ISSN: 2347-467X, Vol. 10, No. (3) 2022, Pg. 1196-1204

Current Research in Nutrition and Food Science

www.foodandnutritionjournal.org

Nutraceutical Prospects and Antioxidant Activity of White and Red Water Lily Stem Available in Bangladesh

MD. AKRAM HOSSAIN KHAN[1], MD. ALTAF HOSSAIN[2,3]*, MOHAMMAD MOZIBUL HAQUE[2], NILUFA YEASMIN[2], ABDUL MATIN[4] and DILSHAD ISLAM[5]

[1]Food and Agriculture Organization of the United Nations (FAO), Dhaka, Bangladesh.
[2]Department of Applied Food Science and Nutrition, Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh.
[3]Department of Animal Science and Technology, Sunchon National University, Suncheon, Republic of Korea.
[4]Department of Food Processing and Engineering, Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh.
[5]Department of Physical and Mathematical Sciences, Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh.

Abstract

Generally, the white and red water lily cultivars' stems are consumed as vegetables in Bangladesh. However, data on the nutritional benefits and antioxidant properties of these aquatic plant's stem are scant. Considering the aforementioned possibilities, the goal of this study was to evaluate the nutritional value, anti-nutritional factors, and antioxidant activity of two common Nymphaea species such as *Nymphaeanouchali* (white variety), and *Nymphaea rubra* (red variety) grown in Bangladesh. The proximate analysis and mineral content were determined by following AOAC methods. Crude fiber content was significantly higher in the white lily (19.56±0.68%) compared to the red cultivar (16.24±1.05%). Conversely, the red lilyshowed ahigher value (18.52±1.01%) for ash content than the white species (15.44±0.79%). However, the varieties had no significant differences in moisture, protein, fat, and carbohydrate content. Both stems were rich in essential minerals, whereas Na and Cu were significantly higher in white water lily (WWL), however, the red water lily (RWL) stem possessed significantly copious amounts of K and P. On the other hand, there was no significant difference between two species in the case of Ca, Mg, Fe, and Zn content. The assay showed that the total phenoliccontents (mg GAE/g)

Article History
Received: 17 August 2022
Accepted: 27 September 2022

Keywords
Antioxidant; DPPH;
Mineral content;
Phytochemical;
Water lily.

CONTACT Md. Altaf Hossain altaf@cvasu.ac.bd Department of Applied Food Science and Nutrition, Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh.

Doi: http://dx.doi.org/10.12944/CRNFSJ.10.3.32

and total flavonoid contents (mg QE/g) were16.51±0.05and 15.48±02,476±0.1 and 7.846±0.1 in white and red water lily stems respectively. In addition, the anti-nutrient screening revealed that both species were positive for saponin and tannin.Furthermore, the extract of RWL stem showed more antioxidant activity (IC_{50} value 28.48±0.12µg/ml) against DPPH than WWL (IC50 value 36.67±0.09 µg/ml).Thus, water lily stems can be incorporated into our diet as a potential natural source of antioxidants and vital elements.

