

Nutritional Composition and Bioactive Properties of Four Duckweed Varieties in Sri Lanka

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Abstract

Duckweed is well known for its high protein content and is gaining attention as a sustainable food source due to its rapid growth and excellent nutritional properties. This study on four duckweed varieties in Sri Lanka; *Spirodela polyrhiza* (SP), *Lemna minor* (LM), *Lemna perpusilla* (LP), and *Landoltia punctata* (LaP) revealed their nutritional composition and some bioactive properties. The carbohydrate, protein, fat, ash, and crude fiber content in these duckweed varieties ranged from 5.26–9.49%, 17.34–26.45%, 3.69–3.92%, 8.03–9.55% and 5.26–9.49% (DW), respectively. K, Na, and Ca content varied from 45.62–20.17 mg/g, 5.61–37.73 mg/g, and 11.03–25.46 mg/g, respectively. High levels of omega-3 fatty acids (44.42–50.38%) were also found. FTIR analysis showed five distinct absorption bands associated with amides and carbohydrates. Among the varieties, *Spirodela polyrhiza* and *Landoltia punctata* demonstrated significant ($P \leq 0.05$) α -amylase inhibition ($IC_{50} = 0.14 \mu\text{g/mL}$), while *Spirodela polyrhiza* exhibited the highest ($P \leq 0.05$) lipase inhibition ($IC_{50} = 1.39 \mu\text{g/mL}$). Additionally, *Spirodela polyrhiza* showed notable inhibition ($P \leq 0.05$) against *A. niger* and *E. coli*, and *Landoltia punctata* showed notable inhibition against ($P \leq 0.05$) *C. albicans*, *A. niger*, and *S. aureus*. Rutin content is relatively more affluent than the other polyphenols analyzed (2.9612–3.0588 $\mu\text{g/mg DM}$). These duckweed varieties showed low to moderate toxicity ($LC_{50} > 4000 \text{ ppm}$), highlighting their potential as nutrient-dense food sources with therapeutic properties.

Introduction

Duckweeds, members of the *Lemnaceae* family, are small, free-floating aquatic plants gaining attention as a protein-rich and sustainable food source. Known for their rapid growth and high nutritional value, they thrive in diverse aquatic environments and can be easily cultivated in nutrient-rich waters. These plants are commonly found in many geographic and climatic regions, except in arid deserts, frozen polar areas, and regions with excessive rainfall. (Crawford et al., 2006). This adaptability makes them a promising candidate for enhancing food security and promoting environmental sustainability (Baek et al., 2021; de Beukelaar et al., 2019; Zhou et al., 2023). Sri Lanka's diverse aquatic ecosystems, including wetlands and inland waters, along with its tropical climate, offer optimal conditions for cultivating duckweed. Exploring its continuous growth and biomass yield throughout the year could support scalable solutions to global food and energy issues. Additionally, duckweed varieties adapted to local conditions may have unique nutritional profiles or bioactive compounds that offer potential advantages for human health and animal nutrition.

Compared to other sustainable food sources, duckweed stands out due to its rapid biomass accumulation, high protein content, and low resource demands. Under optimal conditions, it can yield 6–10 times more protein per hectare than soybeans (Roman et al., 2017; Baek et al., 2021). Furthermore, duckweed cultivation does not require arable land, making it a valuable resource for enhancing food security without competing with traditional agriculture. With the rising global demand for bio-protein, duckweed-based extracts are gaining attention in international markets. As a developing country struggling with protein deficiency, Sri Lanka has much to gain from harnessing its locally available duckweed varieties. Exploring their nutritional composition and bioactive properties is crucial to positioning duckweed as a viable alternative protein source, contributing to national and global food security efforts.

Duckweeds have a well-balanced amino acid profile that aligns with the WHO's recommendations, positioning them as a viable protein source for human consumption (Klaus J. Appenroth, K. Sowjanya Sree, Volker Böhm, Simon Hammond, Walter Vetter, Matthias Leiterer, 2017; Zhou et al., 2023). In addition to protein, duckweeds offer carbohydrates (4–38% DW) and fats (2–11% DW), as well as a range of beneficial secondary metabolites, including phenolic compounds and flavonoids. These components contribute to their antioxidant, anti-diabetic, and anti-obesity properties (Appenroth et al., 2018; Baek et al., 2021; Pagliuso et al., 2020; Zhou et al., 2023). Duckweeds, with their nutrient-rich profile, hold great promise in tackling obesity and associated health conditions like cardiovascular diseases and type 2 diabetes. Their therapeutic benefits are gaining recognition in the creation of functional foods. The antioxidant properties of duckweed play a crucial role in neutralizing harmful free radicals, making it a valuable ingredient for functional foods, nutraceuticals, and natural cosmetics (Baek et al., 2021; Gulcin et al., 2010). A recent study with the Dutch population demonstrates that when duckweed is introduced as a human food in a meal context that aligns with consumer expectations, and assuming that its sensory properties, such as taste, are satisfactory, consumers seem to have no significant objections to its large-scale adoption. (de Beukelaar et al., 2019)

Additionally, duckweeds exhibit notable antibacterial and antifungal properties (Zhang et al., 2010), further enhancing their role as a functional food source. These properties contribute to overall health by preventing infections and supporting the immune system. The nutritional content of duckweed can vary depending on species, clone, and growth conditions. Therefore, optimizing cultivation parameters such as light, temperature, pH, and nutrients is essential to improve their nutritional value. Generally, laboratory-grown duckweeds show higher nutrient levels compared to wild-harvested samples (Miltko et al., 2024)

Duckweed's potential extends beyond just human nutrition. Its high starch content and rapid growth make it an excellent candidate for biofuel production and heat generation (Baek et al., 2021; Thingujam et al., 2024). Duckweed is recognized as a model system for the stable production of biological products such as vaccines and antibodies due to its quick growth and adaptability (Cox et al., 2006; Firsov et al., 2015). This positions duckweed as a promising option for the sustainable manufacturing of pharmaceuticals (Thingujam et al., 2024).

Sri Lanka, as a developing nation facing protein deficiency, holds great promise for utilizing duckweed as an alternative protein source. While duckweed is globally recognized for its sustainability and high protein content, limited studies have been conducted on the nutritional, functional, and bioactive characteristics of duckweed species native to Sri Lanka. The nutritional composition and bioactivity of these local varieties remain largely unexamined. Understanding these characteristics is crucial for positioning duckweed as a functional food with potential health-promoting properties. This research gap presents a valuable opportunity to investigate the nutritional, functional, and therapeutic potential of indigenous duckweed species. Such studies could support the integration of duckweed into food systems, helping to combat protein and micronutrient deficiencies in developing regions (Baek et al., 2021; Zhou et al., 2023).

Materials and methods

Chemicals and reagents

Sodium chloride, Potassium chloride, Magnesium chloride, Magnesium sulfate, Sodium bicarbonate, Ethanol, α -amylase, Porcine pancreatic lipase, glucose oxidase (GOD), Acarbose, Orlistat, PNPB (p-nitro phenylbutyrate), phosphate-buffered saline (PBS), Triton x-100, Acetonitrile, Hexane, Dichloromethane, Methanol, glacial acetic acid was purchased from Sigma Aldrich™, USA and multi-elemental standard solutions for ICP-OES analysis, FAME (Fatty Acid Methyl Esters) standard, Phenolic reference standards were purchased from Sigma-Aldrich™, USA.

Sample collection and preparation

Duckweed varieties (Fig 1) *Spirodella polyrhiza* (Fig. 1a), *Lemna minor* (Fig. 1b), *Lemna perpusilla* (Fig. 1c), and *Landoltia punctata* (Fig. 1d) were collected from the Puttalam, Soragune, Peradeniya, and Bolgoda regions in Sri Lanka (Table 1). The samples were packed in polythene bags, labeled, and transported in temperature-controlled containers to the greenhouse at NIFS, Kandy, within 24 hours of collection. They were cultivated under natural sunlight in a controlled environment, maintaining a room temperature of 25–30 °C and a relative humidity of 70–80 %. Once sufficient biomass had been obtained, the duckweeds were harvested and air-dried for further analysis.

Proximate analysis

All duckweed samples were analyzed in triplicate for moisture, crude fat, crude protein, ash, crude fiber, and carbohydrate content using the AOAC (2000) standard procedures.

Mineral analysis

Mineral analysis was conducted using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES; Thermo Scientific, iCAP 7000 series, Germany), following the method outlined by (Larrea Marin et al., 2010) with slight modifications. Duckweed samples were oven-dried at 105°C for 4 hours until a consistent weight was achieved. The dried samples were then finely ground, and 0.25 g of each sample was digested in 10 mL of 65% HNO_3 using a commercial high-pressure laboratory microwave oven (CEM™ Corporation, BR601050, USA). The microwave was programmed for 15 minutes of ramping time, a 10 minute holding time at 180°C during digestion, and 15 minutes of cooling time. The digested samples were transferred to 50 mL volumetric flasks, diluted to the mark with deionized water, filtered, and stored at 4°C. To correct for potential matrix effects and instrumental drift, 100 $\mu\text{g/L}$ of Rh and Re were added to the test solutions as internal standards. For calibration, multi-elemental standard solutions with concentrations of 0, 5, 10, 30, and 50 mg/L were prepared for Na, Mg, K, Ni, Cr, Pb, Cd, Mo, Cu, Mn, Zn, and Fe. Samples were analyzed in triplicate, and method accuracy was validated using TM 25.4 (Environment Canada) as a certified reference material.

