Nutritional values of wild edible freshwater macrophytes

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Abstract

Background. The social acceptability of wild freshwater macrophytes as locally consumed vegetables is widespread. Freshwater macrophytes have several uses; for example, they can be used as food for humans. This study determined the proximate composition and mineral content of three freshwater macrophyte species, i.e., *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 Kampung Alur Selibong, Langgar (06° 5' 50.9" N, 100° 26' 49.8" E), Kedah, Peninsular Malaysia. The nutritional values of these macrophytes were analysed by using a standard protocol from the Association of Official Analytical Chemists. Eight replicates of *E. crassipes* and *L. flava* and four replicates of *N. oleracea* were used for the subsequent analyses.

Results. In the proximate analysis, *N. oleracea* possessed the highest percentage of crude protein (29.61%) and energy content (4,269.65 cal g^{-1}), whereas *L. flava* had the highest percentage of crude fat (5.75%) and ash (18.31%). The proximate composition trend for each species was different; specifically, all of the species possessed more carbohydrates and fewer crude lipids. All of the species demonstrated a similar mineral trend, with high nitrogen and potassium and

40   lower copper contents. Nitrogen and potassium levels ranged from 12,380-40,380 mg kg^{-1} and
41   from 11,212-33,276 mg kg^{-1}, respectively, and copper levels ranged from 16-27 mg kg^{-1}. The
42   results showed that all three plant species, i.e., *E. crassipes*, *N. oleracea*, and *L. flava* are plant-
43   based sources of macro- and micronutrient beneficial supplements for human consumption.
44
45   **Keywords** Freshwater macrophytes, Mineral content, Nutrients, Proximate analysis, Wild plants
46

Introduction

48   One of the top ten factors contributing to mortality was the low intake of vegetables and fruits
49   (Ezzati et al. 2002). Vegetables are sources of vitamins and minerals for the antioxidant activity
50   that is needed in the diet to meet the daily micronutrient requirements (Gupta & Bains, 2006). To
51   reduce individual risk and cardiovascular disease, humans and animals require optimal intakes of
52   minerals such as potassium, sodium, calcium, magnesium, copper, manganese, iodine, and zinc
53   (Mertz, 1982). To perform physiological functions, micronutrients such as copper, zinc, and iron
54   obtained from food are required in the human body in limited amounts (typically less than 100
55   micrograms per day). Micronutrient deficiency (e.g., zinc deficiency) causes decreased taste
56   acuity, slow wound healing, impaired development, decreased sexual maturity, impaired immune
57   system function, and impaired metabolism and homeostasis disorders of the thyroid gland
58   (Almatsier, 2006).
59
60   Freshwater macrophytes are aquatic plants submerged, emerging, or floating on the water surface
61   (Lacoul & Freedman, 2006). They occur in swamps, peatlands, lakes, streams, ponds, rice fields,
62   and drainage canals (Den Hartog, 1981; Muta Harah et al., 2005). Wild plants have played a
63   crucial role in the human diet, and some communities still depend on these wild foods (Tbatou et
64   al., 2018). According to Grubben, Siemonsma & Kasem (1994), of the 225 vegetables in
65   Southeast Asia, approximately 100 species are wild weeds. In East Malaysia (i.e., Sarawak),
66   some 43-48 species of wild freshwater macrophytes belonging to 28 families that are considered
67   as weeds are collected and used as edible food and food preparation, medicine, household items
68   for pillows and mats, and even used to make souvenirs (Muta Harah et al., 2005, Muta Harah,
69   Japar Sidik & Suzalina Akma, 2014). The local collectors also offer freshwater macrophytes for
70   sale in the local markets. Wild freshwater macrophytes are slowly being well received as
71   consumed vegetables (Muta Harah et al., 2005; Saupi, Zakaria & Bujang, 2009; Muta Harah,
72   Japar Sidik & Suzalina Akma, 2014, Noorasmah et al., 2015; Noorasmah et al., 2016). In
73   addition to being cheaper, vegetarian products are also important as sources of essential minerals
74   in human nutrition (Saupi, Zakaria & Bujang, 2009; Noorasmah et al., 2015; Caunii et al., 2010).
75   Some indigenous leafy vegetables, nuts, and wild fruits also provide energy and are food
76   supplements with good levels of carbohydrates and other nutrients (Achinewhu, Ogbonna &
77   Hart, 1995).
78

