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Green Approach to Valorize Abundant Aquatic Weeds for Nutrient-Rich Edible Paper Sheets Production in BANGLADESH

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Abstract: Three edible aquatic weeds, helencha (*Enhydra fluctuans*), malancha (*Alternanthera philoxeroides*), and kalmi (*Ipomoea aquatica*), were used to produce edible paper sheets. The composition of the raw and paper sheet samples was analyzed, including proximate composition, amino acid content, minerals and heavy metal contents, and bioactive compounds. The dried raw and paper sheets showed similar proximate composition, with carbohydrates being the highest (50.38-64.63%), followed by crude protein (15.25-19.13%), ash (9.30-15.88%), and lipid (1.549-3.43%). The weeds and paper sheets were rich in essential minerals like Na, Ca, Zn, and Fe. Acceptable levels of heavy metals, including Ni, Pb, and Cu, were found. The paper sheets contained seven essential and eight non-essential amino acids. *A. philoxeroides* sheets showed the highest amino acid content (16146.81mg/100g), while *I. aquatica* showed the lowest (13118.67 mg/100g). The aquatic weeds paper sheets were rich in containing bioactive compounds and the number in *E. fluctuans*, *A. philoxeroides*, and *I. aquatica* paper sheets were 31, 33, and 40, respectively. There were no significant changes in the nutritional content of aquatic weeds in paper sheet form compared to the raw weeds which suggests promising prospects for the production and consumption as a source of nutrition and bioactive compounds.

Keywords: aquatic weeds; edible paper; proximate composition; biofunctional compounds; minerals; amino acids

1. Introduction

Bangladesh possesses an enormous area of wetland that supports naturally occurring aquatic and semi-aquatic plants, providing a cost-effective source of food for fish, wildlife, and people [1]. These aquatic weeds are renewable biomass and hold immense medicinal value. They contain a variety of nutritional compounds, including protein, amino acids, lipids, polysaccharides, minerals, vitamins, polyphenols, and other bioactive compounds. Leafy vegetables, such as aquatic weeds, are known to be low in fat and calories while providing various vitamins, minerals, and fiber [2]. They are also delicious and have export potential. Dried seaweed paper, particularly Nori, made from the seaweed species *Porphyra sp*., is popular in many Asian countries, including Japan, Korea, and China. Nori is highly valued in local and international markets due to its high nutrition, unique texture, compactness, and pleasant taste. It is also possible to produce artificial nori using other seaweeds or non-seaweed materials [3]. The production of edible paper sheets from aquatic weeds could be a feasible approach to attract consumers and promote the consumption of aquatic weeds throughout the country across all classes of the population. Further research can focus on investigating the nutritional properties of aquatic weeds and the paper sheets, as well as exploring the production methods for edible paper sheets. In Bangladesh, among the different available aquatic weeds, helencha (*Enhydra fluctuans*), malancha (*Alternanthera philoxeroides*), and kalmi (*Ipomea aquatica*) are consumed as important vegetables.

A. philoxeroides, commonly known as alligator weed or Malanchashak in Bangladesh, is not only used as a food but also as a medicine. It is consumed as leafy vegetables and has traditional uses in treating various conditions such as hazy vision, night blindness, malaria, postnatal complaints, diarrhoea, dysentery, and puerperal fever [4]. *A. philoxeroides* is also rich in iron content and can be used in salads [5]. *E. fluctuans* is an aquatic plant naturally found in marshy areas in tropical countries like India, Bangladesh, Malaysia, China, Southeast Asia, and tropical Africa [6]. Traditionally, the leaves and stems of *E. fluctuans* have been used to treat various diseases including gastric ulcers, diabetes, skin diseases, smallpox, kidney stones, and inflammation [7]. The leaves also possess antimicrobial properties [8,9]. Phytochemical analysis of *E. fluctuans* has shown the presence of flavonoids, triterpenes, carbohydrates, reducing sugars, saponins, phenols, diterpenes, proteins, and tannins [10]. *I. aquatica*, commonly is a highly nutritious plant that offers benefits to the body, skin, and brain. It naturally grows in tropical and subtropical countries and is widely cultivated in China, Indonesia, Thailand, Vietnam, Myanmar, the Philippines, Bangladesh, and India [11]. In Southeast Asia, *I. aquatica* is used to treat conditions like piles, nosebleeds, and high blood pressure. In Ayurveda, leaf extracts are used to treat jaundice and nervous weakness [12]. In Sri Lanka, water spinach is known for its insulin-like properties, and extracts of I. aquatica have shown blood sugar-lowering effects [13]. Bioactive compounds and antimicrobial substances have also been detected in *I. aquatica* [14,15]. This plant exhibits anticancer, antioxidant, anti-inflammatory, diuretic, antitumor, chemopreventive, antimicrobial activities, and is used in vaccine formulations [15].

