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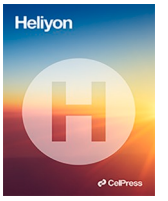

Research article

Nutritional and biofunctional characterizations of four novel edible aquatic plants of Bangladesh

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A R T I C L E I N F O

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A B S T R A C T

Aquatic plants are a cheap and renewable biomass rich in bioactive and biofunctional compounds, holding valorization prospects for use in food and pharmaceuticals. Four commonly found edible aquatic plants in Bangladesh, namely red water lily (*Nymphaea nouchali*), white water lily (*Nympheae alba*), malancha (*Alternanthera philoxeroides*), and red seaweed (*Gracilaria tenuistipitata*), were compared in terms of proximate composition, bioactive compounds, antioxidant activity, mineral and heavy metal contents, and amino acid composition. The crude protein content was the highest in *A. philoxeroids* (26.96 %), followed by *G. tenuistipitata* (25.21 %), *N. nouchali* (25.14 %), and *N. alba* (23.54 %). The sequence of crude lipid content of four aquatic plants was *A. philoxeroids* (4.8 %) > *N. nouchali* (4.0 %) > *G. tenuistipitata* (3.4 %) > *N. alba* (2.4 %). The aquatic plants were rich in carbohydrates, with *G. tenuistipitata* having 37.02 %, significantly ($P < 0.05$) lower than *N. alba* (46.12 %), *N. nouchali* (45.73 %), and *A. philoxeroids* (42.88 %). The ash content in the studied plants varied between 14.63 % and 24.97 %. Substantial numbers of bioactive compounds were identified in these plants: 42 in *N. alba,* 41 in *N. nouchali*, 40 in *A. philoxeroides,* and 36 in *G. tenuistipitata,* as determined by GC-MS analysis. *G. tenuistipitata* showed the highest amount of total phenolic (121.05 ± 2.43 mg gallic acid equivalent/g) and flavonoid (128.03 ± 0.79 mg quercetin equivalent/g) content. The DPPH, hydrogen peroxide, and ferric reducing power assays showed the free radical scavenging ability increased in a dose dependent manner. These aquatic plants contained substantial amounts of minerals, namely Ca ranging from 42.05 ± 2.34 to 441.65 ± 4.67 mg/kg, K ranging from 80.15 ± 1.82 to 97.81 ± 1.74 mg/kg, and Na ranging from 41.16 ± 1.32 to 53.37 ± 1.64 mg/kg. The heavy metal contents of Cu, Ni, and Pb were 0.93 ± 0.06 to 1.25 ± 0.09 mg/kg, 0.44 ± 0.02 to 3.86 ± 0.56 mg/kg, and 0.22 ± 0.02 to 0.67 ± 0.05 mg/kg, respectively. Thirteen different amino acids were identified, with leucine, glycine, alanine, lysine, and phenylalanine dominating, and their contents varying by species. Therefore, regular consumption of these aquatic plants might be a healthy approach to addressing malnutrition and enhancing biofunctional activities.

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1. Introduction

Aquatic plants grow abundantly in aquatic environments, such as ponds, lakes, and waterways worldwide. As a low-lying riverine country, Bangladesh supports a large number of aquatic plants, which provide many benefits to fish, wildlife, and people. These plants are promising sources of diverse bioactive compounds, including polyphenols, flavonoids, carotenoids, and polysaccharides. *Nymphaea nouchali*, known as red water lily, is a member of the Nymphaea genus and is widely grown in Bangladesh and India. *Nymphaea alba,* commonly known as white water lily, belongs to the Nymphaeaceae family and is native to the North Africa, temperate Asia, Europe, and tropical Asia. Nymphaea is the largest and most extensive distributed genus of water lilies, with 35 species. Many *Nymphaea* species are considered functional medicinal plants in Nepal, India, and China. Extracts from *Nymphaea,* including rhizome and flower extracts, are used as therapeutic plant materials with anti-diabetic and anti-inflammatory properties. Flower extracts have an astringent, cardiotonic, demulcent, antipyretic, and free radical scavenging properties, making them useful in treating insomnia, anxiety, and similar disorders [1]. *Alternanthera philoxeroides*, commonly referred to as alligator weed, is locally known as 'malancha shak' in Bangladesh. This plant is considered a leafy vegetable and is used as food for humans and cattle due to their nutritional and medicinal properties. Extracts from the leaves and stems of this plant are consumed to treat night blindness and to stop vomiting. This plant is also effective in treating night blindness, hazy vision, post-natal complaints, diarrhea, dysentery, malaria, and puerperal fever [2]. *A. philoxeroides* has been found to be quite rich in iron content and may be used in salads [3].

The genus *Gracilaria* belongs to red algae (Rhodophyta) is considered the third-largest among the approximately 150 red algae worldwide, with over 28 *Gracilaria* species identified along the Indian coast [4]. It is well known for its economic value as an agarophyte and as well as a food source for humans and various shellfish species. Different members of the genus are farmed in Asia, South America, Africa, and Oceania. Consuming edible marine plants improves human health by lowering blood cholesterol levels, reducing the risk of coronary heart disease, and control obesity [5]. Red algae are commercially significant for their valuable products, such as agar, vitamins, macro and micronutrients, bioactive compounds, protein, vitamins, fatty acids, and fiber. *G. tenuistitata* is used as a sea vegetable in the Philippines and is widely cultured in Japan, Korea, China, and Taiwan for edible purposes. Several species, such as *G. changii* and *G. tenuistipitata*, are consumed as salads and for agar extraction in Malaysia. Agar is widely used in the manufacture of jam, jelly, cosmetics, pharmaceuticals, and in microbiological studies.

Nowadays, people are suffering from various diseases due to stress, pollution, smoking, and poor dietary habits. These diseases are caused by the production of free radicals through oxygen metabolism and other biochemical reactions. Bioactive compounds from edible aquatic plants scavenge free radicals and help to prevent different diseases. Many of these aquatic plants are also traditionally used in the treatment of diseases. About 25 % of all prescribed medicines today are derived from plants [6]. Although many aquatic plants are available in some tropical and sub-tropical regions, the people in these areas are not accustomed to consuming them. Additionally, there are very few reports available that demonstrate the various bio-functional and nutritional properties of the aquatic plants found in Bangladesh. Research on numerous bioactive phytochemicals in crude and fractionated extracts of the aquatic weed *I. aquatica* reported the highest content of ellagic acid, resulting in high antioxidant activity [7]. Among the three seaweeds studied in Bangladesh- *Gracilaria longissimi* (red seaweed), *Ulva intestinalis* (green seaweed), and *Padina tetrastromatica* (brown seaweed), *G. longissimi* showed the most potential for incorporation into food due to its high levels of protein, essential amino acids, essential fatty acids, and overall phenolic and flavonoid contents [8]. An investigation into the secondary metabolites and antioxidant activities of solvent extracts from *Padina tetrastromatica* and *Gracilaria tenuistipitata* found that the methanolic extract of *P. tetrastromatica* exhibited the highest total phenolic content, flavonoid content, and *in vitro* free radical scavenging ability compared to the extracts of *G. tenuistipitata* [9]. There are some research reports on the bioactive compounds and biofunctional roles of freshwater aquatic plants in Bangladesh [10,11]; however, no reports are available on the broad spectrum and comparative analysis of the nutritional and biofunctional properties of both freshwater and marine aquatic plants in Bangladesh. Therefore, the objectives of the present study were to evaluate the proximate composition, nutritional properties such as phenolics and flavonoid, bioactive compounds, amino acids and minerals contents, and bio-functional activities of four commonly available freshwater and marine plants in Bangladesh.

