Nutritional values of wild edible freshwater macrophytes

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Background. The social acceptability of wild freshwater macrophytes as locally consumed vegetables is widespread. Freshwater macrophytes have several uses, and among them are food for humans. This study determines the proximate composition and mineral content of three freshwater macrophytes species, *Eichhornia crassipes*, *Limnocharis flava*, and *Neptunia oleracea*.

Methods. Young shoots of *E. crassipes*, *L. flava*, and *N. oleracea* were collected from shallow channels of Puchong (3° 00’ 11.89” N, 101° 42’ 43.12” E), Ladang 10, Universiti Putra Malaysia (2° 58’ 44.41” N, 101° 42’ 44.45” E) and Kg. Alur Selibong, Langgar (06° 5’ 50.9” N, 100° 26’ 49.8” E), Kedah, Peninsular Malaysia. The nutritional values of these macrophytes were analyzed using a standard protocol of the Association of Official Analytical Chemists. Eight replicates of *E. crassipes* and *L. flava* and four replicates of *N. oleracea* were used for subsequent analyses.

Results. In the proximate analysis, *N. oleracea* possessed the highest percentage of crude protein (29.61%) and energy content (4269.65 cal g^{-1}), while *L. flava* had the highest percentage of crude fat (5.75%) and ash (18.31%). The proximate composition trend for each species is different; all species have more carbohydrates and fewer crude lipids. All species showed a similar mineral trend, with high nitrogen and potassium and fewer copper contents. Nitrogen and potassium ranged from 12380-40380 mg kg^{-1} and 11212 -33276 mg kg^{-1}, respectively, and copper ranged from 16-27 mg kg^{-1}. The results showed that all three plant species, *E. crassipes, N. oleracea,* and *L. flava,* are plant-based sources of macro- and micro-nutrient beneficial supplements for human consumption.

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Abstract

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ranged from 12380-40380 mg kg^{-1} and 11212 -33276 mg kg^{-1}, respectively, and copper ranged from 16-27 mg kg^{-1}. The results showed that all three plant species, *E. crassipes, N. oleracea,* and *L. flava,* are plant-based sources of macro- and micro-nutrient beneficial supplements for human consumption.

Keywords Freshwater macrophytes, Minerals content, Nutrients, Proximate analysis, Wild plants

Introduction

One of the top ten factors contributing to mortality was the low intake of vegetables and fruits (Ezzati et al. 2002). Vegetables are sources of vitamins and minerals for antioxidant activity needed in the diet to meet daily micronutrient requirements (Gupta & Bains, 2006). To reduce individual risk and cardiovascular disease, humans and animals need optimal intakes of minerals such as potassium, sodium, calcium, magnesium, copper, manganese, iodine, and zinc (Mertz, 1982). To perform physiological functions, micronutrients such as copper, zinc, and iron obtained from food are required in the human body in limited amounts, typically less than 100 micrograms per day. Micronutrient deficiency, e.g., zinc, causes decreased taste acuity, slow wound healing, impaired development, decreased sexual maturity, impaired immune system function, and impaired metabolism and homeostasis disorders of the thyroid gland (Almatsier, 2006).

Freshwater macrophytes are aquatic plants submerged, emerging, or floating on the water surface (Lacoul & Freedman, 2006). They occur in the swamp, peatland, lake, stream, pond, rice field, and drainage canal (Den Hartog, 1981; Muta Harah et al., 2005). Wild plants have played a crucial role in the human diet, and some communities still depend on these wild foods (Tbatou et al., 2018). According to Grubben, Siemonsma & Kasem (1994), of the 225 vegetables in Southeast Asia, about 100 species are wild weeds. In East Malaysia, i.e., Sarawak, some 43-48 species of wild freshwater macrophytes, belonging to 28 families considered weeds, are collected and used as edible food and food preparation, medicine, and used as household items for pillows, mats, and even made as a souvenir (Muta Harah et al., 2005, Muta Harah, Japar Sidik & Suzalina Akma, 2014). The local collectors also offer freshwater macrophytes for sale in the local markets. Wild freshwater macrophytes are slowly being well-received as consumed vegetables (Muta Harah et al., 2005; Saupi, Zakaria & Bujang, 2009; Muta Harah, Japar Sidik & Suzalina Akma, 2014, Noorasmah et al., 2015; Noorasmah et al., 2016). Besides being cheaper, vegetarian products also have importance, e.g., source of essential minerals, in human nutrition (Saupi, Zakaria & Bujang, 2009; Noorasmah et al., 2015; Caunii et al., 2010). Some indigenous leafy vegetables, nuts, and wild fruits also give energy and are food supplements with good carbohydrates and other nutrients (Achinewhu, Ogbonna & Hart, 1995).

Global food problems have challenged all organizations and researchers to investigate the possibility of using wild plant species as supplementary sources of nutrients (Abubakar et al., 2021). Wild plants can provide minerals, vitamins, protein, phenolics, carotenoids, and carbohydrates (Seal, Pillai & Chaudhuri, 2017, Ghanimi et al., 2022). Studies on the nutritional potential of some wild edible plants have revealed their comparability or even superiority to domesticated crops (Shad, Shah & Bakht, 2013). Global dietary guidelines recommend increased consumption of fruits and vegetables to mitigate the threat of diet-related diseases, including metabolic disorders, cancer, and cardiovascular diseases (Stratton et al., 2021). Therefore, promoting these plants will ensure important nutritional sources for food security and sustainable development.

Macrophytes have been used locally in Malaysia as food sources (Saupi, Zakaria & Bujang, 2009; Muta Harah, Japar Sidik & Suzalina Akma, 2014; Noorasmah et al., 2015; Noorasmah et al., 2016). The common macrophyte species highly devoured by the locals include *Eichhornia crassipes* (Mart.) Solms, *Limnocharis flava* (L.) Buchenau, and *Neptunia oleracea* Lour. (Figure 1). *Eichhornia crassipes*, known as Water hyacinth, is a floating freshwater macrophyte with broad leaves above the water surface and spongy stalks (Figure 1a). Meanwhile, *Limnocharis flava* (Yellow velvetleaf), is a plant with pale green leaves and stalks with triangular-shaped petiole leaves (Figure 1b). The leaf blade is papery and broadly ovate-elliptic. Water mimosa, *N. oleracea* has sensitive leaves when touched. It has white spongy air tissues on stems floating on the surface of freshwater (Figure 1c). Under uncontrolled conditions, these species cause problems in human-made water bodies. Due to their fast reproduction in vegetative and generative states, these plants are commonly referred to as noxious weeds. Rather than destroying them with herbicides, which might affect the ecology, it is preferable to collect and consume them or use them as feed.

Figure 1 Freshwater macrophytes in study sites: (a) *E. crassipes* from a shallow channel of Puchong, Selangor, Malaysia, (b) *L. flava* from a shallow channel of Ladang 10, Universiti Putra Malaysia, Selangor, Malaysia. Young shoots and inflorescences of (a) and (b) are consumed cooked as vegetables, (c) *N. oleracea* from a shallow channel of Kg. Alur Selibong, Langgar, Kedah. The young leaves' shoot tips are usually consumed blanched or cooked as a vegetable.