The extent of micronutrient deficiency in many countries exceeds that of energy malnutrition. Aquatic weeds are a valuable source of proteins, fats, carbohydrates, vitamins, minerals, and fiber, which are essential for maintaining good health and preventing diseases such as cancer, coronary heart attacks, and diabetes. Despite the availability of numerous aquatic weeds in Bangladesh, the population of this country is not accustomed to consuming aquatic plants. Furthermore, there is a scarcity of reports that highlight the various biofunctional and nutritional properties of aquatic weeds. Therefore, creating edible paper sheets from the aquatic weeds found in Bangladesh can provide an easier way for people, including children, to consume them. The objectives of the present study were to assess the feasibility of preparing edible paper sheets from three available aquatic weeds in Bangladesh, namely Helencha (*E. fluctuans*), Malancha (*A. philoxeroides*), and Kalmi (*I. aquatica*). The study also aimed to evaluate the nutritional composition (proximate composition, amino acids, minerals, heavy metals) and perform GC-MS analysis of both the aquatic weeds and the edible paper sheets made from them.

2. Materials and Methods

2.1. Collection of samples and preparation of edible paper sheet

Helencha (*Enhydra fluctunas*), malancha (*Alternanthera philoxeroides*) and kalmi (*Ipomoea aquatica*) were collected from different location of Jashore district in Bangladesh. The aquatic weeds were properly rinsed with clean water. Then, the weeds were ground using a blender (WBL-13EC25N, Walton, Bangladesh). A mixture of aquatic weeds and water was added to the blender in a ratio of 2:1 (aquatic weeds to water) and mashed to create a paste. Subsequently, the aquatic weed paste was boiled using a gas oven. The mashed paste was then poured into a non-sticky stainless steel pan. While boiling, 0.5% testing salt and 1% corn flour were added according to the quantities listed in Table 1, to enhance the taste and make the paste sticky like glue. The paste was boiled at approximately 100ºC for 10 minutes. The mixture was then poured into a rectangular-shaped sieve covered with cheesecloth. Afterward, it was left to sit for 10 minutes to allow the excess water to seep through and placed in a hot air oven for drying at 65ºC for 12 h. Once dried, the paper was carefully pulled away from the cheesecloth. The raw aquatic weeds were also dried and ground to compare the variations in nutritional properties in the edible paper sheets

2.2. Analysis of proximate composition

The proximate composition such as moisture, protein, lipid, and ash content was analyzed following the methods of AOAC (2005) [16]. The carbohydrate content of raw and aquatic weeds paper sheets was determined indirectly by subtracting the moisture, protein, and ash content from a hundred [17].

2.3. Determination of amino acid composition

An amino acid analyzer (LA 8080, Hitachi, Japan) equipped with a high-performance cation-exchange column and operated at a column temperature of 57 °C was used to determine the amino acid content, following the method described by Islam et al. (2020) with some modifications [18]. For pre-treatment, 1 g of the sample was added to 25 mL of 6 M HCl in a glass tube. The tube was then placed in a sand bath and heated to 110 °C for 24 hours. The resulting solid was dried using an HCl evaporation process, mixed with 6 mL of distilled water, and filtered using a 0.45 μm syringe filter.

2.4. Determination of mineral and heavy metals content

The minerals and heavy metals contents of the samples were determined by using ICP-OES optima 2000 DV (Perkin Elmer, MA, USA) following the method of Islam et al. (2020) with minor alternations [18]. Initially, 1.0 g of each dried sample was placed in a muffle furnace at 600°C for 6 h to facilitate ash formation. After the ash was formed, 4 mL of HNO_3 and 1 mL of H_2O_2 were added to the ash powder, and distilled water was added to create a 60 mL solution. The solution was 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 bring the solution to a total volume of 50 mL, which was subsequently filtered through a 125 mm filter paper. Filtering was done for twice and after that the solution was ready to be tested for minerals and heavy metals analysis. The operational conditions of the instrument were maintained as described by [19].