2. Materials and methods

2.1. Sample collection and preparation

The aquatic plant samples were collected from four different locations such as *N. nouchali* from Gopalganj, *N. alba* from Munshiganj, *A. philoxeroides* from Jashore, and *G. tenuistipitata* from Cox's Bazar district of Bangladesh. The samples were collected manually, placed in a polyethylene bag with native water (plant mass: water = 1:5 w/v), labeled, and transported to the laboratory for analysis. All of the plants were rinsed in clean water and dried in the sun for 5–6 days. The sundried samples were pulverized using a high-capacity electric grinder (Model: WBL-VK01, 1.5 L, Walton, Bangladesh) and sieved. The powdered samples were then kept at –60 °C in airtight plastic containers until further analysis.

2.2. Analysis of proximate composition

The proximate composition, including moisture, protein, lipid, and ash content, was analyzed following the methods of AOAC [12]. Protein content was determined using the basic Kjeldahl procedure, which includes digestion, distillation, and titration. The lipid content was measured using the traditional Soxhlet extraction method with *n*-hexane as the solvent [13]. The carbohydrate content of

aquatic plants was determined indirectly by subtracting the moisture, protein, lipid, and ash content from the total [14].

2.3. Gas Chromatography Mass Spectrophotometry analysis for bioactive compounds

The extract for Gas Chromatography Mass Spectrophotometry (GC-MS) analysis was prepared by taking 10.0 g of pulverized aquatic plant powder in a 100 mL glass beaker. Then, 50 mL of ethanol was added to the sample and stirred using a magnetic stirrer for 5 h at a rotation speed of 400 rpm. The beaker was properly covered with aluminum foil to inhibit solvent evaporation. After that, the sample was filtered with the double filter paper. The filtered sample was then re-filtered with a syringe filter (0.45 μm) and stored in glass vial.

The analysis was conducted using a Clarus 690 Gas Chromatograph, which included a column (Elite-35, 30 m × 0.25 mm, 0.25 μm film thickness; PerkinElmer, USA) and a Clarus@ SQ 8C Mass Spectrophotometer (PerkinElmer, USA). First, 1 μL of the sample solution was injected, and pure helium (99.99 %) was used as the carrier gas, maintained at a flow rate of 1 mL/min for a 40-min run time. The material was evaluated at high energy (70 eV) in electron ionization (EI) mode. The inlet temperature was kept constant at 280 °C; however, the oven temperature was set at 60 °C for 0 min, then increased at 5 °C/min to 240 °C. The compounds in the sample were identified using the database of National Institute of Standards and Technology (NIST).

2.4. Determination of total phenolic content

Total phenolic content of the sample was determined following the previous method [15] using the Folin-Ciocalteu reagent. The dried extract was dissolved in ethanol and combined with 5 mL of 10 % Folin-Ciocalteu reagent. Then, 4 mL of Na_2CO_3 (75 g/L) was added to the mixture. The sample with reagents was then incubated at 40 °C for 30 min. The absorbance of the mixture was measured at 765 nm using a spectrophotometer (UV-1800, Shimadzu UV–Vis spectrophotometer, Japan). For standard curve preparation, several concentrations of gallic acid (31.25–500 μg/mL) were used. Total phenolic content was measured and presented as mg gallic acid equivalent (GE)/g of dried extract.

2.5. Total flavonoids content assay

The aluminum chloride colorimetric test was performed to measure the total flavonoid content of the sample as described by Chang et al. [16]. First, 4 mL distilled water was added to the extract solution, followed by 0.3 mL of $NaNO_3$ (5 % w/v). After 5 min, 0.3 mL of $AlCl_3$ (10 % w/v) was added. Subsequently, 2 mL of 1 M NaOH was added to make the final volume 10 mL, and the absorbance was recorded at 510 nm. The standard calibration curve for this assay was prepared with quercetin (31.25–500 μg/mL) and the total flavonoid content of the sample was presented in mg quercetin equivalent (QE)/g of dried extract.

2.6. Preparation of extracts for antioxidant analysis

The extracts for antioxidant analysis were prepared following the method of Islam et al. [17] with some modifications. A powdered sample (50.0 g) was taken in a 1 L glass beaker and 600 mL of 95 % ethanol was added. The beaker was kept under conditions of regular shaking and stirring for 15 days. The mouth of the beaker was sealed with aluminum foil to prevent solvent evaporation. The entire extract was filtered through a piece of clean, white cotton fabric. Then, Whatman No. 1 filter paper was used for further filtration. The solvent was evaporated using a rotary evaporator at 45 °C to obtain the crude extract, which was stored at 4 °C for future use.

2.6.1. DPPH radical scavenging assay

Free radical scavenging activity of the samples was evaluated following the method described by Villaño et al. [18] with some modification using 2, 2-diphenyl-1-picryl hydrazyl (DPPH) solution. Various concentrations of sample (3.93–500 μg/mL) were prepared from the stock extract. A 3 mL solution of 0.004 % (w/v) DPPH was added to 1 mL of sample solution and incubated in the dark for 30 min at room temperature. The absorbance was then measured at 517 nm using a spectrophotometer. Ascorbic acid, a natural antioxidant, was used as a standard. The DPPH free radical scavenging activity percentage of each plant extract and the standard was calculated using the following Eq. (1):

$$\text{DPPH radical scavenging activity (\%)} = [A_0 - A/A_0] \times 100 \quad (1)$$

Where, A_0 is the absorbance of control and A is the absorbance of extract or standard. Ascorbic acid was used as a positive control in this study.

2.6.2. Hydrogen peroxide scavenging assay

Hydrogen peroxide scavenging activity of the aquatic plant samples was performed following the method described by Halliwell et al. [19] with some modifications. The sample solution (3.93–500 μg/mL), 6 mL of H_2O_2 (2 mM in 50 mM phosphate buffer), and 4 mL phosphate buffer (50 mL, pH 7.4) were mixed together and the absorbance was measured at 230 nm after 10 min. The hydrogen peroxide scavenging capacity was calculated using the following Eq. (2):

$$\text{Hydrogen peroxide scavenging activity (\%)} = [A_0 - A/\ A_0] \times 100 \quad (2)$$

Where, A_0 is the absorbance of control and A is the absorbance of extract or standard. Ascorbic acid was used as a positive control in this study.

