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Research Article
Protein and Lipid Composition of Duckweeds (*Landoltia punctata* and *Wolffia arrhiza*) Grown in a Controlled Cultivation System

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Abstract

Background and Objectives: Duckweed is a common aquatic angiosperm and is consumed by humans in Southeast Asia. Due in part to their high protein content, duckweeds have been identified as a potential human food source. Here, we conducted a preliminary study to quantify and characterize the protein and lipid content of two species of duckweed, *Wolffia arrhiza* and *Landoltia punctata*. **Materials and Methods:** The plants were grown for 9 days in a controlled cultivation system and proteins were extracted into n-hexane with a Soxhlet extractor. Amino acids were analyzed using high-performance liquid chromatography and all eight essential amino acids were present in both species at concentrations comparable to those in legumes. Lipids from the protein extraction residue were recovered and the fatty acids analyzed by gas chromatography. **Results:** The protein content was 0.14±0.03 and 0.17±0.01 g protein/g dry biomass and the total lipid content was 0.06±0.01 and 0.09±0.01 g lipid/g dry biomass for *L. punctata* and *W. arrhiza*, respectively. Most of the FAs were polyunsaturated, specifically, 58.38% of the total FA Methyl Esters (FAME) for *L. punctata* and 44.46% of the total FAME for *W. arrhiza* were polyunsaturated. Among these, most were omega-3 FAs, representing 44.93 and 22.17% of the total FAME in *L. punctata* and *Wolffia arrhiza*, respectively. The FAs obtained were mostly long-chain (13-22 C). **Conclusion:** *L. punctata* and *W. arrhiza* have a high content of both proteins and FAs, contain the essential amino acids and a considerable amount of omega-3. We consider that these results support the potential for these duckweed species to serve as a source of proteins and lipids in human food.

Key words: Duckweed, protein, amino acids, lipid, fatty acids, *Landoltia*, *Wolffia*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Duckweed is a tiny, aquatic angiosperm that grows on the surface of freshwater. The diameter of the plants typically ranges from 3.5-6.5 mm depending on the species, but never exceeds 10 mm[1]. Duckweed is common worldwide except in very cold climates. Its family, the Araceae, consists of five genera: *Landoltia*, *Lemna*, *Spirodela*, *Wolffia* and *Wolffiella*[2]. Duckweed contains a high concentration of primary metabolites, such as carbohydrates, proteins and lipids and is consumed by humans in some parts of the world. For example, *Wolffia arrhiza* is eaten in Myanmar, Laos and Thailand, where it is known as Khai-nam or "Eggs of the Water"[3,4].

This background has led to the consideration of duckweed as a potential new food source for humans. In an evaluation of its nutritional value, it was noted that on a percentage of dry mass basis, duckweed is typically 10-30% protein, 1-5% lipid, 20-25% crude fibre and 10-15% carbohydrate in the basis of dry weight[5]. The plants have a short doubling time, an important advantage in the production of a potential food source as it permits rapid biomass accumulation. Specifically, under controlled conditions, *L. punctata* and *W. arrhiza* had doubling times of 3.6±0.02 and 3.9±0.04 days, respectively[6].

We have undertaken this study to evaluate the potential of duckweed for development as a food source. Their potential for conversion to biofuels has also been considered[6,7]. We selected *L. punctata* for this study because it is extremely abundant in almost all regions of America, Southeast Asia and Australia[8]. We recognize that a preliminary investigation of the nutritional value of duckweeds is required as the first step in their development as a food source. These would be related to legal concerns as they are intended for human consumption[9]. The legal concerns were related to the market introduction policy and food safety legislation[10]. A comprehensive assessment of the quality and nutritional value of duckweeds would follow this preliminary study before industrial-scale production systems could be established. Here, we have undertaken this preliminary investigation. Two duckweed species, *L. punctata* and *W. arrhiza*, were cultivated in a controlled environment to obtain the optimal biomass yields, harvested and extracted with Soxhlet and n-hexane as a solvent to quantify the proteins and lipid content. The amino acid and fatty acid contents of the plants were also measured.

