine is a semi-compact plant type with yellow colour of leaf and stem. The leaf shape is like a palm.

Samples were collected from three different regions in Taiwan. We prepared all varieties of WE-FD from 2–3 bundles (each bunch containing 9–12 leaves) of sweet potato leaves from each region. Regions of samples were prepared and combined. Before preparation, representative samples were verified and randomly picked by Dr. Lai who is an expert in sweet potato breeding. Briefly, the fresh leaves (without stems) were washed, air-dried, weighed, and ground to small pieces. Then samples were immersed in double-distilled water (leaves to water ratio 1:3) and boiled at 100 °C for 20 min. Sample vacuum-filtration was done through Whatman No. 1 paper after boiling .The water extracts were then completely freeze-dried (WE-FD) and stored at −20 °C. Samples were dissolved in double-distilled water at a concentration of 10 mg/ml as a stock solution before use.

2.3. Total phenolic content

The total phenolic content was determined by Taga's method (Taga, Miller, & Pratt, 1984). Different concentrations (0.125, 0.25 and 0.5 mg/ml) of 0.3 ml of WE-FD solutions were mixed with 2.4 ml of distilled water and 0.3 ml Folin–Ciocalteu reagent. Double-distilled water was used as vehicle control. Sodium carbonate (20%, 0.6 ml) was added to the reaction mixture and allowed to stand for 30 min. The absorbance at 730 nm was measured and compared to a gallic acid calibration curve and expressed as mg of gallic acid per gramme of sample (GAE).

2.4. DPPH free radical-scavenging assay

The scavenging activity of sweet potato extracts on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined using the method described by Huang et al. (2010). Fifty microlitre of different concentrations (0.05, 0.1, 0.25, 0.5 and 1 mg/ml dissolved in double-distilled water) of sweet potato extracts were mixed with 150 μl of freshly prepared 1 mM DPPH**.** in ethanol. Double-distilled water was used as vehicle control and ascorbic acid was used as positive control. The mixture was kept in the dark for 30 min. DPPH**.** absorbance was then measured at 517 nm, using an ELISA reader (TECAN, Austria). Percent activity was calculated using the following equation:

$$\%\,\text{Activity} = (1 - (A_{\text{Sample}}/A_{\text{Blank}}) \times 100$$

The EC_{50} value, which is the sample concentration at 50% activity, was determined by interpolation. Each test was carried out in triplicate.

2.5. Flavonoids content

The flavonoids content was determined using the $AlCl_3$ method (modified from Quettier-Deleu et al., 2000). Each WE-FD extract (0.25 mg/ml; 0.5 ml) was mixed with 1 ml of 2% of methanolic $AlCl_3{\cdot}6H_2O$, and absorbance was measured 10 min later at 430 nm. Double-distilled water was used as vehicle control. The flavonoids content was calculated, using the calibration curves of quercetin, and expressed as mg of quercetin (QE) per gramme of sample.

2.6. Reducing power assay

The Singh and Rajini (2004) method was used to determine the reducing power of the extracts. One hundred microlitre of sweet potato extracts at different concentrations (0.05, 0.1, 0.25, 0.5 and 1 mg/ml dissolved in double-distilled water) were mixed with 100 μl of 0.2 M phosphate buffer, pH 6.6, and 100 μl of 1% (w/v) $K_3Fe(CN)_6$. Double-distilled water was used as vehicle control and ascorbic acid was used as positive control. The mixture was incubated at 50 °C for 20 min in a water bath. Ten percent (10% w/v) trichloroacetic acid (100 μl) was added and the resulting mixture was centrifuged at (3000 rpm) for 10 min. One hundred microlitre of the supernatant were combined with 100 μl of distilled water and 20 μl of 0.1% (w/v) $FeCl_3$ solution. The absorbance was measured at 700 nm, using the V630 UV–Vis Spectrophotometer (JASCO Co. Ltd., Japan). Interpolation from the linear regression analysis of Huang, Chang, and Shao (2005) revealed that the absorbance was 0.5 for EC_{50} of reducing power.