2.6.3. Ferric reducing antioxidant power assay

The ferric reducing antioxidant capacity of different aquatic plants was determined using the FRAP reagent with some modifications of previous method [20]. The FRAP reagent was formulated by mixing acetate buffer (pH 3.6), TPTZ solution (10 mM), and $FeCl_3$ (20 nM) in a 10:1:1 ratio. The extract solution of different concentrations (100–500 μg/mL) was then mixed with 3 mL of the FRAP solution. The mixture solution was incubated at 37 °C for 30 min, and the absorbance was recorded at 593 nm using a spectrophotometer. Ascorbic acid was used as a control in this study.

2.7. Determination of minerals and heavy metals content

The minerals and heavy metals contents of the samples were determined using ICP-OES optima 2000 DV (PerkinElmer, MA, USA) following the previously described method [21] with minor modifications. The RF power was set to 15 W, with a plasma gas flow rate of 15 L/min, and the auxiliary gas flow rate was 0.2 L/min. The sample uptake rate was maintained at 1 mL/min and the integration time were set between 1 and 5 s per wavelength. The LOD for the elements was ppb and accuracy levels were maintained within 100 ± 5 %. Periodic verification against quality control samples was conducted to maintain the precision of the measurements. Calibration was carried out with multiple standards with known concentrations. First, 1.0 g of each dried sample was placed in a muffle furnace at 600 °C for 6 h. After ash formation, 4 mL of HNO_3 and 1 mL of H_2O_2 were added to the ash powder, followed by the addition of distilled water to make a 60 mL solution. The solution was then heated on a hot plate and reduced to half of its original volume (30 mL). Subsequently, 4 mL of distilled water and 1 mL of H_2O_2 were added, and the solution (35 mL) was heated again to reduce it to half of its original volume (17.5 mL). Distilled water was then added to make a 50 mL solution, which was then filtered through a 125 mm filter paper. The solution was filtered twice, and after that, it was ready to be tested for minerals and heavy metals analysis.

2.8. Determination of amino acid composition

An amino acid analyzer (LA 8080, Hitachi, Japan) with a high-performance cation-exchange column, maintained at a temperature of 57 °C, was used to determine the amino acid content following the method describe by Zhao al. [22], with some modifications. One g of the sample was added to 25 mL of 6 M HCl in a glass tube for pre-treatment. The tube was then placed in a sand bath and heated to 110 °C for 24 h. The solid was then dried using an HCl evaporation process, mixed with 6 mL of distilled water, and filtered with a 0.45 μm syringe filter.

2.9. Statistical analyses of experimental data

All experiments were repeated for three times, and results are presented as mean ± standard deviation. Statistical analysis was carried out by one-way analysis of variance (ANOVA) using IBM SPSS software (Version 20.0, SPSS Inc., Chicago, IL, USA). Significant differences between means were determined by Duncan's Multiple Range Test and $P < 0.05$ was considered as significant. Microsoft Office (2010) was used to create the graph and table.

3. Results and discussion

3.1. Proximate composition of aquatic plants

The proximate composition, such as moisture, ash, protein, lipid, and carbohydrate contents of the aquatic plants was found to vary depending on the species (Fig. 1). The highest moisture content was found in *N. alba* (11.24 %), followed by *N. nouchali* (10.49 %), *A. philoxeroides* (9.14 %) and *G. tenuistipitata* (8.86 %). However, since the samples were sundried, the moisture content varied due to extent of drying and the nature of plants tissues in holding water. The moisture content and moisture retention of aquatic plants are related to the morphology and structure of the species [23]. Low moisture content helps retain the nutritional components as high moisture content promotes susceptibility to microbial growth and enzyme activity [24]. The crude protein content was highest in *A. philoxeroids* (26.96 %), followed by *G. tenuistipitata* (25.21 %), *N. nouchali* (25.14 %), and *N. alba* (23.54 %). The sequence of crude lipid content in the four aquatic plants was *A. philoxeroids* (4.8 %) > *N. nouchali* (4.0 %) > *G. tenuistipitata* (3.4 %) > *N. alba* (2.4 %). Seaweed is considered a high-protein biomass [8] that plays important roles in repairing worn-out tissues, supporting enzymes, hormones, antibodies, and other substances, and synthesizing new cells. The crude protein content is aquatic weeds are affected by the species, season, habitat, and environmental factors. Carbohydrate content in *G. tenuistipitata* was 37.02 %, which was significantly lower than *N. alba* (46.12 %), *N. nouchali* (45.73 %), and *A. philoxeroids* (42.88 %). Carbohydrates are a vital component of aquatic plants, making them a crucial part of metabolism by providing energy. Various factors such as species, season, habitats, etc. can influence in the variation of carbohydrate content in aquatic plants [25]. The highest ash content was found in *G. tenuistipitata* (24.97 %), followed by *N. alba* (16.69 %), *A. philoxeroides* (15.89 %), and *N. nouchali* (14.63 %). Aquatic weeds are generally rich in ash content [8] and consumption of ash, i.e., minerals from aquatic plants can prevent various illness, including constipation, arthritis fluid retention, gout, bladder issues etc. Additionally, it is used to support colon health maintenance and to treat constipation [26].

The moisture, protein, lipid, ash, and carbohydrate content in white and red water lily was reported to 12.83 ± 1.10 % and 12.64 ±

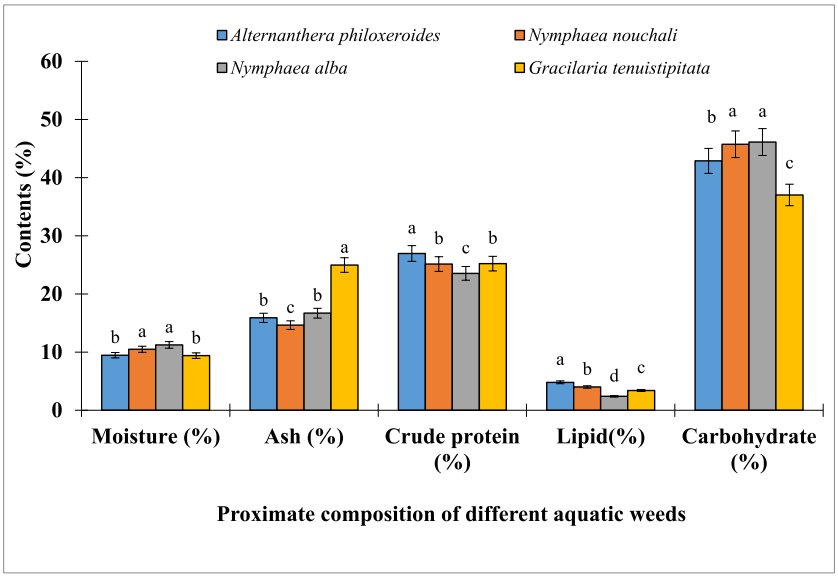

Fig. 1. Proximate composition of different aquatic weeds from Bangladesh. Different small letters on each column bar for each composition indicates significant difference ($P < 0.05$)

