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Nutritional profile and antioxidative properties of selected tropical wild vegetables

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Abstract

Five underutilized wild vegetables namely *Limnophila aromaticoides*, *Ceratopetris thalictroides*, *Crassocephalum crepidioides*, *Etlingera elatoir* and *Monochoria vaginalis* were analyzed for nutritional values, phenolic components and antioxidant activities. These wild greens were found to have high fibre (11.3-19.8 g/100g) and ash (13.0-17.6 g/100g) contents as compared to commercialized species, *Brassica juncea*. The iron content of *Monochoria vaginalis* is four times higher than *Brassica juncea* (33.1 mg/g dry weight). *Crassocephalum crepidioides* demonstrated remarkable lipid peroxidation inhibition (90.4%). The phenolic content of *Etlingera elatoir* is two times higher than *Brassica juncea*. Thus, it is of both great free radical scavenger and iron chelators with the lowest EC_{50} values of 1.8 mg/ml and 2.3 mg/ml respectively. As a conclusion, these wild vegetables could be potentially used in alleviating micronutrients deficiency especially for the rural populace and as a potent source of natural antioxidants.

Introduction

Wild leafy vegetables, which are usually ignored by people in the developed nations, are subsistence agriculture in developing countries especially in the food insecure regions. Indigenous people in remote areas are endowed with deep knowledge concerning the use of these wild species as food especially during period of drought, famine, and civil unrest. The knowledge on these wild species could be considered as the most important determinant as to whether an individual or family could maintain nutritional well-being, become malnourishment, or succumb. Besides, wild vegetables have an important socio-economic impact through their uses in medicines, foods, fibers and cultural ceremonies (Flyman and Afolayan, 2006).

In recent decades, a resurgence of interest has focused on wild plant species for their possible nutritional and medicinal values to broaden the diversity of human diet (Flyman and Afolayan, 2007; Afolayan and Jimoh, 2009). This is because people today are more concern about the effects of modern agricultural technology and marketing, which only cultivate plant types that have high productivity and consequently caused massive lost of biodiversity. Approximately two-thirds of total dietary energy intake is obtained from twelve domesticated species: eight cereals (barley, maize, millet, rice, rye, sorghum, sugar cane, and wheat) and four tubers (cassava, potato, sweet potato and yam) (FAO, 2010). Besides the reduction of genetic diversity of plants species in human diets, the high dietary selectivity practice has become another factor that cause difficulty in getting full complement of essential nutrients through daily diet which consequently lead to malnutrition and under-nutrition (Milton, 2003). Furthermore, most of the foods consumed by people have been "upgraded" to an extreme through refined and modified processes using various food preparation techniques such as cooking, crushing, leaching, and husking that causes inadvertently reduction or removal of certain essential nutrients from the food (Legwaila *et al*., 2009).

On the other hand, increasing research on underutilized vegetables in different regions showed that most of these wild greens have great nutritional values and antioxidant properties, which are comparable to those commercially cultivated vegetables (Afolayan and Jimoh, 2009; Glew *et al*., 2005; Maisuthisakul *et al*., 2007). Glew *et al*. (2005) reported that three usually consumed edible wild plants by the indigenous people in Niger (West Africa's Sahara region) have great influence on the nutritional status of local people due to their proportions of essential amino acids favorably adhering to the World Health Organization (WHO)

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standard. Besides, Maisuthisakul *et al*. (2007) found that indigenous chewing plants in Thailand contained great amount of phenolics and flavonoids, which responsible for the outstanding antiradical activities of these chewing plants. In other words, wild leafy vegetables which regularly deprecate by policy makers and often considered to be the 'weed of agriculture' are now not as simply as famine foods or wild foods in developing country, but yet they could serve as a source of functional nutrients.

In Malaysia and most of the developing countries, a wide range of wild edible native plants are used by indigenous people as part of their daily diets or as traditional medicines. For example, Tutan (*Solanumnigrum* L.) which is used to kill intestinal worms and reduce high blood pressure. In addition, nutritional composition study on the indigenous fruits and vegetables in Sarawak also showed that most local fruits are high in protein and potassium, while the nutritional value of indigenous vegetables are comparable to those commercialized species (Voon and Kueh, 1999). However, the beneficial properties of many other wild vegetables are yet to be proven since most of the previous studies only focused on the ethnobotanical knowledge while chemical analyses upon these edible plants are rare. This nutritional information could serve as a basis for national intervention strategies and dietary planning for the nutritional deficiency populace in rural areas. Therefore, the study aimed to investigate the nutritive values as well as the antioxidant properties of several wild vegetables that are commonly consumed by the indigenous people in East Malaysia.

Materials and Methods

Sample collection

Wild vegetables termed in this study refer to wild or non-domesticated plants that are normally collected from paddy fields, vegetable gardens, and forest. Five different species of edible wild plants have been selected and identified, namely *Limnophila aromaticoides* (finger grass), *Ceratopetris thalictroides* (Indian fern), *Crassocephalum crepidioides* (fireweed), *Etlingera elatoir* (torch ginger), and *Monochoria vaginalis* (pickerelweed). The wild vegetables were authenticated by an expert from the Forest Research Centre, Sandakan and voucher specimens were deposited in Herbarium. One commercially cultivated vegetable, namely *Brassica juncea* (mustard greens) was used as a standard for comparison. Three samples from each species were obtained from several native markets in the interior of Sabah, East Malaysia where subsequently samples were kept in plastic bags and transported in an insulated icebox to the laboratory for immediate preparation.