79   Global food problems have challenged all organizations and researchers to investigate the
80   possibility of using wild plant species as supplementary sources of nutrients (Abubakar et al.,
81   2021). Wild plants can provide minerals, vitamins, proteins, phenolics, carotenoids, and
82   carbohydrates (Seal, Pillai & Chaudhuri, 2017, Ghanimi et al., 2022). Studies on the nutritional
83   potential of some wild edible plants have demonstrated their comparability or even superiority to
84   domesticated crops (Shad, Shah & Bakht, 2013). Global dietary guidelines recommend increased
85   consumption of fruits and vegetables to mitigate the threat of diet-related diseases, including
86   metabolic disorders, cancer, and cardiovascular diseases (Stratton et al., 2021). Therefore, the
87   promotion of these plants will ensure important nutritional sources for food security and
88   sustainable development.
89
90   Macrophytes have been used locally in Malaysia as food sources (Saupi, Zakaria & Bujang,
91   2009; Muta Harah, Japar Sidik & Suzalina Akma, 2014; Noorasmah et al., 2015; Noorasmah et
92   al., 2016). The common macrophyte species that are highly ingested by local residents include
93   *Eichhornia crassipes* (Mart.) Solms, *Limnocharis flava* (L.) Buchenau, and *Neptunia oleracea*
94   Lour. (Figure 1). *Eichhornia crassipes*, which is known as water hyacinth, is a floating
95   freshwater macrophyte with broad leaves above the water surface and spongy stalks (Figure 1a).
96   *Limnocharis flava* (yellow velvetleaf) is a plant with pale green leaves and stalks with triangular
97   petiole leaves (Figure 1b). The leaf blade is papery and broadly ovate-elliptic. Moreover, water
98   mimosa, which is known as *N. oleracea*, has sensitive leaves when touched. It has white spongy
99   air tissues on stems floating on the surface of freshwater (Figure 1c). Under uncontrolled
100 conditions, these species cause problems in human-made water bodies. Due to their fast rate of
101 reproduction in vegetative and generative states, these plants are commonly referred to as
102 noxious weeds. Rather than destroying them with herbicides, which may affect the ecology, it is
103 preferable to collect and consume them or to use them as for feed.
104
105 **Figure 1** Freshwater macrophytes in study sites: (a) *E. crassipes* from a shallow channel of
106 Puchong, Selangor, Malaysia, (b) *L. flava* from a shallow channel of Ladang 10, Universiti Putra
107 Malaysia, Selangor, Malaysia. Young shoots and inflorescences of (a) and (b) are consumed and
108 cooked as vegetables. Meanwhile, (c) *N. oleracea* from a shallow channel of Kampung Alur
109 Selibong, Langgar, Kedah. Young leaf shoot tips are usually consumed either blanched or
110 cooked as vegetables.
111
112 Local people have been harvesting the young leaves with petioles and inflorescences of *E.*
113 *crassipes* and *L. flava*, young leaf shoot tips, and immature pods of *N. oleracea* to be consumed
114 as blanched or cooked vegetables, as well as to be sold in local markets to earn income. This
115 scenario is also supported by studies on ethnobotanical information (Saupi, Zakaria & Bujang,
116 2009; Muta Harah, Japar Sidik & Suzalina Akma, 2014; Saupi et al. 2020) that investigated the
117 most consumed wild aquatic plant species, particularly in the Bintulu and Sarawak communities.
118 Based on the data, young shoots of *L. flava* and *N. oleracea* were commonly used in dish

119   preparations due to their good palatability and sweet taste with great nutritional quality; however,
120   these species are the least frequently available in the market. Due to the fact that the chemical
121   compositions of aquatic macrophytes vary greatly depending on their species, seasons, habitat,
122   and geographic location, proximate analyses and mineral studies are crucial in determining their
123   nutritional value for future usage possibilities. In addition, a lack of documentation has been
124   published on the nutritional profiling of these macrophytes. Therefore, the present study aimed to
125   determine the proximate composition and mineral content of the commonly consumed wild
126   freshwater macrophytes of *E. crassipes, L. flava*, and *N. oleracea*. The findings of this study may
127   also suggest that these species should be commercially grown as new vegetable crops.
128

Materials & Methods

Sample collection and preparation

131   Plants of *E. crassipes*, *L. flava*, and *N. oleracea* were collected from shallow channels of
132   Puchong (3° 00' 11.89" N, 101° 42' 43.12" E), Ladang 10, Universiti Putra Malaysia (2° 58'
133   44.41" N, 101° 42' 44.45" E), and Kampung Alur Selibong, Langgar (06° 5' 50.9" N, 100° 26'
134   49.8" E), Kedah, Peninsular Malaysia, from January to March 2020. The complete specimen of
135   each species was directly arranged on the drawing block, labelled, and pressed as soon as
136   possible by using wooden presses and ensuring that the straps were tight enough to bind the press
137   together. The herbarium samples were examined to verify the identity of the specific plant that
138   was used in a study. The morphological characterization of the aquatic macrophytes was
139   performed following the guidelines based on Weeds of rice in Indonesia by Soerjani, Kostermans
140   & Tjitrosoepomo (1987).
141

142   Young shoots (light green with the tender shoot) were sampled and stored in a zip-lock plastic
143   bag accordingly before being kept in an ice chest for transportation to the laboratory. Any
144   adhering materials were removed from the plant samples by washing them with distilled water.
145   Approximately 500 g of fresh samples were cut into small pieces and oven-dried at 60 °C until
146   they reached a constant weight. Dry samples were ground by using an IKA A11® Basic
147   Analytical Mill and passed through a 0.2 mm laboratory sieve. The sample powder was labelled
148   and kept inside of an airtight container at room temperature prior to proximate composition (ash,
149   moisture, crude lipid, crude protein, crude fibre, and energy) analysis and mineral content
150   (nitrogen [N], phosphorus [P], potassium [K], calcium [Ca], magnesium [Mg], copper [Cu],
151   manganese [Mn], zinc [Zn], and iron [Fe] analysis). Eight replicates (petiole and leaves) of *E.*
152   *crassipes* and *L. flava* and four replicates of the whole pinnately compound leaf of *N. oleracea*
153   were used for the subsequent analyses.
154

Proximate composition analysis of the wild edible freshwater macrophytes

156   Proximate analyses of crude fibre composition, crude lipid, crude protein, ash, and moisture
157   amount for the freshwater macrophytes were identified through the standard approaches of the
158   Association of Official Analytical Chemists (2000). The young shoot moisture was identified by