Local people have been harvesting the young leaves with petioles and inflorescences of *E. crassipes* and *L. flava*, young leaves shoot tips, and immature pods of *N. oleracea* to be consumed as blanched or cooked vegetables as well as to be sold in the local markets to earn income. This is also supported by studies on ethnobotanical information (Saupi, Zakaria & Bujang, 2009; Muta Harah, Japar Sidik & Suzalina Akma, 2014; Saupi et al. 2020) to investigate the most consumed wild aquatic plants species, particularly in Bintulu, Sarawak communities. Based on the data, it shows that young shoots of *L. flava* and *N. oleracea* were commonly used in dish preparation due to their good palatability and sweet taste with great nutritional quality, but these species are the least frequently available in the market. Since aquatic macrophytes' chemical composition varies greatly depending on their species, seasons, habitat, and geographic location, proximate their species, seasons, habitat, and geographic location, proximate analysis, and mineral study are crucial in determining their nutritional value for future usage possibilities. In addition, a lack of documentation has been published on the nutritional profiling of these macrophytes. Therefore, the present study aims to determine the proximate composition and mineral content of the commonly consumed wild freshwater macrophytes of *E. crassipes, L. flava*, and *N. oleracea*. The finding of this study could also suggest that these species to be grown commercially as new vegetable crops.

Materials & Methods

Sample collection and preparation

Plants of *E. crassipes*, *L. flava*, and *N. oleracea* were collected from shallow channels of Puchong (3° 00’ 11.89” N, 101° 42’ 43.12” E), Ladang 10, Universiti Putra Malaysia (2° 58’ 44.41” N, 101° 42’ 44.45” E) and Kg. Alur Selibong, Langgar (06° 5’ 50.9” N, 100° 26’ 49.8” E), Kedah, Peninsular Malaysia from January to March 2020. The complete specimen of each species was arranged directly on the drawing block, labeled, and pressed as soon as possible using wooden pressed and ensuring the straps tight enough to bind the press together. The herbarium samples were examined to verify the identity of the specific plant used in a study. The morphological characterization of the aquatic macrophytes was carried out following the guidelines based on Weeds of rice in Indonesia by Soerjani, Kostermans & Tjitrosoepomo (1987).

Young shoots (light green with the tender shoot) were sampled and stored in a zip-lock plastic bag accordingly before being kept in an ice chest for transportation to the laboratory. Remove any adhering materials from the plant samples by washing them with distilled water. Approximately 500 g of fresh samples were cut into small pieces and oven-dried at 60°C until constant weight. Dry samples were ground using IKA A11® Basic Analytical Mill and passed through 0.2 mm laboratory sieved. The samples powder was labeled and kept inside an air-tight container at room temperature prior to proximate composition (ash, moisture, crude lipid, crude protein, crude fibre, and energy) analysis and mineral content (nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), manganese (Mn), zinc (Zn) and iron (Fe) analysis. Eight replicates (petiole and leaves) of *E. crassipes* and *L. flava* and four replicates of the whole pinnately compound leaf of *N. oleracea* were used for subsequent analyses.

Proximate composition analysis of the wild edible freshwater macrophytes

Proximate analyses of crude fibre composition, crude lipid, crude protein, ash, and moisture amount for the freshwater macrophytes were identified through the standard approaches of the Association of Official Analytical Chemists (2000). The young shoot’s moisture was identified by placing the weighed fresh samples in an oven overnight or until a fixed weight at 60 ºC, and the dried mass was determined. For ash determination, the initial crucible weight which was already labeled and oven-dried (105 °C for 30 minutes) was taken. Samples (2 g) were put into the crucible and oxidized inside a muffle furnace at 600 °C for 6 hours. Then, the samples could cool down overnight before putting into the desiccator for 15 minutes and weighed until constant weight is achieved. The ash amount was calculated following method 930.05.

Crude protein content was identified by placing 0.2 g of samples inside a digestion tube, mixed with one tablet of Kjeltec Cu, 5 g K2SO4 + 0.5 g CuSO4.H2O and 6 ml of concentrated sulphuric acid. The tube was inserted into the Turbotherm Digestor (Gerhardt, Germany) inside a fume hood and digested for 2 hours. The tube was left cool for 30 minutes before being inserted into the Protein Analyzer (Foss Tecator 2300 Kjeltec Analyzer Unit). the protein concentration was calculated as the percentage of nitrogen by using a conversion factor of 6.25 following method 955.04.

The petroleum ether from the samples was used to obtain crude lipids. Crude lipid was identified using 2055 Soxtec Avanti Manual System, Sweden (method 920.39), while crude fibre was calculated by acid-base digestion according to method 993.19. Estimating the present carbohydrate was performed in terms of difference by subtracting the overall percentage of crude protein, crude lipid, crude fibre, ash, and moisture from 100% dry weight (DW) basis.

Mineral content analysis of the wild edible freshwater macrophytes

The mineral content of five macronutrients, nitrogen (N), potassium (K), phosphorus (P), calcium (Ca), and magnesium (Mg), and four micronutrients, zinc (Zn), iron (Fe), copper (Cu), and manganese (Mn) concentrations were analyzed following the AOAC (2000) method. All the nutrient content was determined using AAS (Perkin Elmer 200 Flame Atomic Absorption Spectrophotometer, Massachusetts, United States). The dried samples were milled to less than 1 mm in diameter. The digestion tube was filled with 0.25 g of sample, and 5 ml of sulfuric acids (H_2SO_4) was added. The tube was rotated until all the plant material was moistened. The mixture was allowed to stand for at least 2 hours. After that, 2 ml of 30 – 35% hydrogen peroxide (H_2O_2) was added. Then the tube was placed into a port in a digestion block for 45 minutes at 285ºC. After 45 minutes, the tube was taken out from the block and cooled for 10 minutes. 2 ml H_2O_2 was added if the sample was cloudy, and this process was repeated until the samples were transparent. The samples were later placed in a volumetric flask, filled with distilled water until 100 ml, and mixed. The sample solution was then transferred into 100 ml plastic vials as a stock solution before analyzing it using AAS.

Statistical analysis

The results were reported as mean±standard error. The data for proximate composition (Table 1) and mineral content (Figures 2 and 3) were statistically analyzed using SPSS, Statistical

Software Program (IBM corporation, New York, USA). Means were compared using single-factor analysis of variance (ANOVA). Post hoc Duncan's Multiple Range Test (DMRT, $p<0.05$) (Zar, 1999) was performed if the ANOVA result was significant. Multiple correlation analysis was performed to determine relationships between the above variables and freshwater macrophyte species. Principal Component Analysis (PCA) based on the Bray Curtis similarity index was statistically analyzed using XLSTAT version 2014 software (Addinsoft, 2015, New York, USA) to obtain the relationship between proximate composition and mineral content of freshwater macrophyte species in this study and available data for other edible indigenous species.