Sample preparation

Samples were washed and cleaned by using running potable water to remove visible dirt and only edible portions of the wild vegetables were used in the analysis. The samples were dried at 45-50 ^{0}C to a consistent moisture level before ground into fine powder and used for the subsequent proximate composition and minerals determination. The methanolic extract of fresh sample was used for the determination of total phenolic content, total flavonoids and the antioxidant activities. Fresh samples were blended and extracted 24 hours at room temperature with 80% methanol on a mechanical shaker (GFL, Model 3005, Germany). The supernatant, after filtered through Whatman No. 1 filter paper, was evaporated using rotary evaporator (BŰCHI, Model R-114, Switzerland) at 40 ^{0}C. The methanolic extract was collected and the yield of the sample was calculated. All analyzes were carried out in triplicate.

Proximate analysis

The moisture, dry matter, ash, fat, protein, and fibre content of the sample were determined on dry weight basis according to AOAC (2000). The moisture content of the dry and fresh samples was determined by using oven-drying method. Fat content was determined by Soxhlet method. All defatted residues were used for fibre, protein, and ash content determination. Fiber was determined by sequential extraction with boiling 1.25% of sulphuric acid (H_2SO_4) and 1.25% of sodium hydroxide (NaOH). Protein in the sample was determined by using Kjeldahl method and 6.25 was used as the factor of conversion. Carbohydrates were determined by difference. All samples were analyzed in triplicate.

Vitamins determination

Ascorbic acid (Vitamin C) content

Ascorbic acid was analyzed according to the method described by Zhang and Hamauzu (2004) with some modifications. A 10 g of fresh sample was homogenized with 15 ml of ice-cold 5% meta-phosphoric acid (MPA). The homogenate was filtered with muslin cloth and the residue was treated with 10 ml of ice-cold 5% MPA for two successive extractions. The filtrates was combined and centrifuged at 2500 g for 10 minutes (min) by using Centrifuge 2100 (KUBOTA, Kyoto, Japan). The supernatant was collected and made up to 50 ml

and then filtered before injected into a reverse phase C18 (150 X 4.6 mm 5 µm, Atlantis; Waters, Ireland) high performance liquid chromatography (HPLC) column. The mobile phase consisted of acetonitrile and acidified deionized water (0.1% phosphoric acid) in the ratio of 75:25 with the flow rate of 1.0 ml/min. The samples and standard L(+) – ascorbic acid (MERK, Germany) was detected at 254 nm on a Shimadzu SPD-10AV, UV-Visible detector. The total amount of ascorbic acid in sample was expressed in mg/100 g of fresh weight (FW).

Alpha-tocopherol and γ- tocopherol (Vitamins E) content

Alpha-tocopherol and γ- tocopherol were extracted through a same extraction method where the plant samples were saponified using alcoholic potassium hydroxide and ascorbic acid solution was used as antioxidant agent. Approximately 5 g of finely chopped sample was mixed with 30 ml of ethanol (~99.9%), 10 ml of 10% ascorbic acid solution, and 10 ml of 60% potassium hydroxide (KOH). The mixture was boiled and then refluxed for 5 min and cooled in running water. The saponified sample was filtered and the remaining residue was then added with 30 ml of petroleum ether and stirred for 30 min. The sample was centrifuged and the supernatant was used for liquid-liquid partition. The organic layer was kept in a conical flask, the aqueous layer was re-extracted with 30 ml petroleum ether, and all petroleum ether extracts were combined before being concentrated by rotary evaporator (Rotavapor R-114, BŰCHI, Switzerland) at 45 ^{0}C to dryness before dissolved with 2 ml of HPLC-grade acetonitrile. The quantification was done by HPLC using acetonitrile and water, 50:50 (v/v), as mobile phase with Atlantis, Waters C18 column and a UV detector at 268 nm. Different concentrations of α-tocopherol (SIGMA, Switzerland) and (+)-γ-tocopherol (SIGMA, Japan) standard were prepared for calibration and sample peak was identified through retention time and absorbance spectra in comparison with standards. The total amount of α-tocopherol and γ- tocopherol in sample was expressed in mg/100 g of FW.

Thiamin (Vitamin B1) and riboflavin (Vitamin B2) content

Thiamin was extracted simultaneously with Riboflavin according to Kumar and Aalbesberg (2006) with some modifications. Ten to fifteen grams of finely chopped sample was digested with 60 ml of 0.1N hydrochloric acid (HCl) before heated at 120 ^{0}C for 30 min. The acidic extract was then adjusted to pH 4.3 – 4.7 with 1M of sodium acetate and kept at 37 ^{0}C for 16 h. Two milliliter of 50% trichloroacetic acid (TCA) was added and heated at 50 ^{0}C for 10 min prior to top up to 100 ml with distilled water. The diluted extract was then filtered and the filtrate was used in HPLC analysis for the determination of riboflavin. On the other hand, for thiamin, 5 ml of the filtrate was took and oxidized to thiochrome by adding 3 ml of 1% potassium ferricyanide followed by 0.5 ml of phosphoric acid and 1.5 ml of water. Prior to HPLC injection, all sample extracts were filtered through a 0.45 µm Polytetrafluoroethylene (PTFE) syringe filter. Acetonitrile and deionized water (70:30) were employed as mobile phase with the flow rate of 1 ml/min and a UV detector at 268 nm. Thiamin and riboflavin standards (Sigma, Switzerland) were used for calibration and quantification of sample by comparing the integrated chromatographic peak areas from the test samples to the peak areas of known standards. The total amount of thiamin and riboflavin in sample was expressed in mg/100 g of FW.