1.07 %, 8.16 ± 1.07 % and 8.46 ± 0.72, 15.44 ± 0.79 and 18.52 ± 1.01, and 42.40 ± 1.98 and 42.79 ± 1.11 %, respectively [27]. The seaweed *Gracilariopsis longissima* from Bangladeshi coast showed the protein, lipid, ash, and carbohydrate content 30.63 ± 0.90 %, 1.49 ± 0.05 %, 22.25 ± 1.10 %, and 30.45 ± 0.80 %, respectively [8]. In a previous study, *A. philoxeroides* showed the highest composition of carbohydrates at 60.89 %, followed by protein at 21.66 %, ash at 10.58 %, moisture at 9.36 %, and lipids at 2.72 % [10]. Hasan et al. [28] determined the proximate composition of four commonly found edible aquatic plants such as helencha (*E. fluctuants*), malancha (*A. philoxeroides*), shapla (*N. nouchali*), and kochu (*C. esculenta*) from the Southwestern coastal region of Bangladesh. In this research, the moisture, protein, lipid, and ash content of *A. philoxeroides* was found to be 70.19 ± 1.0 %, 16.50 ± 0.4 %, 1.81 ± 0.1 %, and 14.73 ± 0.5 % for steam, and 60.19 ± 1.0 %, 19.97 ± 0.4 %, 1.12 ± 0.2 %, and 13.93 ± 0.5 % for leaf, respectively. Umate and Marathi [29] analyzed the proximate value of *A. sessilis* and found 82.50 % moisture, 36.16 % carbohydrates, 16.60 % protein, 4.0 % fat, and 13.06 % ash. Othman et al. [30] determined protein, ash, moisture, fat, and carbohydrates of *A. sessilis* red. They found moisture content to be 23.33 ± 0.44 %, ash content to be 10.22 ± 0.08 %, protein content to be 24.11 ± 0.21 %, fat content to be 2.67 ± 0.05 %, and total carbohydrate content to be 39.17 ± 0.46 %. The proximate analysis of *N. nouchali* showed the content of dry matter, crude protein, ash, crude fat, crude fiber, and nitrogen-free extract were 8.4 %, 16.8 %, 18.7 %, 2.8 %, 26.3 %, and 35.4 %, respectively [31]. In another study, the proximate analysis of *N. nouchali* found protein 10.76 %, fat 2.40 %, ash 3.0 %, moisture 9.07 %, and carbohydrate 76.5 % [32]. Aziz et al. [33] reported the moisture protein, lipid, and ash content of *G. tenuistipitata* to be 12.10 ± 0.25 %, 25.55 ± 0.18 %, 0.16 ± 0.30 %, and 10.61 ± 0.69 %, respectively. The reported previous investigations agree with the values obtained in the present study.

3.2. Bioactive compounds in aquatic plants determined by GC-MS analysis

GC-MS analysis identified 42 compounds in *N. alba,* 41 compounds in *N. nouchali*, 40 compounds in *A. philoxeroides,* and 36 compounds in *G. tenuistipitata*. Table S1 shows the retention time, compounds name, peak area (%), molecular weight, and formulas of the bioactive compounds in the studied aquatic plants. The highest number of compounds was identified in *N. alba*, while the lowest number was identified in *G. tenuistipitata.* Major compounds in *N. alba* include 5 alpha-estran-3-one, 2 beta-isopropyl; phthalic acid, di (2-propylpentyl) ester; tetraethyl silicate; agarospiro; and silane, diethoxydimehoxy. The major compounds in *A. philoxeroides* were acetamide, 2-(2-thiophenyl)-n-ethyl-n-2-ethylhexyl at 43.30 %, followed by phytol at 13.33 %, 2 -oxepanol, 5 -(1,1 -dimethylethyl) – at 8.89 %, tetraethyl silicate at 8.09 %, and acetamide, 2-(2-thiophenyl)-n-ethyl-n-nonyl at 2.4 %. Tetraethyl silicate was one of the most dominating and common compounds among the studied aquatic plants and the contents in *A. philoxeroides, N. nouchali, N. alba,* and *G. tenuistipitata* was 8.09 %, 9.79 %, 7.11 %, and 6.30 %, respectively. The highest content of bioactive compound in *N. nouchali* was bis(2-ethylhexyl) phthalate at 9.89 %, followed by tetraethyl silicate at 9.79 %, phthalic acid, di(3-ethylphenyl) at 9.22 %, methyl 2,6-dimethyltridecanoate at 8.49 %, and nonadecane, 2,6,10,14-tetramethyl at 8.41 %. The highest content of individual compound in *N. alba* was 5-alpha-estran - 3 -one, 2-beta -isopropyl and the content was 53.25 %. The other major compounds are phthalic acid, tetraethyl silicate, di(2-propylpentyl) ester, agarospirol and silane, diethoxydimehoxy with the contents of 8.24 %, 7.11 %, 2.09 %, and 2.09 %, respectively. The highest amount of individual compound in *G. tenuistipitata* was 2,4,6-trichlorophenol, diphenylmethylsilyl ether and the content was 57.49 %. The other notable compounds were tetraethyl silicate; acetic acid, chloro-, octadecyl ester; 5-t -butyl-cycloheptene; phthalic acid, di(2-propylpentyl) ester; phytol; 2,4,5,6,7 -pentamethoxyheptanoic acid, methyl ester; and 1,3-

benzenedicarboxylic acid, bis(2-ethylhexyl) ester.

In a previous study, the major compounds in *A. philoxeroides* identified were tamoxifen (26.8 %) and 11,14,17-eicosatrienoic acid [10]. A total of 51 bioactive compounds were identified in the edible paper sheets produced from *A. philoxeroides* and *H. molitrix* where the key compounds included N-hexadecanoic acid, 1,2,3,4-tetrahydro-3-(phenylacetamido) quinolone, tetradecanoic acid, pentadecanoic acid, 14-bromo-, 6-octadecenoic acid, *trans*-2-methyl-4-n-butylthiane, s,s-dioxide, and 9-octadecenoic acid. Other common compounds included carbonic acid, 2-dimethylaminoethyl ethyl ester, hentriacontane, 10,13-dimethyl-pentadecanoic acid, methyl ester, and 1,2,3,4-tetrahydro-3-(phenylacetamido) quinolone [34]. Akbar [35] investigated the GC-MS analysis of *A. philoxeroides* and reported that acetic acid, 2-(2-methoxycarbonylamino-5 nitrophenylthio), and methyl ester comprised 31.90 % of the content, followed by 1,4-benzenediol, and 2,5-bis (1,1-dimethyl) (15.06 %). It was also concluded that the biofunctional properties of the plant might be due to these compounds. Turan et al. [36] reported that high levels of vitamin E in *N. alba,* which varied in containing methyl substituents on 6-chromanol head group and the extent of double bonds in the side chain. Tocopherol, the potent antioxidant, functioned as a radical scavenger. The previous reports and the present findings reveal that *N. alba* and *G. tenuistipitata* could be a potent candidate ingredient in dietary, pharmaceuticals, and cosmeceutical applications. The GC-MS analysis of edible aquatic plants *A. philoxeroides* and *A. bettzickiana* exhibited low and high molecular weight bioactive compounds [37]. Five compounds were commonly found in both plants: hexadecanoic acid, 9, 12-octadecanoic acid, ar-tumerone, bicyclo heptane, phenol, 5-(1,5-dimethyl-4-hexen), and 2,6,6-trimethyl phenol. There was 25 volatile compounds comprising 99.94 % of total volume was identified in *G. lemaneiformis,* with the major constituents being *n*-hexadecanoic acid (38.57 %), oleic acid (25.48 %), arachidonic acid (12.84 %), cholesterol (4.90 %), and tetra-decanoic acid (2.52 %) [38]. Among these, tetra-decanoic acid and *n*-hexadecanoic acid were non-reducing fatty acids, while arachidonic acid, oleic acid, and cholesterol were reducing. The results obtained from the present study agreed with previous findings. Rafiquzzaman et al. [39] reported that freshwater and seaweed extracts were rich in various high and low molecular weight bioactive compounds. These compounds were considered to be responsible for the observed biological activity in the study [34].