MATERIALS AND METHODS

Study area: This study was conducted at Laboratory of Biomass Production, School of Life Sciences and Technology, Institut Teknologi Bandung from July, 2020 to August, 2021.

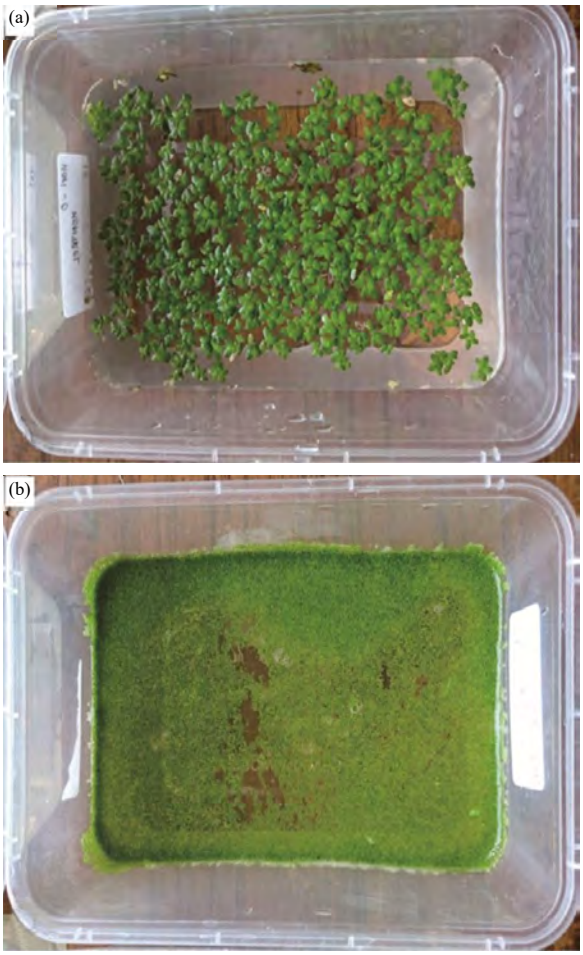

Fig. 1(a-b): Two duckweed species used in this study, (a) *L. punctata* and (b) *W. arrhiza* cultivated in trays

Cultivation of duckweeds: Duckweed plants, *L. punctata* and *W. arrhiza* were collected in Central Java, Indonesia. Representative plants are shown in Fig. 1. The plants were placed in trays (40.5×31.5×15.5 cm) with a nutrient solution (AB-mix Hydro J Indonesia, consisting of macro-and micronutrients) and acclimatized for 3 days irradiated with sunlight, after which they were transferred to a growth chamber. The cultivation experiment was started when the plants were transferred to the growth chamber. Plants were grown in the growth chamber for 9 days in the same trays described. The trays contained 4 L of 10% Hoagland's nutrients, pH 6.5-7.0[6]. The initial biomass was 30 g of plant material per tray and fresh weight was measured daily to produce a growth curve and estimate specific growth rate and doubling time[6]. The light was supplied continuously by Tubular Lamps (TL) with cool-white bulbs, with an intensity of 4000-7000 lux. The temperature in the growth chamber was 24.5-25.5°C and relative humidity was 66-70%.

Biomass harvesting and preparation: After 8 days in the growth chamber, the plants were harvested and weighed, then spread out on filter paper and dried in an oven for 6 hrs at 50°C. When dry, the biomass was ground in a blender and sieved to achieve uniform consistency[6].

Lipid extraction: The dried plant material was wrapped in filter paper, tied with string to keep it together, placed in a Soxhlet extractor (Electrothermal EM1000/CE, UK) with n-hexane (25 mL/g dry weight)[11] and extracted for 6 hrs. The solvent was removed from the lipid extract with a rotary evaporator. The dry weight of the final separated lipids as compared to the dry weight of the initial biomass to obtain lipid yield data (g g^{-1}). The remaining biomass was used in the extraction of protein.