Brine shrimp lethality assay

The brine shrimp cytotoxicity assay was performed to evaluate the cytotoxic effects and determine the lethal concentration (LC_{50}) of various crude extracts, following the protocol described by (Gadir, 2012). Brine shrimp (*Artemia salina*) eggs were hatched in aerated, filtered seawater over a 48 hour incubation. Different concentrations (62.5, 125, 250, 500, 1000, 2000, 4000 ppm) of each crude extract (H_2O , 60% EtOH, 70% EtOH) from the four duckweed varieties were prepared, and 2 mL of each concentration was added to individual wells. Ten shrimp nauplii were then introduced into each well using a glass capillary. The control group consisted of 2 mL of seawater with 10 nauplii. After 24 hours, the number of surviving larvae was counted. All experimental assays were conducted in triplicate, and the LC_{50} was calculated accordingly.

Fatty acid analysis

Lipids were extracted from duckweed powder as described by (Elena Cequier-sanchez, Covadonga Rodriguez, 2008) with some modifications. Briefly, 3 g of duckweed powder with 50 mL hexane was shaken on a wrist-action shaker (BURRELL™, USA) for 30 minutes at room temperature and ultra-sonicated (CL-188, USA) for 15 minutes. The supernatant was obtained after centrifugation at 1500 rpm for 10 minutes. Crude oil was obtained after rotary evaporation (40°C under vacuum conditions), and the total crude oil content of the duckweed samples was weighed and calculated. Fatty acid methyl esters (FAME) were prepared according to (Christie, 1988). Briefly, an oil sample (0.4 g) was weighed into a 15 mL screw-capped methylation tube. Then, 0.3 mL dichloromethane and 2 mL of 0.5 M sodium methoxide were added to the oil and mixed well. Subsequently, the prepared mixture was put in the hot water bath at 50°C for 30 minutes until it reached room temperature. After cooling, 5 mL of distilled water was added drop by drop, and glacial acetic acid (0.1 mL) and 0.5 mL of hexane were added and mixed well. The contents were kept at room temperature for 30 minutes, and the top hexane layer was separated into a 2 mL GC vial. Finally, the vials were capped and sealed further with Parafilm™ and kept immediately at – 20°C until analysis by GC (GC system, US 16,443,037, USA). The column used for analysis was Agilent J&W CP-Sil 88 for FAME (100 m, 250 μm , 0.2 μm), and running conditions in GC were: injection volume (1 μL), carrier gas (hydrogen), pressure mode (constant); inlet: split/spitless 260°C, split ration 50:1; oven conditions: 100°C (5 minutes), 8°C /minute to 180°C (9 minutes), 1°C /minute (15 minutes). FID was adjusted for 260°C, and airflow was: hydrogen 40 L/minute, air 400 mL/minute, makeup gas 25 mL/ minute.

FTIR measurements

FTIR measurements of the dried duckweed powder were carried out as described by (Mittal et al., 2020) with slight modifications. Around 1.5 mg of each dried powder was mixed with 90 mg of KBr (FT-IR grade, $\geq 99\%$ trace metals basis, Sigma Aldrich) and turned into a pallet using a hydraulic press. The spectra were recorded using an FTIR Nicolet iS50 spectrometer (Thermo Nicolet, Madison, WI) equipped with deuterated triglycine sulfate (DTGS) detector and KBr beam splitter. The data were collected in the mid-infrared region of 4000–500 cm^{-1} by co-adding at 64 scans, resolution of 8 cm^{-1} . All spectra were ratioed against the blank background spectrum of pure KBr pallet and recorded as absorbance values at each data point.

Spectral analysis

The spectral data were processed and analyzed using the OMNIC software (version 7.0, Thermo Nicolet). Pre-processing steps included baseline correction and scale normalization for each spectrum. The processed spectra were then used for qualitative analysis of the chemical composition of the duckweed powder samples.

Preparation of water and Ethanol extracts

Powdered samples (0.2 g) were extracted in 20 mL of H₂O, 60% EtOH, and 70% EtOH solvents using an ultrasound-assisted extraction method at 40 kHz for 30 minutes. The mixture was then centrifuged for 15 minutes at 7500 rpm, and the supernatant was collected. The sample extracts were subsequently stored under freezing conditions until further use.

α -amylase inhibition assay

With minor modifications, this test followed the method of (Visvanathan et al., 2016). Initially, 50 μ L of the sample was combined with 50 μ L of α -amylase (13 U/mL in 0.02 M phosphate buffer, pH 6.9, containing 0.006 M NaCl). After incubating for 30 minutes at 37°C, 40 μ L of 1% w/v starch solution (prepared in 0.02 M phosphate buffer, pH 6.9) was added. The mixture was then incubated for 30 minutes at 37°C, followed by 50 μ L of DNS color reagent. The reaction was terminated by heating the mixture in a boiling water bath for 5 minutes. After cooling to room temperature, the absorbance was measured at 540 nm using a microplate reader. Acarbose tablets served as the positive control for preparing the standard solution. A concentration series was prepared using 170 mg of the tablet (50 mg of acarbose). The percentage inhibition of α -amylase was calculated using the following formula:

$$\text{Inhibition of } \alpha\text{-amylase \%} = 100 \times [\text{AC} - (\text{AS} - \text{AB})] / \text{AC}$$

Where, Actual absorbance value of control (AC) = Absorbance of the control (C) - Absorbance of the control blank (CB)

Actual absorbance value of test sample = Absorbance of the test sample (AS) - Absorbance of the test sample blank (AB)

Pancreatic Lipase Inhibition Assay

The lipase inhibitory assay was conducted according to the method described by (Chedda et al., 2016) with minor adjustments. A 100 mM phosphate buffer (pH 7.4) was prepared, utilizing sodium chloride, potassium chloride, potassium dihydrogen phosphate, disodium hydrogen phosphate, and Triton-X-100 as the reagents. Additionally, a p-NPB working solution was prepared by mixing 10 μ L of p-NPB with 10 mL of acetonitrile. A concentration series of duckweed plant extracts was created, ranging from 10 to 0.625 μ g/mL. The procedure began with 100 μ L of phosphate buffer (pH 7.4) to a 96-well microplate, followed by 25 μ L of the sample solution. Next, 50 μ L of enzyme solution was added, and the plate was incubated for 15 minutes at 37°C. After incubation, 25 μ L of the p-NPB working solution was added, and the plate was incubated again for 30 minutes at 37°C. Absorbance was then measured at 400 nm. Orlistat (Orslim tablet) was used as a positive control in the experiment. The percentage of lipase inhibitory activity was calculated, and IC₅₀ values were determined graphically by plotting the percentage inhibition of lipase against the sample concentration for each extract.

Anti-microbial activity

In vitro, antibacterial and antifungal activities were evaluated using three different solvents (water, 70% EtOH, and 60% EtOH) for extracts from each variety of duckweed. The antibacterial and antifungal properties of these plant extracts were tested against two pathogenic bacteria (one Gram-positive and one Gram-negative) and two pathogenic fungi using the agar disk diffusion method (Hudzicki, 2012). The crude extracts were dissolved in dimethyl sulfoxide (DMSO) and stored at 4°C until use. For the determination of the inhibition zone, pure strains of Gram-positive and Gram-negative bacteria, as well as fungal strains, were used as standards for comparison. The extracts were tested for their antibacterial and antifungal activities against *Escherichia coli*, *Staphylococcus aureus*, and the fungi *Candida albicans* and *Aspergillus niger*. Three different concentrations (5, 10, 20 mg/mL) of each extract and standard drug were prepared using dimethyl sulfoxide (DMSO). Control experiments were conducted under similar conditions using amoxicillin (500 ppm, 1000 ppm) for antibacterial activity and itraconazole (125 ppm, 250 ppm) for antifungal activity as standard drugs. The zones of inhibition around the discs were measured after a 24 hour incubation at 37°C for bacterial cultures and 24 hours at 35°C for fungal cultures. The sensitivities of the microorganism species to the plant extracts were determined by measuring the sizes of the inhibitory zones (including the disk diameter) on the agar surface around the disks.

Phenolic Profile

The polyphenolic profile of four duckweed varieties in dried powder form was analyzed following the method of (Alakolanga et al., 2014), with minor modifications. Specifically, 50 mg of each duckweed powder sample was dissolved in 2.5 ml of 70% aqueous methanol (HPLC-grade methanol in ultra-pure water) and subjected to 15 minutes of sonication. The resulting solution was then filtered through a 0.45 μ m membrane filter and collected in an auto-sampler vial. Both qualitative and quantitative analyses of phenolic compounds were carried out using an LC-MS system (UltiMate™ ACC-3000), equipped with a quaternary pump (LPG-3400SD) and a diode array detector (DAID-3000), recording signals at 224 nm, 254 nm, 280 nm, and 360 nm wavelengths.

