

Antioxidant activity and total phenolic content in aqueous extracts of selected traditional Malay salads (*Ulam*)

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Abstract

The total phenolic contents (TPC) and antioxidant activities of five popular Malay raw salads or *Ulam* were investigated using DPPH radical scavenging and reducing ferric ion antioxidant power (FRAP) assays. The *Ulam* studied were the leaves of *Cosmos caudatus* (*Ulam Raja*), *Oenanthe javanica* (*Selom*), *Murraya koenigii* (*Curry Leaf*), *Centella asiatica* (*Pegaga*) and the seeds of *Parkia speciosa* (*Petai*). Ranking order of TPC (mg gallic acid equivalent per gram of plant on dry basis), TEAC_{DPPH} (μmol Trolox equivalent per gram of plant on dry basis) and TEAC_{FRAP} (μmol Trolox equivalent per gram of plant on dry basis) values were: Curry Leaf (33.18), *Selom* (31.8), *Ulam Raja* (31.3) > *Pegaga* (11.16) > *Petai* (6.45); *Ulam Raja* (212.8) > *Selom* (185.9) > *Curry Leaf* (82.1), *Petai* (67.62) > *Pegaga* (32.4); *Selom* (199.96) > *Ulam Raja* (183.11) > *Curry Leaf* (108.34) > *Pegaga* (65.99) > *Petai* (44.67), respectively. No significant correlation ($p > 0.05$) was observed between antioxidant activities and TPC which could be due to steric hindrance or presence of other reducing agents. Interestingly, *Selom* showed antioxidant activities that are comparable to *Ulam Raja*.

Keywords

Traditional Malay salads

Ulam

total phenolic content (TPC)

antioxidant activity

DPPH scavenging

Ferric reducing antioxidant power (FRAP)

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Introduction

Ulam is a fresh green salad that may be tossed in a blend of fermented sauces, aromatic herbs or spices eaten by Malays as side dishes with rice. *Ulam* has been moved out of rural areas and spread to towns where it is embraced by other races as well. An *Ulam* may constitute shoots, leaves, and seeds of certain local plants that are rich in taste and unique in texture. However, *Ulam* are not only flavorful popular side dish; they have been receiving special attention because of their history in folk medicinal uses either for preventative or even curative purposes. Perhaps, more than 120 species of traditional vegetables have been regarded as *Ulam* from various plant families in South East Asia. Most of these herbs are believed to be associated with antioxidant activities (Tee, 1985; Jayamalar *et al.*, 1998; Mohd Zin *et al.*, 2002; Noriham, *et al.*, 2004; Zainol *et al.*, 2003). Antioxidants have the ability to scavenge free radicals and therefore reduce oxidative stress. Antioxidative compounds oppose reactive oxygen species (Lu *et al.*, 1995), hinder oxidative degradation of lipids and consequently enhance the nutritional value of food (Kahkonen *et al.*, 1999). The inverse relationship between consuming fruits and vegetables and cancer, age-related and heart diseases (Ames *et al.*, 1993;

Halliwell, 1996; Van *et al.*, 2000; Temple *et al.*, 2003) can be partly attributed to wide range of antioxidant compounds in them (Halliwell, 1996; Zainol *et al.*, 2003; Shui *et al.*, 2005; Andarwulan *et al.*, 2010; Bolling *et al.*, 2010; Sulaiman *et al.*, 2011).

There is no systematic study that ranks most popular *Ulam* that are served in local eateries and restaurants. However, five types of *Ulam* that are often served in these eateries include the leaves of *Cosmos caudatus* (*Ulam Raja*), *Oenanthe javanica* (*Selom*), *Murraya koenigii* (*Curry Leaf*), *Centella asiatica* (*Pegaga*) and the seeds of *Parkia speciosa* (*Petai*). These *Ulam* are typically served as a side dish to be taken with rice, or used as ingredient in specialty dishes in many restaurants and eateries throughout Malaysia.