Protein extraction: The residual biomass after lipid extraction was dried in an oven at 50°C for 30 min to remove residual solvent, dissolved in 5% NaCl (20 mL per g dry weight) and the pH of the resulting suspension was raised to 10 with 1 M NaOH[12]. The suspension was then stirred and heated on a hotplate for 30 min at 50°C, cooled to room temperature and left undisturbed for 6 hrs, during which time the suspension separated into a supernatant phase and a residual biomass phase. The supernatant was collected, its pH adjusted to 4 with 1 M $C_6H_8O_7$ (citric acid) and stirred thoroughly while heating on a hotplate for 15 min at 50°C. The supernatant was allowed to cool to room temperature and left undisturbed for 12 hrs to precipitate the protein. The precipitated proteins were collected by centrifugation for 15 min at 5,300 rpm. The dry weight of the precipitated protein was compared to the dry weight of the initial biomass to obtain protein yield data (g/g).

Determination of fatty acids using gas chromatography (GC): Lipid samples (20-30 mg) were mixed with 1 mL of 0.5 N NaOH and heated in a water bath at 100°C for 20 min, after which 2 mL of 20% BF_3 was added. The solution was reheated for 20 min and cooled to room temperature. Next, 3 mL of the solution of saturated NaCl iso-octane (2,2,4-trimethylpentane) (2:1 v/v) was added and the resultant solution was mixed until it was homogenous. This addition results in the formation of two phases. One gram of anhydrous Na_2SO_4 was added to the upper phase, which was then left undisturbed for 15 min. The organic phase from this mixture was separated and injected into a GC (GC-17V3 Shimadzu, Japan)[9]. The results from the GC analysis were compared to the Fatty Acid Methyl Esters (FAME) standard and are presented as a percentage of total FAME.

Determination of amino acids using high-performance liquid chromatography (HPLC): Protein samples (3 mg) were mixed with 1 mL 6 N HCl, placed in an oven at 110°C for 24 hrs, dried with a rotary evaporator and mixed with 6 mL 0.01 N HCL. The solution was filtered with Millipore paper (0.22 µm MF-Millipore™ Merck, NJ, USA) to obtain the extract, which was mixed with BK_3O_3 (1:1, v/v). Aliquots (50 µL) of this mixture were reacted with 250 µL of o-phthaldialdehyde (OPA) reagent for 1 min before being injected into an HPLC (CBM-20A Shimadzu, Japan) with a Hypersil ODS-2 column (Thermo Fisher Scientific, USA). The mobile phases were Buffer A (0.025 M $C_2H_3NaO_2$, 0.05% Na-EDTA, 9% CH_3OH, 1% C_4H_8O (in 1 L distilled water) and Buffer B (95% CH_3OH in distilled water)[9]. The data have been presented as g/100 g protein.

Determination of nitrate contents in medium: Nitrate contents in the medium on the initial and final days of cultivation were determined. Analysis was performed using the Hydrochloric (HCl) method[13]. A total of 10 mL of samples were added with 0.2 mL of 0.2 N HCl, then allowed to stand for 20 min at room temperature. The samples were then measured their absorbance using a spectrophotometer (Shimadzu UV-Vis Spectrophotometer UV-1280, Japan) at a wavelength of 220 and 275 nm. The absorbance data would be compared with the standard curve of Nitric Acid (HNO_3).

RESULTS AND DISCUSSION

Plant growth characteristics: The increase in the fresh weight of the duckweed plants over the time of the experiment is shown in Fig. 2. From this growth curve, we calculated specific growth rates and doubling times. The specific growth rates were 0.16±0.01 and 0.15±0.01 per days for *L. punctata* and *W. arrhiza*, respectively. The doubling times were 4.38±0.23 and 4.47±0.17 days for *L. punctata* and *W. arrhiza*, respectively. These values are consistent with our previous findings[6].