Statistical analysis

The results were presented as mean $\pm$ standard deviation and analyzed using SPSS (version 26). A multivariate analysis of variance (MANOVA) followed by Tukey's test was performed to evaluate significant differences between treatment levels. Significance was determined at $p < 0.05$ and $p < 0.01$.

Results and Discussion

Proximate analysis

In the present study, among the four duckweed varieties, SP showed significantly higher ($P \leq 0.05$) fat, carbohydrate, and crude fiber contents, and LM had a significantly higher ($P \leq 0.05$) moisture content compared to the other duckweed varieties. Additionally, the ash content was significantly higher ($P \leq 0.05$) in SP and LP compared to the remaining species. The findings for SP differ from those reported by (Said et al., 2022), but the fat, ash, and crude fiber content were consistent with the results of (Yu et al., 2011). The protein content across the duckweed species ranged from 17.34% to 26.45%, with LaP having a significantly higher ($P \leq 0.05$) protein content compared to the other varieties (Table 2). The carbohydrate content in duckweed varies by species and environmental conditions, typically ranging from 14.1% to 43.6% dry weight (DW), which aligns with the carbohydrate content found in SP in the present study (Obinna Ben et al., 2021).

According to the results, LP exhibited lower percentages of crude fat and ash, while its crude protein content is consistent with the findings (Andriani et al., 2022). The carbohydrate content in LM, LaP, and SP aligns with Andriani et al. (2019), although crude protein and fat content vary. The ash content in LaP is comparable to the values reported by (Pagliuso et al., 2020), which typically range between (1-8)%. The ash content of LM (9.55%) matches the findings of (Chakrabarti et al., 2018). Generally, the ash content in LP falls within the (7-36)% range, as indicated by (Debora Pagliuso, Adriana Grandis, Janaina Silva Fortirer, Plinio Camargo, 2022), and this is in agreement with the present study's data (8.26%). For LaP, the fat content varies from (1-5)% on a dry weight basis, according to (Sembada & Faizal, 2022), and this is similar to the present study's result, which reports a fat content of 3.84% for LaP.

The crude protein value obtained for SP shows a minor variation from the previously reported value for the same species, approximately 25.6% w/w (Said et al., 2022). In the current study, the protein content of SP differed from that of LP and LaP, consistent with the findings reported by (Debora Pagliuso, Adriana Grandis, Janaina Silva Fortirer, Plinio Camargo, 2022). The protein content for LaP in this study is relatively similar to previous studies on the same species, where the range was 20% to 28.7%. Similarly, the protein content of LP in the present study was lower than the 29.20% reported for the same species by (Klaus J. Appenroth, K. Sowjanya Sree, Volker Böhm, Simon Hammannd, Walter Vetter, Matthias Leiterer, 2017). These findings suggest that duckweed has the potential for developing duckweed-based bio-protein, which could help reduce protein malnutrition in developing countries.

Several factors can lead to varying proximate values in different duckweed species. For instance, salt stress has a significant impact on the proximate analysis of the plant, affecting components such as protein, lipid, carbohydrate, and mineral content, as highlighted by (Ullah et al., 2021). Additionally, the type of culture media, whether organic or inorganic fertilizers, influences proximate composition. It was observed by (Chakrabarti et al., 2018) that duckweeds cultivated with organic manure had reduced carbohydrate content, yet significantly elevated levels of protein, lipid, and ash compared to those grown with inorganic fertilizers ($P \leq 0.05$). Furthermore, a study by (Debora Pagliuso, Adriana Grandis, Janaina Silva Fortirer, Plinio Camargo, 2022) noted that duckweeds cultivated in low-nutrient water exhibited higher fiber, ash, and fat content, coupled with lower protein levels. Conversely, when grown in water rich in ammonia and minerals, duckweed showed elevated protein and ash levels but reduced fiber content. The protein content of duckweed varies with growth conditions, ranging from (7–20)% in natural water bodies, while it increases to (30-40)% in mineral media or effluents (Debora Pagliuso, Adriana Grandis, Janaina Silva Fortirer, Plinio Camargo, 2022).

The nutrient content of the growth medium is a key factor influencing the proximate composition of duckweed, as noted by (Obinna Ben et al., 2021). According to (Meers et al., 2021), crude fiber content tends to be lower (7-10% DW) in plants grown in nutrient-rich water than those in nutrient-poor water (11-17% DW). These variations can be attributed to duckweed species, environmental conditions, growth medium, growth stages, temperature, light intensity, and harvesting methods. Furthermore, studies by (Khandaker et al., 2007) suggest that the protein content of duckweed is influenced by nutrition availability, temperature, and the plant's age. Additionally, protein synthesis in duckweed decreases when exposed to phosphorus deficiency (Debora Pagliuso, Adriana Grandis, Janaina Silva Fortirer, Plinio Camargo, 2022; Reid & Bielecki, 1970). Genetic differences among species, along with environmental factors like temperature, light intensity, relative humidity, growth media, and cultivation conditions, can lead to variations in moisture content. (G. Chen et al., 2018). High ash content in duckweed may be due to large amounts of silt or CaCO_3 on its surface, as noted by (Khandaker et al., 2007; Mbagwu & Adenniji, 1988), who found that duckweed grown under ideal conditions and harvested regularly can have a fiber content of (5-15)%. Finally, the fat content of duckweed is influenced by several factors, including temperature, light intensity, nutrient availability, water quality, and species-specific characteristics, as reported by (Debora Pagliuso, Adriana Grandis, Janaina Silva Fortirer, Plinio Camargo, 2022; Khandaker et al., 2007).

Mineral analysis

Macro and microelements

In the present study, K was found to have the highest concentration among the examined microelements, with values ranging (from 50.07-20.17 mg/g) in the four studied duckweed varieties on a dry weight (DW) basis (Table 3). The variety LP exhibited the highest ($P \leq 0.05$) levels of both K and Na. The measured concentrations of K and Na in this study were significantly higher ($P \leq 0.05$) than those reported by (Klaus J. Appenroth, K. Sowjanya Sree, Volker Böhm, Simon Hammannd, Walter Vetter, Matthias Leiterer, 2017). In comparison, another study (Meers et al., 2021) found K levels in duckweeds to be 23.08 and 18.98 g/kg (DW), which closely aligns with the findings of the present study. For Mg and Ca, the content ranged from (2.36-5.06 mg/g) and (25.56 -11.03 mg/g), respectively (Table 3), with the highest ($P \leq 0.05$) levels of both elements observed in SP. These results for the studied macro elements, except for Na in LP, are consistent with the findings of the GADING Final Report (Hetty Busink-van den Broeck, Hanny HakkertRic de Vos, 2016). Additionally, these results are inconsistent with those reported by (Marcin Sonta et al., 2023) for LM under different concentrations of pig slurry.

In the analyzed duckweed varieties, Mo was found to be the most abundant microelement compared to other examined microelements. There was no significant variation in Mo content across the studied duckweed varieties, except for LM. The amounts of microelements such as Fe, Mn, Cu, Zn, and Mo were found to be in the ranges of 0.21-1.05 mg/g, 0.05-0.34 mg/g, 0.02-1.11 mg/g, 0.17-0.38 mg/g, and 5.09-23.50 mg/g in dry weight (DW), respectively (Table 3). The Zn and Mo content in LM was slightly elevated, whereas the Fe and Mn content was somewhat reduced in the results (Vladimirova &

Georgiyants, 2014). Another study by (Ullah et al., 2021) on the impact of salinity on the macro and microelement composition of LM indicated that the overall accumulation of both macro and micronutrients was higher in plants exposed to lower salt concentrations. The Zn and Cu values observed in the present study differ from those reported by (Marcin Sonta et al., 2023). Another finding by (Khellaf & Zerdaoui, 2009) revealed that LM is highly sensitive to Cu and Cd pollution, leading to reduced frond growth when these minerals are present in high concentrations.

In the present study, SP showed the highest ($P \leq 0.05$) concentrations of Cd, Pb, Cr, and Ni among the examined duckweed varieties. Additionally, there was no significant difference in Cd levels between SP and LaP. According to the FAO/WHO, the maximum permissible levels for Cd, Pb, Ni, Fe, Cu, and Zn in vegetables are 0.2, 0.3, 67.9, 425.5, 73.3, and 99.4 mg/kg, respectively (Mensah, 2017). Among these, the concentrations of all elements in the studied duckweed varieties were below the permissible limits, except for Cd and Pb. The FAO (2024) established permissible limits for Pb and Cd in edible plants at 0.43 and 0.21 mg/kg, respectively. The present study found that the Pb and Cd content in all examined duckweed varieties slightly exceeded these WHO-recommended levels (Bhowmik et al., 2012).

