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Phytochemical content and antioxidant activity of selected wild ulam/vegetables consumed by indigenous Jakun community in Taman Negara Johor Endau Rompin (TNJER), Malaysia

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Abstract

This study aimed to investigate the phytochemical content and antioxidant activity of selected wild ulam/vegetables consumed by indigenous Jakun Community in Taman Negara Johor Endau Rompin (TNJER), Malaysia. Five ulam/vegetables were collected from TNJER, Malaysia namely *Dillenia suffruticosa* (pucuk simpuh), *Amischotolype griffithii* (tebu gogok), *Paederia foetida* (pucuk sekentut), *Gnetum gnemon* (pucuk empeng), and *Barringtonia macrostachya* (pucuk putat). Total phenolic content (using Folin-Ciocalteu) and total flavonoid content (using aluminium chloride colorimetric), as well as antioxidant activity using 2,2-diphenylpicrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assays were determined spectrophotometrically. For the phytochemical study, *D. suffruticosa* showed the highest value of total phenolic content (93.87±0.10 µg gallic acid equivalent/mL) while *B. macrostachya* displayed the highest value of total flavonoid content (115.06±21.54 µg rutin equivalent/mL) as compared to all samples tested. For the antioxidant activity, *G. gnemon* showed the lowest EC_{50} value in DPPH assay with the value of 3.92±1.52 µg ascorbic acid/mL, indicated the strongest antioxidant potential. Meanwhile, *D. suffruticosa* showed the highest scavenging activity using ABTS assay (EC_{50} value, 7.93 µg/mL). In conclusion, ulam/vegetables of TNJER are rich in phytochemicals and antioxidant which can be a great potential for use in complementary and alternative medicines as well as functional food applications.

1. Introduction

Being a tropical country, Malaysia is well-known for its richness in the biodiversity of many indigenous ulam/vegetables which grow naturally in the wild (Aralas *et al.,* 2009). In Malaysia, the consumption of raw ulam/vegetable is considered as a common traditional healthy diet by many people due to their health-promoting properties (Chan *et al.,* 2014). Majority of Malay restaurants in this country also serve ulam as a side dish to be eaten with rice (staple food) and sometimes, ulam may also be blanched, sautéed, curried or fried (Husain *et al.,* 2004; Chan *et al.,* 2014). Historically, ulam is used for many years in folk medicines either to prevent or cure the diseases which make them to receive great attention in spite of their flavourful popular side dishes (Reihani and Azhar, 2014). Currently, over 120 species of traditional vegetables from various plant families in Malaysia have been identified and regarded as ulam (Aralas *et al.,* 2009). Recent research has identified a vast variety of antioxidant from wild ulam/vegetables throughout the world especially Malaysia (Reihani and Azhar, 2014). Phytochemical (from plants) elements in the diet play a major role in contributing to human health (Nahak *et al.,* 2014). Consumption of ulam/vegetables are good for health as they lower the risk of getting diseases such as cancer, diabetes, cardiovascular diseases, Alzheimer's disease and atherosclerosis due to presence of various forms of phytochemicals and antioxidants; polyphenol compounds including flavonoids and total phenolic compounds (Abdul Karim *et al.,* 2014; Ahmad *et al.,* 2014). In general, vegetables are considered to be among the top four common daily diets by Malaysians as they offer various health benefits to humans (Norimah *et al.,* 2008). To date, there is still little information on the phytochemical content and antioxidant activity of Malaysian wild ulam/vegetables

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consumed by indigenous local community. Hence, this study aimed to investigate the phytochemical content and antioxidant activity of 80% methanolic extracts from selected wild ulam/vegetables consumed by Jakun Community in Taman Negara Johor Endau Rompin (TNJER).