Nitrogen as the major constituent of the element in the medium would be absorbed and converted into biomass during the cultivation as depicted in Fig. 3. The conversion rate of nitrate into biomass for *L. punctata* and *W. arrhiza* were comparable in this study, 3.35±0.35 and 3.47±0.25 mg L^{-1} g biomass, respectively. The nutrients uptake in *L. punctata* could be through the roots or fronds with the same absorption rate level[14], but not for *W. arrhiza* due to its rootless morphology. Several studies showed that duckweeds had good ability in the nitrate removal ranging from[15,16] 72-98 up to 98%.

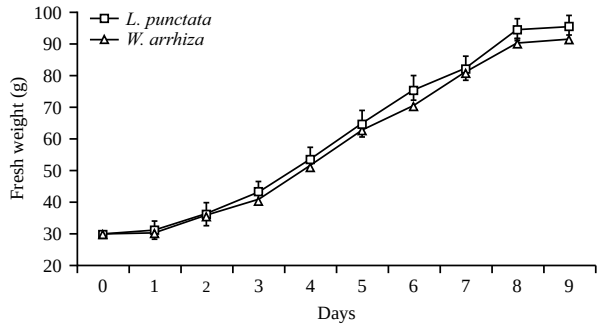

Fig. 2: Increase in fresh weight of *L. punctata* and *W. arrhiza* during the cultivation experiment, initial biomass was 30 g

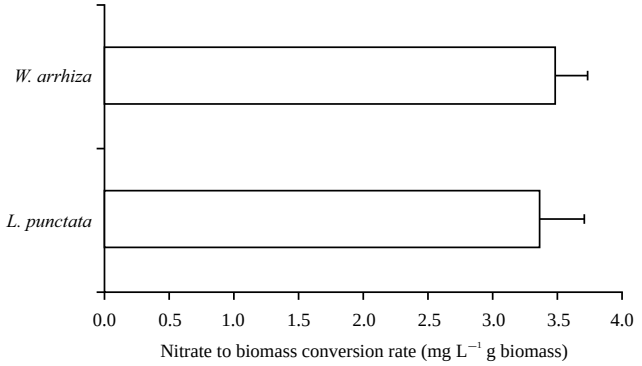

Fig. 3: Conversion rate from nitrate into the biomass of *L. punctata* and *W. arrhiza* during cultivation

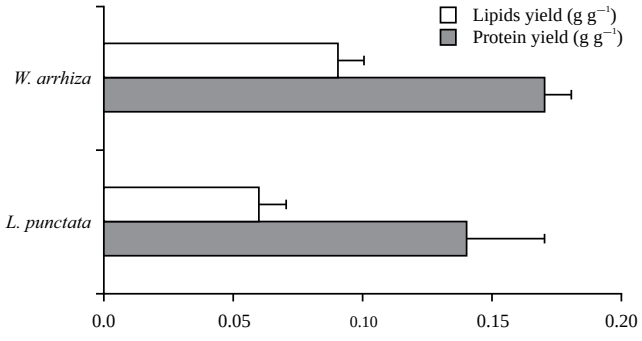

Fig. 4: Proteins and lipids yield of *L. punctata* and *W. arrhiza*

Yield of proteins and lipids: The amount of total protein and lipid from the two species are presented in Fig. 4. We detected 0.14±0.03 g protein/g dry weight and 0.06±0.01 g lipids/g dry weight in *L. punctata*. For *W. arrhiza*, these values were 0.17±0.01 g protein/g dry weight and 0.09±0.01 g lipid/g dry weight.