2.5. Gas chromatography mass spectrometry (GC-MS) analysis

The extract for GC-MS analysis was prepared as follows: 10.0 g of pulverized aquatic plant powder was taken in a 100 mL glass beaker. Subsequently, 50 mL of ethanol was added to the sample, and the mixture was stirred using a magnetic stirrer for 5 hours at a rotation speed of 400 rpm. To prevent solvent evaporation, the beaker was covered with aluminum foil. The sample was then filtered using double filter paper. Finally, the filtered sample was passed through a syringe filter (0.45 μm) and stored in a glass vial. For the analysis, the Clarus 690 gas chromatograph was employed. This instrument utilized a column (Elite-35, 30 m × 0.25 mm, 0.25 μm film thickness; PerkinElmer, USA) and the Clarus@ SQ 8C mass spectrometer (PerkinElmer, USA). Initially, a 1 μL sample solution was injected, and pure helium (99.99%) was used as the carrier gas with a flow rate of 1 mL/min for a runtime of 40 minutes. The analysis was performed in electron ionization (EI) mode at high energy (70 eV). The inlet temperature was maintained at a constant 280°C, while the oven temperature was programmed to start at 60°C for 0 minutes and then increase at a rate of 5°C/min until reaching 240°C. The compounds present in the sample were identified using the database of the National Institute of Standards and Technology (NIST). *2.6. Statistical analyses of experimental data*

Microsoft Excel was utilized to generate the graph and table for the study. All experiments were performed in triplicate, and the results are reported as the mean ± standard deviation (SD). Data analysis was conducted using IBM SPSS software version 20, and a significance level of 5% ($p < 0.05$) was employed. The difference between the means was done by Duncan's Multiple range test.

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3. Results and Discussion

3.1. Proximate composition analysis

The proximate composition of the studied edible paper sheets and raw aquatic weeds is presented in Figure 1.

3.1.1. Moisture content of edible aquatic weed paper sheets

In this study, the moisture content of different paper sheets was evaluated, and it was found that *E. fluctuans* exhibited the highest moisture content (10.62%), while *I. aquatica* had the lowest moisture content (8.61%). Therefore, the sequence of moisture content among the paper sheets was as follows: *E. fluctuans* (10.62%) > *A. philoxeroides* (9.36%) > *I. aquatica* (8.61%). When considering the raw samples, the sequence of moisture content was *I. aquatica* (11.06%) > *E. fluctuans* (10.51%) > *A. philoxeroides* (9.82%). Thus, it can be observed that moisture content was higher in the raw samples compared to the processed paper sheets due to the thermal treatment. A previous study by Hossain et al. (2021) reported moisture content values of 10.30% for *I. aquatica* and 10.06% for *E. fluctuans [20]*. Hasan et al. (2016) discovered that the moisture content of *E. fluctuans* varied from 9.9% to 13.4% depending on seasonal variations [21]. According to Pulipati et al. (2015), the moisture content of *A. philoxeroides* was 10% [22]. These findings align closely with the results obtained in the present study, suggesting a similar maximum moisture content across the different plant species.

3.1.2. Ash content of edible aquatic weed paper sheets

Ash content in foods determine the mineral content consists of inorganic minerals such as calcium, potassium, phosphorus, sodium, magnesium, and trace elements [23]. The ash content of paper sheets made from aquatic weeds with the following sequence: *A. philoxeroides* (10.58%) > *E. fluctuans* (10.16%) > *I. aquatica* (9.3%). In terms of the raw aquatic weeds, the sequence of ash content was *A. philoxeroides* (15.88%) > *E. fluctuans* (13.28%) > *I. aquatica* (11.98%). These results indicate a decrease in ash content in the paper sheets compared to the raw samples due to the heat processing. Among the different plant species, *A. philoxeroides* exhibited the highest ash content, while *I. aquatica* had the lowest, both in the raw and paper forms. In a study conducted by Hasan et al. (2016) on the proximate composition of commonly found edible aquatic plants in Bangladesh, including *E. fluctuans* and *A. philoxeroides*, the ash content was determined to be 17.28% in the stem and 16.13% in the leaf for *E. fluctuans*, and 14.73% in the stem and 13.93% in the leaf for *A. philoxeroides [21]*. Another study by Hossain et al. (2021) focused on the biochemical analysis of *I. aquatica*, revealing an ash content of 13% [20]. Umar et al. (2007) analyzed the nutritional composition of water spinach (*I. aquatica*) and found an ash content of 10.83±0.83% [24]. Datta et al. (2019) reported an ash content of 16.37% for *E. fluctuans* and 15.15% for *I. aquatic [25]*. These findings show both similarities and differences when compared to the present study. Overall, the present study demonstrates close similarities and some variations in ash content compared to other relevant studies in the field.