159 placing the weighed fresh samples in an oven overnight or until a fixed weight was obtained at
160 60 °C, and the dried mass was determined. For ash determination, the initial crucible weight,
161 which was already labelled and oven-dried (105 °C for 30 minutes), was measured. Samples (2
162 g) were put into the crucible and oxidized inside of a muffle furnace at 600 °C for 6 hours.
163 Afterwards, the samples were cooled overnight before being placed into the desiccator for 15
164 minutes and weighed until a constant weight was achieved. The ash amount was calculated
165 following method 930.05.
166
167 Crude protein content was identified by placing 0.2 g of samples inside of a digestion tube and
168 mixed with one tablet of Kjeltec Cu, 5 g K2SO4 + 0.5 g CuSO4, H2O, and 6 ml of concentrated
169 sulfuric acid. The tube was inserted into the Turbotherm Digestor (Gerhardt, Germany) inside of
170 a fume hood and digested for 2 hours. The tube was left to cool for 30 minutes before being
171 inserted into the Protein Analyser (Foss Tecator 2300 Kjeltec Analyser Unit). The protein
172 concentration was calculated as the percentage of nitrogen by using a conversion factor of 6.25
173 following method 955.04.
174
175 Petroleum ether from the samples was used to obtain crude lipids. Crude lipid was identified by
176 using the 2055 Soxtec Avanti Manual System, Sweden (method 920.39), whereas crude fibre
177 was calculated via acid-base digestion according to method 993.19. The estimation of the present
178 carbohydrate level was performed regarding the difference by subtracting the overall percentage
179 of crude protein, crude lipid, crude fibre, ash, and moisture from the 100% dry weight (DW)
180 basis.
181
182 **Mineral content analysis of the wild edible freshwater macrophytes**
183 The mineral contents of five macronutrients, including nitrogen (N), potassium (K), phosphorus
184 (P), calcium (Ca), and magnesium (Mg), and four micronutrients, including zinc (Zn), iron (Fe),
185 copper (Cu), and manganese (Mn), were analysed following the Association of Official Analytical
186 Chemists (AOAC, 2000) method. All of the nutrient contents were determined by using AAS
187 (Perkin Elmer 200 Flame Atomic Absorption Spectrophotometer, Massachusetts, United States).
188 The dried samples were milled to less than 1 mm in diameter. Additionally, the digestion tube was
189 filled with 0.25 g of sample, and 5 ml of sulfuric acid (H_2SO_4) was then added. The tube was
190 rotated until all of the plant material was moistened. The mixture was allowed to stand for at least
191 2 hours. Afterwards, 2 mL of 30–35% hydrogen peroxide (H_2O_2) was added. Subsequently, the
192 tube was placed into a port in a digestion block for 45 minutes at 285 °C. After 45 minutes, the
193 tube was removed from the block and cooled for 10 minutes. H_2O_2 (2 mL) was added if the sample
194 was cloudy, and this process was repeated until the samples were transparent. The samples were
195 then placed into a volumetric flask, filled with distilled water until a level of 100 mL was reached,
196 and mixed. The sample solution was subsequently transferred into 100 mL plastic vials as a stock
197 solution before analysis via AAS.
198

199 **Statistical analysis**

200 The results are reported as the mean±standard error. The data for proximate composition (Table

201 1) and mineral content (Figures 2 and 3) were statistically analysed by using SPSS, Statistical

202 Software Program (IBM corporation, New York, USA). Means were compared by using single-

203 factor analysis of variance (ANOVA). Post-hoc Duncan's Multiple Range Test (DMRT, $p<0.05$)

204 (Zar, 1999) was performed if the ANOVA result was significant. In addition, a multiple

205 correlation analysis was performed to determine relationships between the abovementioned

206 variables and freshwater macrophyte species. Principal component analysis (PCA) based on the

207 Bray–Curtis similarity index was statistically analysed by using XLSTAT version 2014 software

208 (Addinsoft, 2015, New York, USA) to obtain the relationship between the proximate

209 composition and mineral content of freshwater macrophyte species in this study and for available

210 data for other edible indigenous species.

211

212 # Results

213 **Proximate compositions of edible freshwater macrophytes**

214 The proximate composition of freshwater macrophytes is shown in Table 1. *Limnocharis flava*

215 had significantly higher ash and crude lipid contents of 18.31±0.92% and 5.75±0.84%,

216 respectively. In contrast, *N. oleracea* had a significantly higher crude protein of 29.61±0.11%

217 and an energy value of 4,269.65±31.08 cal g⁻¹, whereas the crude fibre content was observed to

218 be high in *E. crassipes* (21.34%). The moisture and carbohydrate contents were comparable

219 between *E. crassipes* and *N. oleracea,* ranging from 10.34-10.82% and 50.76-54.42%,

220 respectively. Generally, carbohydrates had a higher concentration in all of the species, along with

221 lower crude lipids. *Eichhornia crassipes* was categorically represented as carbohydrate > crude

222 fibre > ash > moisture > protein > lipid, and this trend was contradictory to that of *L. flava*

223 (carbohydrate > ash > protein > fibre > moisture > lipid) and *N. oleracea* (carbohydrate > protein

224 > moisture > fibre > ash > lipid).

225

226 **Table 1** Proximate composition of freshwater macrophyte species and comparison of the

227 proximate composition (given as the mean) of freshwater macrophyte species and other edible

228 plant species.

229

230 **The mineral content of edible freshwater macrophytes**

231 Figure 2 shows the macronutrient content in the mineral analysis of the three edible freshwater

232 macrophyte species. All of the macronutrient contents were significantly different ($p < 0.05$)

233 between species (except for the K content). *Neptunia oleracea* had the highest N content

234 (40,380.0±730.6 mg kg⁻¹), followed by *L. flava* (23,970.0±3,022.0 mg kg⁻¹) and *E. crassipes*

235 (12,380.0±1,129.9 mg kg⁻¹). *Eichhornia crassipes* had the highest Ca content (11,863.5±316.4

236 mg kg⁻¹), whereas higher Mg values were demonstrated by *N. oleracea* at 2,616.0±68 mg kg⁻¹.