These results were consistent with previous research, in which protein levels ranges from 10-37% and the lipids ranged from[5,9,17] 4-9%. Proteins and lipids content in duckweeds could easily change adjusting with the environmental conditions in which they lived. A study conducted by Yu *et al.*[18] showed that the proteins content reached 0.34 g g^{-1} of dry weight, it can be concluded that cultivation conditions became one of the trade-offs for gaining high proteins and lipids content.

Characterization of duckweed fatty acids (FAs): Table 1 shows the characteristics of the FAs isolated from both duckweed species. The FAs that were present in the greatest amount in both species were α-linolenic acid, palmitic acid and linoleic acid. Tang *et al.*[19] showed that palmitic acid (C16:0), linoleic acid (C18:2) and linolenic acid (C18:3) were the dominant components of FAs in duckweeds which agreed with our findings. Most of the FAs in both species were polyunsaturated FAs and of these, most were omega-3 and omega-6 FAs. No short-chain fatty acids were detected in either species. The polyunsaturated FAs (total FAME (%)) from both species were similar to those from legumes, notably soya (59.7), chickpea (58.7), lupin (34.6) and green pea (56.6)[20].

The omega-6 FAs were mostly linoleic acid and arachidonic acid while the omega-3 FAs were mostly α-linolenic acid and docosahexaenoic acid. The omega-6 to omega-3 (n6/n3) ratios in *L. punctata* and *W. arrhiza* were 0.23 and 0.72, respectively, considerably lower than the values reported for soya (8.2), chickpea (21.8), lupin (4.6) and green pea (5.7)[20]. Based on the recommendations for a healthy diet in some countries and organizations, the n6/n3 ratio should be between 2 and 3[21]. The low n6/n3 ratio in duckweeds was due to their high α-linolenic acid contents[9]. When cultivated under artificial conditions, the values of n6/n3 for *W. arrhiza* and *W. microscopia* were 0.69 and 0.61, respectively[22], this is consistent with our results.

Characterization of duckweed amino acids: Table 2 summarizes the amino acid content of the protein in both duckweed species. The proteins of both *L. punctata* and *W. arrhiza* contained the eight essential amino acids. Essential amino acids could not be made by the human body so they must be met from food[23,24]. The amino acid content of the protein from both duckweed species was comparable to those from legumes such as soya, chickpea, lupin, green pea[20,25] and water hyacinth[26]. In the pilot-scale applications, duckweeds had greater potential than water hyacinth due to their better

Table 1: Characteristics of fatty acids from *L. punctata* and *W. arrhiza*

	L. punctata (Total FAME (%))	*W. arrhiza* (Total FAME (%))
Saturated fatty acids	31.44	39.19
Monounsaturated fatty acids	10.18	16.35
Polyunsaturated fatty acids	58.38	44.46
Omega-3	44.93	22.17
Omega-6	10.36	16.04
Omega-9	4.87	8.02
Ratio of Omega-6/Omega-3	0.23*	0.72*
Medium-chain fatty acids (C6→C12)	1.30	1.22
Long-chain fatty acids (C13→C22)	95.44	94.49
Very-long-chain fatty acids (C22<)	3.26	4.29
Most prevalent fatty acids, in order of prevalence		
α-Linolenic acid	43.72	20.82
Palmitic acid	18.62	16.11
Linoleic acid	8.31	13.47
Trans-fatty acids	62.66	50.46
Cetylated fatty acids	5.90	10.35

*No unit, FAME: fatty acid methyl esters

Table 2: Characteristics of amino acids from the proteins of *L. punctata* and *W. arrhiza*

Amino acid	*L. punctata* (g/100 g protein)	*W. arrhiza* (g/100 g protein)
Alanine	5.6	6.2
Arginine	4.2	4.4
Aspartate	8.0	8.2
Glutamate	8.9	8.7
Glycine	3.7	4.1
Histidine*	1.4	1.1
Isoleucine*	5.2	4.9
Leucine*	6.9	6.8
Lysine*	5.9	5.5
Methionine*	2.0	2.1
Phenylalanine*	4.9	4.6
Serine	3.4	3.5
Threonine*	4.3	4.3
Tyrosine	3.5	4.0
Valine*	5.4	5.2

*Essential amino acids

ability in recovering nutrients from wastewater and their higher production of crude protein and amino acids[26]. This finding, then, allows these duckweeds to be considered a potential source of high-quality protein or a source of functional food raw ingredients.