Introduction

Malnutrition and nutritional problems are current issues of grave concern in third-world nations. Dietary deficiencies of both macro- and micro-nutrients or in combined cause the aforementioned lethal issues.[1] Although malnutrition has decreased steadily in Bangladesh, micronutrient deficiencies and non-communicable diseases (NCD) like cardiovascular disease, atherosclerosis, cancer, constipation, and irritable bowel syndrome (IBS) have increased over the last decade. Furthermore, youngsters are now inclined to processed food, rich in fat and saltbut less in fiber resulting in being highly prone to NCDs.[2] This food habit is amiable to produce more reactive oxygen species (ROS) or free radicals thatlead to numerous disordersin the body. To prevent this provocative reaction, a sufficient daily amount of antioxidantis required to attenuate the damage in the body by ROS. There is no standard guideline for the daily dietary intake of antioxidants. However, physicians and nutritionists suggest for an adult person about 8000 to11000 antioxidant units daily.[3] Even though artificial antioxidants are usedto avert the gamble of free radicals, some studies revealed that these possess carcinogenic properties.[4,5] In this view, the natural sources of antioxidants are intriguing to recent research and the neglected natural plants are entailed to look into as a source of antioxidants. The WWL and RWL cultivars are abundant and grown as a mixed population in all water bodies of Bangladesh. Some ethnic and village people considered water liliesin their diets without knowing its nutritive and medicinal value. Recently, some studies revealed that water lily leaves are used in the treatment of cancer,[6] controlling blood pressure, and erectile function,[7] seeds are used as dietary supplements for diabetes.[8,9].Theabove research work showed the importance of this aquatic plant in terms of nutritional and medicinal value in daily diet. However, data on water lily stem grown in Bangladesh are limited. Hence, in this present study, we investigated the quantification of nutraceuticals and antioxidant properties of both species of water lily (WL). In addition, phytochemical assays such as total phenol and flavonoid as well as screening of tannin and saponin provides a clear understanding of the health benefits of WL stem.

Materials and Methods

Sample Preparation

Both types of water lily were collected from the Halishahar and Patiya areas of Chattogram, Bangladesh. Then the stems were thoroughly washed andcut into 4 to 5 inches and dried in a cabinet dryer at 60 ºC for around 24 hours. Afterward, fine powder samples were prepared by grinding and sieving through a 2mm mesh sieve.

Proximate Composition Analysis

The proximate composition,notably carbohydrate, fiber, crude protein, fat, ash content, and moisture content of water lily stems was done by following the standard Association of Official Agricultural Chemists (AOAC, 2016)method.[10]

Mineral Elements

Inorganic substances were analyzed following a standard method described by Uddin Zim . (2021).[11] Briefly, a 5g sample was acid digested (HNO_3: $HClO_4$=3:1) and then heated at 95°C for 10 minutes to facilitate digestion. After cooling, digestate was filtered (0.45 µm membrane paper) and diluted to 100 ml with deionized water. Finally, mineral contents (mg/100g) were assessed using Biochemical analyzer (Humalyzer 3000, Germany) and commercial kit (Randox®, England).

Total Phenol Content

The Folin-Ciocalteu technique12 was employed to calculate the total phenol value. Succinctly, 1 g of powder sample was mixed with 10 ml methanol (80%) and then supernatant collected after sonication. Subsequently, 1 ml extract was mixed with 2 ml diluted Folin–Ciocalteu reagent and added 10 ml sodium carbonate (20%), then kept for 24 hours at room temperature. Finally, absorbance of the solution was measured by UV-visible spectroscopy (Shimadzu -2600, Germany) at 765 nm. To quantitate total phenol in sample, standard gallic acid curve ($R^2 = 0.9986$) was used, and unit was evinced as mg of gallic acid equivalent (GAE) per gram sample based on dried weight (DW).

Total Flavonoid Content

To evaluate the total flavonoidcontent, aluminum chloride colorimetric method was used.[13] In short, 50 g dried sample was extracted three times by ethanol (3 × 200 ml) and then dried. The powder was successively extracted with a mixture solvent (hexane, chloroform, ethyl acetate, and butanol) and then dried. Afterward, 4 mg of extracted powder was mixed with 50 ml of deionized water. Then 0.5 ml solution took into a test tube and added 2.8 mL of distilled water, 1.5 mL of methanol, 0.1 mL of aluminum chloride (10%), 0.1 mL of potassium acetate (1 M), and incubate 30 minutes at room temperature. Flavonoid content in samples was quantified against standard curve of quercetin ($R^2 = 0.9985$), and the values were calculated as mg of quercetin equivalent (QE) per gram sample based on DW.

Anti-Nutrient Screening

The presence of saponin and tannin in lily stems was carried out by following standard detection methods.[14] At first, 1 g of sample was taken in a beaker and then boiled with 10ml of 50% aqueous methanol solution. After boiling, 3 ml extracted aqueous solution was taken into a test tube. Subsequently, the test tube was shaken by hand, and foam formationwas considered existence of saponin in sample.Comparably, 1 g of sample was boiled with 3 ml of butanol-HCl reagent (95:5= n-butanol: concentrated HCL), and the color of solution change to pink was considered the presence of tannin in sample.