237 The trend of macronutrient content shows that *E. crassipes* and *L. flava* are high in K > N > Ca >

238 Mg > P, and *N. oleracea* is high in N > K > Ca > Mg > P.

239

240   **Figure 2** Macronutrient content in the three wild edible freshwater macrophytes.

241

242   The micronutrients were significantly different (p < 0.05) between the species. *Eichhornia*

243   *crassipes* had the highest Cu, Mn, and Zn contents, whereas *N. oleracea* had a higher Fe content,

244   as shown in Figure 3. The trend of macronutrient content shows that *L. flava* and *N. oleracea*

245   have similar trends with Fe > Mn > Zn > Cu, whereas the *E. crassipes* trend is Mn > Fe > Zn >

246   Cu.

247

248   **Figure 3** Micronutrient content in the three wild edible freshwater macrophytes.

249

250   ## Discussion

251   **Proximate composition and comparative analysis with previous studies**

252   Table 1 shows the proximate composition of freshwater macrophytes, and other edible plants

253   listed as vegetables, spices, and medicinal plants that are commonly consumed. The reference

254   species were selected to examine the variation among the freshwater macrophytes in this study

255   and previous studies (no 4-7), as well as among commonly consumed species. The objective of

256   comparing freshwater macrophytes and other commonly consumed species is to demonstrate the

257   potential of aquatic macrophytes as an alternative food to be consumed in daily life. The

258   proximate composition in Table 1 was ordinated with a principal component analysis (PCA). The

259   results in Figure 4 (based on the Bray–Curtis similarity index at 50% similarity) showed that the

260   total variance of the first two components was 65.71% (PC1 at 50.13% and PC2 at 15.58%). The

261   low value of variance explained by F2 in the PCA is due to the separation of the plants based on

262   their moisture content as defined by their positioning along F1, which represented more than

263   50% of the total variance of the analyses. The correlation matrix (Table 2) demonstrated a

264   significant moderate correlation between ash and crude lipid (r = 0.616) and a strong correlation

265   between ash and crude fibre (r = 0.767). In contrast, a moderate negative correlation (r = -0.525)

266   was detected between ash and moisture.

267

268   **Figure 4** Principal component analysis of freshwater macrophytes with other edible plant species

269   based on their proximate composition. (a) Plot of proximate composition, and (b) position of PC

270   (principal component) score of species tested with other edible plants according to PC1 and PC2.

271   No. 1-20 represents the assigned number of edible plant species (as in Table 1) and corresponds

272   to Groups 1, 2, 3, 4, and 5.

273

274   **Table 2** Correlation matrix for all of the variables in freshwater macrophyte species with other

275   edible plant species.

276

277   There were five distinct groups (1, 2, 3, 4, and 5) displayed in the PCA (Figure 4). Group 1

278   consisted of *N. oleracea* in the present study and clustered together with spice plants (*Z.*

279 *officinali*s and *Piper guineense*) and *Moringa oleifera*, due to higher carbohydrate and protein
280 contents. The current study grouped *E. crassipes* and *L. flava* with *I. aquatica, Myrianthus*
281 *arboreus, Tribulus terrestris,* and *Chrozophora tinctoria* in Group 2 due to the comparable
282 values of ash and crude lipids. Group 3 had plant species with higher fibre contents, including *M.*
283 *balsamina, Fagonia cretica,* and *Ricinus communis*. In contrast, Group 5 consisted of freshwater
284 macrophytes (*L. flava,* and *N. oleracea*)*,* which had higher moisture content. The remaining
285 species were grouped in Group 4 in the positive part of F1 with lower crude protein and
286 carbohydrate contents.
287
288 The moisture content ranged from 7.99-10.82% (Table 1) of freshwater macrophytes in this
289 study, which was much lower than the moisture content of 65.02-85.58% and 73.46-77.52% in
290 grasses and sedges, respectively (Furch & Junk, 1997). The moisture content of freshwater
291 macrophytes was lower than that of other Nigerian indigenous vegetables, such as *Amaranthus*
292 *hybridus* (59.30%), *Telfaria occidentalis* (58.70%), and *B. alba* (54.80%) (Mepba, Eboh &
293 Banigo, 2007).
294
295 The ash content, which is an index of mineral contents in biota, was high in *L. flava* (18.31%)
296 compared to the values reported in young shoots and inflorescence *L. flava* (0.79%) (Saupi,
297 Zakaria & Bujang, 2009). However, a similar value was observed for *M. balsamina* leaves
298 (18.00%) (Hassan & Umar, 2006). In addition, the crude lipid content was also high in *L. flava*
299 (5.75%), which was higher compared with the same species of *L. flava* at 1.22%, as reported by
300 Saupi, Zakaria & Bujang (2009), as well as for *M. balsamina* leaves at 2.66% (Hassan & Umar,
301 2006). However, the content was lower than in some indigenous wild spices, herbs, fruits, and
302 leafy vegetables, as reported by Achinewhu, Ogbonna & Hart (1995). The crude fibre content of
303 *E. crassipes* was 21.34%, which was slightly lower than that of the green vegetables *M.*
304 *balsamina* (29.00%) and *Myrianthus arboreus* (11.60%), both of which are consumed as soup in
305 West Africa (Hassan & Umar, 2006).
306
307 The protein content in *N. oleracea* (29.61%) was comparable to that in *Moringa oleifera*
308 (27.51%) (Oduro, Ellis & Deborah, 2008) and *Piper quineense* (26.6%) (Achinewhu, Ogbonna &
309 Hart, 1995); additionally, it was higher than that in *M. arboreus* (18.75%) and *M. balsamina*
310 leaves (11.29%) (Hassan & Umar, 2006). Higher protein in *N. oleracea* can be related to the
311 excellent source of protein in the Leguminosae species. Plants of the legume family have root
312 nodules in which symbiotic bacteria fix nitrogen to ammonia. This ammonia contains large
313 amounts of proteins and amino acids (Roos et al., 2020). The crude protein content of edible *N.*
314 *oleracea* was 46.37% of the recommended dietary allowance (RDA) (Institute of Medicine IOM,
315 2005). According to Adeyemi & Osubor (2016), *E. crassipes* contains higher nutritional values,
316 especially in their leaf parts, which consist of concentrated forms of proteins. In addition, water
317 hyacinth leaf protein contains many unsaturated fats, carotenes, xanthophylls, carbohydrates, and
318 minerals, including calcium, iron, and phosphorus (Kateregga & Sterner, 2007). Hence, this wild
319 vegetable can be considered a good protein supplement.
320