3.1.3. Crude protein content of edible aquatic weed paper sheets

The study revealed the respective crude protein contents of three raw aquatic weeds: *A. philoxeroides* (21.66%), *E. fluctuans* (19.13%), and *I. aquatica* (18.12%). Similarly, in the paper sheets, the protein contents were observed in the following order: *A. philoxeroides* (17.12%), *E. fluctuans* (16.23%), and *I. aquatica* (15.25%). The protein content in the paper sheets decreased compared to the raw aquatic weeds due to the cooking and heat processing involved. Hasan et al. (2016) reported the protein content of two aquatic weeds in Bangladesh, with *E. fluctuans* showing values of 19.64% for steam, 20.58% for leaf, and *A. philoxeroides* exhibiting 19.97% for leaf and 16.5% for stem [21]. Suraiya et al. (2022) found the protein content in *A. philoxeroides* to be 26.96% [26]. Hossain et al. (2021) conducted a study on the biochemical analyses of *I. aquatica*, revealing that young shoots contained 16.8% protein [20]. Furthermore, Satter et al. (2016) explored the nutritional quality and safety aspects of wild vegetables consumed in Bangladesh, reporting crude protein contents of 16.69% for Helencha

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(*E. fluctuans*) and 21.45% for Kalmishak (*I. aquatica*) [27]. Protein is a vital nutrient that plays numerous essential roles in the human body including tissue growth and repair, enzyme and hormone production, immune function, transport and storage in animal body [28–30].

3.1.4. Lipid content of edible aquatic weed paper sheets

The results of the present study indicate the lipid content of raw aquatic weeds as follows: *E. fluctuans* (2.98%), *A. philoxeroides* (2.72%), and *I. aquatica* (3.43%). Similarly, the lipid content in the edible paper sheets was observed in the following order: *E. fluctuans* (1.95%), *A. philoxeroides* (1.59%), and *I. aquatica* (2.11%). Among the raw samples, *I. aquatica* exhibited the highest lipid content (3.43%), while *A. philoxeroides* had the lowest (2.72%). In contrast, among the paper sheet samples, *A. philoxeroides* had the lowest lipid content (1.59%), while *I. aquatica* showed a slightly higher content (2.11%). Therefore, the study suggests that there is a slight difference in lipid content between raw and paper samples. It is worth noting that aquatic weeds generally contain a low amount of lipids in their proximate composition. Hasan et al. (2016) investigated four edible aquatic weeds in Bangladesh and reported lipid contents in *E. fluctuans* and *A. philoxeroides* leaves ranging from 1.12% to 2.96%, and in stems ranging from 1.11% to 2.02% [21]. Satter et al. (2016) analyzed various wild vegetables, including Dhekishak, Helencha, Kalmishak, Patshak, and Shapla stems, which contained crude fat contents of 2.27%, 2.66%, 3.34%, 4.76%, and 1.45%, respectively [27]. Dutta et al. (2015) reported lipid contents of *I. aquatica* and *E. fluctuans* as 2.19% and 1.10%, respectively [31]. These findings align closely with the combination of results from previous studies and the present study. Lipids of aquatic weeds are importance due to various health benefits such as provide essential fatty acids which are known for their positive effects on cardiovascular health, brain function, inflammation regulation etc. [21,31].