2. Materials and methods

2.1 Sample preparation

The selected ulam/vegetables were *Dillenia suffruticosa* (family name: Dilleniaceae; local name: pucuk simpuh), *Amischotolype griffithii* (family name: Commelinaceae; local name: tebu gogok), *Paederia foetida* (family name: Rubiaceae; local name: pucuk sekentut), *Gnetum gnemon* (family name: Gnetaceae; local name: pucuk empeng), and *Barringtonia macrostachya* (family name: Lecythidaceae; local name: pucuk putat). The fresh samples were collected from the Jakun Community in TNJER, Malaysia which was then cleaned and stored at -80°C overnight, followed by lyophilised for 48 hrs. Then, the dried vegetables were ground into powder and stored at -20°C in darkness for further analysis.

2.2 Sample extraction

Sample of 0.1 g was mixed with 25 mL of 80% methanol. The mixture was agitated on an orbital shaker set at 200 rpm for 2 hrs followed by filtration by using Whatman filter paper and the supernatant was stored at -20°C in a freezer. The samples were extracted for phytochemical and antioxidant activity analysis. Each test was done in triplicate to ensure the accuracy of the results.

2.3 Determination of phytochemical content

2.3.1 Total phenolic content

Total phenolic content was determined using Folin-Ciocalteu reagent done by Velioglu *et al.* (1998) with some modifications. The absorbance values were obtained spectrophotometrically at 750 nm against zero absorbance (T60 Aurora Oasis Scientific, Inc., China). Gallic acid was used as a standard and the results were expressed as µg gallic acid equivalents/ml (µg GAE/ mL).

2.3.2 Total flavonoid content

Total flavonoid was measured using aluminium chloride colorimetric method (Zhishen *et al.,* 1999). The absorbance was measured spectrophotometrically at 510 nm against zero absorbance (T60 Aurora Oasis Scientific, Inc., China). Rutin was used as a standard and results were expressed as µg rutin equivalent/ml (µg RE/ mL).

2.4 Determination of antioxidant activity

2.4.1 DPPH (2, 2 –diphenyl-2-pycrylhydrazyl) free radical scavenging action

Free radical model, 2, 2-diphenyl-2-pycrylhydrazyl (DPPH) was used to determine the antioxidant activity (Mensor *et al.,* 2001). The absorbance was measured spectrophotometrically at 518 nm against blank (T60 Aurora Oasis Scientific, Inc., China). The radical scavenging activity was calculated as below:

Antioxidant activity (%) = 100 - (Absorbance sample-absorbance blank/absorbance control) x 100

The scavenging percentage of all samples was plotted, and the final result was expressed as an EC_{50} value (µg/mL).

2.4.2 ABTS (2,2'-azinobis (3-ethylbenzthiazoline)-6-sulphonic acid) radical scavenging assay

ABTS free radical decolorization assay was carried out according to Re *et al.* (1999) with slight modification. The absorbance values were measured spectrophotometrically at 734 nm (T60Aurora Oasis Scientific, Inc., China) and the radical scavenging was expressed as µg ascorbic acid equivalent antioxidant capacity /mL (µg AEAC/mL).

2.5 Statistical analysis

Statistical analysis was done using Analysis of Variance (ANOVA) and the results were expressed as mean ± standard deviation. In ANOVA, the differences between the mean values were considered significantly different when the value of $p<0.05$ was obtained.

3. Results

3.1 Total phenolic content (TPC)

The results showed that the total phenolic content of ulam/vegetables ranged from 17.08±1.02 to 93.87±0.10 µg gallic acid equivalent/mL. Pucuk simpuh had the highest total phenolic content with the value of 93.87±0.10 µg gallic acid equivalent/mL, followed by pucuk empeng, pucuk putat, pucuk sekentut and tebu gogok with the values of 89.58±3.76, 65.89±0.82, 37.02±0.10 and 17.08±1.02 µg gallic acid equivalent/ mL, respectively (Table 1) ($p<0.05$).