Results

Proximate compositions of edible freshwater macrophytes

The proximate composition of freshwater macrophytes is shown in Table 1. *Limnocharis flava* has significantly higher ash and crude lipid of 18.31±0.92% and 5.75±0.84%, respectively. In contrast, *N. oleracea* has a significantly higher crude protein of 29.61±0.11%, and an energy value of 4269.65±31.08 cal g^{-1} whereas crude fiber content was observed high in *E. crassipes* (21.34%). Moisture and carbohydrate contents were comparable between *E. crassipes* and *N. oleracea* ranging from 10.34-10.82% and 50.76-54.42% respectively. Generally, carbohydrate has a higher concentration in all species with lower crude lipid. *Eichhornia crassipes* was categorically represented as carbohydrate > crude fibre > ash> moisture > protein > lipid, and this trend was contradictory to the *L. flava* (carbohydrate > ash > protein > fibre > moisture > lipid) and *N. oleracea* (carbohydrate > protein > moisture > fibre > ash > lipid).

Table 1 Proximate composition of freshwater macrophyte species and compares proximate composition (given as mean) of freshwater macrophyte species and other edible plant species

The mineral content of edible freshwater macrophytes

Figure 2 shows the macronutrient content in mineral analysis of the three edible freshwater macrophytes species. All macronutrient contents were significantly different ($p < 0.05$) between species except for K content. *Neptunia oleracea* has the highest N content (40380.0±730.6 mg kg^{-1}), followed by *L. flava* (23970.0±3022.0 mg kg^{-1}) and *E. crassipes* (12380.0±1129.9 mg kg^{-1}). *Eichhornia crassipes* have the highest Ca (11863.5±316.4 mg $kg^{-1)}$ while higher Mg values were depicted by *N. oleracea* with 2616.0±68 mg kg^{-1}. The trend of macronutrient content shows that *E. crassipes* and *L. flava* are high in K > N > Ca > Mg > P, and *N. oleracea* is high in N > K > Ca > Mg > P.

Figure 2 Macronutrient content in the three wild edibles freshwater macrophytes.

The micronutrient has significant differences ($p < 0.05$) between species. *Eichhornia crassipes* have the highest Cu, Mn, and Zn, while *N. oleracea* has a higher Fe, as shown in Figure 3. The trend of macronutrient content shows that *L. flava* and *N. oleracea* have similar trends with Fe > Mn > Zn > Cu, while *E. crassipes* trend is Mn > Fe > Zn > Cu.

Figure 3 Micronutrient content in the three wild edibles freshwater macrophytes.

Discussion

Proximate composition and comparative analysis with previous studies

Table 1 shows the proximate composition of freshwater macrophytes, and other edible plants listed as vegetables, spices, and medicinal plants commonly consumed. The reference species were selected to study the variation among the freshwater macrophytes in this study and previous studies (no 4-7), and also commonly consumed species. The objective of comparing freshwater macrophytes and other commonly consumed species is to show the potential of aquatic macrophytes as an alternative food to be consumed in everyday life. The proximate composition in Table 1 was ordinated with principal component analysis (PCA). The results in Figure 4, based on the Bray-Curtis similarity index at 50% similarity, showed that the total variance of the first two components is 65.71% (PC1 has a 50.13% and PC2 15.58%). The low value of variance explained by F2 in the PCA is due to separating plants based on their moisture content defined by their positioning along F1, representing more than 50% of the total variance of the analyses. The correlation matrix (Table 2) shows a significant moderate correlation between ash and crude lipid ($r = 0.616$), while a strong correlation between ash and crude fiber ($r = 0.767$). On the other hand, a moderate negative correlation, $r = -0.525$, was detected between ash and moisture.

Figure 4 Principal component analysis of freshwater macrophytes with other edible plant species based on their proximate composition (a) plot of proximate composition (b) position of PC (Principle component) score of species tested with other edible plants according to PC1 and PC2. No. 1-20 represents the assigned number of edible plant species as in Table 1 and corresponds to Groups 1, 2, 3, 4, and 5.

Table 2 Correlation matrix for all variables in freshwater macrophyte species with other edible plant species.

There were five distinct groups, 1, 2, 3, 4, and 5, displayed in the PCA analysis (Figure 4). Group 1 consists of *N. oleracea* of the present study clustered together with spices plants (*Z. officinali*s and *Piper guineense*) and *Moringa oleifera* due to higher carbohydrate and protein contents. The present research grouped *E. crassipes* and *L. flava* with *I. aquatica, Myrianthus arboreus*, *Tribulus terrestris,* and *Chrozophora tinctoria* in Group 2 due to the comparable value of ash and crude lipid. Group 3 had plant species with higher fibre contents, *M. balsamina, Fagonia cretica,* and *Ricinus communis*. In contrast, Group 5 consisted of freshwater macrophytes, *L. flava,* and *N. oleracea,* which had higher moisture content. The rest were grouped in Group 4 in the positive part of F1 with lower crude protein and carbohydrate content.

Moisture content ranged from 7.99-10.82% (Table 1) of freshwater macrophytes in this study were much lower than the moisture content of 65.02-85.58% and 73.46-77.52% in grasses and sedges, respectively (Furch & Junk, 1997). The moisture content of freshwater macrophytes was lower compared with other Nigerian indigenous vegetables such as *Amaranthus hybridus* (59.30%), *Telfaria occidentalis* (58.70%), and *B. alba* (54.80%) (Mepba, Eboh & Banigo, 2007).

The ash content, which is an index of mineral contents in biota, is high in *L. flava* (18.31%) compared to the values reported in young shoots and inflorescence *L. flava* (0.79%) Saupi, Zakaria & Bujang (2009). However, a similar value with *M. balsamina* leaves (18.00%) (Hassan & Umar, 2006). In addition, crude lipid content was also high in *L. flava* (5.75%), which was high compared with the same species of *L. flava*, 1.22% reported by Saupi, Zakaria & Bujang (2009) and *M. balsamina* leaves, 2.66% (Hassan & Umar, 2006). However, the content is lower than in some indigenous wild spices, herbs, fruits, and leafy vegetables, as reported by Achinewhu, Ogbonna & Hart (1995). The crude fiber content of *E. crassipes* is 21.34%, slightly lower than the green vegetables *M. balsamina* (29.00%) and *Myrianthus arboreus* (11.60%), both were consumed as soup in West Africa (Hassan & Umar, 2006).

Protein content in *N. olearcea* (29.61%) was comparable with *Moringa oleifera* (27.51%) Oduro, Ellis & Deborah (2008) and *Piper quineense* (26.6%) (Achinewhu, Ogbonna & Hart, 1995), and higher compare to *M. arboreus* (18.75%) and *M. balsamina* leaves (11.29%) (Hassan & Umar, 2006). Higher protein in *N. oleracea* can be related to the excellent source of protein in the Leguminosae species. Plants of the legume family have root nodules in which the symbiotic bacteria fix nitrogen to ammonia. This ammonia contains a large amount of proteins and amino acids (Roos et al., 2020). The crude protein of edible *N. oleracea* was 46.37% of the recommended dietary allowance (RDA) (Institute of Medicine IOM, 2005). According to Adeyemi & Osubor (2016), *E. crassipes* contains higher nutritional values, especially in their leaf parts which consist of concentrated form of proteins. Aside from that, water hyacinth leaf protein contains a lot of unsaturated fats, carotenes, xanthophylls, carbohydrates, and minerals including calcium, iron, and phosphorus (Kateregga & Sterner, 2007). Hence, this wild vegetable could be considered a good protein supplement.