3.3. Total phenolic and flavonoid contents

The total flavonoid contents of the four aquatic plants are shown in Table 1. The highest phenolic content was found in *G. tenuistipitata* extract, with a value of 121.05 ± 2.43 mg GE/g of extract, and the lowest in *N. alba* extract, with a value of 61.04 ± 1.64 mg GE/g of extract. The highest flavonoid content was obtained in the *G. tenuistipitata*, with a value of 128.03 ± 0.79 mg QE/g of extract, and the lowest content was found in the *A. philoxeroids*, with a value of 16.26 ± 0.45 mg QE/g of extract. Among the four aquatic plants, the marine-originated plant showed the highest phenolic and flavonoid contents. This might be due to the intense physical, chemical, and biological challenges in the marine environment [40] compared to freshwater habitats. Phenolics and flavonoids are known for their free radical scavenging activity, neutralizing harmful free radicals, and protecting from UV radiation in the marine environment. Additionally, these compounds deter harmful microorganisms and pathogens, thus support defense mechanisms in marine plants. Phenolics and flavonoids possess a broad spectrum of biological and chemical activities and protect against cardiovascular problems, hepatotoxicity, and ulcer syndrome [41].

The methanolic extract of *G. tenuistipitata* showed total phenolic contents of 68.20 ± 0.92 mg GA/g of extract and flavonoid contents of 36.17 ± 2.38 mg QE/g of extract [40]. The phenolics and flavonoids contents in a Bangladeshi seaweed *Gracilariopsis longissimi* was reported to be 88.70 ± 2.19 mg GE/g dry weight and 71.46 ± 2.17 mg QE/g dry weight, respectively [42]. The total phenolic and flavonoid contents of the studied marine plant, *G. tenuistipitata*, were higher compared to the reference values; however, the contents were lower in freshwater aquatic plants. The total phenolic and flavonoid contents in the crude extract of the Bangladeshi freshwater aquatic weed *Ipomoea aquatica* were reported to be 94.93 ± 1.24 mg GE/g and 373.30 ± 1.32 mg QE/g dry crude extract, respectively [7]. The phenolic and flavonoid contents of aquatic plants are influenced by factors such as their sources, seasonal variations, extraction techniques, storage conditions of raw biomass, and the presence of interfering components such as pigments or fatty acids [43]. In the ethanolic extract, the total phenolic content of *A. sessilis* red and *A. sessilis* green were 35.92 ± 0.70 and 3.70 ± 0.21 g GE/100 g fresh weight, respectively [30]. Shaheen et al. [44] investigated the polyphenols and antioxidant capacity of medicinal plants and reported that polyphenols in many plant foods play a protective role against certain diseases like cancer, neurodegenerative, and cardiovascular diseases. Cudalbeanu et al. [45] reported 19.42 mg GE/100 mg extract of phenolic content and 0.97 mg QE/100 mg extract of flavonoid content in *N. alba*. Among the 27 phytochemical compounds identified in *N. alba*, rutin, and *p*-coumaric acid were found as the major components. The total phenolic and flavonoid contents were found to be 18.70 ± 0.3 mg GE/g extract and 21.0 ± 6.8 mg QE/g extract [46]. Hossain et al. [47] reported the total phenolic content in *Gelidium, Hypnea*, and *Ipomea* to be 48.37 mg GA/g, 11.37 mg GA/g, and 9.40 mg GA/g, respectively. The polyphenol content in *Sargassum thunbergii*,

Table 1
Total phenolic and flavonoid contents in aquatic weeds from Bangladesh.

Name of the aquatic weeds	Total phenolic content (mg gallic acid equivalent/g extract)	Total flavonoid content (mg quercetin equivalent/g extract)
A. philoxeroides	73.61 ± 0.96^{b}	16.26 ± 0.45^{c}
N. nouchali	75.42 ± 1.01^{b}	18.92 ± 1.68^{b}
N. alba	61.04 ± 1.64^{c}	22.14 ± 2.79^{b}
G. tenuistipitata	121.05 ± 2.43^{a}	128.03 ± 1.98^{a}

Values with the same letter in each row are not significantly different ($P < 0.05$). Mean ± SD (n = 3).

S. miyabei, and *Undaria pinnatifida* was reported to be 34.99 mg/g, 23.26 mg/g, and 25.34 mg/g, respectively [48].

3.4. Antioxidant test analysis

3.4.1. DPPH radical scavenging ability

In this process, free radical DPPH is scavenged by antioxidant compounds, causing the purple color of DPPH solution to clear. The highest DPPH free radical scavenging activity was found 99.09 % at 500 μg/mL in *N. alba,* which was quite similar to the activity of L-ascorbic acid, at 98.84 %. The DPPH free radical scavenging activity in *G. tenuistipitata, N. nouchali, N. alba,* and *A. philoxeroids* was 43.90 %, 36.56 %, 19.26 %, and 12.04 %, respectively at a concentration of 3.93 μg/mL (Fig. 2). The findings of the present study showed that the antioxidant activity of each crude extract increased dramatically as the concentration of the extract increased ($P < 0.05$).) The DPPH antioxidant activity of methanolic extracts of *P. tetrastromatica* and *G. tenuistipitata* was 77.07 % and 68.54 %, respectively [9]. They also reported that the antioxidant activity of crude seaweed extracts increased dramatically with increasing concentrations of seaweed extracts. The DPPH free radical scavenging activity of the aquatic plant, *Scirpus mucronatus* extract was reported to be 55.62 % at 250 μg/mL [49]. The DPPH radical sequencing activity of the aquatic plants in the present study was higher than in previous reports. The DPPH scavenging activity of methanolic *Hypnea musciformis* extract was reported to be 79.34 % [39]. The methanolic extract of *A. philoxeroides* exhibited dose-dependent inhibition of DPPH radical scavenging capacity of 67.90 % in 60 mg/mL extract [50]. The aqueous extract of *G. tenuistipitata* contained a high amount of antioxidant compounds, which showed protective effects against several reactive oxygen species (ROS) and related DNA damages [46].