Antioxidant Activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging method was used to determine the antioxidant capability of water lily stems described by Akter .(2021).[15] Initially, 10 mg of methanolic extracted powder was mixed with 10 ml of methanol and serial dilution to prepare the same concentrated solution as reference solutions (ascorbic acid solution). Afterward, 1 ml of each diluted solution and 4 ml of DPPH solution took in a test tube, shook vigorously, and allowed to stand at room temperature in the dark for 30 minutes. Finally, the absorbance was measured by UV-visible spectroscopy (Shimadzu -2600, Germany) at 517 nm. DPPH and methanol solution was used as a negative control and blank solution respectively. The DPPH free radicals scavenging capacity was measured by the following equation-

% of inhibition = $[(A_c - A_s)/A_c \times 100]$, where, A_c = absorbance of control; A_s = absorbance of sample. The half inhibition concentration (IC_{50}) value was calculated from % inhibition graph (Figure 1) by following equation-

$IC_{50} = (50-b)/m$, where, b = intercept value, m = slope of line

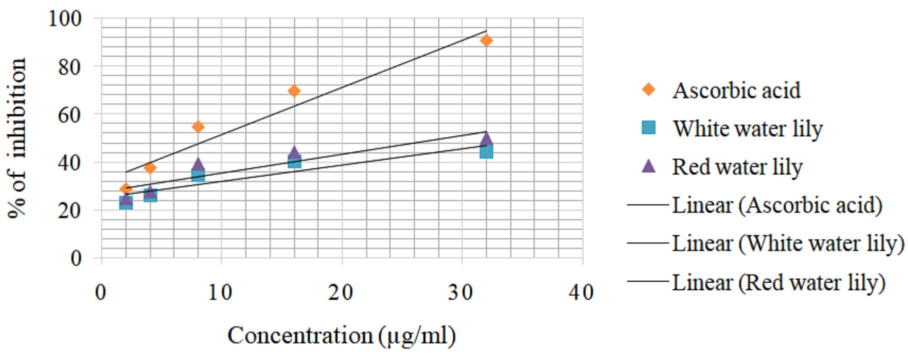

Fig. 1: DPPH radical scavenging capability (%) of sample and reference

Data Analysis

One-Way ANOVA and Tukey's test was performed to evaluate and compare the data by using Statistical package for socialsciences (SPSS) version 25 software. All values are exhibitedas mean value ± Standard deviation (SD). Level of significance was considered at P<0.05.

Results

Proximate Composition

The nutritive value of water lily stems is depicted in Table 1. RWL stem contains a significantly higher amount of ash content than WWL stem. On the contrary, crude fiber content in WWL was significantly higher compared to RWL. Both species possessan almost similar amount of moisture, protein, crude fat, and carbohydrate.

Minerals Content

The stems of both species carried a rich amount of five essential minerals (Na, K, Ca, Mg, and P) display in Figure 2. The highest amount of macro-mineral content (mg/100 g) was K in both species, 871.21 ± 6.17 in WWL and 980.73 ± 5.35 in RWL respectively. Micro-minerals such as zinc (Zn), iron (Fe), and copper (Cu) were also sufficient amount in both species.

Table 1: Nutritional composition of water lily

Sample	Moisture (%)	Ash (%)	Protein (%)	Crude fiber (%)	Crude fat (%)	CHO (%)
White lily	12.83±1.10	15.44±0.79	8.16±1.07	19.56±0.68	1.54±0.21	42.40±1.98
Red lily	12.64±1.07	18.52±1.01	8.46±0.72	16.24±1.05	1.55±0.25	42.79±1.11
P-value	0.838	0.014	0.702	0.010	0.960	0.784

All samples were replicated three times and the values represented as mean value ±SD,
P value <0.05 was considered as statically significant