Based on the carbohydrate content, higher carbohydrate was in *E. crassipes*, followed by *N. oleracea* and *L. flava*. In a study by Madsen, Luu & Getsinger (1993), water hyacinth roots are actively respiring tissues without any modifications for storing carbohydrates. Therefore, carbohydrates such as sugar accumulated in upper parts such as leaf laminae, leaf petioles, and inflorescences, which explained the higher carbohydrate in *E. crassipes* compared to others. The carbohydrate content in all freshwater macrophytes (44.03-54.42 %) of the present study was higher compared with *L. flava* young leaves and inflorescence (14.56%) studied by Saupi, Zakaria & Bujang (2009), *M. balsamina* leaves (39.05%) by Hassan & Umar (2006), and some Nigerian edible leafy vegetables such as *Basella alba* (34.30%), *Amaranthus hybridus* (27.40%) and *Lycopersicon esculentum* (33.20%) by Mepba, Eboh & Banigo (2007). Leafy vegetables are low lipid-containing food, thus beneficial health-wise, e.g., to avoid obesity (Lintas, 1992). Vegetables usually contain low lipid content in a range of 0.10 – 0.20%, as reported by Hazra and Som (2005).

Fibre is one of the essential elements when consuming vegetables. Dietary fiber is part of an overall healthy diet to reduce blood cholesterol levels and lower heart disease risks and obesity. The fibre content was within the range of herbs, 18.71–42.74%, as reported by Furch & Junk (1997). The benefit of consuming vegetables in human nutrition is their high fibre content (Vadivel & Janardhanan, 2000). Besides, consuming large quantities of plant vegetables can provide adequate nutrients. *Eichhornia crassipes*, with high fibre content, are like cellulosic wood and other lignocellulosic plants. They are also used as raw materials for papermaking (Wang et al. 2004; Saijonkari-Pahkala, 2001). A previous study by Banerjee & Matai (1990) reported that the leaf part normally had higher fibre content, especially in the floating and emergent plants because they required more strength to support the aerial vegetation. Fibre content is an important diet component widely utilized as a value indicator in poultry and feeding animal diets. As for food consumption high fibre content help to increase stool volume and decreases the time waste products spend in the gastrointestinal tract (Enyi, Uwakwe & Wegwu et al. 2020). It is also reported that a calorific value of more than 12% can be provided by plant food with a good source of crude protein (Pearson, 1976). Therefore, *N. oleracea* (29.61%), *L. flava* (16.58%), and other plants such as *Alisma plantago-aquatica* (14.83%) and *N. nucifera* (14.05%) also provide this requirement.

Based on National Diets and Nutrition Survey (NDNS) 2014, Bates et al. (2014) stated that the average percent of carbohydrate uptake is 50% of the food consumed. Carbohydrates (starches) from cereals, roots and tubers constitute primary energy-giving food, according to Achinewhu, Ogbonna & Hart (1995). Some indigenous leafy vegetables, nuts, and wild fruits are energy, giving food supplements since they are also excellent carbohydrates. The energy value in *N. oleracea* (4269.65 cal g^{-1}) was high, comparable with local vegetables *Ipomoea aquatica* (3009.40 cal g^{-1}) (Umar et al., 2007). In contrast, Umar et al. (2007) reported that most vegetables are low in energy value, within the range of 1250-2090 cal g^{-1}. According to SACN (2011), the energy requirement for 19-75 years old female adults is 1840-2175 kcal per day, while for 19-75 years old male adults is 2272-2294 kcal per day.

Mineral content and comparative analysis with previous studies

Table 3 compared the mineral contents (macro- and micro-nutrient) of freshwater macrophyte species and other edible plants. The micronutrients (Cu, Mn, and Zn) in Table 4 were ordinated with principal component analysis (PCA), as in Figure 5. Some of the mineral elements were not included to be compared in the PCA analysis as no data from previous studies were available. The correlation matrix (Table 5) shows a significant moderate correlation between copper and zinc (r = 0.626).

Table 3 Comparison of mineral contents (macro- and micro-nutrient) of freshwater macrophyte species and other edible plant species.

Table 4 Comparison of mineral contents of freshwater macrophyte species and other edible plant species.

Figure 5 Principal component analysis of freshwater macrophytes with other edible plant species based on their mineral contents (a) plot of mineral contents (b) position of PC score of species tested and other edible plants according to PC1 and PC2. No. 1-12 represents the assigned number of edible plant species as in Table 1 and corresponds to Groups 1, 2, 3, and 4.

Table 5 Correlation matrix for Ca, Mg, Cu, Mn, and Zn in freshwater macrophyte species with other edible plant species.

Minerals are essential in the diet, even at the content of 4-6% of the human body. The daily body (per day) macro minerals requirement is higher than 100 mg. They served as structural components of tissues, functional cellular, and basal metabolism (Macrae, Robinson & Sadler, 1993). The content of N, P, Mg, Ca, Cu, Mn, Fe, and Zn were different among the three plant species studied except for K. The protein and mineral content of the water in which the plants are grown are strongly reliant on the composition of the water: for example, the protein and phosphorus content of the plant is directly proportional to the nutrient loading rate of the water (Boyd, 1970). As stated above, higher ash was observed in *L. flava* (18.31%) and *E crassipes* (13.23%), contributing to their higher mineral composition. Nitrogen values from the present study ranged from 12380-40380 mg kg^{-1}. They were higher compared to *M. balsmina* (1224.9 mg kg^{-1}) and *P. quineense* (10800 mg kg^{-1}) but comparable with *M. arboreus* (33800 mg kg^{-1}), which showed that the freshwater macrophytes are the nitrogen-rich vegetables. Phosphorus and potassium contents of *L. flava* (2734.5 mg kg^{-1} and 33276.0 mg $kg^{-1,}$ respectively) had higher values compared to *Melochia corchorifolia* (1018.9 and 72.5 mg kg^{-1})*, M. balsmina* (1304.6 and 13200 mg kg^{-1}), and *P. quineense* (300 and 700 mg kg^{-1}). However, its P value was lower than freshwater, *N. oleracea* (4059.2 mg kg^{-1}) and vegetable, *M. arboreus* (5000 mg kg^{-1}) from the previous studies by Saupi, Zakaria & Bujang (2009) and Hassan & Umar (2006). Calcium content in *L. flava* was higher (8066.5 mg kg^{-1}) compared to the same species of the prior research (7708.7 mg kg^{-1}) and other freshwater macrophytes species, *N. oleracea* (3814.2 mg kg^{-1}) and *M. corchorifolia* (7503.7 mg kg^{-1}) and most of the vegetables except for *M. balsmina* (9410 mg kg^{-1}). The Mg values of the present study ranged from 1198.5 to 2716.0 mg kg^{-1}. They were comparable with freshwater macrophytes from previous studies (range between 1083 to 2281 mg kg^{-1}) and vegetables of *M. balsmina* and some medicinal plants such as *A. fragrantissima*, *A. graveolens,* and *C. bonariensis* (1200, 1050 and 1092 mg kg^{-1}, respectively).