321 Based on the carbohydrate content, higher carbohydrate content was observed in *E. crassipes*,
322 followed by *N. oleracea* and *L. flava*. In a study by Madsen, Luu & Getsinger (1993), water
323 hyacinth roots were actively respiring tissues without any modifications for storing
324 carbohydrates. Therefore, carbohydrates (such as sugar) accumulated in upper plant parts, such
325 as leaf laminae, leaf petioles, and inflorescences, which can explain the higher carbohydrate
326 content in *E. crassipes* compared to others. The carbohydrate content in all freshwater
327 macrophytes (44.03-54.42%) of the current study was higher compared with *L. flava* young
328 leaves and inflorescence (14.56%) that were examined by Saupi, Zakaria & Bujang (2009), as
329 well as *M. balsamina* leaves (39.05%) examined by Hassan & Umar (2006) and some Nigerian
330 edible leafy vegetables, such as *Basella alba* (34.30%), *Amaranthus hybridus* (27.40%), and
331 *Lycopersicon esculentum* (33.20%), which were examined by Mepba, Eboh & Banigo (2007).
332 Leafy vegetables are low lipid-containing foods; thus, they are beneficial in several health
333 aspects, such as for avoiding obesity (Lintas, 1992). Vegetables usually contain low lipid content
334 in a range of 0.10–0.20%, as reported by Hazra and Som (2005).
335
336 Fibre is one of the essential elements when consuming vegetables. Dietary fibre is part of an
337 overall healthy diet to reduce blood cholesterol levels and to decrease heart disease risks and
338 obesity. The fibre content was within the range of herbs (18.71–42.74%), as reported by Furch &
339 Junk (1997). The benefit of consuming vegetables in human nutrition is represented by their high
340 fibre content (Vadivel & Janardhanan, 2000). In addition, consuming large quantities of plant
341 vegetables can provide adequate nutrients. *Eichhornia crassipes*, with a high fibre content, is
342 similar to cellulosic wood and other lignocellulosic plants. They are also used as raw materials
343 for papermaking (Wang et al. 2004; Saijonkari-Pahkala, 2001). A previous study by Banerjee &
344 Matai (1990) reported that the leaf part normally had higher fibre content (especially in floating
345 and emergent plants) because they required more strength to support the aerial vegetation. Fibre
346 content is an important dietary component that is widely utilized as a value indicator in poultry
347 and feeding animal diets. For food consumption, high fibre content helps to increase stool
348 volume and decreases the time that waste products spend in the gastrointestinal tract (Enyi,
349 Uwakwe & Wegwu et al. 2020). It has also been reported that a calorific value of more than 12%
350 can be provided by plant food with a good source of crude protein (Pearson, 1976). Therefore, *N.*
351 *oleracea* (29.61%), *L. flava* (16.58%), and other plants, such as *Alisma plantago-aquatica*
352 (14.83%) and *N. nucifera* (14.05%), also meet this requirement.
353
354 Based on the National Diets and Nutrition Survey (NDNS) 2014, Bates et al. (2014) stated that
355 the average percent carbohydrate uptake is 50% of the food consumed. Carbohydrates (starches)
356 from cereals, roots, and tubers constitute primary energy-giving food, according to Achinewhu,
357 Ogbonna & Hart (1995). Some indigenous leafy vegetables, nuts, and wild fruits constitute
358 energy and can be provided as food supplements because they are also excellent carbohydrates.
359 The energy value in *N. oleracea* (4,269.65 cal g^{-1}) was shown to be high, comparable to that of
360 the local vegetable *Ipomoea aquatica* (3,009.40 cal g^{-1}) (Umar et al., 2007). In contrast, Umar et