Minerals determination

Atomic absorption spectrophotometric method was used to determine seven minerals that are ubiquitous in plants including potassium (K), sodium (Na), calcium (Ca), zinc (Zn), magnesium (Mg), copper (Cu) and iron (Fe) (AOAC, 2000). Samples were digested using wet ashing method with the mixture of concentrated nitric acid and perchloric acid. Flame atomic absorption spectrophotometer (Model Z5000, Hitachi, Japan) with air-acetylene flame was employed and the mineral contents were quantified by using plot of absorption (nm) against concentration (µg/ml) of standard solution with known concentrations. The entire analysis was carried out in four replicates and the average values were reported. The amount of element in each gram of the sample was calculated using formula below:

Amount of Element, ppm (µg/g) = (µg/ml) X F/ g sample

where, F = (ml original dilution X ml final dilution)/ ml aliquots if original 100 ml is diluted.

Antioxidant activity determination

Total antioxidant activity

Total antioxidant activity of the plant methanolic extracts were determined by using β-Carotene bleaching assay as described by Amin *et al*. (2006). One microliter of β-carotene solution (0.2 mg/ml chloroform) was pipetted into a round-bottom flask containing 20 µl linoleic acid and 200 µl Tween 20. The mixture was then evaporated under vacuum at

40°C for 10 min before 100 ml of distilled water added and vigorously agitated to form an emulsion. Five milliliters aliquot of the emulsion was transferred into different test tubes containing 200 µl of extract and vortexed before incubated at 50°C for 2 hours. Absorbance of the sample was measured every 15 min for 120 min at 470 nm using spectrophotometer. Blank solution (without β–carotene but containing sample of same concentration) was prepared; 0.5 mg of BHA is used as control. All determinations were performed in triplicate. The antioxidant activity (ANT) was expressed as the percent inhibition of β-carotene decolourization in comparison to the control

$$ANT\ (\%) = [1- (A_0 - A_t / Å_0 - Å_t)]\ X\ 100$$

where A_0 and A_t are the sample absorbance value measured at initial time and at t = 120 min respectively; $Å_0$ and $Å_t$ are the control absorbance value measured at initial time and at t = 120 min respectively.

Free radical scavenging activity.

The 2,2,-diphenyl-1-picrylhydrazyl• hydrate (DPPH•, SIGMA, Germany) free radical scavenging activity of plant extracts was determined spectrophotometrically according to the method of Brand-William *et al*. (1995) with some modifications. Briefly, 1 mM solution of DPPH in methanol was prepared fresh daily. The DPPH solution (0.5 ml) was added to 1 ml of extract (in different concentration from 2 to 10 mg/ml). The mixtures were shaken vigorously and left to stand at room temperature for 30 min. BHA was used as positive control while water was used as negative control. The change of absorbance was read at 517 nm and ability to scavenge DPPH free radical was calculated from the absorbance of the control (A(-ve) and A(+ve)) and of the sample (As) by using equation below:

$$Scavenging\ activity\ (\%) = [\ (A_{(-ve)} - A_s) / (A_{(-ve)} - A_{(+ve)})]\ X\ 100$$

where A_s is the absorbance of the sample, $A_{(-ve)}$ and $A_{(+ve)}$ are the absorbance values of negative and positive controls, respectively.

Ferrous ion chelating capacity

The ferrous ion chelating capacity of all wild vegetable extracts were determined according to the method of Hsu *et al*. (2003). A 0.1 ml of 2 mM ferrous chloride ($FeCl_2 \cdot 4H_2O$, System), 0.2 ml of 5 mM of ferozine (3-(2-pyridyl)-5, 6-diphenyl-1,2,4-triazine-4',4''-disulfonic acid sodium salt, SIGMA) and 3.7 ml of ethanol was added sequentially into test tubes contained 1 ml of methanolic wild plant extracts (from 2 to 10 mg/ml). The mixtures were left to react for 10 min before the absorbance was measured at 562 nm. Ethylenediamine-tetraacetic acid (EDTA, SIGMA), 2 mg/ml was used as positive control and the ferrous ion chelating capacity of plant extract was calculated with the following equation,

$$Ferrous\ ion\ chelating\ capacity\ (\%) = [1- (A_s / A_c)]\ X\ 100$$

where A_s is absorbance of the sample and A_c is the absorbance of the control sample.

Total phenolic content determination

The total phenolic content in plant methanolic extracts was determined using Folin-Ciocalteu assay, according to the method described by Djeridane *et al*. (2006). A 100 µl of the sample was dissolved in 500 µl (1/10 dilution) of the Folin-Ciocalteu reagent and 1000 µl of distilled water. The solutions were mixed and incubated at room temperature for 1 min before 1500 µl of 20% sodium carbonate solution was added. The final mixture was shaken and then incubated for 2 h in the dark at room temperature before determined using spectrophotometer at 760 nm. Quantification was done based on the standard curve of gallic acid and result was expressed in milligram of gallic acid equivalents (GAE) per 100 g of FW.

Total flavonoids content determination.