Sodium, potassium, phosphorus, and magnesium are macronutrients that play an essential role in calcium economy and bone status (Heaney, 2015). Further explained that fruits and vegetables rich in potassium have an alkaline ash characteristic important in the diet. Adequate phosphorus means enough protein content in a healthy diet. Both calcium and phosphorus play a role in the growth and maintenance of muscles, bones, and teeth (Turan et al., 2003). According to RDI (2005), the calcium intake for men and women 19-29 years old was 800 mg, while for pregnant women was 1000 mg. Therefore, the value of calcium in this plant indicates that this plant may serve as a calcium source.

Based on the Bray-Curtis similarity index at 50% similarity, Figure 5, showed that the total variance of the first two components is 62.97% (PC1 has a total variance of 36.16% and PC2 26.81%). *Eichornia crassipes* were grouped with a medicinal plant, *A. virdis,* in Group 1 due to higher Mn (444.0 and 108.1 mg kg^{-1}, respectively) and similar Cu values (27.0 mg kg^{-1} *E. crassipes* and 26.8 mg kg^{-1} *A. graveolens*). Group 2 consisted of medicinal plants, *A. fragrantissima,* and *A. graveolens,* with higher Zn contents (400 and 544 mg kg^{-1}, respectively). Recommended zinc uptake for men and women (19-29 years old) is 6.70 mg and 4.90 mg, respectively, while for pregnant women is 5.50-10.00 mg RDI (2005). Deficiency of zinc results in retarded growth and delayed sexual maturation (Berminas, Charles & Emmanuel, 1998). Both *L. flava* and *N. oleracea* were clustered in Group 3 with *N. oleracea,* and *M. balsmina. C. album* and C. *bonariensis* were in Group 4, with a comparable Mg value ranging between 1092 to 8900 to 196 mg kg^{-1}.

Furthermore, iron (Fe) is one of the essential micronutrients in forming hemoglobin or the functioning of the central nervous system in the body (Adeyeye & Otokiti, 1999). The results showed *L. flava* and *E. crassipes* possessed iron at 314.0 mg kg^{-1} and 377.5 mg kg^{-1}, respectively, compared with iron values in medicinal plants, *C. bonariensis* and C. *album,* which ranged from 255.1 to 380 mg kg^{-1}. The high iron content in the plant could be why iron requirements are higher in pregnancy than in the non-pregnant state.

Conclusions

Although claimed as aquatic weeds due to their fast growth and causing a nuisance to the aquatic environment, freshwater macrophytes are also consumed food, especially by the locals, for their subsistence. The results showed that species of the present study had higher and comparable protein (*L. flava* and *N. oleracea*) and carbohydrate (*E. crassipes* and *N. oleracea*) contents with other edible vegetables suitable for human consumption as energy sources. The adequate amount of ash (*L. flava* and *E. crassipes*) and mineral analysis in each plant species can be served as alternatives in food nutrient supplements. These aquatic plants are also useful staple foods since they are simple to cultivate, spread fast, and require minimal care.

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Figure 1

Freshwater macrophytes in study sites

(a) *E. crassipes* from a shallow channel of Puchong, Selangor, Malaysia, (b) *L. flava* from a shallow channel of Ladang 10, Universiti Putra Malaysia, Selangor, Malaysia. Young shoots and inflorescences of (a) and (b) are consumed cooked as vegetables, (c) *N. oleracea* from a shallow channel of Kg. Alur Selibong, Langgar, Kedah. The young leaves' shoot tips are usually consumed blanched or cooked as a vegetable

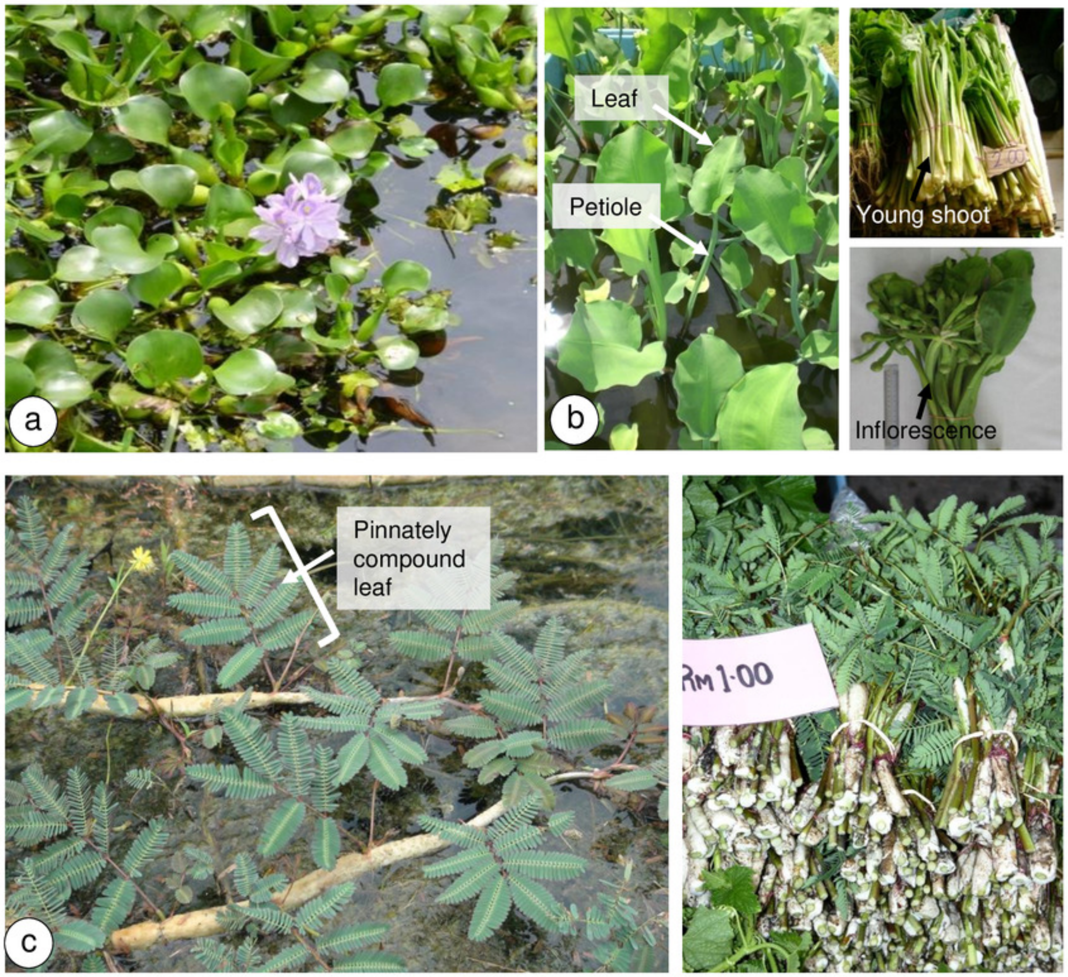
a
b
Leaf
Petiole
Young shoot
Inflorescence
c
Pinnately compound leaf
RM 1·00