Careful management is essential to minimize heavy metal accumulation in duckweed, ensuring its safe use in food production and animal feed. Certain duckweed species demonstrate strong metal uptake abilities, with *Lemna minor* capable of removing 82.5% of cadmium from water within seven days (Ol et al., 2023; Yang et al., 2023) while *Spirodela polyrhiza* exhibits greater tolerance to chromium. To reduce heavy metal absorption in duckweed, (Markou et al., 2020) highlighted that anaerobic digestion and post-treatment of agro-industrial waste and wastewater can significantly lower associated risks. Additionally, chemical precipitation and adsorption filters can pre-remove heavy metals from highly polluted water, limiting duckweed exposure to only residual, manageable levels (Markou et al., 2020). Integrating duckweed with microbial consortia or algae further enhances heavy metal removal efficiency, as bacteria can immobilize metals in the rhizosphere, reducing their uptake by duckweed (Kaur & Kanwar, 2022; Markou et al., 2020). A study by (Huebert & Shay, 1992; Srivastava, 1995) also found that chelators like ethylenediaminetetraacetic acid (EDTA) can significantly decrease or even prevent heavy metal absorption in duckweed. The safest approach for utilizing duckweed in food or feed applications is to cultivate it in uncontaminated water sources to eliminate the risks associated with residual heavy metals (Markou et al., 2020).

The regulation of the European Commission (Kleissler & Leist, 1976) specifies the maximum levels of heavy metals, in various groups of food products including leafy vegetables and seaweed, the highest permissible levels are 0.10 for Pb and 0.20 mg/kg fresh weight for Cd. The current study indicates that the levels of Pb and Cd are marginally above the permissible limits in the duckweed varieties (Kleissler & Leist, 1976; Marcin Sonta et al., 2023). According to European legislation on heavy metals in feed, the maximum content for Pb is 10 mg/kg in animal feed, and the value for Cd is 0.5 mg/kg. The results of the present study (Cd and Pb) are not aligned with the permissible level (Hetty Busink-van den Broeck, Hanny HakkerRic de Vos, 2016).

Duckweed is known for its capacity to absorb heavy metals such as cadmium (Cd), lead (Pb), mercury (Hg), and arsenic (As) from contaminated aquatic environments. (Xu et al., 2021; Zhou et al., 2023). Earlier studies have shown that the levels of heavy metals and pesticides in fish raised in sewage stabilization ponds, where duckweed was used as feed, remained within safe limits, posing no risk to human or animal health (Xu et al., 2023). However, the concentration of macro-nutrients in duckweed can fluctuate based on the growth conditions and the culture medium used (Sonta et al., 2020; Ullah et al., 2021). According to (Kekina, 2020), the biological accumulation factor (BAF) of macro-elements in duckweed is considerably lower in polluted environments compared to unpolluted areas. Additionally, another study found that the content of macro and micro-elements (minerals) in duckweed is influenced not only by the cultivation conditions but also by the genetic background of the species (Appenroth et al., 2018).

Strict monitoring and risk assessment are essential to manage heavy metal contamination in duckweed before it can be widely consumed by humans (Xu et al., 2021). Given the species variation and the different growth media used, controlling heavy metal levels in commercially produced duckweed is critical. A study by (Zhou et al., 2023) found that duckweed species show different tolerance and capacity to accumulate heavy metals. Mixing different species or co-culturing with microorganisms might help mitigate heavy metal toxicity. While duckweed has the potential to be a nutritious food source, the lack of WHO guidelines on heavy metal limits and the variability in accumulation across species and conditions highlight the need for further study and standardization to ensure its safety for human consumption. Proper cultivation practices, careful handling, and thorough testing are vital.

Brine shrimp lethality assay

Duckweed is widely used as animal feed due to its rapid growth and high protein content; however, its potential toxicity and adverse effects are still being explored (Ziegler et al., 2016). The brine shrimp lethality assay is a valuable tool for isolating bioactive compounds from plant extracts (Jerry L. McLaughlin, 2016). In this study, an increase in *A. salina* mortality was observed with rising concentrations of the tested extracts. Water and ethanolic extracts of duckweed exhibited moderate bioactivity, as indicated by their impact on brine shrimp. Potassium dichromate ($K_2Cr_2O_7$) is commonly used as a positive control in brine shrimp lethality assays, demonstrating a high toxicity level with a mortality rate of $76.67 \pm 4.71\%$ at concentrations exceeding $7.8125 \mu\text{g/mL}$. Even at the lowest tested concentration ($1.953 \mu\text{g/mL}$), $K_2Cr_2O_7$ caused mortality, with an LC_{50} value of $30.00 \pm 0.00\%$. The 50% mortality threshold for $K_2Cr_2O_7$ was observed between concentrations of 7.8125 and $3.9061 \mu\text{g/mL}$. Meanwhile, the negative control provided a suitable growth environment for *A. salina*.

According to previous studies, ethanol exhibits moderate toxicity to *Artemia salina*, with an LC_{50} of 3.4% (v/v) and a maximum safe working concentration of 1.25% in brine shrimp assays. Similarly, Dimethyl sulfoxide (DMSO) has an LC_{50} of 8.5%, with a maximum tolerable concentration of 1.25% (v/v) (Geethaa, 2013). In the present study, the ethanol concentration remained below the 1.25% threshold, minimizing its influence on the brine shrimp lethality assay (BSLA). Similarly, 1% DMSO remained within the safe range and is unlikely to cause any significant toxicity that would affect the assay results. Both ethanol and DMSO are individually considered safe at concentrations $\leq 1.25\%$. When combined, their overall toxicity is expected to remain below this threshold. Therefore, maintaining solvent concentrations at or below these maximum tolerable levels in BSLA should prevent false-positive results in experimental findings (Geethaa, 2013).

In the present study, most extracts exhibited moderate activity, except for the 60% and 70% EtOH extracts of LM and the water and 60% EtOH extracts of SP. The results indicated that the 60% and 70% EtOH extracts showed a significantly higher response ($P \leq 0.05$) in the BSLA than the water extracts. The highest brine shrimp mortality ($P \leq 0.05$) was observed at 4000 ppm. These findings align with previous research by (Karchesy et al., 2016), which reported that alcohol or organic solvent extracts generally exhibit higher toxicity than aqueous extracts. However, this is not always the case. Studies by (Bussmann et al., 2011; Karchesy et al., 2016) have shown that toxicity can vary significantly depending on factors such as harvest time, collection location, plant organ or tissue, and the solvent used for extraction. In the present study, among the tested extracts, *Spirodela polyrhiza* (SP) and *Landoltia punctata* (LaP) exhibited the highest toxicity against *Artemia salina* compared to other duckweed varieties (Table 4), with the highest brine shrimp mortality ($P \leq 0.05$) observed at 4000 ppm. The heightened toxicity of SP and LaP could be due to their natural ability to accumulate heavy metals and environmental toxins from water sources. Among the collected data, the 60% EtOH extract of *Lemna minor* (LM) resulted in a mortality rate of $43.33 \pm 5.77\%$ at 4000 ppm, indicating notable toxicity, although its LC_{50} value exceeded the tested concentration range.

Similarly, 70% EtOH extract of LM exhibited a mortality rate of $43.33 \pm 5.77\%$ at 4000 ppm, indicating significant toxicity, although its LC_{50} remained above the assessed limits. The heightened toxicity observed in *Lemna minor* may be attributed to heavy metal accumulation from its aquatic environment, as mineral analysis revealed slightly elevated levels of heavy metals. Since the samples were collected from natural water bodies, duckweed grown in contaminated environments, such as thermal mineral waters, can accumulate potentially hazardous levels of heavy metals like lead (Pb), sometimes exceeding the European Union's (EU) maximum allowable limits for food supplements (Ujong et al., 2025). Mineral content analysis in this study revealed that SP had the highest ($P \leq 0.05$) concentrations of Cd, Pb, Cr, and Ni among the examined duckweed varieties. Additionally, there was no significant difference in Cd levels between SP and LaP. These heavy metals may have been extracted into ethanol and water during the lethality assay, contributing to the observed toxicity. This contamination presents a significant risk to animal feed, as incorporating duckweed with elevated levels of heavy metals (Cd, Pb, Cr, Ni) could result in bioaccumulation in livestock tissues. Over time, this may negatively impact animal health by impairing growth, reproduction, and immune function.

Furthermore, heavy metals accumulated in livestock can be transferred to humans through meat, milk, or eggs, potentially causing long-term health issues such as kidney damage, neurotoxicity, and carcinogenic effects. In addition to health risks, heavy metal contamination can reduce the nutritional value of duckweed by interfering with the bioavailability of essential nutrients, thus diminishing its effectiveness as a high-protein feed ingredient. These findings highlight the importance of closely monitoring cultivation environments to ensure the safety of duckweed-based products.

A study by (Tan et al., 2018) reported an LC_{50} of 140.64 ppm for methanol extracts of LM, differing significantly from this study. None of the extracts reached LC_{50} within the tested range, indicating that higher concentrations would be necessary to determine the lethal dose for 50% mortality. Research by (Karchesy et al., 2016) highlights that bioactive plant extracts can exhibit varying toxicity due to differences in experimental conditions, methodologies, and biological factors. Additionally, (Osman & Omar, 2019) suggest that variations in toxic phytochemicals, such as alkaloids, terpenoids, or phenolic compounds, can influence toxicity levels among plant varieties. This study provides valuable insights, as research on LM cytotoxicity is limited, and no data exist for the other three duckweed species. These findings are essential for evaluating the toxicological potential of duckweed extracts and their suitability for applications in aquaculture and other biological systems.