The flavonoid content was measured using a colourimetric assay developed by Zhishen *et al*. (1999). One millilitre extract was added to a 10 ml volumetric flask. Distilled water was added to make a volume of 5 ml. At zero time, 0.3 ml of 5% sodium nitrite solution was added to the flask. After 5 min, 0.6 ml of 10% aluminium chloride solution was added and after 6 min, 2 ml of 1M NaOH was added to the mixture followed by the addition of 2.1 ml distilled water. The mixture was thoroughly vortexed and the absorbance of the mixture was read at 510 nm. Rutin was used as standard and the result was reported as rutin equivalents (RE)/100 g FW.

Statistical analysis

All measurements were performed in triplicate and recorded as mean ± standard deviation (SD). Results obtained were then analyzed by using Statistical Package for Social Science (SPSS) for Windows version 12.0. One-way analysis of variance (ANOVA) and Tukey's test were used to determine the significant differences between the five wild

vegetable and cultivated vegetable at p-values ≤0.05. The EC_{50} values were calculated from linear regression analysis while Pearson correlation was used to determine the relationship between antioxidant components (phenolic content and flavonoid content) and antioxidant activity.

Results and Discussion

Proximate composition

The moisture content of the selected fresh wild vegetables ranged from 92.6 to 97.5 g/100 fresh weight [FW] (Table 1). The high moisture content of these plants give great impact on energy density (amount of energy in a given weight of food (kcal/g)) as water adds substantial weight to the food without adding energy and this may give the consumers a better satiety without increase their energy intake. All underutilized vegetables analyzed were found to be poor sources of fat with *Limnophila aromaticoides* has the highest fat content (3.3 g/100g dry weight [DW]), only able to contribute 12% of the Dietary Reference Intakes (DRI) for fat (20-35 g/day). However, the wild vegetables used in this study showed great fibre content as compared to *Brasicca juncea* (10.6 g/100g DW), the commercialized vegetable with the highest in *Etlingera elatoir* (19.8 g/100g DW). Interestingly, the fiber content of *Etlingera elatoir* is five times higher than *Momordica balsamina* L (an underutilized vegetable) from Botswana (Flyman *et al*., 2007). In addition, the average fibre content of the analyzed wild vegetables (15.84 g/100g DW) is two times higher than the four common leafy vegetables (*Vernonia amygdalina*, *Solanum Africana*, *Amaranthus hybridus* and *Telfaria occidentalis*) found in Nigeria as reported by Aletor *et al*. (2002). Hence, these wild greens could potentially be a source of high fibre and low fat diet for those who concern about their weight or on weight management program.

The protein content of the wild vegetables ranged from 10.0 to 21.2 g/100g DW, most of them have lower protein content compared to the cultivated vegetable, *Brasicca juncea* (20.5 g/100g DW). It is obvious that these plants are not a good source of protein but it provides almost 65% of the world supply of edible protein especially in developing countries. Therefore, in order to improve the low concentration and incompleteness of plant proteins, consumption of a mixture of plant proteins food are suggested to obtain well balanced of amino acids that is equivalent to animal protein. The ash content of wild vegetables in this study (13-18g/100g DW) was higher than *Brassica juncea* (10.7 g/100g DW) indicating that consumption of these wild greens might contribute higher minerals content. The energy contribution ranged from 225 to 240 kcal/100 g DW, which is slightly lower than their commercialized counterpart (277 kcal/100 g DW).

Minerals content

The major and trace minerals content of the studied vegetables are shown in Table 1. The wild vegetables contained comparable potassium to other cruciferous vegetables (Miller-Cebert *et al*., 2009) and the potassium content of *Etlingera elatoir* is about two times higher than *Brassica juncea* (22 mg/g DW).). In addition, the potassium content of *Etlingera elatoir* was found greater than some wild leafy vegetables in South Africa which included *Sonchu asper*, *Solanum*

Table 1. Proximate composition (g/100 g DW), energy (kcal/100 g DW), major minerals and trace minerals (DW basis) of five different species of edible wild vegetables and one commercialized green vegetable that usually consumed by indigenous people in East Malaysia