3.2 Total flavonoid content (TFC)

The results for the total flavonoid content of wild ulam/vegetables in this study ranged from 24.90±5.30 to 115.06±21.54 µg rutin equivalent/mL. Pucuk putat showed the highest total flavonoid content with the value of 115.06±21.54 µg rutin equivalent/mL, followed by pucuk empeng, pucuk simpuh, pucuk sekentut and tebu

Table 1. Phytochemical and antioxidant activity of ulam/vegetables

Sample	TPC (µg/mL)	TFC (µg/mL)	DPPH (µg/mL) EC_{50}	ABTS (µg/mL)
Pucuk simpuh	93.87±0.10[a]	55.38±5.85[c]	5.482±0.32[d]	7.93±0.02[a]
Pucuk putat	65.89±0.82[c]	115.06±21.54[a]	10.67±0.26[c]	7.71±0.03[a]
Pucuk empeng	89.58±3.76[b]	94.11±13.21[b]	3.92±1.52[e]	7.80±0.01[a]
Tebu gogok	17.08±1.02[e]	24.90±5.30[e]	48.68±1.47[a]	7.35±0.03[a]
Pucuk sekentut	37.02±0.10[d]	50.78±9.6[d]	40.66±0.06[b]	4.51±0.03[b]

Values are presented in mean ± standard deviation (n=3). Different alphabet superscript in the same row are significantly different ($p<0.05$).

gogok with the value of 94.11±13.21, 55.38±5.85, 50.78±9.62 and 24.90±5.30 µg rutin equivalent/mL, respectively (Table 1) ($p<0.05$).

3.3 DPPH (2, 2 –diphenyl-2-picrylhydrazyl) free radical scavenging action

The EC_{50} value (equivalent concentration to give 50% effect) was determined to better quantify the radical scavenging activity in the samples, the lower EC_{50} value indicated the stronger DPPH radical scavenging activity. Pucuk empeng showed the lowest EC_{50} value with 3.92±1.52 µg/mL, indicated the stronger DPPH radical scavenging activity while tebu gogok showed the highest EC_{50} value with 48.6±1.47 µg/mL. EC_{50} values for pucuk sekentut, pucuk putat and pucuk simpuh were 40.66±0.06 µg/mL, 10.67±0.26 µg/mL and 5.482±0.32 µg/mL, respectively. The results showed that they were statistically significant difference between all samples for scavenging free-radical activity ($p<0.05$) (Table 1).

3.4 ABTS radical scavenging

The results showed that pucuk simpuh had the highest value with 7.93±0.02 µg ascorbic acid/mL followed by pucuk empeng, pucuk putat, tebu gogok and pucuk sekentut with the values of 7.80±0.01, 7.71±0.03, 7.35±0.03, and 4.51±0.03 µg ascorbic acid/mL, respectively (Table 1).

4. Discussion

The health-promoting effect of vegetables, fruits, whole grains, and nuts may be contributed by polyphenols and antioxidant constituents (Pandey and Rizvi, 2009). For instance, phenolic compounds are secondary metabolites commonly found in plants such as vegetables, fruits and spices (Chua *et al.,* 2014). They are natural antioxidants and play a role as scavengers of free radicals and hence, they form an essential part of the human diet as they may contribute directly to antioxidative action in the body (Duh *et al.,* 1999; Shahidi and Ambigaipalan, 2015). It is believed that their radical scavenging ability is due to their hydroxyl groups (Hatano *et al.,* 1989). The level of total phenolic contents in wild ulam/vegetables varied from 17.08±1.02 to 93.87±0.10 µg gallic acid equivalent/mL. *Dillenia suffruticosa* (pucuk simpuh) demonstrated the highest phenolic content with the value of 93.87±0.10 µg gallic acid equivalent/mL. Previous study demonstrated that *Cosmos caudatus* (ulam raja) had the highest amount of total phenols as compared to other vegetables such as *Polyscias pinnata, Pluchea indica, Nothopanax scutellarius, Talinum triangulare, Pilea melastomoides* and *Etlingera elatior* (Andarwulan *et al.,* 2010).

Flavonoids are the most diverse group of natural compounds which possess a broad spectrum of chemical and biological properties (Prasad *et al.,* 2010). Not only for the antioxidant activity, but they also have the properties as anti-carcinogenic and anti-arteriosclerosis (Wong *et al.,* 2006). In this study, the total flavonoid content of wild ulam/vegetables varied from 24.90±5.30 to 115.06±21.54 µg rutin equivalent/mL. The previous study reported that the flavonoid content of the vegetables ranged from 0.3 to 143 mg/100 g fresh weight, with the highest value was found in *Sauropus androgynus* (L) Merr or known as cekur manis in Malay. This might be due to the presence of quercetin and kaempferol (Andarwulan *et al.,* 2010). Other factors such as climate and cultivar were also identified as the main factors affecting the structure of phenolics and other bioactive compounds (Bolling *et al.,* 2010).