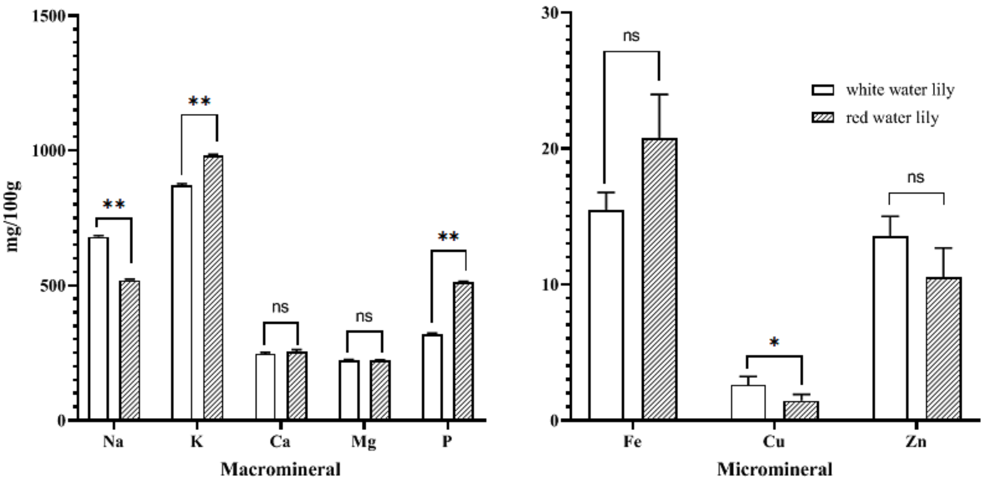

Fig. 2: Macro and micro mineral content in water lily cultivars' stem on dry basis, ** and * indicates the P-value<0.001, and P-value<0.005 respectively, ns = no significance

Total Phenolic and Flavonoid Content

WWL contains a slightly higher amount of total phenol content (16.51±0.05 mg GAE/g) than RWL (15.48±0.2 mg GAE/g) represented in Table 2. The results revealed that both types of stems contain amost same amount of total flavonoid.

Anti-nutrients Screening

In this study, we screened both saponin and tannin in stem extract. Both species gave a positive result throughout the test shown in Table 3.

Table 2: Phenolic and Flavonoid Content of water lily stems

Phytochemical	WWL	RWL	Significant
Total phenol (mg GAE/g)	16.51±0.05	15.48±0.2	NS
Total flavonoid (mg QE/g)	7.476±0.1	7.846±0.1	NS

The values are expressed as mean ± SD of three biological replicates.
NS indicates Not significant (P> 0.05).

Table 3: Saponin and Tannin screening of water lily

Sample	Tannin	Saponin
WWL	+	+
RWL	+	+

Legends: + indicates the presence of anti-nutrient

Antioxidant Activity

Free radical scavenging capacity of samples were assayed against DPPH solution. IC_{50} value of ascorbic acid (AA), WWL, and RWL was 9.22, 36.67, and 28.48 µg/ml respectively depicted in Table 4.

Table 4: DPPH scavenging activity of water lily stem extracts

Sample	IC_{50} (µg/ml)
AA	9.22±0.17^{a}
WWL	36.67±0.09^{c}
RWL	28.48±0.12^{b}

Results are expressed as mean value ± SD (n=3), different superscripts within column describe significant difference.