CONCLUSION

We conclude that *L. punctata* and *W. arrhiza* have the potential to be developed as functional food sources. The finding that they have a high content of both proteins and FAs, contain the essential amino acids and contain a considerable amount of omega-3 and polyunsaturated fatty acids allow them to be considered, along with other duckweed species, as a potential food source for humans.

SIGNIFICANCE STATEMENT

This study discovered information about the nutritional values of primary metabolites in the form of amino acids and fatty acids from duckweeds under controlled cultivation conditions that could be beneficial for future various applications in several fields. This study will help the researcher to uncover the critical areas of functional food production from new potential resources.

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REFERENCES

1. Ullah, H., B. Gul, H. Khan and U. Zeb, 2021. Effect of salt stress on proximate composition of duckweed (*Lemna minor* L.). Heliyon, Vol. 7. 10.1016/j.heliyon.2021.e07399.
2. Yang, G.L., D. Feng, Y.T. Liu, S.M. Lv, M.M. Zheng and A.J. Tan, 2021. Research progress of a potential bioreactor: Duckweed. Biomolecules, Vol. 11. 10.3390/biom11010093.
3. Ruekaewma, N., S. Piyatiratitivorakul and S. Powtongsook, 2015. Culture system for *Wolffia globosa* L. (Lemnaceae) for hygiene human food. Songklanakarin. J. Sci. Technol., 37: 575-580.
4. Sirirustananun, N., 2018. Appropriate proportion of water meal (*Wolffia arrhiza* (L.)) and commercial diet in combined feeding for tilapia fingerlings rearing. Int. J. Agric. Technol., 14: 249-258.