The health authority of Malaysia has been promoting the consumption of *Ulam*, even though the types and quantity have not been specified. Thus, an effort to rank popular *Ulam* on the basis of antioxidant activities should be encouraged. Considering the supporting information on their potential nutraceutical properties, choice of *Ulam* for meals should be based on the antioxidant contents and activities rather than taste and flavor. Although there are various reports of antioxidant activities performed by different authors, it is difficult to compare their results due to vastly

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differing methods. Besides, it is recommended to use more than one method to evaluate antioxidant activity in order to consider different possible mechanisms of action (Jara *et al.*, 2008) as the composition and condition of the test system can affect antioxidant activities. In this study, antioxidant activity and total phenolic content are given as quantities relative to those of trolox and gallic acid respectively. These are common as references for antioxidant activity and antioxidative compounds (Perez-Jimenez *et al.*, 2008; Van Den Berg *et al.*, 2000). By using a reference antioxidant, it is possible to compare the antioxidant activities of the *Ulam* with other plants. The objectives of this study were to determine the radical scavenging activity, total phenolic contents (TPC) and ferric ion reducing power of aqueous extracts of selected *Ulam* and to rank them according to their antioxidant activities and TPC.

Materials and Methods

Plants

Five types of *Ulam* namely *Ulam* Raja (*Cosmos caudatus*), Selom (*Oenanthe javanica*), Curry Leaf (*Murraya koenigii*), Asian pennywort/Pegaga (*Centella asiatica*) and the seeds of Petai (*Parkia speciosa*) were obtained fresh from an *Ulam* supplier in Jitra, Kedah, Malaysia, and wet-markets in Penang, Malaysia on three different occasions ($n=3$). All *Ulam* materials were washed upon arrival to the processing laboratory with tap and deionized water and damaged portions were removed. Then, the leaves (*Ulam* Raja, Selom, Pegaga and Curry) and seeds (Petai) were stripped and dried according to the method described by Mohd Zin *et al.*, (2002) in a convection dryer (AFOS Mini Kiln, England) at 45°C for 48 h. The dried *Ulam* were stored in sealed polyethylene bags at 4°C before use. For extraction, dried *Ulam* were ground using a domestic blender and 0.5 g of the powdered sample was extracted in 25 ml of deionized water at room temperature. The mixture was allowed to stand for 1 h at room temperature in the dark with agitation. Aqueous extract was obtained by filtering the mixture through Whatman No 43 filter paper and used for analysis (Wong *et al.*, 2006).

Total phenolic contents (TPC) determination

The TPC of the extracts were determined by using the Folin–Ciocalteu method (Wong *et al.*, 2006). An aliquot (100 μ l) of an *Ulam* extract was mixed with 2.5 ml of Folin–Ciocalteu phenol reagent (10 x dilutions). After 5 min, 2.5 ml of saturated Na_2CO_3 solution was added and allowed to stand for 1 h before the absorbance of the reaction mixture was read at 740 nm. All samples were analyzed in triplicates and

results averaged. The total phenolic contents (TPC) of the *Ulam* extract was calculated using a gallic acid calibration curve (five different concentration within the range of 1.7–3 mM, $R^2 = 0.98$) and expressed as mg gallic acid equivalent per gram of plant on dry basis (g db).

DPPH free radical scavenging assay

DPPH scavenging activity was measured using the method described by Brand-Williams *et al.*, (1995) with some modifications. This assay is based on the determination of the concentration of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) methanolic solution, after adding the antioxidants. DPPH concentration is reduced by the existence of an antioxidant at 515 nm and the absorption gradually disappears with time. The Ultraspec 1601 UV/vis spectrophotometer (Shimadzu) was used to determine the antioxidant activity of each sample. A 0.1 mM methanolic solution of DPPH was prepared. The initial absorbance of the DPPH solution was measured at 515 nm and the absorbance was the same throughout the period of assay. An aliquot (40 μ l) of *Ulam* extract (with appropriate dilution) was added to 3 ml of methanolic DPPH solution. The change in absorbance at 515 nm was measured at 30 min intervals until the reaction curve reaches the plateau. All samples were analyzed in triplicates and results averaged. The antioxidant capacity was expressed as μ mol Trolox equivalent per gram of plant on dry basis (g db).