Edible wild plants	Finger grass (*Limnophila aromaticoides*)	Indian fern (*Ceratopetris thalictroides*)	Fireweed (*Crassocephalum crepidioides*)	Torch ginger (*Etlingera elatoir*)	Pickerelweed (*Monochoria vaginalis*)	Mustard greens (*Brassica juncea*)
Proximate Composition (/100g DW)						
Moisture (g/ 100g FW)	94.9±0.1^{d}	92.6±0.1^{e}	95.7±0.1^{c}	94.9±0.2^{d}	97.5±0.1^{a}	96.8±0.11^{b}
CHO (g)	44.6±1.4^{a}	37.0±0.6^{bc}	34.8±0.0^{c}	36.4±0.5^{bc}	38.5±1.1^{b}	43.6±0.2^{a}
Protein (g)	10.0±0.1^{e}	21.2±0.0^{a}	18.1±0.4^{c}	16.2±0.1^{d}	19.6±0.0^{b}	20.5±0.2^{a}
Fat (g)	3.3±0.1^{a}	2.1±0.0^{bc}	3.2±0.2^{c}	1.6±0.0^{c}	0.9±0.1^{d}	2.4±0.2^{b}
Fibre (g)	16.7±0.5^{b}	17.7±0.4^{b}	13.7±0.2^{c}	19.8±0.3^{a}	11.3±0.4^{d}	10.6±0.0^{d}
Ash (g)	13.3±0.1^{b}	13.0±0.3^{b}	16.7±0.1^{a}	17.6±0.1^{a}	13.2±0.5^{b}	10.7±0.3^{c}
Energy (kcal)	248	252	240	225	240	277
Mineral Composition						
Major Mineral (mg/g)						
Na	10.5±0.3^{b}	3.3±0.3^{c}	3.3±0.2^{c}	5.2±0.2^{c}	18.8±2.1^{a}	4.7±0.9^{c}
K	21.8±0.3^{b}	24.0±2.3^{b}	19.9±0.3^{b}	39.0±3.1^{a}	20.2±5.0^{b}	22.1±0.0^{b}
Mg	3.13±0.0^{ab}	2.89±0.1^{b}	2.17±0.0^{c}	2.91±0.0^{b}	1.64±0.1^{d}	3.28±0.1^{a}
Ca	11.9±0.1^{ab}	11.0±0.3^{c}	3.9±0.1^{d}	11.5±0.6^{c}	4.4±0.0^{d}	12.9±0.0^{b}
Trace Mineral						
Fe (mg/100g)	11.3±0.4^{b}	7.1±0.1^{c}	11.9±0.0^{b}	3.6±0.0^{d}	33.1±0.5^{a}	8.0±0.5^{c}
Zn (µg/g)	216.4±0.7^{a}	70.3±1.9^{e}	116.5±2.7^{c}	183.8±2.5^{b}	37.8±1.2^{f}	100.3±5.6^{d}
Cu (µg/g)	61.3±0.0^{d}	113.0±4.2^{b}	231.9±1.0^{a}	107.7±2.1^{b}	67.1±1.0^{cd}	74.2±1.1^{c}

* Data presented as mean ± standard deviation. Mean values with different letters within row are significantly different ($p< 0.05$). DW, dry weight; FW, fresh weight; CHO, carbohydrate; Na: Sodium; K: Potassium; Mg: Magnesium; Ca: Calcium; Fe: Iron; Zn: Zinc; Cu: Copper.

Table 2. The vitamin composition, total phenolic and total flavonoid content and EC_{50} values of methanolic extract from five different species of wild vegetables and one commercialized green leafy vegetable

Edible wild plants	Finger grass (*Limnophila aromaticoides*)	Indian fern (*Ceratopetris thalictroides*)	Fireweed (*Crassocephalum crepidioides*)	Torch ginger (*Etlingera elatoir*)	Pickerelweed (*Monochoria vaginalis*)	Mustard greens (*Brassica juncea*)
Vitamins (µg/g FW)						
Ascorbic acid	844.6±7.3^{b}	551.8±2.9^{d}	733.2±3.1^{c}	272.2±2.8^{f}	438.1±3.1^{e}	872.8±7.7^{a}
γ-tocopherol	7.0±1.2^{b}	12.2±0.9^{a}	4.5±0.7$_{b}$	11.5±0.2^{a}	ND	ND
α-tocopherol	14.8±0.4^{a}	3.5±0.1^{cd}	5.8±0.3^{c}	12.3±0.4^{b}	1.1±0.2^{d}	ND
Thiamin	16.6±0.1^{a}	9.0±0.1^{b}	ND	3.2±0.0^{c}	ND	ND
Riboflavin	13.7±0.0^{a}	8.9±0.0^{c}	12.4±0.1^{b}	4.4±0.1^{d}	2.3±0.1^{e}	2.3±0.2^{e}
Antioxidant components						
Total phenolic (mg GAE/g FW)	3.8±0.1^{a}	2.1±0.2^{c}	2.0±0.3^{b}	4.1±0.3^{a}	1.9±0.1^{d}	1.8±0.3^{d}
Total Flavonoid (mg RE/g FW)	1.3±0.0^{a}	1.0±0.1^{c}	0.6±0.0^{e}	1.4±0.4^{b}	0.4±0.1^{f}	0.9±0.1^{d}
EC_{50} value (mg extract/ml)**						
DPPH radical scavenging ability	2.85±1.58^{cd}	4.61±0.02^{c}	3.80±0.07^{c}	1.80±0.66^{d}	7.18±0.36^{b}	12.06±0.53^{a}
Ferrous ion Chelating ability	10.80±0.25^{b}	6.59±0.24^{d}	3.80±0.07^{e}	2.26±0.06^{f}	18.74±1.04^{a}	8.17±0.28^{c}

* Each value is expressed as mean± standard deviation. Mean values with different letters within the same row are significantly different ($p< 0.05$).
FW, fresh weight; GAE, gallic acid equivalent; RE, rutin equivalent; ND, not detected, which means that the value is less than 0.35X10-5 absorbance unit (au).
** EC_{50} value, the efficient concentration of plant extracts at which DPPH radicals were scavenged by 50%; and ferrous ion were chelated by 50%

nigrum and *Urtica urens* but not for *Chenopodium album* which demonstrated much higher potassium content (Afolayan *et al*., 2009). Regular consumption of these wild vegetables may assist in preventing hypertension and cardiovascular diseases, as dietary potassium is an important cation in regulating blood pressure and attenuating platelet reactivity, which is the major causative factor of vascular occlusion (He and MacGregor, 2008). Furthermore, consumption of wild vegetables with high potassium content enhances the bioavailability of calcium in body and promotes bone health by preventing the occurrences of calciuria.