Antioxidant inhibits the cellular damage by its known mechanism, free radical scavenging. DPPH free radical scavenging is one of the methods used to evaluate antioxidant activities with a very limited period of time compared to other methods. The EC_{50} value was determined to better quantify and determine the radical scavenging activity in the samples. *Gnetum gnemon* or pucuk empeng showed the lowest EC_{50} value with 3.92±1.52 µg/mL as shown in Table 1. Therefore, it possessed the strongest DPPH radical scavenging activity. There was also significant difference for all samples for 80% methanolic extract ($p< 0.05$). The previous study also reported that the stick of *G. gnemon* extracted with methanol gave the highest scavenging activity with a value of 38.679 ± 0.745% (Wazir *et al.,* 2011). Other study demonstrated that *C. caudatus* (ulam raja) extract inhibited linoleic acid oxidation which resulted in greatest DPPH free radical scavenging activity (Andarwulan *et al.,* 2010). Many studies showed

that the extract with a high amount of polyphenol content was able to exhibit high antioxidant activity (Bolling *et al.,* 2010; Reihani and Azhar, 2012). In this study, the extract of pucuk empeng corresponded with this suggestion but not in pucuk simpuh since it showed a relatively low antioxidant activity but higher total phenolic content. Poor specificity of the total phenolic content assay might be the reason for this disagreement (Singleton *et al.,* 1999; Escarpa and González, 2001). Besides, the number of phenolic compounds present may affect its respond towards Folin–Ciocalteu reagent (Singleton *et al.,* 1999).

ABTS radical scavenging assay is a method used to study the antioxidant potential and the results varied from that of DPPH radical scavenging activity method. In this assay, the highest scavenging activity was found in pucuk simpuh with the value of 7.93±0.02 µg ascorbic acid/mL. However, the results from ABTS assay showed that pucuk simpuh had the highest scavenging activity and it was varied from DPPH method whereby pucuk empeng had the highest scavenging activity. The difference in the trend of the results might be due to the different mechanism of action in both methods.

Phytochemicals such as the phenolic and flavonoid compounds contributed to the antioxidant activity in the wild ulam/vegetables extracts when correlation analysis was performed. Some correlations were observed between antioxidant activity and phytochemical content for several samples. From this analysis, DPPH free radical scavenging activity of pucuk putat was highest, moderately positively correlated with the phenolic content of the extracts (r = 0.615) while tebu gogok showed the lowest positive correlation with total phenolic content (r = 0.264). The results were in agreement with the previous studies which showed a strong correlation between the total phenolic content and antioxidant activity (Deighton *et al.,* 2000; Martínez *et al.,* 2012).

Meanwhile, total flavonoid content of pucuk putat showed the strongest positive correlation with antioxidant activity in all samples tested (r = 0.999), which was in agreement with an earlier study which also reported that antioxidant activity was closely related to the phenolic and flavonoid content (Sabli *et al.,* 2012; Ling *et al.,* 2015). Furthermore, ABTS radical scavenging of the extracts of pucuk putat was positively correlated to the total phenolic (r = 0.982) and pucuk empeng showed the lowest correlation with total phenolic content (r = 0.480). However, total flavonoid content of the pucuk simpuh extract was highly correlated with ABTS free radical scavenging (r = 0.987), while pucuk putat showed lowest correlation with ABTS free radical scavenging (r = 0.718).

5. Conclusion

Wild ulam/vegetables in Taman Negara Johor Endau Rompin (TNJER) are rich in phytochemicals and antioxidant which can be a great potential for use in complementary and alternative medicines as well as functional food applications.

Conflict of Interest

The authors do not have any conflicts of interest regarding the content of the present work.

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