Figure 2

Macronutrient content in the three wild edibles freshwater macrophytes

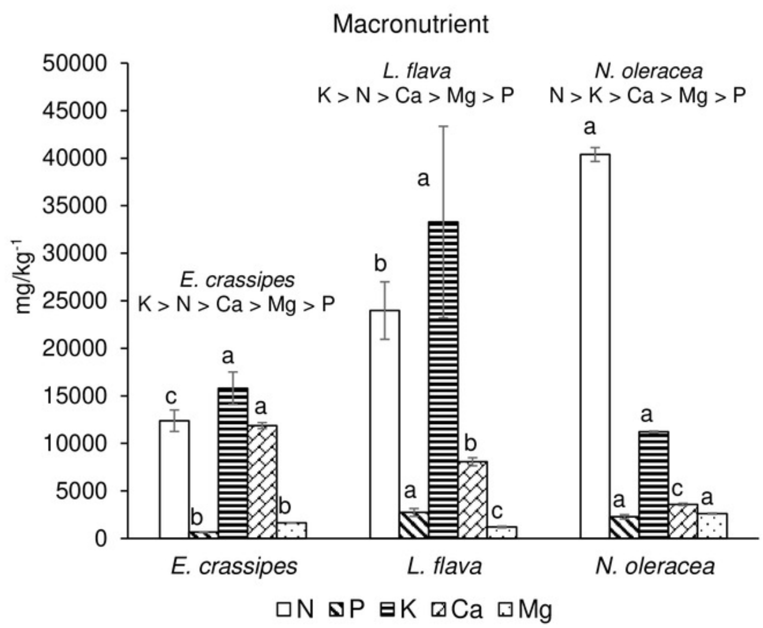
Macronutrient
mg/kg-1
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
E. crassipes
K > N > Ca > Mg > P
L. flava
K > N > Ca > Mg > P
N. oleracea
N > K > Ca > Mg > P
E. crassipes
L. flava
N. oleracea
N P K Ca Mg

Figure 3

Micronutrient content in the three wild edibles freshwater macrophytes

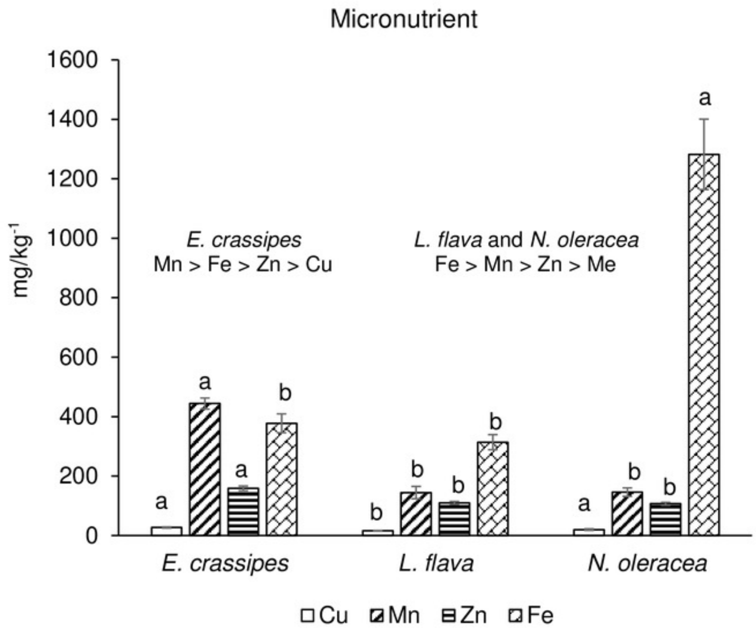
Micronutrient
1600
1400
1200
1000
800
600
400
200
0
mg/kg-1
E. crassipes
Mn > Fe > Zn > Cu
L. flava and N. oleracea
Fe > Mn > Zn > Me
a
b
E. crassipes
L. flava
N. oleracea
Cu
Mn
Zn
Fe

Figure 4

Principal component analysis of freshwater macrophytes with other edible plant species based on their proximate composition

(a) plot of proximate composition (b) position of PC (Principle component) score of species tested with other edible plants according to PC1 and PC2. No. 1-20 represents the assigned number of edible plant species as in Table 1 and corresponds to Groups 1, 2, 3, 4, and 5

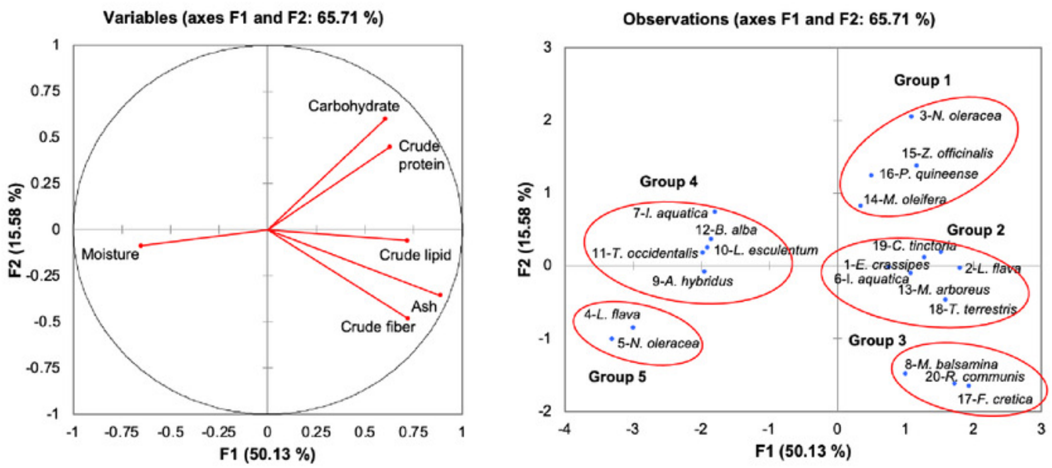
Variables (axes F1 and F2: 65.71 %)
Carbohydrate
Crude protein
Moisture
Crude lipid
Ash
Crude fiber
F1 (50.13 %)
F2 (15.58 %)
Observations (axes F1 and F2: 65.71 %)
Group 1
3-N. oleracea
15-Z. officinalis
16-P. quineense
14-M. oleifera
Group 2
19-C. tinctoria
1-E. crassipes
6-I. aquatica
2-L. flava
13-M. arboreus
18-T. terrestris
Group 3
8-M. balsamina
20-R. communis
17-F. cretica
Group 4
7-I. aquatica
12-B. alba
11-T. occidentalis
10-L. esculentum
9-A. hybridus
Group 5
4-L. flava
5-N. oleracea