3.1.5. Carbohydrate content of edible aquatic weed paper sheets

The present study revealed the carbohydrate content of edible paper sheets, with the highest content found in *I. aquatica* paper (64.63%), followed by *A. philoxeroides* paper (60.89%), and the lowest content in *E. fluctuans* paper (60.04%). In comparison, the carbohydrate content in raw aquatic weeds followed the sequence: *I. aquatica* raw (55.41%) > *E. fluctuans* raw (54.09%) > *A. philoxeroides* raw (50.38%). These findings indicate that the carbohydrate content in paper samples was higher than in raw samples, possibly due to the cooking and heat processing involved. In a study conducted by Satter et al. (2016) on wild edible plants, the carbohydrate content in Dhekishak, Helencha, Kalmishak, Patshak, and Shapla stems was reported as 57.69%, 61.61%, 52.78%, 60.21%, and 76.34%, respectively [27]. Umar et al. (2007) analyzed the nutritional composition of water spinach (Ipomoea analyzed the nutritional composition of water spinach (*I. aquatica*) leaves and found an available carbohydrate content of 54.20±0.68% [24]. Igwenyi et al. (2011) conducted a proximate analysis of *I. aquatica* and reported a carbohydrate content of 42.18% [32]. Dutta et al. (2015) found the carbohydrate content of *I. aquatica* (10.51%) and *E. fluctuans* (9.64%) based on weight moisture process [31]. Sree et al. (2018) studied *Alternanthera sessilis* and reported a carbohydrate content of 74.56% [33]. These previous studies demonstrate both similarities and variations in carbohydrate content compared to the present study's findings.

3.2. Minerals and heavy metal content of edible aquatic weed paper sheets

In this study, the mineral content of various aquatic weed samples was analyzed. Among the paper samples, *A. philoxeroides* paper had significantly higher calcium (Ca) content (489.65±5.55 mg/100g) compared to *E. fluctuans* (433.3±4.94 mg/100g) and *I. aquatica* (126.8±2.17 mg/100g). Similarly, among the raw aquatic weeds, *A. philoxeroides* had a higher Ca content (442.85±4.53 mg/100g) compared to *E. fluctuans* (397.1±2.49 mg/100g) and *I. aquatica* (281.9±4.09 mg/100g) (Table 2). Regarding sodium (Na) content, in paper samples, *E. fluctuans* had significantly higher Na (30.4±0.36 mg/100g) compared to *A. philoxeroides* (29.3±0.36 mg/100g) and *I. aquatica* (29.2±0.24 mg/100g). In raw aquatic samples, *E. fluctuans* (29.1±0.52 mg/100g) had significantly higher Na

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compared to *A. philoxeroides* (27.7±0.38 mg/100g) and *I. aquatica* (28.9±0.55 mg/100g). When examining zinc (Zn) content, among the edible paper sheets, *A. philoxeroides* paper (12.55±0.92 mg/100g) had significantly higher Zn compared to *I. aquatica* paper (4.55±0.13 mg/100g) and *E. fluctuans* paper (5.92±0.21 mg/100g). Among the raw aquatic weeds, *A. philoxeroides* (16.29±0.81 mg/100g) had the highest Zn content compared to *E. fluctuans* (6.01±0.27 mg/100g) and *I. aquatica* (6.11±0.32 mg/100g). Thus, the study demonstrated that the paper samples contained higher levels of minerals (Ca, Na, and Zn) compared to the raw samples (Table 3).

Furthermore, in the heavy metal test, copper (Cu), nickel (Ni), lead (Pb), and arsenic (As) were detected. Among the samples, *I. aquatica* raw had the highest Ni content (1.39±0.26 mg/100g) compared to *A. philoxeroides* raw (1.15±0.37 mg/100g) and *E. fluctuans* raw (1.24±0.12 mg/100g). Among the paper sheets, *A. philoxeroides* paper (1.22±0.32 mg/100g) had the highest Ni content compared to *E. fluctuans* paper (0.34±0.08 mg/100g) and *I. aquatica* paper (0.77±0.17 mg/100g). However, the raw weed samples contained higher Ni levels than the paper sheets. Regarding other metals, *A. philoxeroides* raw had the highest Cu content (4.39±0.33 mg/kg), while the lowest was found in *I. aquatica* paper (2.16±0.39 mg/100g). Palladium (Pd) was detected in *E. fluctuans* paper (0.04±0.002 mg/100g), *I. aquatica* raw (0.14±0.03 mg/ 100 g).