Discussion

Nutraceutical composition such as proximate composition, minerals, total phenol and flavonoid, and anti-nutrients (saponin and tannin) were measured in this study. The results revealed that both WWL and RWL species are rich inprotein 8.16±1.07% and 8.46±0.72% respectively. Previous research work reported about 16.4% crude protein in the stem of Nymphaea (*Nymphaea alba*).[16] This variation in value is may be due to the species variation, water bodies' composition, and locations' variation where water lily grown. In Table 1, the crude fiber content is significantly higher in WWL (19.56±0.68%) compared with RWL stems(16.24±1.05%), which are higher than the *Nymphaea alba* ranging from 9.39 – 9.50 %, 11.56 – 12.30 % and 11.84 – 15.1 % in the leaves, flowers and leaf stalk respectively.[17] This high range of fiber in stems may be due to its buoyancy properties, which require fiber and lignin in tissues of floated aquatic plants.[15] In concord withthis explanation, it is believed that the stems of WL contain more buoyant structural material. A previous study reported that crude fiber is related to lowering the risk of colon cancer, diabetes, obesity, and heart disease.[18] The ash content of RWL (18.52±1.01%) is significantly higher than WWL (15.44±0.79%). These values are within the ranges reported by Adelakun. *et al.* (2016).[19] The moisture content in both species of WL wasan average of 13%, quite higher to compare with *Nymphaea lotus* species (9%).[20] Fat is one of the essential nutrientsthat can increase palatability of food and also carry out the fat-soluble vitamins. The quantity of fat is the lowest amount in both species about 1.5% on an average, inter changeable to the findings reported earlier by Shah. *et al.* (2010).[21] The probable reason for lower level of fat content in stem maybe for its buoyancy properties and soaking with water. Low-fat foods are recommended forweight-reducing diets and also suggested in diets with high cholesterol levels.[22] As shown in Table 1 both species of WL are good sources of energy as they contain carbohydrates of about 43% and the result resembles the findings of Shah. *et al.* (2010).[21]

Minerals regulate the physiological reactions in the body. However, a number of studies have indicated consumption of suboptimal micronutrients may lead to numerous nutrition-related chronic diseases and micronutrient deficiencies (MNDs).[23,24,25]

In Bangladesh, children and women of reproductive age are prevalent to micronutrient deficiencies, where 41% of women[26] and 51% of children under the age of five suffer Iron deficiency anemia (IDA), zinc insufficiency affects both women and children at 45% and 51%, respectively.[27] From Figure 2 it is vividly observed that both species contain a wide range of macro and trace elements. Among macronutrients Na, K and P were in substantial amounts in both species. The sodium content (mg/100g) was significantly higher in WWL (680.08±4.78) compared to RWW (520.24±3.77), conversely, RWL contained higher amount of K (980.73±5.35 mg/100g) than WWL (871.24±6.17 mg/100g), which is higher than of *Nymphaea lotus*(742.89mg/100g).[19] This higher mineral content in stem may be due to water qualities where the WL has grown up as the aquatic plants accumulate the nutrients from water bodies.[28] Both K and Na are responsible for regulating blood pressure, water balance, muscle contraction, and the nervous system.[29] The content of calcium and magnesium were satisfactory in both species,three times higher than *Nymphaea lotus.*[19] However, this specie contains a higher amount of phosphorus (635mg/100g)than the findings of the present study where the phosphorus was 320.08±3.15 and 511.58±4.51mg/100g in WWL and RWL respectively. These essential minerals play a crucial role in bone tissues, blood coagulation, neuromuscular irritability, and enzyme activity.[30] The water lily stemscontain amoderate amount of iron,15.44±1.33 and 20.72±3.25 mg/100g in white and red species those are higher than some vegetables consumed by rural people as curry such as cauliflower, cabbage, okra, pumpkin, etc. Iron is another essential micronutrient, responsible for iron deficiency anemia (IDA), highly prevalent (41%) in women of Bangladesh.[26] It is essential for the transportation of oxygen, enzyme activity, regulating cell growth and cell differentiation, and boosting the immune system.[31] The copper content of RWL (2.79±0.43 mg/100g) was about doubled that of WWL (1.62±0.42 mg/100g) and zinc levels were13.52±1.49 and 10.51±2.17 mg/100g respectively, whereas Adelakun. *et al.*(2016) reported that *Nymphaea lotus* content. 8.16mg/100g of zinc that was lower than our findings.[19] Both copper and zinc trace elements are essential for innate immune function, DNA and RNA reproduction, maintenance of cell membrane integrity, and destruction of free radicals.[32]