Figure 5

Principal component analysis of freshwater macrophytes with other edible plant species based on their mineral contents

(a) plot of mineral contents (b) position of PC score of species tested and other edible plants according to PC1 and PC2. No. 1-12 represents the assigned number of edible plant species as in Table 1 and corresponds to Groups 1, 2, 3, and 4

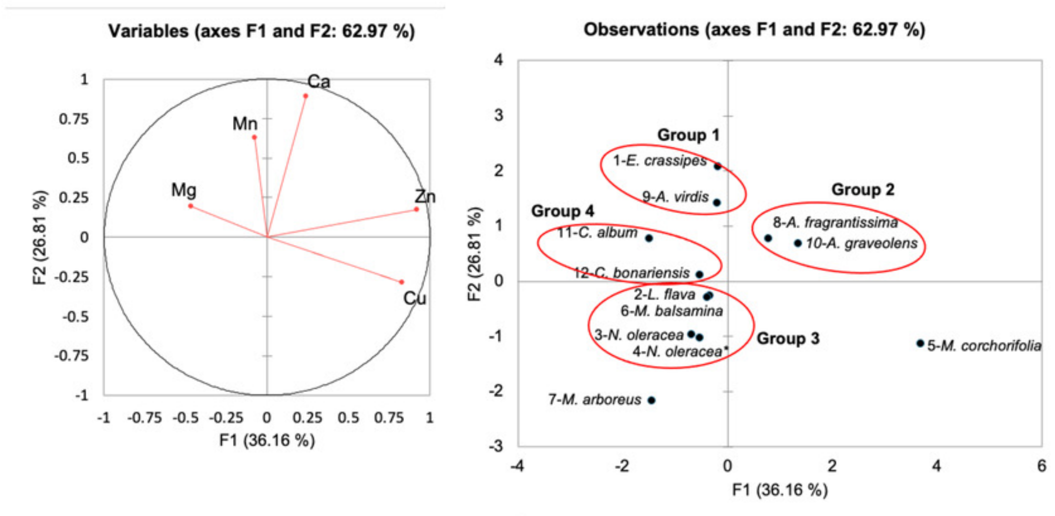
Variables (axes F1 and F2: 62.97 %)
Ca
Mn
Mg
Zn
Cu
F2 (26.81 %)
F1 (36.16 %)
Observations (axes F1 and F2: 62.97 %)
Group 1
1-E. crassipes
9-A. virdis
Group 2
8-A. fragrantissima
10-A. graveolens
Group 4
11-C. album
12-C. bonariensis
2-L. flava
6-M. balsamina
3-N. oleracea
4-N. oleracea
Group 3
5-M. corchorifolia
7-M. arboreus
F2 (26.81 %)
F1 (36.16 %)

Table 1(on next page)

Proximate composition of freshwater macrophyte species and compares proximate composition (given as mean) of freshwater macrophyte species and other edible plant species

No.	Species	Moisture (%)	Ash (%)	Crude lipid (%)	Crude fiber (%)	Crude protein (%)	Carbohydrate (%)	Energy (cal g^{-1})	Trend
Freshwater macrophytes									
1	*Eichhornia crassipes*	10.34±0.76 [a] (7.90-12.70)	13.23±0.30 [b] (11.93-14.14)	1.43±0.26 [c] (0.56-2.39)	21.34 ± 0.33 [a] (20.16-22.58)	9.58±0.73 [c] (7.53-11.71)	54.42±0.42 [a] (52.89-56.05)	3395.15±26.61 [b] (3253.20-3481.90)	C>F>A>M>P>L
2	*Limnocharis flava*	7.99±0.46 [b] (6.60-9.70)	18.31±0.92 [a] (15.70-20.83)	5.75±0.84 [a] (2.62-8.07)	15.33±1.18 [b] (11.48-19.50)	16.58±2.01 [b] (11.21-22.09)	44.03±0.91 [b] (40.62-48.29)	3486.98±151.34 [b] (2996.10-3905.70)	C>A>P>F>M>L
3	*Neptunia oleracea*	10.82±0.51 [a] (9.33-11.50)	7.42±0.04 [c] (7.31-7.51)	3.48±0.12 [b] (3.13-3.64)	8.73±0.30 [c] (8.04-9.39)	29.61±0.11 [a] (29.32-29.87)	50.76±0.29 [a] (49.95-51.20)	4269.65±31.08 [a] (4203.80-4353.10)	C>P>M>F>A>L
df		2	2	2	2	2	2	2	
F-value		5.572	52.334	14.480	44.218	35.564	103.928	14.216	
P-value		0.014	5.4305E-8	0.000213	1.8342E-7	8.4202E-7	2.9351E-10	0.000235	

No.	Species	Moisture (%)	Ash (%)	Crude lipid (%)	Crude fiber (%)	Crude protein (%)	Carbohydrate (%)	Reference (s)
4	*Limnocharis flava*	79.34	0.79	1.22	3.81	0.28	14.56	Saupi, Zakaria & Bujang (2009)
5	*Neptunia oleracea*	86.26	1.05	0.25	2.30	3.23	6.91	Noorasmah et al. (2015)
6	*Ipomoea aquatica*	72.83	10.83	11.00	17.67	6.30	54.20	Umar et al. (2007)
7	*Ipomoea aquatica*	51.36	2.75	0.81	1.20	1.70	42.18	Igwenyi et al. (2011)
Vegetables								
8	*Momordica balsamina*	71.00	18.00	2.66	29.00	11.29	39.05	Hassan & Umar (2006)
9	*Amaranthus hybridus*	59.30	4.10	1.20	3.40	4.60	27.40	Mepba, Eboh & Banigo (2007)
10	*Lycopersicon esculentum*	56.40	3.00	1.60	2.60	3.20	33.20	Mepba, Eboh & Banigo (2007)
11	*Telfaria occidentalis*	58.70	3.10	1.40	2.60	3.20	32.00	Mepba, Eboh & Banigo (2007)
12	*Basella alba*	54.80	3.10	0.90	2.70	4.20	34.30	Mepba, Eboh & Banigo (2007)

13	*Myrianthus arboreus*	83.90	16.40	13.10	11.60	18.75	40.15	Amata (2010)
14	*Moringa oleifera*	76.53	7.13	2.23	19.25	27.51	43.88	Oduro, Ellis & Deborah (2008)
Spices								
15	*Zingiber officinalis*	16.10	9.60	12.10	4.50	14.90	53.10	Achinewhu, Ogbonna & Hart (1995)
16	*Piper quineense*	55.30	4.50	11.70	8.70	26.60	38.90	Achinewhu, Ogbonna & Hart (1995)
Medicinal plants								
17	*Fagonia cretica*	9.30	16.00	10.40	25.10	7.70	31.50	Dastagir et al. (2013)
18	*Tribulus terrestris*	8.70	15.70	9.90	15.50	13.10	37.20	Dastagir et al. (2013)
19	*Chrozophora tinctoria*	9.70	16.00	13.00	6.70	10.50	44.10	Dastagir et al. (2013)
20	*Ricinus communis*	9.80	16.20	12.90	23.80	16.20	21.10	Dastagir et al. (2013)

The varying superscript alphabets in the same column demonstrate the contrasts at $p < 0.05$ (ANOVA, Duncan's Multiple Range Test test).
Values are given as mean ± SE and range in parenthesis.