Fatty acid profile

The fatty acid composition of four duckweed varieties (Fig 2), SP (Fig. 2a), LM (Fig. 2b), LP (Fig. 2c), and LaP (Fig. 2d) was investigated by using the GC method. According to the results, the carbon chain lengths in the studied duckweed varieties ranged from 12 to 24. The total fatty acid analysis showed unsaturated to saturated fatty acid (U: S) ratios ranging from 1.54 (SP) to 2.71 (LM). The current study found that the most abundant fatty acids in the duckweed varieties (Table 5) were linolenic acid (23.53-33.02)%, palmitic acid (21.45-32.26)%, and linoleic acid (10.78-16.76)%, consistent with findings by (Sembada & Faizal, 2022; Tang et al., 2015; Yan et al., 2013). Moreover, omega-3 fatty acids, such as eicosapentaenoic acid (EPA) and α -linolenic acid (ALA), constituted between 50.38%-44.42% of the total fatty acids in all the studied duckweed varieties, aligning with the results of (Sembada & Faizal, 2022).

The findings aligned with earlier studies, confirming that all examined duckweed varieties contain omega-6 fatty acids, including linoleic acid and γ -linolenic acid (Yan et al., 2013). The high levels of omega-3 fatty acids resulted in a favorable n6/n3 ratio, enhancing the nutritional value of the duckweed (Klaus J. Appenroth, K. Sowjanya Sree, Volker Böhm, Simon Hammannd, Walter Vetter, Matthias Leiterer, 2017). Previous research indicated that the omega-3: omega-6 ratio in duckweeds ranges from 5:3 to 4:1, which is considered highly beneficial (Baek et al., 2021), though this result differs slightly from the present study. Increased omega-3 fatty acid intake may help prevent inflammatory diseases, cancer, cardiovascular diseases, and other chronic conditions, making the omega-3: omega-6 ratio in duckweeds particularly significant (Saini & Keum, 2018). Studies have also demonstrated that incorporating duckweed into fish diets can increase the levels of long-chain omega-3 fatty acids, specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in the fish. This suggests that fish can convert the ALA from duckweed into these beneficial long-chain omega-3s (Goswami et al., 2022).

According to the values in (Table 5), duckweeds were highly rich in unsaturated fatty acids (60.72–73.10)% but moderately rich in saturated fatty acids (26.89–39.27)%. These values in SP differ slightly from those in the other duckweed varieties examined. Additionally, polyunsaturated fatty acids (57.54–68.65)% were higher than monounsaturated fatty acids (MUFA) (3.17–6.50)% in the studied duckweed varieties. In a recent study, (Klaus J. Appenroth, K. Sowjanya Sree, Volker Böhm, Simon Hammannd, Walter Vetter, Matthias Leiterer, 2017) reported that the polyunsaturated fatty acid content in various duckweed varieties ranging from 48% to 71%, which is consistent with the findings of the present study. The values of saturated SFA, MUFA, and PUFA of LaP in the present study showed slight differences compared to the research carried out by (Klaus J. Appenroth, K. Sowjanya Sree, Volker Böhm, Simon Hammannd, Walter Vetter, Matthias Leiterer, 2017). On the other hand, the SFA, MUFA, and PUFA values of LM and SP in the present study were aligned with the values reported by (Klaus J. Appenroth, K. Sowjanya Sree, Volker Böhm, Simon Hammannd, Walter Vetter, Matthias Leiterer, 2017). The fatty acid (FA) profile analysis reveals that α -linolenic acid (18:2n-6), a polyunsaturated fatty acid (PUFA), was present in all four duckweed species studied. (Table 5), it is identified as the most abundant fatty acid in LM, LP, and LaP, with values ranging from 29.56% to 46.44%, followed by palmitic acid (21.50–35.92)% and linoleic acid (11.10–16.76)%. In SP, however, palmitic acid is found in a higher proportion than α -linolenic acid. The α -linolenic acid content in LM (46.44%)

observed in this study aligns with the findings (Chakrabarti et al., 2018; Zhao et al., 2014). However, the results differ slightly from those reported by (Klaus J. Appenroth, K. Sowjanya Sree, Volker Böhm, Simon Hammannd, Walter Vetter, Matthias Leiterer, 2017).

Among saturated fatty acid (SFA) types, palmitic acid (21.45-32.26)% was the most available type, followed by stearic acid (3.54-2.11)%, which is consistent with the findings that unsaturated fatty acids (60.72-73.10)% predominate over saturated fatty acids in studied four duckweeds. Palmitoleic acid was the major monounsaturated fatty acid (MUFA) except in SP, existing in the range of (0.61-2.75)% of total fatty acids. Lauric acid (C12:0), myristic acid (C14:0), and heptadecenoic acid (C17:0) were found in relatively low quantities compared to docosadienoic acid in the examined duckweed varieties. The current study found that, of the varieties mentioned, eicosatrienoic acid was detected only in SP, while γ -linolenic acid was not detected. The results obtained for the present study aligned with the study of (Klaus J. Appenroth, K. Sowjanya Sree, Volker Böhm, Simon Hammannd, Walter Vetter, Matthias Leiterer, 2017).

The present study indicates that the fatty acid content and composition of the duckweed varieties examined show slight variations. Previous studies have shown that the fatty acid content and composition of duckweed can differ considerably depending on the species and growth conditions. For instance, (Chakrabarti et al., 2018) found that duckweed cultivated in nutrient-rich organic manure (OM) exhibited higher lipid content compared to those grown with inorganic fertilizers (IF). Another study by (Achoki et al., 2024) demonstrated that fatty acid composition varied between indoor and outdoor environments. Additionally, the study revealed that duckweed grown in nutrient-poor water bodies had a lower lipid content (1.8–2.5)% than the 3–7% lipid content found in duckweed grown in nutrient-enriched water (Chakrabarti et al., 2018).

FTIR Analysis

In the present study FTIR (Fourier transform infrared Spectroscopy) in examined duckweed varieties showed distinct absorption bands related to amides A and B, amides I and II, III, and the carbohydrate component. (Fig. 3, Table 6). The selected duckweed species in this study, SP (18.76%), LaP (26.45%), LM (17.34%), and LP (22.06%), exhibit relatively high protein content. The FTIR spectral characteristics offer insights into the secondary structure and functional groups within the duckweed protein. The analysis revealed that the protein repeats units in duckweed produced five distinct amide absorption bands (Fig. 3). The amide A and B bands appeared at 3400 cm^{-1} and 2950 cm^{-1} , respectively, resulting from NH stretching (N-H stretching). The amide I band, primarily associated with the stretching vibrations of C=O bonds (C=O stretching), is centered at 1650 cm^{-1} and is the most sensitive spectral region for assessing the secondary structural composition and conformational changes in proteins. (Kong & Yu, 2007). The amide II band is found between 1500 cm^{-1} and 1600 cm^{-1} . It is less sensitive than the amide I band and mainly arises from in-plane N-H bending and C-N stretching vibrations. (Krimm & Bandekar, 1986). Amide III was a relatively weak band and appeared at about 1300 cm^{-1} (N-H bending and C-N stretching) (G. Yu et al., 2011). The FTIR absorption observed in the 1550 cm^{-1} was attributed to the carbohydrate component. The obtained results of the present study were aligned with the research carried out by (G. Yu et al., 2011; HU, 2010). This study found that all the examined duckweed varieties exhibit six distinct FTIR spectral bands associated with duckweed protein.

Anti- Diabetic Activity

Duckweed is a rich source of high-quality protein, containing all nine essential amino acids, with methionine (+76%) and tryptophan (+24%) levels exceeding FAO recommendations. These amino acids contribute to improved insulin sensitivity, insulin secretion, and glycemic regulation (Xu et al., 2021). Additionally, duckweeds contain a variety of bioactive compounds, including saponins, phytosterols, carotenoids, fatty acids, terpenoids, and phenolic compounds, which have demonstrated potential in diabetes management (Baek et al., 2021; On-Nom et al., 2023; Xu et al., 2021). Previous studies indicate that duckweeds are rich in flavonoids, particularly luteolin and apigenin derivatives, as well as phenolic compounds like chlorogenic acid derivatives (Pagliuso et al., 2020). Higher total flavonoid (TFC) and total phenolic content (TPC) in duckweeds contribute to their ability to inhibit α -amylase and α -glucosidase enzyme activities (Mustafa et al., 2021). This suggests that phenolic and flavonoid compounds play a direct role in reducing glucose absorption and regulating blood sugar levels. The exact mechanisms of these plant compounds that inhibit these enzymes are still not completely understood. Some studies suggest that flavonoids might cause structural changes in the enzymes, thereby diminishing their activity (Mustafa et al., 2021). Further research is needed to gain a complete understanding of the anti-diabetic mechanisms of duckweed. Additionally, saponins have been shown to enhance insulin sensitivity and glucose uptake by influencing signaling pathways involved in glucose metabolism. For example, they can activate the PI3K/Akt pathway, which is essential for GLUT4 translocation in muscle and adipose tissues, contributing to their potential anti-diabetic effects (Yufan Liua, Shumin Mub, 2021).