3.4.2. Hydrogen peroxide assay

Hydrogen peroxide is a reactive oxygen metabolic byproduct that serves as a key regulator for a number of oxidative stress-related states. The findings showed that the antioxidant activity of crude extracts increased dramatically as the concentration of each extract increased ($P < 0.05$). *A. philoxeroids* showed the highest antioxidant activity (99.15 %) at a 500 μg/mL concentration, which was quite similar to the other extracts and standard L-ascorbic acid, which showed an activity of 99.09 %. At a concentration of 3.93 μg/mL, ascorbic acid showed 72.70 %, whereas *A. philoxeroids* and *N. alba* showed activities of 34.76 % and 19.44 %, respectively (Fig. 3). The aquatic plants showed strong hydrogen peroxide scavenging activity because the hydroxyl groups of phenolic compounds act as hydrogen donors and reacting with radicals to reduce the activities of •O2−, H2O2, •OH, ROO•, 1O2, and other active radicals. Polyphenol compounds also reduce free radical formation by inhibiting the activities of enzymes essential for their production [7]. The H_2O_2 scavenging activity of *P. tetrastromatica* and *G. tenuistipitata* was found to be 67.89 % and 63.28 %, respectively, and the activity was exhibited in a concentration-dependent manner [9].

3.4.3. Ferric reducing power assay

The FRAP assay evaluates antioxidant activity based on the reduction of ferric (Fe^{3+}) to ferrous (Fe^{2+}). At a concentration of 500 μg/mL, the optical density of *N. alba* was 3.77, which was similar to that of ascorbic acid at 3.78. At a concentration of 100 μg/mL, the optical densities were 1.003 for L-ascorbic acid, 0.948 for *G. tenuistipitata*, 0.872 for *N. alba*, 0.80 for *N. nouchali,* and 0.53 for *A. philoxeroides*. Cudalbeanu et al. [45] investigated the antioxidant properties of *N. alba*, which were assessed by ABTS and FRAP assays. The *N. alba* extracts showed strong ferric reducing antioxidant power, with a scavenging activity of 74.6 ± 0.13 % (Fig. 4). The crude methanolic extracts of *P. tetrastromatica* and *G. tenuistipitata* showed reducing power values of 53.24 mg AAE/g and 46.81 mg AAE/g, respectively [9].

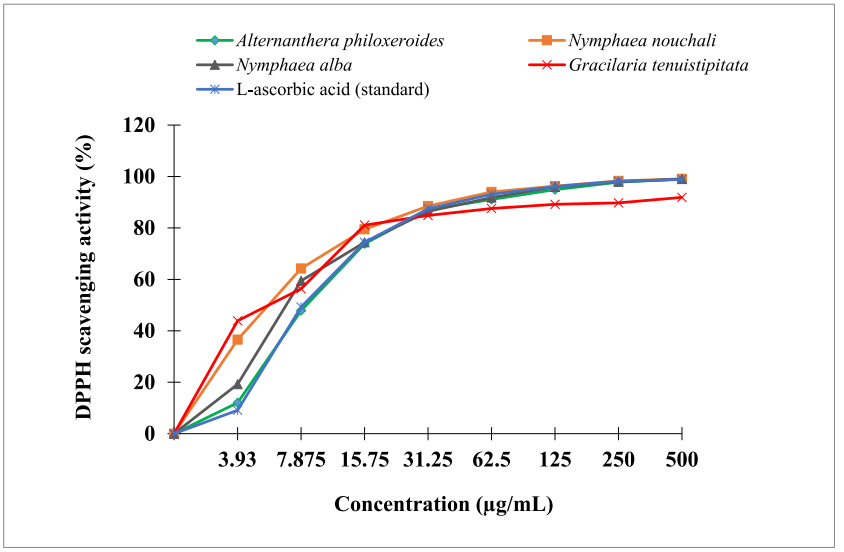

Fig. 2. DPPH (2, 2′-diphenyl-1-picrylhydrazyl) radical scavenging activity of different aquatic weeds from Bangladesh.

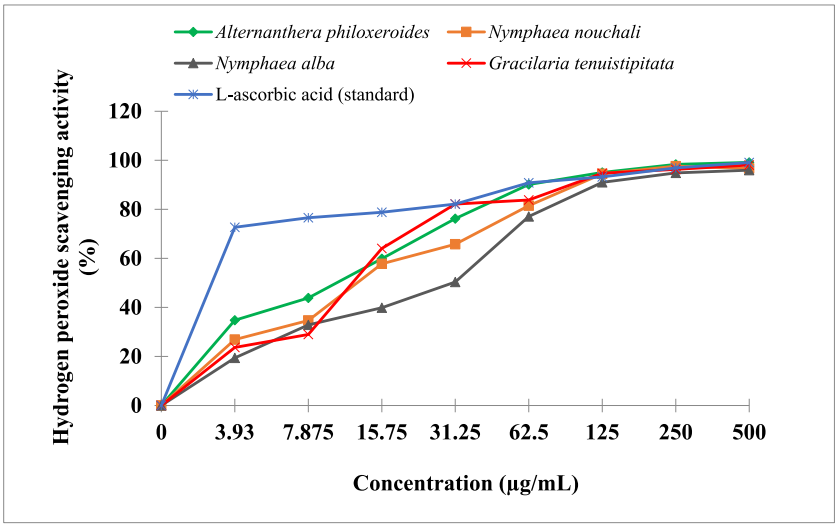

Fig. 3. Hydrogen peroxide scavenging activity of different aquatic weeds from Bangladesh.

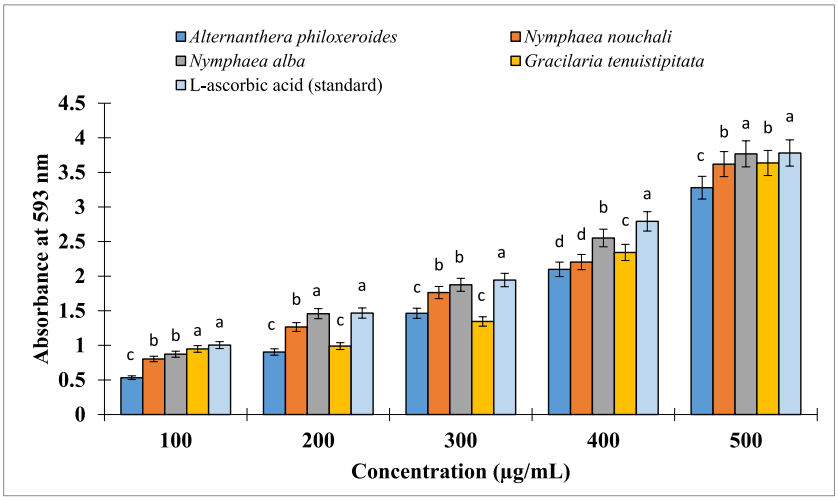

Fig. 4. Ferric reducing power assay of different aquatic weeds from Bangladesh. Different small letters on each column bar for each concentration indicates significant difference ($P < 0.05$).

The FRAP activity of *Scirpus mucronatus* extract showed an enhancement of FRAP scavenging activity in a dose dependent manner [49]. Hossain et al. [47] reported that the FRAP radical scavenging activity of seaweeds was higher compared to freshwater plants. Phenolics and flavonoids in aquatic plants chelate metal ions that facilitate free radical generation, thereby reducing free radical production. Furthermore, polyphenol compounds inhibit oxidation reactions by enhancing the activity of antioxidant enzymes or increasing the

Table 2
Minerals and heavy metal contents (mg/kg) in aquatic weeds from Bangladesh.