361 al. (2007) reported that most vegetables are low in energy value (within the range of 1,250-2,090
362 cal g^{-1}). According to SACN (2011), the energy requirement for 19- to 75-year-old female adults
363 is 1,840-2,175 kcal per day, whereas for 19- to 75-year-old male adults, the requirement is 2,272-
364 2,294 kcal per day.
365
366 **Mineral content and comparative analysis with previous studies**
367 Table 3 compares the mineral contents (macro- and micronutrients) of freshwater macrophyte
368 species and other edible plants. The micronutrients (Cu, Mn, and Zn) in Table 4 were ordinated
369 via a principal component analysis (PCA), as shown in Figure 5. Some of the mineral elements
370 were not included for comparison in the PCA, as no data from previous studies were available.
371 The correlation matrix (Table 5) shows a significant moderate correlation between copper and
372 zinc (r = 0.626).
373
374 **Table 3** Comparison of mineral contents (macro- and micronutrients) of freshwater macrophyte
375 species and other edible plant species.
376
377 **Table 4** Comparison of the mineral contents of freshwater macrophyte species and other edible
378 plant species.
379
380 **Figure 5** Principal component analysis of freshwater macrophytes with other edible plant species
381 based on their mineral contents. (a) Plot of mineral contents, and (b) position of PC score of the
382 tested species and other edible plants, according to PC1 and PC2. No. 1-12 represents the
383 assigned number of edible plant species as in Table 1 and corresponds to Groups 1, 2, 3, and 4.
384
385 **Table 5** Correlation matrix for Ca, Mg, Cu, Mn, and Zn in freshwater macrophyte species with
386 other edible plant species.
387
388 Minerals are essential in the diet, even at contents of 4-6% of the human body. The daily body
389 (per day) macro mineral requirement is higher than 100 mg. In addition, they serve as structural
390 components of tissues and for functional cellular and basal metabolism (Macrae, Robinson &
391 Sadler, 1993). The contents of N, P, Mg, Ca, Cu, Mn, Fe, and Zn were found to be different
392 among the three plant species studied (except for K). The protein and mineral contents of the
393 water in which the plants are grown are strongly reliant on the composition of the water; for
394 example, the protein and phosphorus contents of the plant are directly proportional to the nutrient
395 loading rate of the water (Boyd, 1970). As stated above, higher ash was observed in *L. flava*
396 (18.31%) and *E. crassipes* (13.23%), which contributed to their higher mineral composition.
397 From the current study, nitrogen values ranged from 12,380-40,380 mg kg^{-1}. They were higher
398 than those of *M. balsmina* (1,224.9 mg kg^{-1}) and *P. quineense* (10,800 mg kg^{-1}) but comparable
399 to those of *M. arboreus* (33,800 mg kg^{-1}), which showed that freshwater macrophytes are
400 nitrogen-rich vegetables. Moreover, the phosphorus and potassium contents of *L. flava* (2,734.5

401   mg kg⁻¹ and 33,276.0 mg kg⁻¹, respectively) were higher than those of *Melochia corchorifolia*
402   (1,018.9 and 72.5 mg kg⁻¹, respectively)*, M. balsmina* (1,304.6 and 13,200 mg kg⁻¹,
403   respectively), and *P. quineense* (300 and 700 mg kg⁻¹, respectively). However, its P value was
404   lower than that of freshwater *N. oleracea* (4,059.2 mg kg⁻¹) and the vegetable *M. arboreus* (5,000
405   mg kg⁻¹) from previous studies by Saupi, Zakaria & Bujang (2009) and Hassan & Umar (2006).
406   Additionally, the calcium content in *L. flava* was higher (8,066.5 mg kg⁻¹) than that in the same
407   species in prior studies (7,708.7 mg kg⁻¹), as well as other freshwater macrophyte species,
408   including *N. oleracea* (3,814.2 mg kg⁻¹) and *M. corchorifolia* (7,503.7 mg kg⁻¹), and most of the
409   vegetables (except for *M. balsmina*) (9,410 mg kg⁻¹). The Mg values of the current study ranged
410   from 1,198.5 to 2,716.0 mg kg⁻¹. Furthermore, they were comparable with freshwater
411   macrophytes from previous studies (range between 1,083-2,281 mg kg⁻¹) and vegetables of *M.*
412   *balsmina* and some medicinal plants, such as *A. fragrantissima*, *A. graveolens,* and *C.*
413   *bonariensis* (1,200, 1,050, and 1,092 mg kg⁻¹, respectively).

415   Sodium, potassium, phosphorus, and magnesium are macronutrients that play an essential role in
416   calcium homeostasis and bone status (Heaney, 2015). Furthermore, fruits and vegetables that are
417   rich in potassium have an alkaline ash characteristic that is important in the diet. Adequate
418   phosphorus indicates sufficient protein content in a healthy diet. Both calcium and phosphorus
419   play a role in the growth and maintenance of muscles, bones, and teeth (Turan et al., 2003).
420   According to the RDI (2005), the calcium intake for men and women aged 19-29-years-old was
421   800 mg, whereas for pregnant women, this intake was was 1,000 mg. Therefore, the value of
422   calcium in this plant indicates that this plant may serve as a vital calcium source.

424   Based on the Bray–Curtis similarity index at 50% similarity, Figure 5 shows that the total
425   variance of the first two components was 62.97% (PC1 had a total variance of 36.16%, and PC2
426   had a variance of 26.81%). *Eichornia crassipes* was grouped with a medicinal plant (*A. virdis*) in
427   Group 1 due to higher Mn (444.0 and 108.1 mg kg⁻¹, respectively) and similar Cu values (27.0
428   mg kg⁻¹ for *E. crassipes* and 26.8 mg kg⁻¹ for *A. graveolens*). Group 2 consisted of medicinal
429   plants, including *A. fragrantissima,* and *A. graveolens,* with higher Zn contents (400 and 544 mg
430   kg⁻¹, respectively). The recommended zinc uptake for men and women (19-29-years-old) is 6.70
431   mg and 4.90 mg, respectively, whereas for pregnant women, this intake is 5.50-10.00 mg RDI
432   (2005). Zinc deficiency results in retarded growth and delayed sexual maturation (Berminas,
433   Charles & Emmanuel, 1998). Both *L. flava* and *N. oleracea* were clustered in Group 3 with *N.*
434   *oleracea* and *M. balsmina.* Moreover, *C. album* and *C. bonariensis* were clustered in Group 4,
435   with comparable Mg values ranging from 1,092-8,900 to 196 mg kg⁻¹.