In a previous study by Othman et al. (2016), the mineral composition of *A. sessilis* red (ASR) and A. *sessilis green* (ASG) was examined. ASR was found to contain 236.36 ± 4.47 mg/100g of calcium, 7.02 ± 0.01 mg/100g of potassium, 68.14 ± 8.00 mg/100g of sodium, and 6.67 ± 0.35 mg/100g of zinc. On the other hand, ASG had 7.02 ± 0.01 mg/100g of calcium, 199.02 ± 0.18 mg/100g of potassium, 0.67 ± 0.07 mg/100g of sodium, and 0.50 ± 0.00 mg/100g of zinc. Copper content was 1.13 ± 0.12 mg/100g in ASR and 0.85 ± 0.01 mg/100g in ASG [34]. The mineral composition of *A. sessilis* is influenced by soil fertility, as minerals are absorbed from the soil, as well as genetic factors and fertilizer usage, as mentioned by Leterme et al. (2006) [35]. The mineral content per 100g of *A. sessilis* included 285.78 ± 5.95 mg of sodium, 510.35 ± 9.69 mg of calcium, phosphorus, magnesium, manganese, and copper at 1.6 ± 0.02 mg, and zinc at 8.9 ± 2.25 mg [33]. The plant was particularly rich in iron and manganese. Ndamitso et al. (2015) studied on wild *I. aquatica* and found that the leaves of *I. aquatica* exhibited higher levels of magnesium (2300 ± 0.023 mg/kg), phosphorus (225 ± 0.003 mg/kg), iron (155 ± 0.020 mg/kg), zinc (25 ± 0.000 mg/kg), manganese (8 ± 0.002 mg/kg), and copper (36 ± 0.002 mg/kg) compared to the stems [36]. On the other hand, the stems had higher levels of sodium (1000 ± 0.020 mg/kg), potassium (5562.5 ± 0.003 mg/kg), and calcium (65.00 ± 0.01 mg/100g). These minerals play important roles in various biological processes. Calcium, phosphorus, and magnesium are involved in bone growth and turnover, while iron is essential for the formation of hemoglobin [25]. These findings suggest that regular consumption of aquatic weeds paper sheets can help combat malnutrition and other health disorders due to its abundance of macro and microelements.

3.3. Amino acid composition in aquatic weeds paper sheets

The amino acids found in the three raw aquatic weeds and edible aquatic weed paper sheets are presented in Table 3 and Figure 2. Both essential and non-essential amino acids were detected including phenylalanine, histidine, glycine, and glutamic acid. Among all the paper sheets, *A. philoxeroides* paper contained the highest amount of amino acids (16146.81 mg/100g). Similarly, among all the raw aquatic weeds, *A. philoxeroides* raw had the highest amount of amino acids (15967.94 mg/100g). *E. fluctuans* paper and *I. aquatica* paper showed the amounts of amino acids (14018.64 mg/100g) and (14079.43 mg/100g), respectively. On the other hand, *I. aquatica* raw had the lowest amount of amino acids (13118.67 mg/100g). The predominant amino acid in *A. philoxeroides* raw and paper was Alanine (8721.25 ± 14.76 mg/100g) and (7195.54 ± 16.28 mg/100g), respectively. In *I. aquatica* raw and paper sheets, the major amino acid was glycine (6012.12 ± 15.65 mg/100g) and (5609.25 ± 18.75 mg/100g), respectively. The highest amount of phenylalanine (2735.9 ± 14.6 mg/100g) was found in *E. fluctuans* paper sheet.

In the study conducted by Suthari et al. (2017) on *A. philoxeroides* found that glutamic acid (20.86%), serine (8.28%), aspartic acid (7.82%), and arginine (6.52%) were abundant in the plant [37]. Essential amino acids such as leucine (4.3%), isoleucine (4.24%), and lysine (3.88%) were also present.

The plant contained approximately 24% of total nitrogen, indicating its potential as a good dietary source. Ndamitso et al. (2015) identified 18 amino acids in varying proportions in *I. aquatic* [36]. Glutamic acid was found to be the highest amino acid content in both the leaves (11.91 ± 0.015 g/100g protein) and stems (7.27 ± 0.020 g/100g protein). Among the essential amino acids, leucine had the highest concentration in both the leaves (7.54 ± 0.30 g/100g protein) and stems (6.39 ± 0.583 g/100g protein). Cystine and tryptophan were identified as the least abundant essential amino acids in the leaves (0.71 ± 0.002 g/100g protein) and stems (0.53 ± 0.153 g/100g protein), respectively. According to Dewanji et al. (1993), A. philoxeroides contains various amino acids, including lysine, histidine, arginine, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, and phenylalanine [38].