Names	*A. philoxeroids*	*N. nouchali*	*N. alba*	*G. tenuistipitata*
Zn	11.65 ± 1.20^{a}	3.59 ± 0.42^{b}	4.03 ± 0.34^{b}	3.60 ± 0.56^{b}
Ca	441.65 ± 4.67^{a}	177.30 ± 3.01^{b}	176.45 ± 2.87^{b}	42.05 ± 2.34^{c}
K	91.17 ± 1.28^{b}	80.15 ± 1.82^{c}	83.40 ± 1.51^{c}	97.81 ± 1.74^{a}
Na	45.43 ± 1.54^{b}	43.24 ± 1.73^{b}	41.16 ± 1.32^{b}	53.37 ± 1.64^{a}
Fe	18.79 ± 0.52^{d}	33.24 ± 1.10^{b}	26.77 ± 0.61^{c}	153.16 ± 2.86^{a}
Ni	0.44 ± 0.02^{c}	1.69 ± 0.63^{b}	1.78 ± 0.23^{b}	3.86 ± 0.56^{a}
Pb	0.67 ± 0.05^{a}	0.24 ± 0.01^{c}	0.22 ± 0.02^{c}	0.46 ± 0.07^{b}
Cu	1.25 ± 0.09^{a}	0.93 ± 0.06^{c}	1.10 ± 0.08^{b}	0.96 ± 0.04^{c}

Values with the same letter in each row are not significantly different ($P < 0.05$). Mean ± SD ($n = 3$).

expression of antioxidant proteins. They also create synergistic antioxidant effects with other substances [51,52].

3.5. Minerals and heavy metal contents in the aquatic plants

The minerals and heavy metal contents of the aquatic plants are shown in Table 2. Among all the minerals, Ca content was the highest in *A. philoxeroids*, followed by *N. nouchali, N. alba*, and *G. tenuistipitata,* with contents of 441.65 ± 4.67 mg/kg, 177.30 ± 3.01 mg/kg, 176.45 ± 2.87 mg/kg, and 42.05 ± 2.34 mg/kg, respectively. The highest K content (97.81 ± 1.74 mg/kg) was in *G. tenuistipitata*, followed by *A. philoxeroids* (91.17 ± 1.28 mg/kg), *N. alba* (83.40 ± 1.51 mg/kg), and *N. nouchali* (80.15 ± 1.82 mg/kg). The highest Na content (80.13 ± 2.45 mg/kg) was in *G. tenuistipitata*, whereas it was the lowest in *N. alba* (41.16 ± 1.32 mg/kg). *G. tenuistipitata* contained a significantly ($P < 0.05$) higher amount of Fe (153.16 ± 2.86 mg/kg) compared to the other studied aquatic plants. The Zn content was 11.65 ± 1.20 mg/kg in *A. philoxeroids*, followed by *N. alba* (4.03 ± 0.34 mg/kg), *G. tenuistipitata* (3.60 ± 0.56 mg/kg), and *N. nouchali* (3.59 ± 0.42 mg/kg). The highest Ni content was in *G. tenuistipitata* (3.86 ± 0.56 mg/kg) and the lowest in *A. philoxeroids* (0.44 ± 0.02 mg/kg). *A. philoxeroids* contained the highest amount of Pb (0.67 ± 0.05 mg/kg), followed by *G. tenuistipitata* (0.46 ± 0.07 mg/kg), *N. nouchali* (0.24 ± 0.01), and *N. alba* (0.22 ± 0.02 mg/kg). The mineral contents of the studied aquatic plants are of special dietary importance because the Na/K values lie between 0.49 and 0.54 (Table 2), which aligns with the recommended dietary choices for individuals with high blood pressure [53]. Additionally, aquatic plants with low Na/K ratios are considered health beneficial for replacing sodium chloride [54]. Minerals are essential biofactors for the human body, aiding in the formation of body tissues and catalyzing various metabolic reactions. The mineral contents in aquatic plants depend on the species, habitat, wave, environment, seasons, physiological parameters, processing technique, etc. The aquatic plants in this study contain suitable mineral concentrations, suggesting they could be a significant source of dietary mineral supplements for human and animal nutrition.

The calcium, potassium, and sodium contents in *A. sessilis* red (ASR) and *A. sessilis* green (ASG) were reported 236.36 ± 4.47 mg/100 g and 7.02 ± 0.01 mg/100 g, 1094.84 ± 16.87 mg/100 g and 199.02 ± 0.18 mg/100 g, and 68.14 ± 8.00 mg/100 g, and 0.67 ± 0.07 mg/100 g, respectively [30]. The zinc content was 6.67 ± 0.35 mg/100 g and 0.50 ± 0.00 mg/100 g, and copper was 1.13 ± 0.12 mg/100 g and 0.85 ± 0.01 mg/100 g in ASR and ASG, respectively. The minerals composition of *A. sessilis* depended on the fertility of the soil because the minerals are absorbed from the soil. Additionally, the genetic factors of the plants and the extent of fertilizers also determined the mineral content [55]. The mineral composition of *A. Sessilis* was found correlating the Na, K, Ca, P, Mg, Mn, Cu, Zn, Fe, and Cr contents with Recommended Daily Allowance (RDA) values [56]. Among them, Fe and Mn were present in higher proportions. Usually, plants are considered a rich source of sufficient macro and microelements. The findings of the present study revealed that the regular intake of these aquatic plants could combat malnutrition and other health disorders.

Dias et al. [57] reported that the petal extract of *N. nouchali* was rich in minerals for human nutrition and showed immense potential in amelioration various diseases due to its antioxidant compounds. The major minerals found in this study were Ca, P, Fe, K, and Na. The mineral content of *N. nouchalli* was found Na at 1.19, K at 2.23, Ca at 0.52, P at 0.32, and Ca/P ratio of 1.63 [31]. The major minerals potential of *N. alba* was P (1.56 %–4.63 %) and Na (1.97 %–4.21 %), along with Zn, Ca, Cu, and Fe [57]. In *G. tenuistipitata*, the minerals contents were 132.75 ± 3.4, 3.90 ± 1.2, 80.13 ± 2.45, 3.99 ± 1.2, 596.90 ± 10.4 mg/100g dry weight for Ca, Mg, Fe, Cu, and P, respectively [33]. The heavy metals Pb, As, Cd, and Cr contents in *G. tenuistipitata* were found to be 0.031, 0.01, 0.02, and 0.06 mg/kg dry matter basis, respectively. The heavy metal content in aquatic plants varied depending on the species and the environment in which the plants are grown.

3.6. Amino acid composition of aquatic plants

The amino acids content and chromatogram of the aquatic plants are shown in Table 3 and Fig. S1. This research identified total 13

Table 3
Amino acids composition and contents (mg/100 g) of different aquatic weeds from Bangladesh.

