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Antioxidant properties of selected varieties of lettuce (*Lactuca sativa* L.) commercially available in Malaysia

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Abstract

This study aimed to determine total antioxidant and antioxidant activity of selected local varieties of lettuce (*Lactuca sativa* L.). Five varieties (iceberg, butterhead, romaine, green coral and red coral) were subjected to DPPH radical scavenging activity and ferric reducing antioxidant power assay (FRAP) assays for determination of antioxidant activity. Total phenolic content and total flavonoid content were determined as total antioxidant. The EC_{50} values obtained from the DPPH radical scavenging assay ranged from 303.56 to 4485.41 µg/ml. The red coral lettuce had the lowest EC_{50} value indicating it possesses the highest antioxidant activity among the varieties. This variety also showed the highest FRAP value compared with the other varieties, where the values ranged from 48.05 to 2135.82 mM Fe^{2+}/100 g fresh weight. Total phenolic content of samples ranged from 4.85 to 76.05 mg gallic acid equivalent/100 g fresh weight, with the red coral lettuce had the highest value. Total flavonoid content of the lettuce samples ranged from 2.28 to 21.96 mg quercetin equivalent/100 g fresh weight, and were significantly different ($p<0.05$) among the samples. The EC_{50} values of DPPH radical scavenging activity and FRAP values among samples were highly correlated with total phenolic content and total flavonoid content. Among the different varieties of lettuce, red coral lettuce showed the highest total antioxidants and antioxidant activity. Therefore, consumers are encouraged to consume this lettuce more on a regular basis for gaining a better health.

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Introduction

Nowadays, a diet rich in fresh fruits and vegetables are believed to possess the ability to protect the body from degenerative and chronic diseases such as cancer and cardiovascular diseases (Szeto *et al*., 2004). Epidemiologic studies showed that the consumption of diet rich in fruits and vegetables are associated with reduced risk for cardiovascular diseases (Hung *et al*., 2004; Mirmiran *et al*., 2009). The beneficial effects of fruits and vegetables are believed due to the presence of antioxidant compounds obtained from plant including ascorbic acid, carotenoids and flavonoids which play an important role in protecting key biological sites such as membranes, lipoprotein and DNA (Khanam *et al*., 2012).

Use of antioxidant is increasingly popular in the modern society nowadays due to the wide publicity about its health benefits through the mass media (Huang *et al*., 2005). The detection of various bioactive compounds in the food which possess antioxidant activity leads to the increase of interest in the relationship between antioxidant and the risk of diseases (Nilsson *et al*., 2004). Antioxidant functions as an inhibitor for the oxidation of molecule by inhibiting the initiation or propagation of oxidizing chain reaction caused by the free radicals substances (Ismail *et al*., 2004). Plants especially vegetables may contain a wide variety of antioxidant substances that can act as free radical scavenging molecules such as vitamins, terpenoids, phenolic compounds, nitrogen compounds and some other endogenous metabolites (Cai *et al*., 2004). In fact, a previous study has shown the health effect of lettuce on the cardiovascular disease in rats (Nicolle *et al*., 2004).

Lettuces which belong to the Asteraceae family, are considered the most popular vegetables used as ingredient for salad due to the perception of "healthier" food (Llorach *et al*., 2008). Lettuce is a self-pollinated annual plants and it forms a deep taproot with largely horizontal lateral roots in order to maximize the water and nutrient absorption.

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There is considerable diversity in color, shape, surface, margin and texture of leaves among the different varieties of lettuce. Lettuce is a good source of flavonoids. A previous study reported that total flavonoids content of lettuce (4.57 ± 0.17 mg catechin equivalent, CE/100 g fresh weight, FW) was significantly higher than the total flavonoids content in cabbage (1.2 ± 0.18 mg CE/100 g FW) and spinach (1.42 ± 0.19 mg CE/100 g FW) (Chun *et al.*, 2005). Basically, six generally recognized types of lettuce are crisphead, butterhead, romaine, leaf, stem and Latin (Mou, 2008). However, in fruits and vegetables, especially for lettuce, antioxidant micronutrients such as polyphenols and carotenoids played important role in preventive nutrition, but at the same time they are also susceptible to different growth conditions and highly varied among cultivars (Nicolle *et al.*, 2004). Therefore, this study aimed to determine and compare the total antioxidant activity and content of selected local varieties of lettuce available in Malaysia.