Ferric reducing antioxidant potential assay

The ability to reduce ferric ions was measured using a modified version of the method described by Benzie *et al.* (1996). An aliquot (200 μ l) of an *Ulam* extract (with appropriate dilution) was added to 3 ml of FRAP reagent (10 parts of 300 mM sodium acetate buffer at pH 3.6, 1 part of 10 mM TPTZ solution and 1 part of 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution) and the reaction mixture was incubated in a water bath at 37 °C. The increase in absorbance at 595 nm was measured after 30 min. All samples were analyzed in triplicates and results averaged. The antioxidant capacity based on the ability to reduce ferric ions of the extract was expressed as μ mol Trolox equivalent per gram on dry basis (g db).

Statistical Analysis

Experimental data was analyzed using analysis of variance (ANOVA), SPSS version 18 and significant differences among means from triplicate analyses at ($p<0.05$) were determined by Duncan's multiple range test (SAS Institute, 1990). Pearson correlation analysis was also carried out to determine the relationship between TPC, DPPH scavenging ability

and FRAP values of the extracts.

Results and Discussion

Total phenolic Content (TPC)

As can be seen in Figure 1 the leaf extracts of Curry, Selom and *Ulam* Raja had the highest TPC (31-33 mg GAE/g), while the seed extract of Petai showed the lowest (6.5 mg GAE/g). TPC values were ranked as: Curry Leaf, Selom, *Ulam* Raja > Pegaga > Petai. Ningappa *et al.* (2008) and also Sulaiman *et al.*, (2011) reported different TPC levels in Curry Leaf. This could be due to difference in extraction methods. The TPC of Curry Leaf in this study was comparable to that reported by Wong *et al.* (2006), even though, the values for *Ulam* Raja and Pegaga were higher, and the values for Petai was lower than their data. Cultivar and climate can be counted as factors affecting the structure of phenolics and bioactive compounds (Zheng *et al.*, 2001; Bolling *et al.*, 2010). This can partly explains the wide range of variation in TPC values obtained from different studies which used the same evaluation methods.

DPPH free radical scavenging activity

This assay is based on the reduction of DPPH radicals in methanol which causes an absorbance drop at 515 nm. In this study, the antioxidant activity was expressed as Trolox equivalent per gram of plant material on a dry basis (g db). Thus, a direct comparison between the antioxidant activities of samples against trolox is provided. The DPPH free radical scavenging activities of five *Ulam* extracts are shown in Figure 2. Pegaga showed the lowest DPPH free radical scavenging activity while *Ulam* Raja showed the highest. Ranking order of TEAC_{DPPH} values was: *Ulam* Raja > Selom > Curry Leaf, Petai > Pegaga. The values of TEAC_{DPPH} for *Ulam* Raja and Curry Leaf were in good agreement with those reported by Wong *et al.* (2006), nevertheless, Pegaga showed lower whilst Petai showed higher values. Generally it is expected that extracts with a high amount of polyphenol content should also exhibit high antioxidant activity (Zheng *et al.*, 2001; Bolling *et al.*, 2010). The aqueous extracts of *Ulam* Raja and Selom corresponded with this suggestion. On the contrary, Curry Leaf that showed a relatively low antioxidant activity exhibited the highest in TPC. This disagreement could be due to poor specificity of the TPC assay (Singleton *et al.*, 1999; Escarpa *et al.*, 2001). In addition, phenolic compounds, depending on the number of phenolic groups they have, respond differently to the Folin–Ciocalteu reagent (Singleton *et al.*, 1999).