Phenolic compounds (PCs) are secondary metabolites of plants. Although PCs are not essential elements for the body, but can helpto prevent numerous diseases and improve the human health. The total phenol content of both species stem is about 16 mg GAE/g which is within the range reported in *Nymphaea alba* ranging from 5.40 to 19.50 mg GAE/g33.Thus WL stem is a good source of PCs. It showsbioactive properties such as antioxidant activity due to its hydroxyl groups, and antibacterial activity.[34] The stems of both species contain approximately 8 mg QE/g flavonoid compounds, which is lower than other parts of water lilies such as black *Nymphaea lotus* seeds (24.057 ± 0.113 mg EC/g) and *Nymphaea micrantha* seeds (27.026 ± 0.074 mg EC/g). This is due to the stem staying under water whereas, flowers stay in the upper part of a plant and fight against UV radiation,cold, heat, and pathogens, thus stressor parts accumulate more flavonoid than other parts of plants.[35] It plays a crucial role to prevent cancer, inflammation, and viral infections.[36] Moreover, the presence of tannin and saponin in WL stem revealed that it can be a good source of anti-peroxidative, anti-inflammatory, anti-carcinogenic, and anti-allergic.[34]

The antioxidants prevent the oxidation caused by free radicals. During the metabolic process, the body produces free radicals by burning fuels. However, smoking, illness, food adulteration, and stress exacerbate the free radical condition.[37] Our study, divulged that RWL (IC_{50} value= 28.48 µg/ml) possesses more antioxidant properties than WWL (36.67 µg/ml), this is due to RWL containing more flavonoid and polyphenol in their stems.[34] In contrast, according to Baehaki . (2015), *Nymphaea stellate* and *Nelumbonucifera* species showed IC_{50} valuesof 43.21 and 139.84 µg/ml respectively which are higher than our findings.[38] It is evident from the above result that both species of water liliescan be used as a natural source of antioxidants in diet as well as in the medicinal purposes.

To the best of our knowledge,the present study is primitive that analyzed the nutraceutical and antioxidant properties (in vitro) of Bangladeshi white and red WLcultivars' stems. The results of this study showed that the stems of both species are a rich source of protein, crude fiber, and essential macro and micro-minerals. Additionally, these can be incorporated into a normal diet to reduce oxidative stress such as aging, and are potential sources of natural antioxidants. However, further studies are recommended to identify additional phytochemical and bioactive substances in WL stems. Research on how different cooking methods affect the nutritional value of WL is also appreciated. Additionally, extensive in-vitro and in-vivo investigations are encouraged before moving forward with the development of supplements from WL stems.

Acknowledgment

The authors would like to express their gratitude and cordial thanks to the Department of Applied Food Science and Nutrition, and Poultry Research and Training Centre (PRTC), CVASU for providing analytical facilities.

Funding

The authors would like to express their gratitude to the Ministry of Science and Technology, Bangladesh, and Advanced Studies &Research, CVASU, for providing funds for this research work.

Conflict of Interest

All authors declare no conflict of interest.