The amount of calcium ranged between 3.9 and 11.9 mg/g DW with the lowest in *Crassocephalum crepidioides* and the highest in *Limnophila aromaticoides*. The common vegetable, *Brassica juncea* has higher calcium content (12.9 mg/g DW) as compared to other wild vegetables. However, the calcium level was found higher than some of the wild plants from Africa, for example, young shoot of *Borassus aethiopum* (0.2 mg/g DW), fruit of *Tamarindus indica* (1.9 mg/g DW) and *Momordica balsamina* L. (2.2 mg/g DW) (Glew *et al*., 2005; Flyman and Afolayan, 2007). Calcium with the name of "super nutrient" has been proven clinically associated with reduced risk of various non-communicable diseases such as osteoporosis (Nieves 2005), cardiovascular diseases (Vaskonen, 2003) and it also helps to reduce colorectal cancer risk by promoting the apoptosis in human colorectal epithelium that reduce colorectal neoplasms (Flood *et al*. 2005; Fedirko *et al*. 2009). Few researches have promoted the intake of plant-based diet such as soybean and green leafy vegetable as a source of calcium instead of dairy or animal products. This is because animal protein could increase bone loss and risk of fracture through promoting calcium excretion and acid-base metabolism (Park *et al*., 2011).

The magnesium content was low and varied between 1.6 and 2.9 mg/g DW while the zinc content in wild vegetables oscillated from 37.8 µg/g DW (*Monochoria vaginalis*) to 216.4 µg/g DW (*Limnophila aromaticoides*). It seems that the results are much higher than those wild plants found in Nigeria, which ranged between 12.1 and 19.0 µg/g DW (Glew *et al*., 2005). Iron (Fe) is the most abundant trace mineral in these wild plants, ranged 3.6 mg/100 g - 33.1 mg/100 g DW. This result compares favorably with the underutilized vegetables reported by Flyman and Afolayan (2007). The iron level of *Monochoria vaginalis* is 4 times higher than the iron content of the common vegetable (*Brassica juncea*, 8.0 mg/100 g DW) and every serving size of 85 g of fresh *Monochoria vaginalis* (5.5 mg)) contributed 69% and 31% to the DRI of iron for an adult male (8 mg/day) and adult female (18 mg/day) respectively. Consequently, with this level of iron, the locals especially women might benefit from eating *Monochoria vaginalis* during pregnancy as well as after delivery to prevent iron deficiency which may cause anemia and immune system dysfunction. Thus, cultivation of this wild plant can be suggested as a food based strategy to alleviate or improve the unsatisfactory dietary iron intake of adolescents in the low-income areas.

Vitamins Content

Results of the current study demonstrated that the wild species contained valuable amount of vitamins such as riboflavin and ascorbic acid (Table 2). The riboflavin content was the highest in *Limnophila aromaticoides* (13.7 µg/g FW) followed by *Crassocephalum crepidioides* (12.4 µg/g FW) and these contents are of the same magnitude as that generally found in dairy products. Therefore, daily consumption of these vegetables might help to alleviate riboflavin deficiency, which gives

negative impact on the metabolism of other nutrients, especially B-group vitamins, through flavin coenzyme activity. In addition, sufficient intake of riboflavin has proven to have protective effect against proximal colon cancer through preventing the aberrant of DNA methylation (de Vogel *et al*., 2008). The amount of thiamin in Limnophila aromaticoides (16.6 µg/g FW), *Ceratopetris thalictroides* (9.0 µg/g FW) and *Etlingera elatoir* (3.24 µg/g FW) are high compared to the uncommon vegetables studied by Raghuvanshi *et al*. (2001). Nevertheless, plants or vegetables usually contain great amount of heat-stable anti-thiamin compounds such as tannic acid and thiaminase I, which will affect the bioavailability of thiamin in plants (Lonsdale, 2006).

Similar to most other vegetables and fruits, both form of vitamin E were also found in these wild vegetables except *Monochoria vaginalis*. *Limnophila aromaticoides* exhibited the highest α-tocopherol content (14.8 µg/g FW) whereas, the highest γ-tocopherol content was found in Ceratopetris thalictroides (12.2 µg/g FW). However, both γ-tocopherol and α-tocopherol content of these plants were found rather low compared to the edible aromatic plants studied by Gómez-Coronado *et al*. (2004). Wild vegetables contained appreciable amounts of ascorbic acid (Vitamin C) with *Limnophila aromaticoides* (844.6 µg/g FW) contained the highest level whereas the lowest was found in *Etlingera* sp (272.2 µg/g FW). The consumption of every 100 g of *Limnophila aromaticoides* enable to fulfill 112% and 94% of the DRI of ascorbic acid for both adult female (75 mg/day) and male (90 mg/day) respectively. Surprisingly, *Limnophila aromaticoides*, *Crassocephalum crepidioides* and *Ceratopetris thalictroides*, were found to have higher ascorbic acid than that found in broccoli (52.9 mg/100g) (Singh *et al*., 2007). According to the findings of Lui *et al*. (2008), high concentration of ascorbic acid in plant samples might associated with attractive free radical scavenging capacity and health benefit like anti-carcinogenic and anti-atherogenic.