437   Furthermore, iron (Fe) is one of the essential micronutrients in forming haemoglobin and for the
438   functioning of the central nervous system in the body (Adeyeye & Otokiti, 1999). The results
439   showed that *L. flava* and *E. crassipes* exhibited iron levels at 314.0 mg kg⁻¹ and 377.5 mg kg⁻¹,
440   respectively, compared with the iron values in medicinal plants (*C. bonariensis* and C. *album*),

441 which ranged from 255.1-380 mg kg^{-1}. The high iron content in the plant could be the reason as
442 to why iron requirements are higher in pregnancy than in the nonpregnant state.
443

Conclusions

445 Although claimed to be aquatic weeds due to their fast growth and nuisance to the aquatic
446 environment, freshwater macrophytes are also consumed as food, especially by the local
447 residents, for their subsistence. The results of the current study showed that the species had
448 higher and comparable protein (*L. flava* and *N. oleracea*) and carbohydrate (*E. crassipes* and *N.*
449 *oleracea*) contents than other edible vegetables that are suitable for human consumption as
450 energy sources. An adequate amount of ash (*L. flava* and *E. crassipes*) and mineral analysis in
451 each plant species can serve as alternatives in food nutrient supplements. These aquatic plants
452 are also useful staple foods because they are simple to cultivate, spread quickly, and require
453 minimal care.
454