References

1. Müller O, Krawinkel M. Malnutrition and health in developing countries. *Cmaj*. 2005;173(3):279-286.
2. Biswas T, Azzopardi P, Anwar SN, *et al*. Assuring Bangladesh's future: non-communicable disease risk factors among the adolescents and the existing policy responses. *J Heal Popul Nutr.* 2022;41(1):1-10. doi:10.1186/s41043-022-00294-x
3. Greger M. How to Get *Enough Antioxidants* Each Day.; 2021. https://nutritionfacts.org/2014/12/04/how-to-get-enough-antioxidants-each-day/
4. Branen AL. Toxicology and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene. *J Am Oil Chem Soc*. 1975;52(2):59-63. doi:10.1007/BF02901825
5. Kahl R. Synthetic antioxidants: Biochemical actions and interference with radiation, toxic compounds, chemical mutagens and chemical carcinogens. *Toxicology.* 1984;33(3-4):185-228. doi:10.1016/0300-483X(84)90038-6
6. Gueye FK, Niang L, Dieng B, . Phytochemical Screening and Antioxidant Activities of Water Lilies Seeds, Neglected and Underused Species in the Delta and Lower Valley of the Senegal River. *Am J Plant Sci.* 2022;13(06):756-765. doi:10.4236/ajps.2022.136051
7. Kameni PM, Dzeufiet DPD, Bilanda DC, *et al.* Nymphaea lotus Linn. (Nymphaeaceae) Alleviates Sexual Disability in L-NAME Hypertensive Male Rats. Evidence-Based *Complement Altern Med*. Published online 2019:1-9. doi:10.1155/2019/8619283
8. Gueye FK, Ayessou NC, Mbaye MS, Diop MB, Cissé M, Noba K. Nymphaea lotus L. and Nymphaea micrantha Guill. et Perr Seeds as Cereal Substitute in the Delta of Senegal River. *Food Nutr Sci.* 2020;11(05):375-384. doi:10.4236/fns.2020.115027
9. Pokhrel T, Shrestha D, Dhakal K, Yadav PM, Adhikari A. Comparative Analysis of the Antioxidant and Antidiabetic Potential of Nelumbo nucifera Gaertn. and Nymphaea lotus L. var. pubescens (Willd.). *J Chem*. 2022;2022.
10. AOAC International. Official methods of analysis of AOAC International. *Assoc Off Anal Chem Int.* Published online 2016:3172.
11. Uddin Zim AFMI, Khatun J, Khan MF, Hossain MA, Haque MM. Evaluation of in vitro antioxidant activity of okra mucilage and its antidiabetic and antihyperlipidemic effect in alloxan-induced diabetic mice. *Food Sci Nutr.* 2021;9(12):6854-6865.
12. Wojdyło A, Oszmiański J, Czemerys R. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem. 2007;105(3):940-949. doi:10.1016/j.*food chem.* 2007.04.038
13. Shah MD, Hossain MA. Total flavonoids content and biochemical screening of

the leaves of tropical endemic medicinal plant Merremia borneensis. *Arab J Chem.* 2014;7(6):1034-1038. doi:10.1016/j.arabjc.2010.12.033