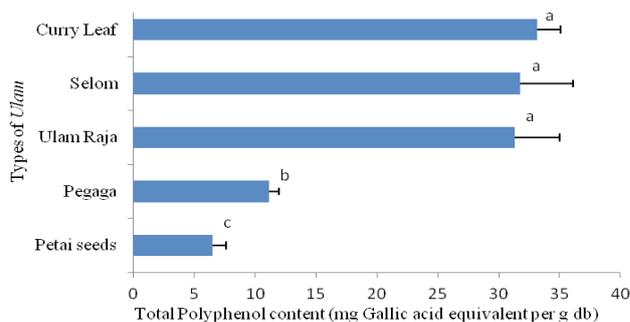


Figure 1. Total phenolic contents of 5 *Ulam*; values with different letters are significantly different ($p < 0.05$), ($n = 3$, error bars represent standard deviation)

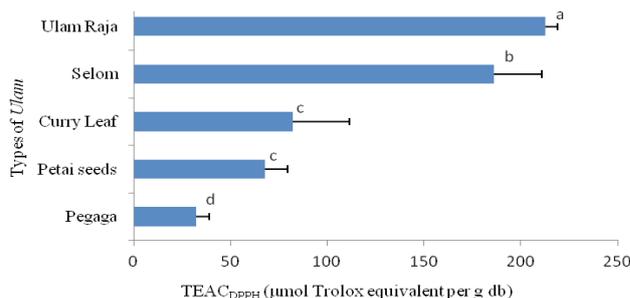


Figure 2. DPPH radical scavenging activities of 5 *Ulam*, values with different letters are significantly different ($p < 0.05$), ($n = 3$, error bars represent standard deviation)

Ferric ion reducing activity

The ability of the *Ulam* extracts to reduce ferric ions was determined using the FRAP assay developed by Benzie *et al.* (1996). An antioxidant capable of donating a single electron to the ferric-TPTZ (Fe(III)-TPTZ) complex would cause the reduction of this complex into the blue ferrous-TPTZ (Fe(II)-TPTZ) complex which absorbs strongly at 595 nm. Compared to other *Ulam* extracts, Selom and *Ulam* Raja showed the highest ferric reducing activities whilst Petai seeds showed the lowest (Figure 3). Ranking order of TEAC_{FRAP} was: Selom, *Ulam* Raja > Curry Leaf > Pegaga > Petai. The values of TEAC_{FRAP} for Curry Leaf and Petai compare favorably to those obtained in the report of (Wong *et al.*, 2006), even though, the TEAC_{FRAP} values of *Ulam* Raja and Pegaga were slightly lower. The trend of DPPH scavenging and ferric reducing activities of the five extracts differ (Figure 2 and Figure 3), however, *Ulam* Raja and Selom showed consistently the highest values in TEAC_{FRAP} and TEAC_{DPPH}. For each of sample, it can be seen that the values of TEAC_{FRAP} were slightly higher than those of TEAC_{DPPH}. Such a trend has been reported in literature, for instance in red onion seeds (Dini *et al.*, 2008) and in selected tropical plants (Wong *et al.*, 2006). This difference in values could be due to different reaction efficiencies of tested compounds towards these two assays (Wong *et al.*, 2006). Lower TEAC_{DPPH} in these reports could

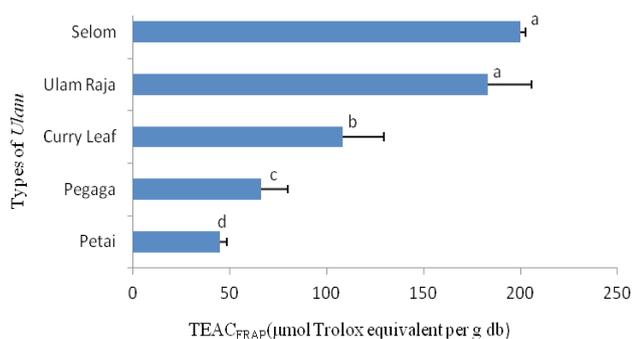


Figure 3. Antioxidant activities of 5 *Ulam* based on their abilities to reduce the ferric ion-TPTZ; values with different letters are significantly different ($p < 0.05$), ($n = 3$, error bars represent standard deviation).