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612

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

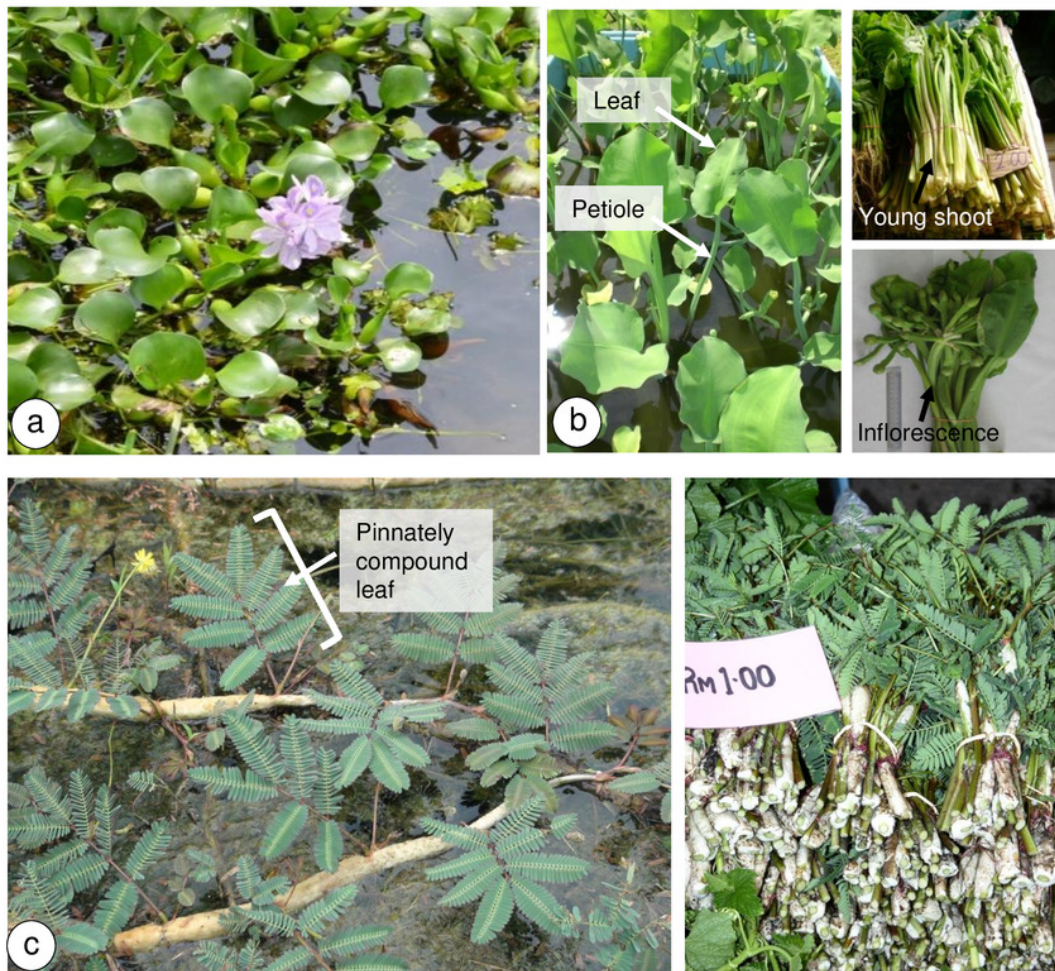



Figure 2

Macronutrient content in the three wild edibles freshwater macrophytes

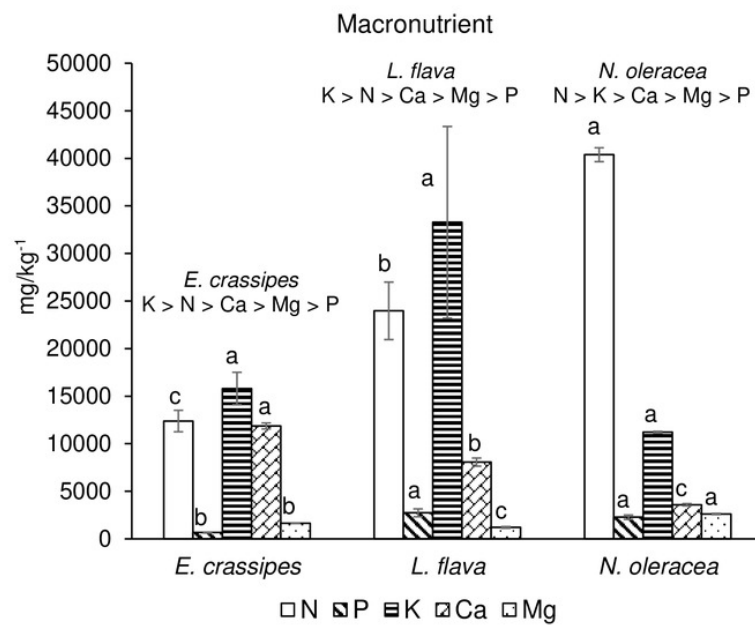



Figure 3

Micronutrient content in the three wild edibles freshwater macrophytes

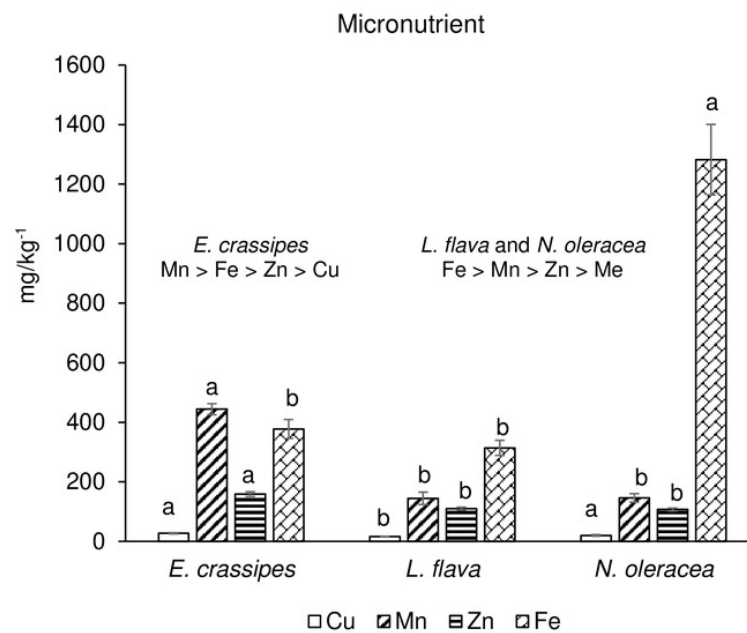



Figure 4

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

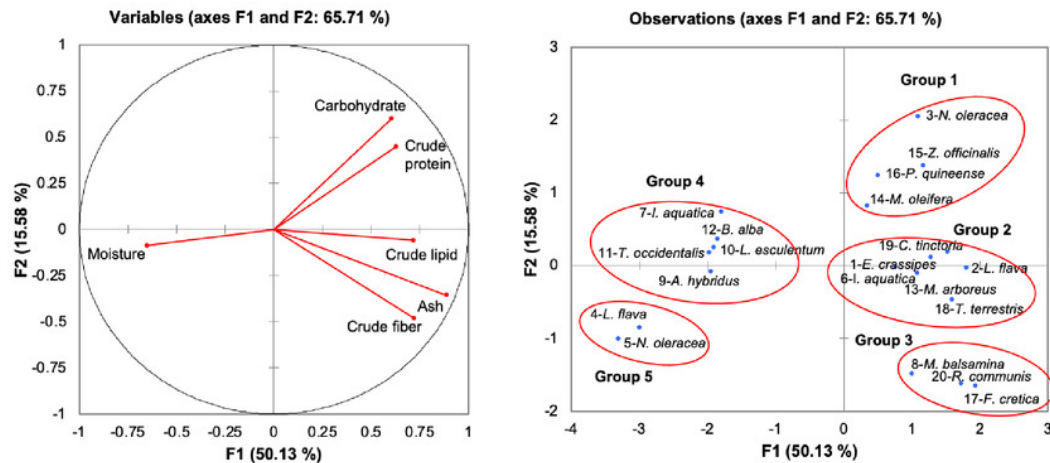
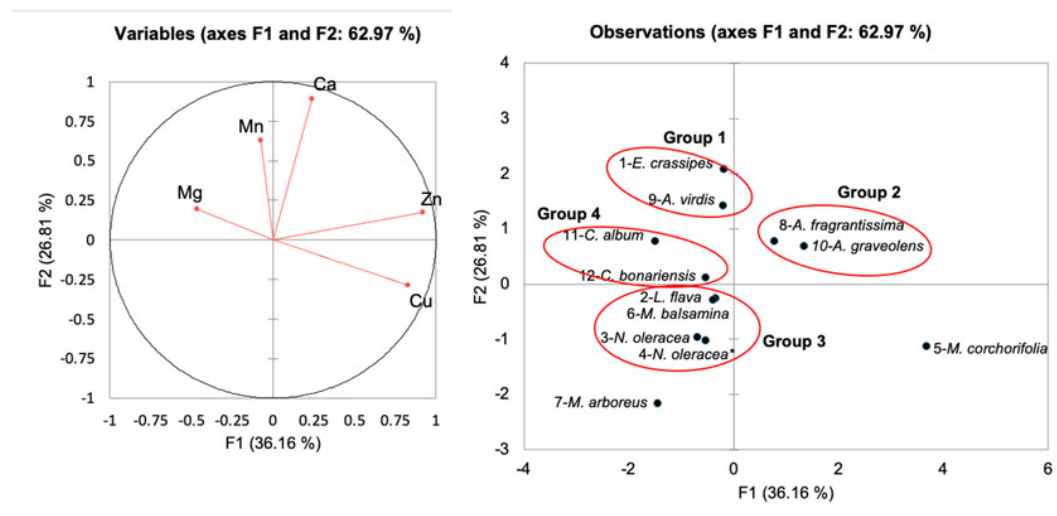



Figure 5

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

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.

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 $p < 0.05$.

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).

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)

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

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 $p < 0.05$.