Materials and Methods

Sample preparation and extraction

One kilogram of each variety of lettuce (*Lactuca sativa* L.), consisted of iceberg, butterhead, romaine, green coral, and red coral were purchased from two local supermarkets in Serdang Selangor, Malaysia in one visit. These five varieties of lettuces were brought to the nutrition laboratory in UPM for cleaning and washing under running tap water. The inedible portions were discarded and the water retained on the lettuces was dripped off using kitchen sieve. The lettuces were then allowed to cooled and dried under a fan for 20 min. The remaining portion of the lettuces were weighed and cut into smaller portions of 2 to 4 cm long. After that, the lettuces were frozen and dried using a freeze dryer. The freeze-dried lettuces were grinded into fine particle size using mortar with a pestle.The lettuces were then homogenated and kept in an air-tight container and stored at -80°C prior to further analyses.

The extract was prepared according to the procedure of Velioglu *et al.* (1998). The ground sample of freeze-dried lettuces was mixed with 70% aqueous ethanol in the ratio of 1 to 25 (g/v) separately. The mixture of sample with solvent was then shaken for 2 h at 50°C using an orbital shaker (Unimax 1010, Heidolph Instruments GmbH and Co. KG, Germany). The extract was followed with a filtration process using Whatman No. 1 filter paper to get a clear solution and the filtrate was kept frozen at -20°C prior to analyses.

Determination of total phenolic content

The total phenolic content (TPC) of each sample of lettuces was determined by Folin-Ciocalteu method as described by Marinova *et al.* (2005). Briefly, in aliquot of 1 ml for the extracts with different concentration (0, 20, 40, 60, 80, and 100 mg/l) was added into a 25 ml volumetric flask which was previously added with 9 ml of deionized water. A reagent blank using the deionized water was prepared. The mixture was added with 1 ml of Folin-Ciocalteu reagent and shaken for 5 min, followed by addition of 10 ml of 7% Na_2CO_3. The solution was then diluted to 25 ml using deionized water and mixed well. The solution was left for incubation for 90 min at room temperature. The absorbance against the reagent blank was determined at 750 nm with an UV-Vis spectrophotometer. Standard solutions of gallic acid were prepared with the same procedure for the extract of the samples for the purpose of standard curve plotting.

Determination of total flavonoid content

Total flavonoid content (TFC) in lettuce extract was determined using the aluminium chloride colorimetric method as described by Khanam *et al.* (2012) which was a modification from Chang *et al*. (2002). About 500 µl of sample extract was transferred into a test tube followed by 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride solution, 0.1 ml of 1 M potassium acetate solution and 2.8 ml of distilled water. After 30 min of incubation at room temperature, the absorbance of the reaction mixture was measured at 415 nm using a UV-vis spectrophotometer. Standard solution of quercetin with different concentrations (0, 20, 40, 60, 80, and 100 mg/l) was prepared with the same procedure for the extract of the samples for the purpose of standard curve plotting.

DPPH radical scavenging activity

Free radical scavenging activity using free radical DPPH was determined according to the method of Zdravković *et al.* (2014) with some modifications. Serial dilutions will be prepared with the stock solution (1 mg/ml) of the extract to produce different concentration (0, 62.5, 125, 250, 500 µg/ml) of sample extract. The lettuce extract (2 ml) was then mixed with 2 ml of methanolic solution containing DPPH and left in the dark for 30 min. The samples were measured for the absorbance at 517 nm using the UV-Vis spetrophotometer. Gallic acid was used as the reference standard and prepared by dissolving it in methanol to obtain the stock solution with the same concentration (1 mg/ml). The control sample was prepared containing the same volume without samples or reference antioxidants while 95% methanol was used as blank. The 50% inhibition concentration (EC_{50}) value, defined as the concentration of the sample that provide to 50% of the DPPH free radical scavenging activity, was calculated as micrograms per millilitre through a linear regression equation.

Ferric reducing antioxidant power assay

The ferric reducing antioxidant power (FRAP) assay was performed according to Llorach *et al.* (2008). The FRAP solution was freshly prepared by mixing 25 ml of 0.3 M acetate buffer (pH 3.6) plus 2.5 ml of 10 mM TPTZ solution in 40 mM HCl (previously prepared) and 2.5 ml of 20 mM ferric chloride ($FeCl_3$•$6H_2O$). For the blank, 950 µl FRAP solution was mixed with 50 µl of the solvent used and immediately measured the absorbance at 593 nm for 0 min using UV-Vis spectrophotometer. On the other hand, 950 µl of FRAP solution was mixed with 50 µl of the sample extract and incubated at room temperature for 45 min. The ferric reducing ability of lettuce extracts was then measured at the absorbance of 593 nm.