Antioxidant properties of wild vegetables

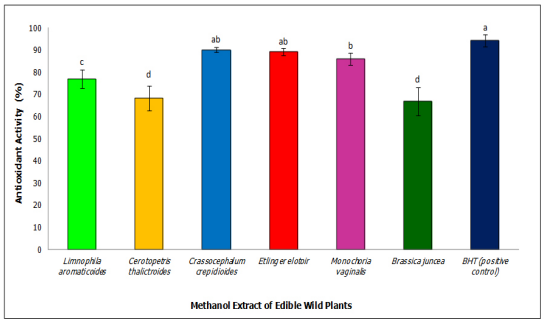
Total phenolic and flavonoid content

The phenolic content of these wild vegetables were significantly higher in comparison to *Brassica juncea*, the commercialized vegetable (Table 2). *Etlingera elatoir* contained the highest phenolic level (4.1 mg gallic acid equivalents [GAE]/g FW), followed by *Limnophila aromaticoides* and *Ceratopetris thalictroides*. The phenolic content of both *Etlingera elatoir* and *Limnophila aromaticoides* are two times higher than *Brassica juncea*. Furthermore, the results are also higher than the commonly consumed food plants from India (0.0-1.2 mg GAE/g raw foodstuff) obtained by Saxena *et al*. (2007). In contrast to the current findings, however some of the plant species found in Amazonion region such as *Bauhinia forficate* (16.6 mg GAE/g FW), *Davilla kunthii* (36.4 mg GAE/g FW) and *Byrsonima crassifolia* (45.5 mg GAE/g FW) have 10 fold higher phenolic contents (Silva *et al*., 2007). Phenolics are nonnutritive secondary metabolites found in plants that promote significant health benefit and prevent various diseases. Study by Delgado *et al*. (2009) showed that phenolic compounds are able to protect human cell against oxidative DNA damage and hence possess great anti-carcinogenic capabilities.

The total flavonoid content of the vegetable samples varied considerably from 0.4 to 1.4 mg rutin equivalents (RE)/g FW of sample. The order of total flavonoid content of the investigated underutilized vegetables is *Monochoria vaginalis* < *Crassocephalum crepidioides* < *Brassica juncea* < *Ceratopetrist halictroides* < *Limnophila aromaticoides* < *Etlingera elatoir*. It seems the total flavonoids content of the studied plants are comparable to some of the medicinal plants (1.6-13.12 mg RE/g DW) and vegetables (0.9-4.9 mg RE/g DW) reported in the previous study (Djeridane *et al*., 2006). Even though flavonoids are important phenolic compounds contributing to the antioxidant activity of the vegetables, apparently it is also possible that the antioxidant activities of these vegetables are due to other phenolic compounds. Furthermore, the type of flavonoids present in the plant samples could be another factor that determines their antioxidant capability. As Tsimogiannis and Oreopoulou (2006) found that antioxidant properties of flavonoids depend on their C-ring structure, flavonoids with full substitution C-ring like rutin and luteolin, which their ether bonded with three oxygen demonstrate better free radical scavenging capacity and higher reaction rate compare to flavonoids that lack of one or more C-ring structural elements.

Antioxidant capacity of the methanolic extracts

The determination of total antioxidant activity by β-carotene-linoleic acid system is widely used due to high susceptibility of β-carotene towards free radical mediated oxidation. Methanolic extracts of the wild vegetables exhibited attractive antioxidant activities in β-carotene-linoleate model system as compared to Brassica juncea (66.78%) (Figure 1). This indicates that wild vegetables extracts can act as an effective lipid peroxidation inhibitor in the β-carotene-linoleate system. *Crassocephalum crepidioides* shows the highest antioxidant activity (90.04%), followed by *Etlingera elotoir* (89.23%), *Monochoria vaginalis*



Data presented as mean ± standard deviation. Mean values with different letters are significantly different ($p < 0.05$)

Figure 1. Antioxidant activity of underutilized vegetables extract (2 mg/ml) in β-carotene-linoleate model system

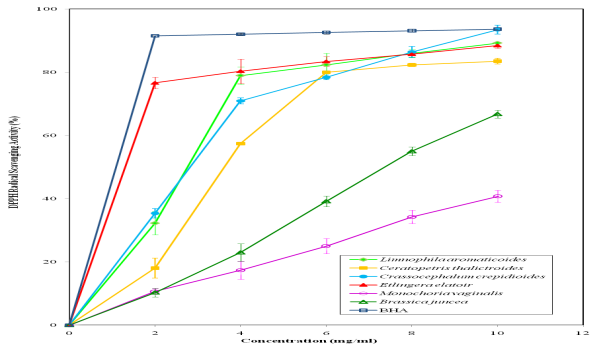


Figure 2. DPPH radical scavenging effect of underutilized vegetables with different concentrations of plant extracts

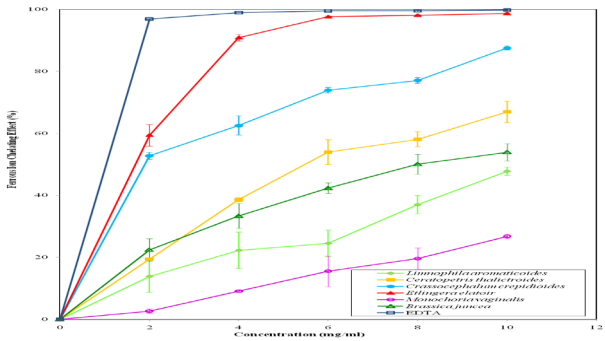


Figure 3. Chelating effect of underutilized vegetables with different concentrations of plant extracts

(88.85%) and *Limnophila aromaticoides* (76.91%) respectively. These findings are comparably higher than the antioxidant activity of four *Amaranthus* species reported by Amin *et al*. (2006) which ranged between 44 and 61.4%. The marked activity of *Etlingera elatoir* extract is partially attributed to its high phenolic content and its outstanding antioxidant activity was in agreement with findings reported by Chan *et al*. (2007). However, *Monochoria vaginalis* and *Crassocephalum crepidioides* with low phenolic content surprisingly showed great antioxidant activity. This could be explained by some individual phenolic units, which may act as efficient antioxidant rather than contributing to high phenolics measured by Folin-Ciocalteu assay.