3.4. Major bioactive compounds in aquatic paper sheets determined by GC-MS

Among the three raw aquatic weeds, 29 compounds were identified in EfR (*E. fluctuans* raw), 36 compounds were identified in ApR (*A. philoxeroides* raw), and 37 compounds were identified in IaR (*I. aquatica* raw). Among the three edible paper sheets, 31 compounds were identified in EfP (*E. fluctuans* paper), 33 compounds were identified in ApP (*A. philoxeroides* paper), and 40 compounds were identified in IaP (*I. aquatica* paper). The highest number of compounds was identified in IaP, and the lowest number of compounds was identified in EfR. The retention time (RT), name of the compounds, peak area (%), molecular weights, and their molecular formulas are shown in Table 4. In EfP, the major compounds identified were Androst-5-En-4-One (41.01%), Kauran-16-Ol (15.43%), 12-Bromododecanoic Acid (7.93%), and 1,4-Dimethyladamantane (7.89%). In EfR, the major compound identified was 6-Hydroxy-4,4,7A-Trimethyl-5,6,7,7A-Tetrahydrobenzofuran-2(4H)-One (8.71%). In ApR, the major compounds identified were Tamoxifen (26.8%) and 11,14,17-Eicosatrienoic Acid, Methyl Ester (12.33%). In ApP, the major compounds identified were Dodecanoic Acid (8.71%) and Phytol (14.02%). In IaP, the major compound identified was N-Hexadecanoic Acid (16.07%). The samples shared common compounds, which included Benzeneacetaldehyde, Quinoline, 2-Ethyl-, Pipradrol, Methyl 11-Methyl-Dodecanoate, N-Hexadecanoic Acid, Phytol, and 11,14,17-Eicosatrienoic Acid, Methyl Ester.

Akbar et al. (2021) reported various compounds in *A. philoxeroides* including the highest concentration acetic acid, 2-(2-methoxycarbonylamino-5-nitrophenylthio)-, methyl ester (31.9%), followed by 1,4-benzenediol, 2,5-bis(1,1-dimethylethyl)- (15.06%) [39]. These compounds were considered to be responsible for the observed biological activity in the study. Bhaigybati et al. (2020) conducted research on the GC-MS analysis of the methanolic extract of *I. aquatica* Forsk. The analysis revealed the identification of 40 compounds from the methanolic extract of the edible parts of *I. aquatica* Forsk. Among these compounds, 11 were identified as major compounds, including 1,5-Heptadiene-3,4-diol, 2,5-dimethyl, 9,12-Octadecadienoic acid, methyl ester (E,E), phytol, hexadecanoic acid, 5,8-Octadecadienoic acid, methyl ester, Ar-tumerone, 7-Tetradecyne, 2-Cyclohexen-1-ol, 3-bromo, 11,14-Eicosadienoic acid, methyl ester, and heptadecanoic acid, methyl ester. Pamila et al. (2017) conducted a GC-MS analysis of *A. philoxeroides* and *A. bettzickiana [40]*. The analysis showed different peaks indicating the presence of various bioactive compounds with both low and high molecular weights. Five compounds were found to be commonly present in both plants: n-Hexadecanoic acid, 9,12-Octadecadienoic acid (Z,Z), Ar-tumerone, Bicycloheptane, 2,6,6-trimethyl, and Phenol, 5-(1,5-dimethyl-4-hexeny), Limonene. Muselli et al. (2000) investigated the dihydroperillaldehydes in the essential oil of *E. fluctuans [41]*. The chemical composition of *E. fluctuans* essential oil from Vietnam was analyzed using GC/retention indices and carbon-13 NMR spectroscopy. The major components identified were myrcene, limonene, trans- and cis-dihydroperillaldehydes. This study reported the presence of these two isomers in nature for the first time.

4. Conclusions

Among the three edible paper sheets made from aquatic weeds, *A. philoxeroides* was the most nutritious, but the other two are also nutritious. Both the raw aquatic weeds and the edible paper sheets contain a significant amount of bioactive compounds. Although there are minor changes in nutrition due to the thermal process, there is a close similarity between the raw aquatic weeds and the edible paper sheets. The paper sheets prepared from the aquatic weeds also provide appreciable quantities of various macro and micro elements. These paper sheets could be a promising source of carbohydrates, proteins, lipids, polyphenols, and other bioactive compounds, making them suitable for individuals suffering from malnutrition. Furthermore, the minerals present in the samples can contribute to meeting dietary requirements. The bioactive compounds derived from aquatic edible plants possess antioxidant properties, helping to scavenge free radicals and prevent various diseases. Since the edible paper sheets retain a good amount of bioactive compounds, we can make the best use of these aquatic weeds in this form.

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