Results and Discussion

Total antioxidant content

TPCs of selected five varieties of lettuce ranged from 4.85 to 76.05 mg gallic acid equivalent per 100 g fresh weight (mg GAE/100 g FW) (Figure 1). The TPC was reported in descending order of lettuce varieties: red coral (76.05 mg GAE/100 g FW) > green coral (30.39 mg GAE/100 g FW) > romaine (9.14 mg GAE/100 g FW) > butterhead (4.92 mg GAE/100 g FW) > iceberg (4.85 mg GAE/100 g FW). Statistical analysis of these results showed significant differences exist among the lettuce varieties ($p<0.05$) except iceberg and butterhead (Figure 1).

TPC determined for the studied samples were different from the TPC values reported in previous studies (Llorach *et al*., 2008; Ozgen and Sekerci, 2011). The TPC of similar lettuce varieties as reported by Llorach *et al*. (2008) were higher than the TPC of the lettuce samples determined in this study. Environmental influences on the growth of lettuce could be the main factors for the variation in TPC of lettuces. Besides, irrigation and soil composition also caused significant effect on phenolic content of the plant and other phenolic physiological disorders (Nicolle *et al.*, 2004). The study done by Zdravković *et al.* (2014) also showed a variation in phenolic content among the lettuce samples grown in different conditions of light exposure.

The results showed that TFC of the selected five lettuce varieties ranged from 2.28 to 21.96 mg quercetin equivalent/100 g fresh weight (mg QE/100 g FW). The TFC values (mg QE/100 g FW) of different varieties of lettuce are presented in descending order: red coral (21.96) > green coral (9.94) > romaine (4.19) > butterhead (3.00) > iceberg (2.28). Red coral lettuce had the highest TFC in addition to TPC while iceberg lettuce had the lowest TFC. Besides that, the mean values of TFC were significantly different between different types of lettuce ($p<0.05$). The exposure to light on different part of lettuce causes uneven production of polyphenols in the lettuce. Anthocyanin is one of the major compounds found in the red lettuce. A study by García-Macías *et al*. (2007) showed that red lettuce has up to 992 µg cyanidin-3-glucoside (C3G)/g FW. The study also reported that low or no exposure to ultraviolet (UV) radiation inhibited the production of anthocyanin in the red lettuce, where some samples have as little as 375 µg C3G/g FW compared with the normal sun exposure. Besides, the outer layer of lettuce leaves has higher polyphenolic compounds than the inner leaves due to the high intensity of sun exposure (Baslam *et al*., 2013). Nevertheless, Mulabagal *et al.* (2010) found that the both red and green lettuces provided good sources of antioxidant and anti-inflammatory effects. Besides, anthocyanin added nutritional value to red lettuce, thus contributed to the higher total antioxidant content than the other lettuce varieties.

Antioxidant activity

In the present study, the result for DPPH radical scavenging activity is presented as EC_{50} values. As shown in Table 1, red coral lettuce possessed the lowest EC_{50} value, followed by green coral, iceberg, butterhead and romaine. A low EC_{50} value indicates a high antioxidant activity of a sample. Therefore, red coral lettuce had the highest antioxidant activity while the romaine variety had the lowest activity. Similar finding was reported previously by Llorach *et al.* (2008) that the red and darker green types of lettuce possess higher antioxidant activity than the green type lettuce. They assumed that the high antioxidant activity of red lettuce was due to the existence of anthocyanin, which was also a strong antioxidant. In addition, Xin *et al.* (2004) revealed that the iceberg lettuce has the lowest antioxidant activity among the salad vegetables they investigated.

In this study, the DPPH radical scavenging activities of samples were significantly different between the lettuce varieties ($p<0.05$) (Table 1), except for iceberg, butterhead, and romaine. These

Table 1. Antioxidant activity of lettuces

Samples	EC_{50} (μg/ml)	FRAP (mM Fe^{2+}/100 g FW)
Iceberg	3991.67 ± 174.7[a]	48.05 ± 6.7[a]
Butterhead	4230.13 ± 401.5[a]	84.38 ± 22.3[a]
Romaine	4485.41 ± 784.4[a]	126.24 ± 25.3[a]
Green coral	775.55 ± 43.7[b]	761.83 ± 61.1[b]
Red coral	303.56 ± 11.3[b,c]	2135.82 ± 119.2[c]

[1]Values are mean ± standard deviation.
[2]EC_{50} value is referring to the value for 50% inhibition concentration from DPPH radical scavenging activity.
[3]Values with different superscript letters in each column are significantly different ($p<0.05$).
[4]FRAP, ferric reducing antioxidant power; FW, fresh weight.

varieties of lettuce had significantly lower DPPH radical scavenging activity than the green and red Corals ($p<0.05$). Moreover, there was no significant difference for the radical scavenging activity between Green and Red Corals ($p\geq0.05$). It proves that the red and green coral lettuces have high antioxidant activity compared with the other three varieties of lettuces.