Plant extract with remarkable free radicals scavenging abilities possess excellent capabilities in preventing oxidative damages mediated by radicals within cellular system as well as in food system (Valko *et al*., 2007). Most of the methanolic extract of wild vegetables studied show appreciable free radical scavenging activities in concentration-dependent manner, where scavenging activity increased with an increase in the concentration of each individual plant extract (Figure 2). Two mg/ml of *Etlingera elatoir* extract able to scavenge almost 80% of the DPPH free radicals in contrast, the scavenging activity of 2 mg/ml of *Monochoria vaginalis* extract is 8 times lower than *Etlingera elatoir* extract, where only 10% of the radicals had been scavenged. For *Crassocephalum crepidioides* extract at 2 mg/ml, the scavenging ability towards free radicals was 35.4%, but it increased to 93.47% when the concentration of the extract was 10 mg/ml. Its scavenging activity surpassed other wild vegetables and comparable with the synthetic antioxidant agent, BHA (93.63%). The superior scavenging activity of this methanolic extract could be also due to its high ascorbic acid content since ascorbic acid has been reported as an excellent DPPH radical-scavenger compared to other polyphenols and synthetic antioxidant agent like BHT (Lui et al., 2008). The effective concentration, EC_{50} value of the wild vegetables extract in DPPH scavenging activity ranged from 1.8 mg/ml to 7.2 mg/ml (Table 2). A higher DPPH scavenging activity is associated with a lower EC_{50} value. The EC_{50} value of *Etlingera elotoir* (1.8 mg/ml) was comparable with BHT (1.0 mg/ml).

Ferrous ion is well known as a reactive pro-oxidant that is commonly found in food systems and will accelerate oxidative changes in lipids, protein and other cellular components by breaking down hydrogen and lipid peroxidase to reactive free radicals via Fenton reaction (Hsu *et al*., 2003). Therefore, in this assay, the effectiveness of wild vegetable in metal chelation was determined by measuring the capability of the plant extract in competing with ferrozine (a chelating reagent) for ferrous ion. At 10 mg/ml, the chelating ability of methanolic extracts was oscillated from 26.75% to 98.64%. *Etlingera elatoir* showed the highest chelating effect (98.64%), comparable to EDTA (99.78%) and about two times higher than the cultivated vegetable, *Brassica juncea* (53.91%) (Figure 3). Furthermore, EC_{50} value (effective concentration of extract required to chelate 50% of ferrous ion) of *Etlingera elotoir* is the lowest (2.3 mg/ml) among all the studied wild vegetables followed by *Crassocephalum crepidioides* (3.8 mg/ml). However, *Limnophila aromaticoides* with high phenolic content and radical scavenging activity surprisingly showed weak ferrous ion chelating ability with EC_{50}

value more than 10 mg/ml. The present findings seem to be consistent with other studies, which reported that there was no significant correlation between the chelating activity and other antioxidant activity for some of their plant extracts (Chan *et al*., 2007; Hinneburg *et al*., 2006). This would mean that only certain compounds are able to exhibit great chelating effect. Compounds like anthraquinone and anthrone has been reported nearly no chelating effect on metal ions. The compounds responsible could be nitrogen or hydroxyl groups (such as –COOH, -NR_2 and –OH) containing compounds which were found in the leaves of Etlingera species are generally better chelators (Chan *et al*., 2007).

The correlation between the antioxidative groups (phenolic and flavonoid content) and antioxidant capacities have been evaluated using Pearson's correlation coefficients. DPPH radical scavenging activity with both total phenolic and flavonoid content exhibited good correlation with the regression coefficient of r^2=0.785, $p<0.01$ and r^2=0.614, $p<0.01$ respectively. On the other hand, a relatively weak relationship was found between ferrous ion chelating ability and phenolic content (r^2=0.470, $p<0.01$). This finding is in accordance to the report by Hinneburg *et al*. (2006). Furthermore, flavonoids content in the plant extract might not be the main contributor to antioxidant activities, as they did not show strong correlation with the antioxidant activity in this study. However, this equivocal correlation between antioxidant activity and flavonoid content can be explained as different phenolic compound respond differently depending on the chemical structure of the phenolic groups in the plant extracts. The number and position of hydrogen donating hydroxyl groups on the aromatic cycles of phenolic molecules could also determine their antioxidant capabilities (Djeridane *et al*., 2006).

Conclusion

The wild vegetables selected in the study could be a good source of micronutrients and natural antioxidants in alleviating malnutrition problems of local societies especially the rural populace. Besides the natural attribute of these underutilized vegetables such as higher resistance against diseases, these vegetables are more adaptive to harsh environments and grown less intensively which enables them to be considered as new food crops in order to broaden the diversity of human diet.

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