Table 2(on next page)

Correlation matrix for all variables in freshwater macrophyte species with other edible plant species

Variables	Moisture	Ash	Crude lipid	Crude fiber	Crude protein	Carbohydrate
Moisture	1.000	–0.525*	–0.378	–0.269	–0.249	–0.366
Ash		1.000	0.616*	0.767*	0.339	0.381
Crude lipid			1.000	0.322	0.399	0.273
Crude fibre				1.000	0.347	0.237
Crude protein					1.000	0.431
Carbohydrate						1.000

1 * = significant at $p < 0.05$.

Table 3 (on next page)

Comparison of mineral contents (macro- and micro-nutrient) of freshwater macrophyte species and other edible plant species

No.	Species	Macronutrients (mg kg-1)					Micronutrients (mg kg-1)			
		N	P	K	Ca	Mg	Cu	Mn	Zn	Fe
Freshwater macrophytes										
1	*Eichhornia crassipes*	12380.0±1129.9[c] (10040-16720)	657.0±44.1[b] (488-824)	15799.0±1708.9[a] (11716-27000)	11863.5±316.4[a] (10764-13736)	1649.0±24.7[a] (1560-1748)	27.0±2.1[a] (20-36)	444.0±18.7[a] (380-504)	158.5±8.1[a] (120-196)	377.5±31.8[b] (292-540)
2	*Limnocharis flava*	23970.0±3022.0[b] (12960-35680)	2734.5±417.1[a] (1964-5240)	33276.0±10073.4[a] (17244-90400)	8066.5±426.0[b] (6172-9284)	1198.5±91.2[b] (980-1608)	16.0±0.8[b] (12-20)	144.5±20.8[b] (92-260)	110.0±4.6[b] (92-128)	314.0±25.4[b] (220-424)
3	*Neptunia oleracea*	40380.0±730.6[a] (38320-41600)	2275.0±232.7[a] (1924-2960)	11212.0±94.3[a] (11008-11440)	3583.0±120.2[c] (3416-3932)	2616.0±68.0[c] (2488-2808)	20.0±2.8[ab] (16-28)	146.0±14.3[b] (124-384)	107.0±4.7[b] (100-120)	1282.0±118.5[a] (984-1508)
	df	2	2	2	2	2	2	2	2	2
	F value	42.76	17.71	2.77	95.10	104.26	7.33	67.56	13.99	69.04
	P value	<0.0001	0.0005	0.1148	0.0001	0.0001	0.0110	0.0001	0.0013	0.0001
4	*Limnocharis flava*[1]	-	-	42020	7708.7	2281	83.1	-	6.6	-
5	*Neptunia oleracea*[2]	-	4059.2	32284	3814.2	1866.7	29.7	142.3	105.3	-
6	*Melochia corchorifolia*[3]	-	1018.9	72.5	7503.7	1083.3	335	96.8	673	199.1
Vegetables										
7	*Momordica balsmina*[4]	1224.9	1304.6	13200	9410	2200	54.4	116	31.8	-
8	*Amaranthus hybridus*[5]	-	60	450	20	40	-	-	80	118
9	*Lycopersicon esculentum*[5]	-	60	580	850	40	-	-	50	80
10	*Telfaria occidentalis*[5]	-	40	280	50	360	-	-	130	800
11	*Basella alba*[5]	-	60	-	10	60	-	-	20	60
12	*Myrianthus arboreus*[6]	33800	5000	20130	540	4600	3.550	6.957	1.82	44.125

Spices										
13	*Piper quineense*[7]	10800	300	700	11500	3500	-	-	-	-
Medicinal plants										
14	*Fritillaria ussuriensis*[8]	-	-	-	355.76	-	3.44	12.91	31.54	103.88
15	*Gastrodia elata*[8]	-	-	-	1415.34	-	3.42	30.09	10.64	126.15
16	*Achillea fragrantissima*[9]	-	-	-	14400	1200	4.44	88.5	400	192
17	*Amaranthus virdis*[9]	-	-	-	15280	8255	12.44	108.1	356	480
18	*Asteriscus graveolens*[9]	-	-	-	13200	1050	26.8	107.1	544	204
19	*Chenopodium album*[9]	-	-	-	11500	8900	14.44	148	21.2	380
20	*Conyza bonariensis*[9]	-	-	-	10200	1092	8.2	152.6	38.8	255.1

The varying superscript alphabets in the same column demonstrate the contrasts at $p < 0.05$ (ANOVA, Duncan's Multiple Range Test test). Values are given as mean ± SE and range in parenthesis. 1-9 are references list; 1&2- Saupi, Zakaria & Bujang (2009); 3- Umar et al. (2007); 4- Hassan & Umar (2006); 5- Mepba et al. (2007); 6- Amata (2010); 7- Achinewhu, Ogbonna & Hart (1995); 8-Yukui et al. (2016); 9- Daur (2015).

Table 4(on next page)

Comparison of mineral contents of freshwater macrophyte species and other edible plant species

No	Species	Ca	Mg	Cu (mg kg^{-1})	Mn	Zn	Reference (s)
1	*Eichhornia crassipes*	11863.5	1649	27.0	444.0	158.5	Present study
2	*Limnocharis flava*	8066.5	1198.5	16	144.5	110.0	Present study
3	*Neptunia oleracea*	3583	2616	20	146	107	Present study
4	*Neptunia oleracea**	3814.2	1866.7	29.7	142.3	105.3	Saupi, Zakaria & Bujang (2009)
5	*Melochia corchorifolia*	7503.7	1083.3	335	96.8	673	Umar et al. (2007)
6	*Momordica balsamina*	9410	2200	54.4	116	31.8	Hassan & Umar (2006)
7	*Myrianthus arboreus*	540	4600	3.55	6.957	1.82	Amata (2010)
8	*Achillea fragrantissima*	14400	1200	4.44	88.5	400	Daur (2015)
9	*Amaranthus virdis*	15280	8255	12.44	108.1	356	Daur (2015)
10	*Asteriscus graveolens*	13200	1050	26.8	107.1	544	Daur (2015)
11	*Chenopodium album*	11500	8900	14.44	148	21.2	Daur (2015)
12	*Conyza bonariensis*	10200	1092	8.2	152.6	38.8	Daur (2015)

**Neptunia oleracea* study by Saupi, Zakaria & Bujang (2009)

Table 5(on next page)

Correlation matrix for Ca, Mg, Cu, Mn, and Zn in freshwater macrophyte species with other edible plant species

Variables	Ca	Mg	Cu	Mn	Zn
Ca	1.000	0.157	-0.110	0.271	0.413
Mg		1.000	-0.246	-0.164	-0.242
Cu			1.000	-0.096	0.626*
Mn				1.000	-0.133
Zn					1.000

1 * = significant at $p < 0.05$.