The different regions of lettuce plantation may give rise to the variation in polyphenolic content, which in turn causing a variation in DPPH radical scavenging of different varieties of lettuce. The DPPH radical scavenging activity is greatly influenced by the total phenolic content of the lettuce samples. As mentioned earlier, in different geographical locations, environmental factors including soil composition could affect the total phenolic content that indirectly altered the antioxidant activity of the lettuce samples (Nicolle *et al.*, 2004).

As shown in Table 1, the FRAP values (Fe^{2+}/100 g FW) were shown in descending order of lettuce varieties: red coral > green coral > romaine > butterhead > iceberg. This finding is in line with previous studies by Llorach *et al.* (2008) and Llorach et al. (2004) that a higher FRAP value was determined for red lettuces than the green type. This observation is also supported by the finding by Llorach *et al.* (2004) that green lettuces have lower FRAP values than the red varieties. The lettuce varieties in the study conducted by Tiveron *et al.* (2012) showed similar result (44.71 mM Fe^{2+}/100 g) compared with the studied lettuce sample (48.05 mM Fe^{2+}/ 100 g). The variation in FRAP values of the lettuce samples is mainly due to the environmental factors (Nicolle *et al.*, 2004) and other factors (cultivar, microclimatic conditions, soil and rational fertilization) that affect the antioxidant activity of these vegetables, which are important for human health after consuming the vegetable (Liu *et al.*, 2007).

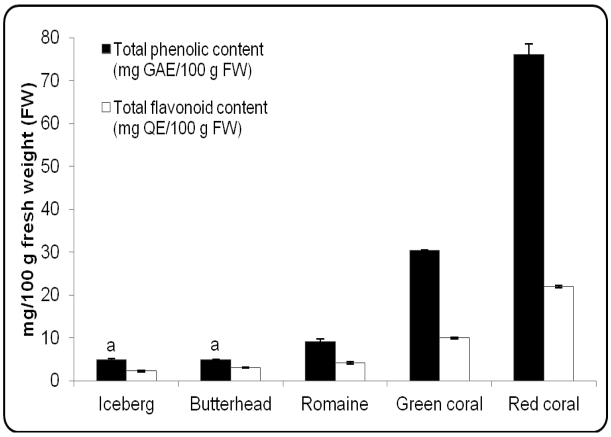

Figure 1. Total phenolic content and total flavonoid content of lettuces.
[1]Same lower case letter shows no significant difference between the two varieties ($p\geq0.05$).
[2]GAE, gallic acid equivalent; QE, quercetin equivalent; FW, fresh weight.

Correlation between total antioxidant contents and antioxidant activity

The EC_{50} values of DPPH radical scavenging activity of the lettuce samples showed negative and high correlations with TPC ($r= -0.879$) and TPC ($r= -0.881$). This shows that the low EC_{50} value of the sample is contributed by the high TPC or TFC. The degree of antioxidant activity might be attributed to the total phenolic content and flavonoids in the sample. The finding is well agreed with the study by Lamien-Meda *et al.* (2008) that a high correlation was found between total antioxidant content (TPC or TFC) and antioxidant activity (DPPH or FRAP).

The FRAP value of the lettuce samples showed a positively high correlation with TPC ($r= 0.999$) and TFC ($r= 0.998$). The result indicates that the higher the FRAP value of lettuce sample, the higher the TPC or TFC. A previous study reported that TPC of lettuce was highly correlated with the antioxidant activity (DPPH or FRAP), regardless of the extraction method (Llorach *et al.*, 2004). Polyphenols are responsible for the antioxidant activities of most botanical extracts, and therefore, phenolic content should be strongly correlated with antioxidant activity (Wong *et al.*, 2006).

Conclusion

Among the different varieties of lettuce, red coral lettuce showed the highest total antioxidant (TPC and TFC) and antioxidant activities for both DPPH radical scavenging activity and FRAP assays. It also had the lowest EC_{50} value of DPPH radical scavenging assay and the highest FRAP value. Iceberg, butterhead and romaine lettuces contained lower amounts of TPC and TFC than the coral varieties. The higher total antioxidant and antioxidant activity observed for red coral lettuce than the other varieties indicate a more beneficial effect of consuming this lettuce as the main ingredient for salad.

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