be due to the presence of antioxidant compounds such as polyphenols that are reactive towards ferric iron but do not react efficiently with DPPH free radicals due to steric resistance, even though, some reports had pointed the opposite direction (Gil *et al.*, 2002; Norhaiza *et al.*, 2009). The exceptionally high level of antioxidant properties in *Ulam Raja* was first reported by Wong *et al.* (2006). Our results confirmed their report. Antioxidant activities of *Ulam* are comparable to those of some fruits known as good sources of antioxidant. For instance, DPPH scavenging activity of some Brazilian cherries were reported to be about 180 μmol Trolox equivalent per g db (Celli *et al.*, 2011) which is close to that of *Ulam Raja* (213 μmol Trolox) and Selom (186 μmol Trolox). As shown in results, apart from *Ulam Raja*, *Oenanthe javanica* (Selom) appeared to be a good source of antioxidants. Although there have been some studies on antioxidant properties of Selom, (Huda-Faujan *et al.*, 2007; Wan-Ibrahim *et al.*, 2010) few of them used the combination of common systematic methods to evaluate its antioxidant activities. FRAP, DPPH, ABTS, ORAC and TPC are the most common assays for determining antioxidant capacity (Perez-Jimenez *et al.*, 2008). Since these assays have different levels of applicability, applying two or three of them is always recommended in order to be able to obtain comprehensive information on the antioxidant capacity.

Correlations

Strong correlation ($p < 0.05$) between TEAC_{DPPH} and TEAC_{FRAP} (Table 1) implies compounds in these plants are capable of donating hydrogen atoms to DPPH• and also reducing ferric iron by single electron donation (Arnous *et al.*, 2000). Lower TEAC_{DPPH} values may be due to presence of other reducing agents which do not react with DPPH•. Antioxidant compounds like polyphenols may not scavenge DPPH• due to steric resistance. On the other hand, there might be reducing agents which are not antioxidants

Table 1. Correlation coefficients for the relationship between the assays

	TEAC _{FRAP}	TPC
TEAC _{DPPH}	0.932 ^a	0.669 ^b
TEAC _{FRAP}	---	0.808 ^b

^aSignificant correlations ($p < 0.05$); ^b No significant correlations ($p > 0.05$)

(Wong *et al.*, 2006). As can be seen in Table 1, there was a positive but no significant correlation between TPC with the TEAC_{FRAP} ($r = 0.808$; $p = 0.098 > 0.05$) and TEAC_{DPPH} ($r = 0.669$; $p = 0.217 > 0.05$). This can be explained by the fact that Folin–Ciocalteu reaction is based on redox reactions (Escarpa *et al.*, 2001). The assay detects not only polyphenolic compounds, but also other biological substances which are reactive towards the Folin–Ciocalteu reagent such as amino acids, carbohydrates and ascorbic acid (Tulipani *et al.*, 2008; Singleton *et al.*, 1999; Escarpa *et al.*, 2001).

Conclusion

Ranking order of TEAC_{DPPH} and TEAC_{FRAP} indicated *Ulam Raja* and Selom as the most potent antioxidant sources. Curry Leaf, Selom and *Ulam Raja* ranked as *Ulam* with highest TPC. A strong correlation was observed between TEAC_{DPPH} and TEAC_{FRAP}, nevertheless, neither TEAC_{DPPH} nor TEAC_{FRAP} showed significant correlation with the TPC. Based on our results, Selom appeared as a good source of antioxidants and phenolic compounds along with *Ulam Raja*.

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