2.7. Fe^{2+}-chelating capacity assay

The iron-chelating capacity of the sample was determined, using the method proposed by Dinis, Maderia, and Almeida (1994). Sample solutions, with a concentration of 0.1–1 mg/ml, were prepared from the stock solution. A 25 μl aliquot was mixed with 175 μl of methanol, 25 μl of 400 μM $FeCl_2^{\cdot}$ $4H_2O$, and 25 μl of 2 mM ferrozine. The mixture was allowed to stand for 10 min, and the absorbance was then measured at 562 nm, using an ELISA reader (TECAN, Austria). EDTA was used as positive control. This test was carried out in triplicate.

2.8. Superoxide radical-scavenging assay

The superoxide-scavenging ability of the extract was assessed, using the method of Nishikimi, Appaji, and Yagi (1972). The phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) system generates superoxide radicals, which reduce nitro blue tetrazolium (NBT) to a purple diformazan. Briefly, a reaction solution, containing 50 μl of various concentrations of sweet potato extract (0.05–1 mg/ml) mixed with PMS (80 μM), NADH (1248 μM), and NBT (200 μM) in phosphate buffer (0.1 M pH 7.4), was incubated at room temperature for 5 min. Double-distilled water was used as vehicle control and quercetin was used as positive control. The absorbance was read at 560 nm against blank samples. The superoxide radical-scavenging capability was calculated, using the following equation:

$$\text{Scavenging effect}(\%) = 1 - (A_{\text{Sample 560 nm}}/A_{\text{Control 560 nm}}) \times 100$$

2.9. Cell culture and experimental treatment

Human keratinocytes (HaCaT cells) were cultured at a density of 5×10^4 cells/ml DMEM medium supplemented with FBS (10%, v/v), streptomycin (100 μg/ml), and penicillin (100 U/ml) and kept at 37 °C in a 5% CO_2 humidified atmosphere. Drug treatments were applied 24 h after seeding the cells. To induce oxidative stress, H_2O_2 was prepared from 8.8 M stock solution prior to each experiment. HaCaT cells were incubated with sweet potato water extract for 3 h before exposure to different concentrations of H_2O_2.

2.10. Determination of protective effect against H_2O_2-induced cytotoxicity

To determine the protective effect of sweet potato water extract against H_2O_2 toxicity, HaCaT cells were seeded in a 96-well plate and treated with various concentrations of sweet potato water extract (0.1–1 mg/ml) after 24 h. Three hours later, cells were incubated with various concentrations of H_2O_2 for 24 h. The viability of HaCaT cells was determined using the MTT assay, which is a colourimetric assay that measures the reduction of yellow MTT by mitochondrial succinate dehydrogenase to an insoluble, coloured (dark purple) formazan product (Vistica et al., 1991). Briefly, 10 μl of MTT (5 mg/ml) were added to each well for 1 h. The supernatant was then removed and the formazone crystals were dissolved in 100 μl of DMSO. The absorbance was read at 570 nm with an ELISA reader (TECAN, Austria). Cell viability was expressed as percentage of surviving cells relative to the control cell samples.

2.11. Statistical analysis

Three samples were prepared for each WE-FD assay. The results were expressed as means and standard deviation. Data analysis included one-way ANOVA, followed by Duncan's multiple range test ($p < 0.05$), and a correlation test using the SigmaStat 3.5 program.

3. Results and discussion

3.1. Total phenolic and flavonoids contents

Among the four sweet potato varieties, TNG 57 had the highest total phenolic content (130 ± 10.1 mg GAE/g sample), followed by TNG10 (104 ± 16.3 mg GAE/g sample), TNG66 (65.3 ± 6.16 mg GAE/g sample), and YSP (28.1 ± 4.07 mg GAE/g sample)(Table 1). The flavonoid contents of the WE-FD samples were in the order: TNG10 > TNG57 > YSP > TNG66 (Table 1). These results demonstrate that, though YSP had the lowest total phenolic content in all four WE-FD samples, YSP (29.6 ± 2.58 mg GAE/g sample) had a significantly higher flavonoid content than had TNG66 (18.0 ± 1.00 mg GAE/g sample). It was reported that sweet potato leaves had the highest antioxidant capacity, due to being rich in total phenolics and flavanols, followed by the fruits and root crops (Lako et al., 2007). In the Lako et al. (2007) results, total phenolics of five varieties of sweet potato leaves were between 240 and 280 mg GAE/100 g of dry sample and the quercetin contents were between 43 and 90 mg/100 g of dry sample (Lako et al., 2007). Based on the current results and Lako et al. (2007) results, sweet potato leaves have high contents of total phenolics and flavonoids that could have profound benefits for combating chronic degenerative disorders (Brunetti et al., 2009; Duračková, 2010).