The current study identified significant differences ($P \leq 0.05$) in the anti-diabetic properties across three solvent extractions and four duckweed samples tested (Fig. 4, Table 7). Among the duckweed varieties, LM exhibited the highest ($P \leq 0.05$) α -amylase inhibition. This finding aligns with (de Beukelaar et al., 2019), who suggest that LM may help manage blood sugar levels due to its lower sugar content and beneficial protein digestibility. SP demonstrates notably higher inhibition activity compared to LaP and LP. Specifically, the 70% EtOH extracts of SP ($0.14 \pm 0.00\text{ }\mu\text{g/mL}$) and LM ($0.14 \pm 0.00\text{ }\mu\text{g/mL}$) exhibited the most significant α -amylase inhibition activity ($P \leq 0.05$) among various extracts. While LaP and LP also exhibit potential anti-diabetic properties, studies have shown that SP and LaP, in particular, are recognized for their robust antioxidant activity, which is attributed to the presence of bioactive compounds such as orientin, vitexin, and luteolin. These flavonoids are known for their health benefits, including anti-diabetic effects (Baek et al., 2021; Pagliuso et al., 2020; Qiao et al., 2011). However, there is currently no direct literature specifically connecting SP, LaP, and LP to anti-diabetic activity.

In this study, both water and ethanol extracts of duckweed demonstrated significant ($P \leq 0.05$) anti-diabetic effects. This activity is likely attributed to the extraction of hydrophilic compounds, such as flavonoids and phenolic acids, by polar solvents, which are known for their anti-diabetic properties. Previous research has also highlighted duckweed's anti-diabetic potential, because of its high flavonoid content (Pagliuso et al., 2020). Moreover, ethanol, particularly when combined with water, generally extracts higher concentrations of bioactive compounds linked to anti-diabetic effects. (El Hosry et al., 2023). Notably, the α -amylase inhibition activity of all tested duckweed extracts exceeded that of the standard drug acarbose ($12.16 \pm 0.10\text{ }\mu\text{g/mL}$), indicating superior IC_{50} values in managing diabetes. This suggests that duckweed could serve as a promising natural alternative with potentially fewer side effects. However, additional study is necessary to determine optimal dosages and the effects of long-term consumption.

Anti-obesity activity

The current study reveals a significant difference ($P \leq 0.05$) in anti-obesity activity among the four different duckweed samples tested. (Fig. 5, Table 8). Notably, LM exhibited significantly higher lipase inhibition activity ($P \leq 0.05$) than the other duckweed varieties, as indicated by IC_{50} values. The results also highlight a substantial variation in anti-obesity activity depending on the type of solvent used. Among the solvent extracts, 60% EtOH showed the highest inhibition activity compared to the other solvents tested. This finding is consistent with the study conducted by (El Hosry et al., 2023).

In this study, SP exhibited the highest lipase inhibition ($P \leq 0.05$) using a 60% EtOH extract, reaching $1.39 \pm 0.02 \mu\text{g/mL}$. Meanwhile, LM, LP, and LaP showed significant inhibition with 70% EtOH extraction, with values of 1.75 ± 0.0 , 2.86 ± 0.02 , and $2.62 \pm 0.12 \mu\text{g/mL}$, respectively. Although these values are slightly above the standard orlistat ($1.33 \pm 0.44 \mu\text{g/mL}$), they highlight the potential of duckweed extracts for obesity management. Previous research indicates that duckweed contains hydroxycinnamic acids with anti-obesity effects (Baek et al., 2021) and is rich in phytochemicals, including flavonoids, which may further contribute to its lipase inhibition properties (On-Nom et al., 2023; Pagliuso et al., 2020). These phytochemicals, such as flavonoids, interact with the active site of the lipase enzyme, altering its structure and reducing its catalytic efficiency. For instance, apigenin induces a conformational shift in lipase from an open to a closed state, thereby blocking its activity (Fuqiang Lianga, Yumeng Shia, Weiwei Caob, 2022)

Anti-microbial activity

In this study, SP demonstrated significant antibacterial activity against *E. coli*, showing the highest ($P \leq 0.05$) inhibition zones at $17.33 \pm 1.15 \text{ mm}$, compared to its activity against *S. aureus* (Tables 9, 10). The 70% EtOH extract of SP also demonstrated significant inhibition against both bacterial strains, consistent with findings from (Das et al., 2012). Specifically, ethanol fractions containing steroids showed the most substantial inhibition zone, as noted by (Das et al., 2012). These results suggest that SP possesses antibacterial properties against *E. coli* and could be explored as a natural antimicrobial agent. The differences in inhibition zones among various duckweed varieties and extraction solvents reflect variations in the composition and concentration of the active compounds responsible for the antibacterial activity.

Studies by (Dorman et al., 2000; Kuete, 2010) have highlighted the antimicrobial properties of various secondary metabolites, including reducing sugars, anthraquinones, flavonoids, terpenoids, steroids, phenols, tannins, and organic acids. However, the specific mechanisms underlying their antimicrobial activity remain largely unexplored. Among these phytochemicals, duckweed species are particularly rich in phytosterols such as β -sitosterol (57–84% of total sterols), campesterol (8.5%), and stigmasterol (7.7%) (Appenroth et al., 2018; Das et al., 2012), as well as phenolic and flavonoid compounds (On-Nom et al., 2023; Pagliuso et al., 2020).

Flavonoids and phenols exhibit antibacterial effects by forming complexes with extracellular and soluble proteins, as well as interacting with bacterial cell walls, ultimately leading to bacterial cell death (Ahidin et al., 2024). Steroids exert antimicrobial activity by integrating into the lipid bilayer of bacterial membranes, disrupting membrane fluidity and permeability, which results in cytoplasmic leakage and cell lysis (Ahidin et al., 2024; Y. Chen et al., 2020). Similarly, anthraquinones have been reported to disrupt bacterial membranes, leading to structural perturbation (Cheng et al., 2014). Other phytochemicals also exhibit antimicrobial properties. Tannins act by contracting bacterial cell walls and altering permeability, which causes cell lysis (Ahidin et al., 2024). Saponins contribute to bacterial destruction by reducing surface tension, leading to membrane leakage and cell breakdown (Ahidin et al., 2024). Terpenoids interfere with cell membrane synthesis, protein prenylation, and carbon source utilization, thereby inhibiting bacterial growth (Nayak et al., 2010). Additionally, reducing sugars has demonstrated antibacterial activity (Dhale & Markandeya, 2011).

The antimicrobial activity observed in the study is likely due to the presence of these bioactive compounds. Specifically, the ethanol extracts demonstrated higher inhibitory effects, which may be attributed to their higher concentrations of steroids, phenols, and flavonoids, known for their potent antimicrobial properties. Although several duckweed species have been tested for their antimicrobial potential, comprehensive investigations into their bioactive compounds and mechanisms of action remain limited. Further research is required to gain a deeper understanding of how duckweed extracts produce their antimicrobial effects.

LaP exhibited the lowest antibacterial activity ($P \leq 0.05$) against *E. coli* while showing the highest activity against *S. aureus* (Tables 11, 12). This indicates that LaP may contain potent antimicrobial compounds specifically effective against *S. aureus*. According to (Baggs et al., 2022), transcriptional analysis indicates that LaP may trigger specific genes in response to bacterial stress, which could help explain its antibacterial properties through its defense mechanisms. In contrast, LM showed significant inhibitory activity against both *S. aureus* and *E. coli*, consistent with findings reported by (Das et al., 2012; Zhang et al., 2010). However, (Tan et al., 2018) observed that methanol extracts of LM did not inhibit *S. aureus* or *E. coli* at a concentration of 1 mg/mL, and (Gulcin et al., 2019) found no inhibition at 1 ppm. These results align with the current study, where LM showed slightly lower ($P \leq 0.05$) activity against both bacterial strains at somewhat higher concentrations. Overall, the results suggest that higher extract concentrations generally lead to larger inhibition zones, indicating that concentration is a crucial factor in antimicrobial efficacy. The study found that LM had significantly higher inhibition activity against *S. aureus* than *E. coli*, possibly because *E. coli* has developed resistance mechanisms to the active compounds in LM. This is consistent with findings by (Mesmar & Abussaud, 1991). Other studies also support that LM demonstrates antibacterial activity against gram-negative and gram-positive bacteria and could be a viable alternative to traditional antibacterial agents for treating various infections (Gonzalez renteria et al., 2020; Mane et al., 2017). There is no existing literature on the antibacterial activity of LaP and LP. However, the current study demonstrates that all examined duckweed varieties exhibit antibacterial properties against *E. coli* and *S. aureus*. The effectiveness of this activity varies depending on the solvent extract used, the duckweed variety, concentration, and most importantly, the type of bacterial strain.

In this study, SP and LaP demonstrated significantly higher antifungal activity ($P \leq 0.05$) against *A. niger* and *C. albicans* compared to other duckweed varieties (Table 11, Table 12). Water and 70% EtOH extracts were more effective ($P \leq 0.05$) against *A. niger* than the 60% EtOH extract.