14. Kumari B, Solanki H. Isolation of Alkaloids, Saponins and Tannins from Plants. I*nt J Plant Environ.* 2019;5(03):223-225. doi:10.18811/ijpen.v5i03.13
15. Akter S, Jahan I, Khatun R, . Pharmacological insights into Merremia vitifolia (Burm.f.) Hallier f. leaf for its antioxidant, thrombolytic, anti-arthritic and anti-nociceptive potential. *Biosci Rep.* 2021;41(1):1-11. doi:10.1042/BSR20203022
16. Akmal M, Hafeez-ur-rehman M, Ullah S, Younus N, Khan KJ, Qayyum M. Nutritive value of aquatic plants of Head Baloki on Ravi River, Pakistan. *Int J Biosci.* 2014;6655:115-122. doi:10.12692/ijb/4.10.115-122
17. Abarike ED, Amenyogbe E, Ampofo-Yeboah A, . Exploring the nutrient potential of Nymphaea alba (water lilly), for use as livestock feed. *UDS Int J Dev.* 2015;2(1):1-11.
18. O'Keefe S. Dietary Prevention of Colon Cancer and Other Noncommunicable Westernized Diseases. *UPMC Physician Resour.* Published online 2022:1-6.
19. Adelakun K, Kehinde A, Amali R, Ogundiwin D, Omotayo O. Nutritional and Phytochemical Quality of Some Tropical Aquatic Plants. *Poultry, Fish Wildl Sci*. 2016;04(02):2-5. doi:10.4172/2375-446x.1000164
20. Stephen EC, Adebisi AK, Chinedu I, Samuel AA. Chemical Composition of Water Lily (Nymphaea lotus) Bulbs. *Am J Food Sci Nutr.* 2017;4(2):7-12. http://www.aascit.org/journal/ajfsn
21. Shah KA, Syed S, Andrabi SA. A Study on Nutritional Potential of Aquatic Plants. *Vetscan* | *Online Vet J*. 2010;5(1):33-38.
22. Chinelo AS, Jega UK. Proximate and Amino Acid Analyses of the Rhizome of Nymphaea lotus (Water Lily). *Mod Chem.* 2019;7(3):54. doi:10.11648/j.mc.20190703.12
23. Trijsburg L, Talsma EF, Crispim SP, . Method for the development of wish, a globally applicable index for healthy diets from sustainable food systems. *Nutrients.* 2020;13(1):93.
24. Afshin A, Sur PJ, Fay KA, . Health effects of dietary risks in 195 countries, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet.* 2019;393(10184):1958-1972.
25. Castela Forte J, Gannamani R, Folkertsma P, Kanthappu S, van Dam S, Wolffenbuttel BHR. A Pilot Study on the Prevalence of Micronutrient Imbalances in a Dutch General Population Cohort and the Effects of a Digital Lifestyle Program. *Nutrients.* 2022;14(7):1426.
26. Jana A, Chattopadhyay A, Saha UR. Identifying risk factors in explaining women ' s anaemia in limited resource areas : evidence from West Bengal of India and Bangladesh. BMC *Public Health.* 2022;22:1-16. doi:10.1186/s12889-022-13806-5
27. USAID. Bangladesh: Nutrition Profile. Published online 2021:1-9. https://www.usaid.gov/sites/default/files/documents/Copy_of_tagged_Bangladesh-Nutrition-Profile.pdf
28. Shardendu S, Sayantan D, Sharma D, Irfan S. Luxury Uptake and Removal of Phosphorus from Water Column by Representative Aquatic Plants and Its Implication for Wetland Management. *ISRN Soil Sci.* 2012;2012:1-9. doi:10.5402/2012/516947
29. Shrimanker I, Bhattarai S. Electrolytes. *Int Stat Pearls.* Published online 2022:1-29.
30. Ciosek Ż, Kot K, Kosik-Bogacka D, Łanocha-Arendarczyk N, Rotter I. The effects of calcium, magnesium, phosphorus, fluoride, and lead on bone tissue. *Biomolecules.* 2021;11(4). doi:10.3390/biom11040506
31. Hsu MY, Mina E, Roetto A, Porporato PE. Iron: An Essential Element of Cancer Metabolism. *Cells*. 2020;9(12):1-28. doi:10.3390/cells9122591
32. Djoko KY, Cheryl-Lynn YO, Mark JW, Alastair GM. The role of copper and zinc toxicity in innate immune defense against bacterial pathogens. *J Biol Chem.* 2015;290(31):1854-1861. doi:10.1074/jbc.R115.647099
33. Cudalbeanu M, Ghinea IO, Furdui B,. Exploring new antioxidant and mineral compounds from nymphaea alba wild-grown in danube delta biosphere. *Molecules.* 2018;23(6):1-16. doi:10.3390/molecules23061247
34. Parimala M, Shoba FG. Phytochemical analysis and In vitro antioxidant acitivity of hydroalcoholic seed extract of Nymphaea nouchali Burm. f. *Asian Pac J Trop Biomed*.

2013;3(11):887-895. doi:10.1016/S2221-1691(13)60174-4

35. José C del V, Buide ML, Casimiro-Soriguer I, Justen BW, Narbona E. On flavonoid accumulation in different plant parts: Variation patterns among individuals and populations in the shore campion (Silene littorea). *Front Plant Sci.* 2015;6(OCTOBER):1-13. doi:10.3389/fpls.2015.00939
36. Ullah A, Munir S, Badshah SL,. Important Flavonoids and Their Role as a Therapeutic Agent. *Molecules.* 2020;25(22):1-39.
37. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn* Rev. 2010;4(8):118.
38. Baehaki A, Lestari SD, Apriyanti W. Phytochemical screening and antioxidant activity of seeds extract of water plant (Nymphaea stellata and Nelumbo nucifera). *J Chem* Pharm *Res*. 2015;7(11):221-224.