3.2. DPPH radical-scavenging activity

The DPPH radical-scavenging activity of all four varieties of WE-FD increased sigmoidally at sample concentrations between 0.05 and 1 mg/ml sample (Fig. 1). These results indicate that the DPPH radical-scavenging activity of TNG10 and TNG57 reached a saturation point at concentrations of 0.5 mg/ml. However, the saturation concentrations of TNG66 and YSP were 1 mg/ml (Fig. 1). The EC_{50} of DPPH radical-scavenging activity of the WE-FD samples was inversely proportional to the order of total phenolic contents (Table 1). The exception to this trend was YSP, which had significantly higher flavonoid content than had TNG66 (Table 1). Previous research indicates that DPPH radical-scavenging is positively correlated with total phenolic content ($r = 0.71$)(Huang et al., 2005). However, the current results indicate that DPPH radical-scavenging activity was more strongly correlated with flavonoids ($r = 0.84$) than with total phenolic content ($r = 0.68$). The DPPH radical-scavenging activity can also be affected by other reducing substances, such as ascorbic acid (Table 1). It was reported that different varieties of sweet potato had different ascorbic acid contents (Huang et al., 2005; Reddy & Sistrunk, 1980) which is also a possible reason why DPPH radical-scavenging activity in our study was less correlated with total phenolics than in other reports (Huang et al., 2005).

3.3. Reducing power

Fig. 2 shows the plot of reducing power for WE-FD samples as a function of sample concentration. The reducing power of TNG10 was significantly higher than those of TNG66 and YSP at concentrations of 0.25, 0.5 and 1 mg/ml ($p < 0.05$) (Fig. 2). The reducing power of TNG57 was significantly higher than those of TNG66 and YSP at concentrations of 0.5 and 1 mg/ml ($p < 0.05$). On the basis of superoxide radical-scavenging activity, TNG10 and TNG57 had higher reducing powers than had TNG66 and YSP (Fig. 2, Table 1). The EC_{50}, by reducing powers, of WE-FD samples were not in the same order as total phenolic content (Table 1). These results are inconsistent with the Huang et al. (2005) study, which indicates that reducing power is positively correlated with phenolic content ($r = 0.98$). This difference may be because YSP had a relatively high flavonoid content in this study (Table 1). The order of reducing power of WE-FD samples was similar to the flavonoid content order. These results also indicate that flavonoids may play a more important role than the other groups of phenolic compounds in reducing power.

3.4. Iron-chelating capacity

The iron-chelating capacity of WE-FD samples was measured by assessing their ability to compete with ferrozine in chelating ferrous ion (Elmastas et al., 2006). This test measures activity as the decrease in absorbance of the red Fe^{2+}/ferrozine complex. Table 2

Table 1
Antioxidant compounds and antioxidative activity of WE-FD samples TNG10, TNG57, TNG66 and YSP.

Variety	Total phenols (mg GAE/g sample)[A]	Flavonoids (mg QE/g sample)[A]	EC_{50} (mg/ml) of WE-FD		
			DPPH·-scavenging[A]	Reducing power[A]	Superoxide radical[A]
TNG10	104 ± 16.1[a]	72.7 ± 2.08[a]	0.27 ± 0.01[b]	0.27 ± 0.02[a]	0.0.10 ± 0.01[a]
TNG57	130 ± 10.1[a]	65.2 ± 1.00[b]	0.19 ± 0.01[a]	0.34 ± 0.02[b]	0.22 ± 0.01[b]
TNG66	65.3 ± 6.16[b]	18.0 ± 2.75[d]	0.41 ± 0.02[d]	0.47 ± 0.02[c]	0.61 ± 0.02[d]
YSP	28.1 ± 4.07[c]	29.6 ± 2.58[c]	0.31 ± 0.02[c]	0.45 ± 0.03[c]	0.40 ± 0.02[c]
Positive control[B]	–	–	0.08 ± 0.01	0.11 ± 0.01	0.07 ± 0.00

[a–d] Means in a column with different small letters are significantly different ($p < 0.05$).
[A] Each value expressed as the mean ± standard deviation ($n = 3$).
[B] Ascorbic acid was used as positive control for DPPH·-scavenging and reducing power assay. Quercetin was used as positive control for superoxide radical assay.