The antimicrobial properties of plant extracts are attributed to their bioactive compounds, which not only protect plants from bacteria and fungi but also exhibit antimicrobial effects against microorganisms (D Tagoe, S Baidoo, I Dadzie, V Kangah, 2009). Therefore, the antifungal activity of these extracts varies depending on their composition and concentration of phenolics, flavonoids, tannins, and alkaloids (Mekam et al., 2019). Ethanol extracts generally yield a wider range of bioactive compounds, including phenolics, flavonoids, tannins, and alkaloids, which contribute to antifungal effects and a study by (Ikegaki, 1988) indicated that 70% EtOH is particularly effective in extracting flavonoids with antifungal properties. These compounds may demonstrate higher activity against *Aspergillus niger* compared to those obtained using 60% EtOH (Jameel et al., 2018). Water, being a highly polar solvent, efficiently extracts polar compounds such as monoterpenes and phenolic compounds, both of which are known for their strong antifungal properties, especially against *A. niger*. Since 60% EtOH has a lower polarity, it may be less effective at extracting certain polar bioactive compounds essential for antifungal activity. This could explain why extracts obtained using 70% EtOH and water were more effective against *A. niger* than those extracted with 60% EtOH (Carla et al., 2023; Mekam et al., 2019). Moreover, different fungal species exhibit varying sensitivities to antifungal compounds. For example, *Candida albicans* may respond differently to specific bioactive molecules compared to *A. niger*. Research suggests that while some extracts are highly effective against *C. albicans*, they may have limited activity against *A. niger*. This variation could be due to differences in cell wall composition or metabolic pathways between these fungi (Carla et al., 2023).

For *C. albicans*, the 70% EtOH extract showed the highest inhibition, though no significant differences were observed among the various extract concentrations for *A. niger*. Notably, the lowest concentration of extracts exhibited the highest inhibition against *S. aureus*, suggesting that active compounds may work synergistically at lower concentrations to enhance antifungal activity against *S. aureus* (Jeong et al., 2021; Ramata-Stunda et al., 2022). The study also found that LM has antifungal activity against both fungal strains tested, aligning with (Das et al., 2012; Mane et al., 2017) similarly showed that SP extract has a broad spectrum of activity against *C. albicans*, supporting our findings. Additionally, (Gulcin et al., 2010) confirmed that LM extract exhibits antifungal activity against *C. albicans*, which is consistent with our results. Although no previous studies have addressed the antifungal activity of LaP and LP, the present study indicates that both varieties possess antifungal properties against the tested fungal strains.

Phenolic Profile

In the current study (Table 13), vanillin acid was detected in all examined duckweed varieties except LaP, with LP showing a relatively high amount (0.45825 $\mu\text{g}/\text{mg DM}$). LaP (0.00615 $\mu\text{g}/\text{mg DM}$) and SP (0.00665 $\mu\text{g}/\text{mg DM}$) contained more sinapic acid than the other varieties. Rutin showed consistently high levels across all varieties, with values ranging from 2.9612 to 3.0588 $\mu\text{g}/\text{mg DM}$, indicating its prominence in these varieties. LaP (0.03885 $\mu\text{g}/\text{mg DM}$) and SP (0.02615 $\mu\text{g}/\text{mg DM}$) showed moderately high amounts of P-Coumaric acid. Gallic acid was detected only in LaP (0.2634 $\mu\text{g}/\text{mg DM}$) and SP (0.39960 $\mu\text{g}/\text{mg DM}$), not LM or LP. Chlorogenic acid was found only in LM (0.002 $\mu\text{g}/\text{mg DM}$) and SP (0.00665 $\mu\text{g}/\text{mg DM}$). (Pagliuso et al., 2020) Revealed that SP displays significant chlorogenic acid derivatives, which could enhance the use of the plant extract for human health applications and aligned with the present study. Catechin acid was detected in LM (0.00850 $\mu\text{g}/\text{mg DM}$), and LP (0.00285 $\mu\text{g}/\text{mg DM}$). Caffeic acid was only detected in LaP (0.01205 $\mu\text{g}/\text{mg DM}$) and SP (0.01765 $\mu\text{g}/\text{mg DM}$). LP (0.00505 $\mu\text{g}/\text{mg DM}$), shows a high content of Ferulic acid compared with the other varieties. The presence of caffeic acid and ferulic acid in SP and LaP are consistent with findings that highlight the importance of these compounds in duckweed for their antioxidant properties and potential health benefits. This result aligned with the study carried out by (Baek et al., 2021; Pagliuso et al., 2020) revealed that particularly SP and LaP, are rich sources of flavonoids and phenolic compounds, including chlorogenic acid derivatives, ferulic acid, and various flavonoids such as luteolin and apigenin According to the results phenolic content was different with the metabolite profiles based on species and environmental conditions. (Baek et al., 2021; Pagliuso et al., 2020)

Limitations of the study

This study has several limitations. It focuses solely on four duckweed varieties found in Sri Lanka, and the findings may not apply to other regions, as duckweed's nutritional and bioactive properties are influenced by environmental factors such as water quality, temperature, and soil composition. Additionally, the bioactivity assessments, including α -amylase, lipase inhibition, and antimicrobial properties, were conducted using in vitro assays. Further research involving animal models or human trials is required to validate their therapeutic potential and bioavailability. Although the study indicates low to moderate toxicity, there is no evidence regarding the long-term effects of duckweed consumption. Thus, extensive toxicological studies are required to assess its safety for long-term use. Additionally, the study does not investigate sensory characteristics such as taste, texture, or odor, nor does it evaluate consumer acceptance. Furthermore, processing techniques aimed at enhancing palatability and minimizing undesirable flavors were not investigated.

Conclusion

In conclusion, the various duckweed species analyzed exhibit rich nutritional profiles, particularly in proteins, carbohydrates, and essential microelements. SP stands out with the highest levels of crude fiber, carbohydrates, and microelements, though caution is advised due to Cd and Pb levels exceeding WHO recommendations. LaP, with its superior crude protein content, and LM, rich in polyunsaturated fatty acids and bioactive compounds, show potential as valuable nutritional sources. The low cytotoxicity and significant anti-diabetic, anti-obesity, and antimicrobial activities further highlight these duckweed varieties as promising candidates to enhance nutritional status, especially in developing countries, where affordable and nutrient-dense food sources are crucial.

Suggestions for future studies

Future studies should focus on evaluating the safety of consuming duckweed, particularly concerning its heavy metal content and potential effects on human health. Investigating methods to reduce heavy metal accumulation in duckweed should also be a priority. Enhancing the nutritional profile of duckweed, especially in terms of protein, essential fatty acids, and phenolic compounds, is another important study area. Further studies should aim to extract and characterize bioactive compounds from duckweed to explore their potential health benefits. While duckweeds offer a promising source of plant-

based protein, efficient extraction methods are needed to make this protein more accessible and usable. Additionally, examining the ecological effects of cultivating duckweed in various environments as a sustainable food source is essential. Comparative studies with other aquatic plants could provide insights into the unique benefits and applications of duckweed in nutrition and health. Ensuring the safety, improving the nutritional value, and understanding the ecological impacts of duckweed products are crucial for fully realizing the potential of this versatile plant.

Abbreviations

SP - *Spirodela polyrhiza*

LM- *Lemna minor*

LP- *Lemna perpusilla*

LaP - *Landoltia punctata*

Declarations

Author Contribution

R.H wrote the main manuscript text and S.S, M.W. and I.R assisted in laboratory analysis.T.M ,B.J ,S.W. and R.L supervised the research and corrected the final manuscript. All authors reviewed the manuscript.

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Tables

Table 1: Geographical Locations of collected Duckweed Varieties in Sri Lanka

Species	Latitude	Longitude	Location
<i>Spirodela polyrriza</i>	7°16'16"N	80°35'44"E	Peradeniya Botanical Garden, Central province, Sri Lanka
<i>Lemna minor</i>	8° 16' 72" N	79° 88' 33" E	Paththayama lake, Northwestern Province, Sri Lanka
<i>Lemna perpusilla</i>	6° 46' 10"N	79° 54' 8" E	Bolgoda lake, Western Province, Sri Lanka
<i>Landoltia punctata</i>	6°74'70"N	80°89'50"E	Soragune devalaya, Western Province,Sri Lanka

Table 2: Proximate composition in four duckweed varieties on a DW basis (%)

Duckweed varieties	<i>Spirodella polyrriza</i>	<i>Lemina cf. minor</i>	<i>Lemina perpusilla</i>	<i>Landoltia punctata</i>
Moisture content*	44.46±0.31 ^a	56.89±0.50 ^d	47.79±0.67 ^c	46.12±0.58 ^b
Fat content	3.92±0.01 ^c	3.72±0.04 ^a	3.69±0.01 ^a	3.84±0.00 ^b
Ash content	8.03± 0.12 ^a	9.55± 0.16 ^c	8.26±0.04 ^a	8.85±0.06 ^b
Protein content	18.76±0.21 ^b	17.34±0.07 ^a	22.06±0.02 ^c	26.45±0.41 ^d
Carbohydrate content	14.55±0.01 ^d	6.74±0.01 ^a	12.92±0.01 ^c	6.95±0.01 ^b
Crude Fiber content	9.49 ±0.26 ^d	5.74 ±0.23 ^a	5.26 ±1.04 ^a	7.78±0.16 ^b

Moisture content* - Fresh weigh basis (FW)

Data represent the mean values ±SD (n=3) of three independent experiments. Means in the same row followed by different letters are significantly different at $p < 0.05$., FW- Fresh weight, DW- Dry weight.