5. Appenroth, K.J., K.S. Sree, M. Bog, J. Ecker and C. Seeliger *et al*., 2018. Nutritional value of the duckweed species of the genus wolffia (Lemnaceae) as human food. Front. Chem., Vol. 6. 10.3389/fchem.2018.00483.
6. Faizal, A., A.A. Sembada and N. Priharto, 2021. Production of bioethanol from four species of duckweeds (*Landoltia punctata*, *Lemna aequinoctialis*, *Spirodela polyrrhiza*, and *Wolffia arrhiza*) through optimization of saccharification process and fermentation with *Saccharomyces cerevisiae*. Saudi J. Bio. Sci., 28: 294-301.
7. Cui, W. and J.J. Cheng, 2015. Growing duckweed for biofuel production: A review. Plant Biol., 17: 16-23.
8. Wiersema, J.H., 2015. Application of the name *Lemna punctata* G. Mey., the type of *Landoltia* Les & D. J. Crawford. Plant Biol., 17: 5-9.
9. Appenroth, K.J., K.S. Sree, V. Böhm, S. Hammann, W. Vetter, M. Leiterer and G. Jahreis, 2017. Nutritional value of duckweeds (lemnaceae) as human food. Food Chem., 217: 266-273.
10. van der Spiegel, M., M.Y. Noordam and H.J.V. Fels-Klerx, 2013. Safety of novel protein sources (insects, microalgae, seaweed, duckweed, and rapeseed) and legislative aspects for their application in food and feed production. Compr. Rev. Food Sci. Food Saf., 12: 662-678.
11. Smyatskaya, Y., N. Politaeva, A. Toumi and L. Olshanskaya, 2018. Influence of extraction conditions on the recovery lipids extracted from the dry biomass of duckweed Lemna minor. MATEC Web Conf., Vol. 245. 10.1051/matecconf/201824518004.
12. Zhang, C., J.P.M. Sanders and M.E. Bruins, 2014. Critical parameters in cost-effective alkaline extraction for high protein yield from leaves. Biomass Bioenergy, 67: 466-472.
13. Faizal, A. and R.T. Putra, 2019. Uniconazole increases starch content in duckweed (*Lemna aequinoctialis* Welw.). 3BIO: J. Biol. Sci. Technol. Manage., 1: 1-6.
14. Fang, Y.Y., O. Babourina, Z. Rengel, X.E. Yang and P.M. Pu, 2007. Ammonium and nitrate uptake by the floating plant *Landoltia punctata*. Ann. Bot., 99: 365-370.
15. Awuah, E., M. Oppong-Peprah, H.J. Lubberding and H.J. Gijzen, 2004. Comparative performance studies of water lettuce, duckweed and algal-based stabilization ponds using low-strength sewage. J. Toxicol Environ. Health Part A: Curr. Issues, 67: 1727-1739.
16. Selvarani, A.J., P. Padmavathy, A. Srinivasan and P. Jawahar, 2015. Performance of duckweed (*Lemna minor*) on different types of wastewater treatment. Int. J. Fish Aquat. Stud., 2: 208-212.
17. Chakrabarti, R., W.D. Clark, J.G. Sharma, R.K. Goswami, A.K. Shrivastav and D.R. Tocher, 2018. Mass production of *Lemna minor* and its amino acid and fatty acid profiles. Front. Chem., Vol. 6. 10.3389/fchem.2018.00479.
18. Yu, G., H. Liu, K. Venkateshan, S. Yan, J. Cheng, X.S. Sun and D. Wang, 2011. Functional, physiochemical, and rheological properties of duckweed (*Spirodela polyrhiza*) protein. Trans. ASABE, 54: 555-561.
19. Tang, J., Y. Li, J. Ma and J.J. Cheng, 2015. Survey of duckweed diversity in Lake Chao and total fatty acid, triacylglycerol, profiles of representative strains. Plant Biol., 17: 1066-1072.
20. Jahreis, G., M. Brese, M. Leiterer, U. Schäfer and V. Böhm, 2015. Legume flours: Nutritionally important sources of protein and dietary fiber. Ernahr. Umsch., 63: 36-42.
21. Arbex, A.K., V.R. Bizarro, J.C.S. Santos, L.M.M. Araújo and A.L.C. de Jesus *et al*., 2015. The impact of the essential fatty acids (EFA) in human health. Open J. Endocr. Metab. Dis., 5: 98-104.
22. Hu, Z., Y. Fang, Z. Yi, X. Tian and J. Li *et al*., 2022. Determining the nutritional value and antioxidant capacity of duckweed (*Wolffia arrhiza*) under artificial conditions. LWT, Vol. 153. 10.1016/j.lwt.2021.112477.
23. Brestenský, M., S. Nitrayová, P. Patráš, J. Heger and J. Nitray, 2015. Branched chain amino acids and their importance in nutrition. J. Microbiol., Biotechnol. Food Sci., 5: 197-202.
24. Liu, Y., H. Xu, C. Yu and G. Zhou, 2021. Multifaceted roles of duckweed in aquatic phytoremediation and bioproducts synthesis. GCB Bioenergy, 13: 70-82.
25. Kaplan, A., H. Zelicha, G. Tsaban, A.Y. Meir and E. Rinott *et al*., 2019. Protein bioavailability of *Wolffia globosa* duckweed, a novel aquatic plant-a randomized controlled trial. Clin. Nutr., 38: 2576-2582.
26. Zhao, Y., Y. Fang, Y. Jin, J. Huang and S. Bao, 2014. Potential of duckweed in the conversion of wastewater nutrients to valuable biomass: A pilot-scale comparison with water hyacinth. Bioresour. Technol., 163: 82-191.