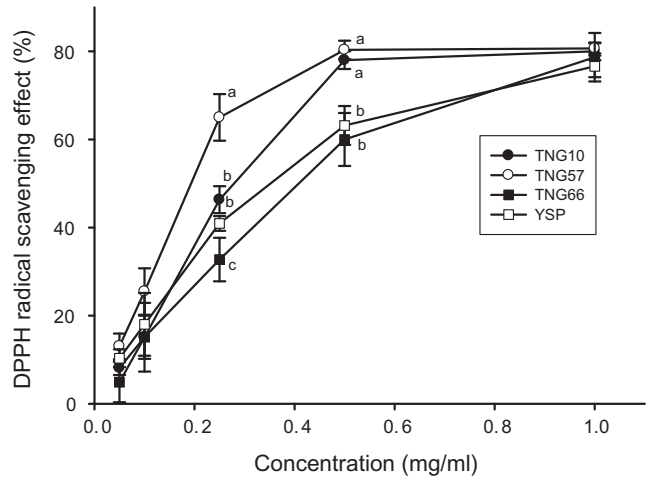

Fig. 1. DPPH radical-scavenging activity of four WE-FD samples. Values are means ± SD (n = 3). The same concentration of four WE-FD samples with different lower case letters indicates a significant difference (p < 0.05).

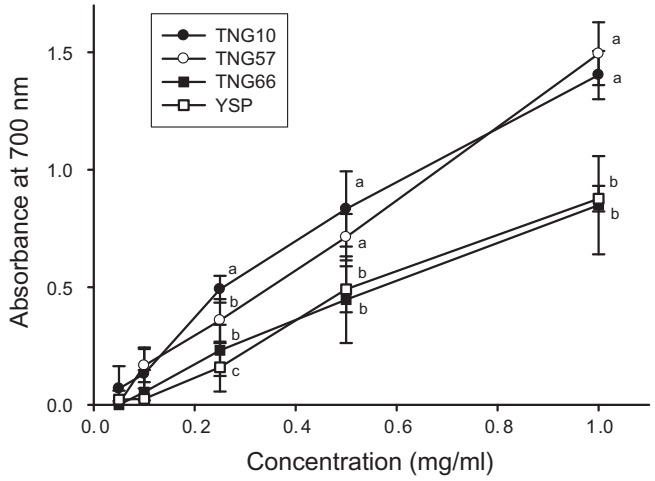

Fig. 2. Reducing power of four WE-FD samples. Values are means ± SD (n = 3). The same concentration of four WE-FD samples with different lower case letters indicates a significant difference (p < 0.05).

presents the iron-chelating capacity as a function of sample concentration. Of all four WE-FD samples, TNG10 had the highest chelating capacity, while TNG66 had the lowest. The chelating capacities of TNG10 (23.3 ± 2.56%) and TNG57 (23.1 ± 1.27%) reached the saturation point at a concentration of 0.5 mg/ml. However, TNG66 (15.6 ± 1.88%) and YSP (14.9 ± 2.68%) only reached the saturation point at a concentration of 1 mg/ml (Table 2). The EC_{50} of EDTA is 0.13 ± 0.01 mg/ml. The chelating effects of WE-FD samples ranged from 3.87% to 23.3% (Table 2). These results

Table 2
Chelating activity of different concentrations of WE-FD samples.