Amino acids	*A. philoxeroids*	*N. nouchali*	*N. alba*	*G. tenuistipitata*
Thr	Nd	Nd	Nd	13.69 ± 1.23
Met	174.70 ± 5.33^{d}	2853.04 ± 3.23^{a}	890.52 ± 5.26^{b}	292.04 ± 3.44^{c}
Leu	5254.73 ± 16.65^{a}	Nd	4704.65 ± 14.33^{b}	4603.01 ± 12.54^{c}
Phe	1101.23 ± 13.32^{d}	4243.08 ± 13.50^{a}	1419.28 ± 17.10^{c}	1647.25 ± 2.45^{b}
Lys	1533.48 ± 12.43^{c}	4769.94 ± 5.52^{a}	1656.98 ± 8.41^{b}	1457.57 ± 3.44^{d}
His	94.93 ± 2.43^{d}	543.31 ± 3.65^{c}	2638.10 ± 8.34^{a}	1917.74 ± 8.43^{b}
Glu	627.31 ± 6.54^{a}	Nd	Nd	200.87 ± 4.24^{b}
Gly	3293.13 ± 15.54^{b}	2917.81 ± 18.37^{c}	1591.56 ± 4.32^{d}	3843.88 ± 16.34^{a}
Ala	10088.90 ± 26.54^{a}	Nd	Nd	5548.26 ± 15.44^{b}
Arg	8.42 ± 0.87^{b}	Nd	9.29 ± 1.09^{a}	Nd
Ile	Nd	4599.33 ± 17.43^{a}	9.49 ± 1.34^{b}	Nd
Tyr	Nd	10.64 ± 0.95^{a}	Nd	Nd
Cys	Nd	Nd	4823.56 ± 13.43^{a}	Nd
Total	**22176.86**	**19937.15**	**17743.43**	**19524.30**

Values are presented as means ± standard deviation of triplicates. Different superscript letters on each row indicates significant differences ($P < 0.05$). Nd = not detected.

both essential and non-essential amino acids including methionine, phenylalanine, lysine, histidine, and glycine. Among the aquatic plants studied, *A. philoxeroides* contained the highest total amount of amino acids (22,176.86 mg/100 g), with alanine being the major amino acid (10,088.90 ± 26.54 mg/100 g). The lowest total amino acids content was found in the *N. alba* (17,743.43 mg/100 g). The individual highest amino acids were alanine, lysine, cysteine, and alanine in *A. philoxeroids, N. nouchali, N. alba,* and *G. tenuistipitata* and the content was 10088.90 ± 26.54 mg/100 g, 4769.94 ± 5.52 mg/100 g, 4823.56 ± 13.43 mg/100 g, and 5548.26 ± 15.44 mg/100 g, respectively. The amino acids contents in the aquatic plants varied substantially and significantly ($P \leq 0.05$) among the samples. This might be due to the samples originating from different geographic locations, habitats, and species. The amino acids content in aquatic weeds depends on species, habitats, growth stage, geographic location, soil and water chemistry, genetic and stress factors. The amino acids found in the studied plants were comprised of essential and non-essential amino acids, making them a suitable source of quality protein for the consumers. The ratio of essential and non-essential amino acids was reported more than 1, and was suggested suitable of the seaweeds for human consumption as a balance source of amino acids [58].

The amino acids such as lysine, histidine, arginine, aspartic acid, serine, threonine, proline, methionine, tyrosine, glutamic acid, glycine, alanine, valine, isoleucine, leucine, and phenylalanine were found in *A. philoxeroides* [59]. Anand et al. [32] investigated the amino acid composition of boiled *N. nouchali* tuber and found that eight of the nine essential amino acids required in the diet (isoleucine, histidine, leucine, methionine, lysine, phenylalanine, threonine, and valine) were present. Non-essential amino acids were also detected in *N. nouchali.* Wen et al. [38] identified valine, lysine, isoleucine, threonine, tyrosine, and phenylalanine in *G. lemaneiformis*. The total amino acid content in *G. lemaneiformis* was 2.27 % on a wet weight basis, with essential amino acids making up 41.0 % of this content. *G. lemaneiformis* contained high amounts of aspartic acid, glutamic acid, alanine, arginine, lysine, and serine. There were found a total 15 amino acids in *G. tenuistipitata*, including 11 essential amino acids, with lysine being the most abundant and histidine the least [33]. In this study, four non-essential amino acids were also found, with glutamate being the most abundant. Torres et al. [60] stated that *Gracilaria* species contained amino acids such as aspartic acid, glutamic acid, alanine, proline, glutamine, glycine, threonine, and serine which contributed to the typical flavor of algae.

4. Conclusion

Among the four aquatic plants studied, *N. alba* and *A. philoxeroides* exhibited superior nutritional properties compared to the others. These aquatic plants contained substantial amounts of essential nutrients, including proteins (ranging from 23.54 % to 26.96 %) and polysaccharides (ranging from 37.02 % to 46.12 %), making them suitable as dietary supplement. They were rich in various macro- and micro-elements that play critical roles in human health. Additionally, *N. alba* and *A. philoxeroides* had higher levels of bioactive compounds compared to *N. nouchali* and *G. tenuistipitata*. The marine plant *G. tenuistipitata* extract showed notably high contents of phenolics and flavonoids, with values of 121.05 ± 2.43 mg GE/g extract and 128.03 ± 0.79 mg QE/g extract, respectively, which are associated with strong free radical scavenging activity. These plants are valuable sources of bioactive compounds, carbohydrate, fats, and proteins, and could be recommended for malnourished communities. Furthermore, all the aquatic plants had low Na/K ratios (0.49–0.54), making them suitable alternatives to sodium chloride and beneficial for meeting dietary requirements. Bioactive compounds from these edible aquatic plants can scavenge free radicals and potentially prevent various diseases. This paper compares marine and freshwater aquatic plants in terms of their bioactive compounds and biofunctional roles. Further studies are recommended to evaluate the *in vivo* effects of these aquatic plants and to identify specific bioactive compounds for targeted applications through molecular docking. In tropical countries, where freshwater aquatic plants are more accessible than seaweeds, it is recommended that people incorporate these freshwater plants into their diets to meet their nutritional needs.

Ethical approval

Not applicable.

Data availability statement

Data included in article/supplementary material/referenced in article.

CRediT authorship contribution statement

Sharmin Suraiya: Supervision, Software, Project administration, Methodology, Conceptualization. **Sadia Jannat Ria:** Writing – original draft, Resources, Investigation, Formal analysis. **Mst. Umme Tanzim Riya:** Software, Resources, Methodology, Data curation. **Farzana Yasmin Ritu:** Writing – review & editing, Validation, Formal analysis. **Ayesha Akhter Sumona:** Visualization, Resources, Investigation, Data curation. **Ashika Banu Rodela:** Validation, Software, Resources, Formal analysis. **Lovely Akter:** Validation, Methodology, Conceptualization. **Md. Salah Uddin:** Validation, Resources, Methodology, Conceptualization. **Md. Nazmul Hasan:** Writing – review & editing, Supervision, Project administration, Methodology.

Declaration of competing interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35538.

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