Variety	Chelating activity (%)		
	0.125 mg/ml[A]	0.5 mg/ml[A]	1 mg/ml[A]
TNG10	12.6 ± 2.50[a]	23.3 ± 2.56[a]	0. 22.0 ± 1.94[a]
TNG57	12.1 ± 1.21[a]	23.1 ± 1.27[a]	19.5 ± 1.13[a]
TNG66	6.37 ± 1.20[b]	12.0 ± 1.43[b]	15.6 ± 1.88[b]
YSP	3.87 ± 1.87[b]	10.1 ± 2.62[b]	14.9 ± 2.68[b]
EDTA[B]	48.2 ± 3.26	89.5 ± 2.62	94.7 ± 3.17

[a,bA] Each value expressed as the mean ± standard deviation (n = 3).
[a,bB] EC50 of EDTA is 0.13 ± 0.01 mg/ml.

support the results of Huang et al. (2010). This discrepancy may be due to different varieties and different tissues of sweet potato investigated. The lack of correlation between the antioxidant components present in the WE-FD samples and their chelating capacity was not consistent with the results of Rumbaoa et al. (2009).

3.5. Scavenging of superoxide radical

Fig. 3 plots the scavenging activity of superoxide radicals. While a marginal inhibition of only 1.26–9.28% appeared at a concentration of 0.05 mg/ml in all WE-FD samples, this inhibition increased from 81.9% to 83.9% inhibition at concentrations of 1 mg/ml WE-FD (Fig. 3). On the basis of EC_{50} of WE-FD samples (Table 1), TNG10 and TNG57 had higher scavenging effects of superoxide radicals than had YSP, while TNG66 had the lowest scavenging effects. There was negative correlation between flavonoid content (r = 0.97), but not phenolic content, on the EC_{50} of scavenging of super oxide radicals (Table 1). Flavonoids are a sub-group of phenolic compounds. In the current results, it appears that flavonoids may play a more important role than the other phenolic compounds in superoxide radical-scavenging activity (Table 1) which is consistent with the result of DPPH radical-scavenging activity.

3.6. Protection against H_2O_2-induced cytotoxicity

Hydrogen peroxide can induce oxidative stress and result in cell damage or cell death (Jeon, Yoon, Park, Lee, & Park, 2009). As Fig. 4A shows, hydrogen peroxide induces dose–response cytotoxicity in HaCaT cells. At a concentration of 250 μM H_2O_2, HaCaT cell viability fell to 52.9 ± 5.18%, which was significantly different from the results of untreated cells (p < 0.05) (Fig. 4A and B). The 250 μM H_2O_2-induced cytotoxicity was significantly attenuated by 1 mg/ml of TNG10 and 1 mg/ml of TNG57, and cell viability improved from 52.9 ± 5.18% to 77.7 ± 2.99% and 83.7 ± 3.02%, respectively (Fig. 4B). However, there was no significant (p > 0.05) protective effect on H_2O_2-induced cytotoxicity in the pretreatment with 1 mg/ml of TNG 66 or 1 mg/ml of YSP (Fig. 4B). In the current results, 1 mg/ml of different WE-FD samples produced no significant cytotoxicity in HaCaT cells (Fig. 4B). At concentrations of less than 1 mg/ml, none of the WE-FD samples prevented H_2O_2-induced cytotoxicity.

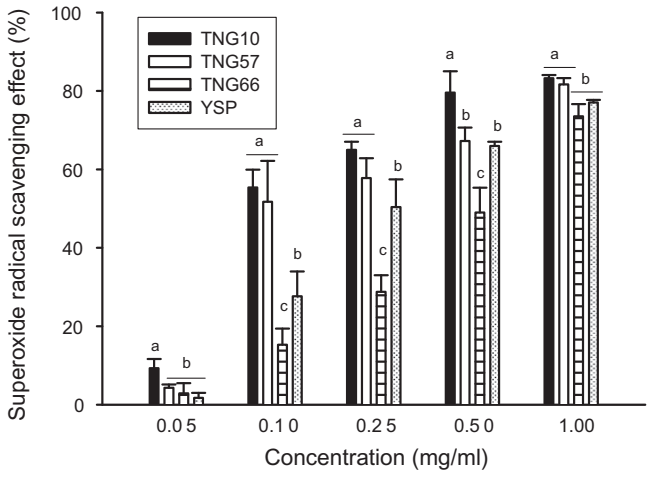

Fig. 3. Scavenging activity of superoxide radical of four WE-FD samples. Values are means ± SD (n = 3). The same concentration of four WE-FD samples with different lower case letters above the bars indicates a significant difference (p < 0.05).

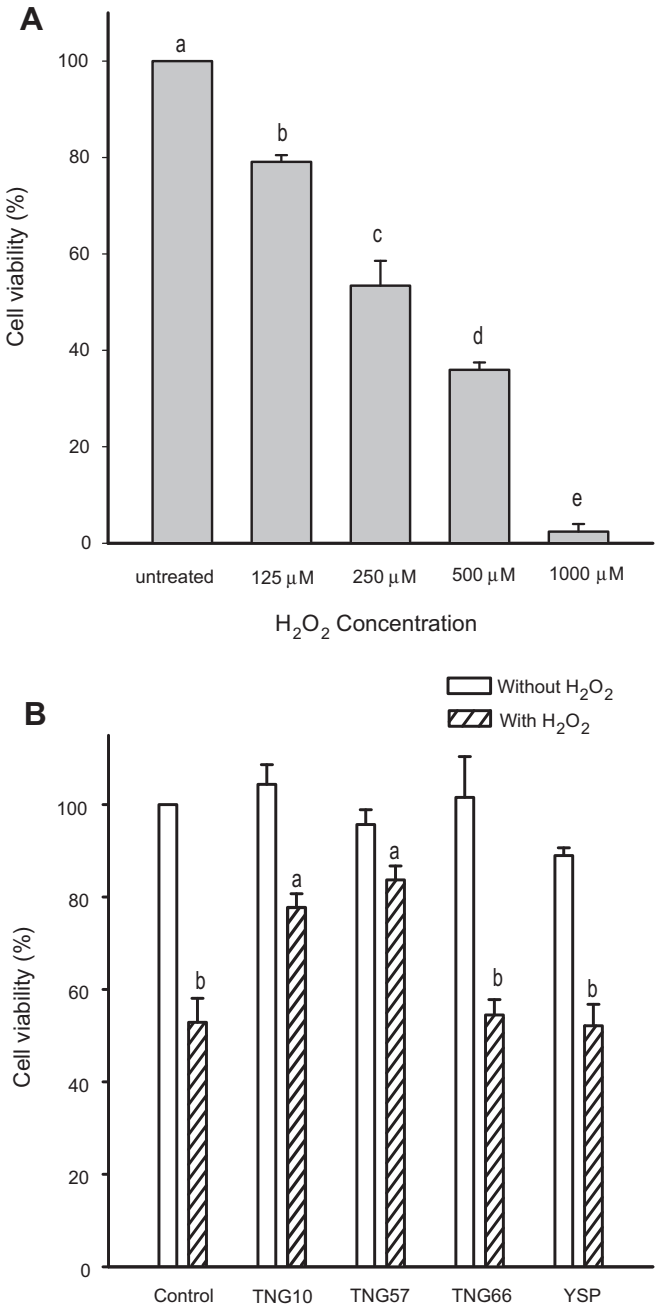

Fig. 4. Protective effects of four WE-FD samples against H_2O_2-inuced HaCaT cytotoxicity. (A) H_2O_2-induced dose response cytotoxicity in HaCaT cells. (B) Four 1 mg/ml WE-FD samples against 250 μM H_2O_2-induced HaCaT cytotoxicity. Values are means ± SD (n = 3). Mean values not sharing the same lower case letter above the bars are significantly different ($p < 0.05$).

4. Conclusion

The results of this study demonstrate that the water extract of sweet potato leaves has potent activity, in many antioxidative assays. In DPPH radical-scavenging, reducing power, hydrogen peroxide radical-scavenging, and chelating effect, both TNG10 and TNG57 showed better antioxidative effects than did TNG 66 and YSP. Protection against H_2O_2-induced cytotoxicity showed similar results. This study indicates that total phenolic and flavonoid contents may play important roles in antioxidative activity. These results suggest that the water extract of sweet potato leaves may help the food and cosmetic industry to alleviate oxidative-induced aging and chronic diseases.

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