Table 3: Macro and micro elements in four duckweed varieties on the DW basis (mg/g)

Macro elements(mg/g)	<i>Spirodela polyrhiza</i> (mg/g DW)	<i>Lemna minor</i> (mg/g DW)	<i>Lemna perpusilla</i> (mg/g DW)	<i>Landoltia punctata</i> (mg/g DW)
K	20.17 ± 0.12 ^d	45.62 ± 0.10 ^b	50.07 ± 0.42 ^a	37.93 ± 0.13 ^c
Mg	5.06 ± 0.04 ^a	3.01± 0.02 ^b	2.92 ± 0.00 ^c	2.36 ± 0.01 ^d
Na	11.82 ± 0.10 ^c	12.44± 0.15 ^b	37.73 ± 0.12 ^a	5.61± 0.03 ^d
Ca	25.56 ±0.06 ^a	20.56± 0.01 ^b	11.03± 0.12 ^d	14.33 ± 0.07 ^c
Micro elements (mg/g)				
Fe	0.21±0.00 ^d	1.05± 0.00 ^a	0.26 ± 0.00 ^c	0.33 ± 0.00 ^b
Mn	0.05 ±0.00 ^d	0.08± 0.00 ^c	0.34 ± 0.00 ^a	0.12 ± 0.00 ^b
Cu	1.11 ± 0.00 ^a	0.03± 0.00 ^b	0.02± 0.00 ^c	0.02± 0.00 ^d
Zn	0.31±0.00 ^b	0.17± 0.05 ^c	0.22±0.00 ^c	0.38±0.00 ^a
Mo	23.50±0.99 ^a	5.09 ± 3.99 ^b	20.52± 1.23 ^a	21.53 ± 0.26 ^a
Heavy Metals (µg/g)				
Cd	1.80 ± 0.01 ^a	1.11± 0.33 ^b	0.52± 0.02 ^c	1.87 ± 0.01 ^a
Pb	2.13± 0.48 ^a	1.29± 0.25 ^b	0.59± 0.04 ^b	1.20 ± 0.15 ^b
Cr	8.91 ± 0.05 ^a	2.39 ± 0.03 ^b	0.61 ± 0.03 ^c	0.68 ± 0.00 ^c
Ni	2.98± 0.04 ^a	4.44±1.87 ^a	0.01±0.02 ^{b, c}	1.15± 0.05 ^b

Data represent the mean values ± SD (n = 3) of three independent experiments. Means followed by the same letters in a row are not significantly different (P < 0.05).

Table 4: Brine Shrimp Mortality and LC₅₀ Values for Different Extracts at Various Concentrations in duckweed varieties

Extract type	Concentration of the extract (ppm)	Brine shrimp motility after 24 hrs	LC ₅₀
SPW	4000	30± 14.14 ^{a,b,B,a}	> 4000
	2000	13.33±4.71 ^{a,b,B,b}	
	1000	10±0.00 ^{a,b,B,c}	
	500	10±0.00 ^{a,b,B,d}	
	250	3.33±4.71 ^{a,b,B,e}	
	125	0± 0.00 ^{a,b,B,e}	
SP60%EtOH	4000	33.33± 4.71 ^{a,b,A,a}	> 4000
	2000	26.67±4.71 ^{a,b,A,b}	
	1000	30±8.160 ^{a,b,A,c}	
	500	20±8.160 ^{a,b,A,d}	
	250	6.67±9.42 ^{a,b,A,e}	
	125	0± 0.00 ^{a,b,A,e}	
SP70% EtOH	4000	36.67±4.71 ^{a,b,A,a}	> 4000
	2000	33.33±4.71 ^{a,b,A,b}	
	1000	23.33±4.71 ^{a,b,A,c}	
	500	26.67±9.42 ^{a,b,A,d}	
	250	23.33±4.71 ^{a,b,A,e}	
	125	13.33±4.71 ^{a,b,A,e}	
LPW	4000	30.00±8.16 ^{b,B,a}	> 4000
	2000	26.67±9.42 ^{b,B,b}	
	1000	23.33±12.47 ^{b,B,c}	
	500	6.67±4.71 ^{b,B,d}	
	250	0± 0.00 ^{b,B,e}	
	125	0± 0.00 ^{b,B,e}	
LP60%EtOH	4000	36.67±4.71 ^{b,A,a}	> 4000
	2000	30±8.16 ^{b,A,b}	
	1000	20.00±16.32 ^{b,A,c}	
	500	6.67±4.71 ^{b,A,d}	
	250	0± 0.00 ^{b,A,e}	
	125	0± 0.00 ^{b,A,e}	
LP70% EtOH	4000	36.67±4.71 ^{b,A,a}	> 4000
	2000	33.33±4.71 ^{b,A,b}	
	1000	26.66±9.42 ^{b,A,c}	
	500	0± 0.00 ^{b,A,d}	
	250	0± 0.00 ^{b,A,e}	
	125	0± 0.00 ^{b,A,e}	
LMW	4000	10.00±0.00 ^{c,B,a}	> 4000

	2000	0± 0.00 ^{c,B,b}	
	1000	0± 0.00 ^{c,B,c}	
	500	0± 0.00 ^{c,B,d}	
	250	0± 0.00 ^{c,B,e}	
	125	0± 0.00 ^{c,B,e}	
LM60%EtOH	4000	40.00±8.16 ^{c,A,a}	> 4000
	2000	25.00±5.00 ^{c,A,b}	
	1000	13.33±4.71 ^{c,A,c}	
	500	10± 0.00 ^{c,A,d}	
	250	0±0.00 ^{c,A,e}	
	125	0±0.00 ^{c,A,e}	
LM70% EtOH	4000	43.33±4.71 ^{c,A,a}	> 4000
	2000	25.00±5.00 ^{c,A,b}	
	1000	13.33±4.71 ^{c,A,c}	
	500	13.33±4.71 ^{c,A,d}	
	250	0±0.00 ^{c,A,e}	
	125	0±0.00 ^{c,A,e}	
LaPW	4000	43.33±4.71 ^{a,B,a}	> 4000
	2000	33.33±4.71 ^{a,B,b}	
	1000	30±8.16 ^{a,A,B,c}	
	500	26.67±9.42 ^{a,B,d}	
	250	23.33±4.71 ^{a,B,e}	
	125	20.00±0.00 ^{a,B,e}	
LaPW60%EtOH	4000	40±0.00 ^{a,A,a}	> 4000
	2000	20±4.71 ^{a,A,b}	
	1000	15±4.71 ^{a,A,c}	
	500	15±4.71 ^{a,A,d}	
	250	15±4.71 ^{a,A,e}	
	125	0±0.00 ^{a,A,e}	
LaPW70% EtOH	4000	23.33±9.42 ^{a,A,a}	> 4000
	2000	20±0.00 ^{a,A,b}	
	1000	10±0.00 ^{a,A,c}	
	500	10±0.00 ^{a,A,d}	
	250	3.33±4.71 ^{a,A,e}	
	125	3.33±4.71 ^{a,A,e}	
Positive control (+) K ₂ Cr ₂ O ₇	62.5	100 ± 0.00	<62.500 ppm
	31.25	100 ± 0.00	<31.250 ppm
	15.625	80.00± 14.14	<15.625 ppm

	7.8125	76.67± 4.71	<7.8125 ppm
	3.9061	30.00 ± 0.00	> 3.9061 ppm
	1.953	16.67± 4.71	>3.9061 ppm
Negative control (-)	Artificial sea water	0.00 ±0.00	0 .00 ppm

Values are expressed as mean ± standard deviation (n=3). Means with simple letters for samples, capital letters for extractions and italic letters for concentrations. Different letters within the column are significantly different (P<0.05)

Table 5: Fatty acid composition as a percentage of total fatty acids of studied duckweed varieties

Component (Methyl ester)	<i>Spirodella polyrhiza</i> (SP)	<i>Lemina cf. minor</i> (LM)	<i>Lemna perpusilla</i> (LP)	<i>Landoltia punctata</i> (LaP)
Lauric acid C12:0	0.7686	0.6014	0.7001	0.2185
Myristic acid C14:0	1.4160	0.9100	1.0648	0.8659
Palmitic acid C16:0	32.2608	21.7121	23.5756	21.4535
Palmitoleic acid C16:1	0.6158	2.7558	2.2961	1.0718
Heptadecenoic acid C17:0	1.9756	1.0766	1.1057	1.7453
Cis-10-Heptadecenoic acid C17:1	2.5580	0.7504	1.1849	1.1342
Stearic acid C18:0	2.8577	2.5939	2.1104	3.5446
linoleic acid C18:2n	10.7808	16.7658	15.9767	11.0835
α-linolenic acid C18:3n3	29.5394	46.4447	33.0209	29.5617
γ-linolenic acid C18:3n6	ND	1.5048	4.5356	6.7434
Cis- 8,11,14- Eicosapentaenoic acid C20:3n6	5.1600	1.1484	ND	2.9850
Cis- 5,8,11,14,17- Eicosatrienoic acid C20:5n3 (EPA)	5.1140	ND	ND	ND
Cis- 13,16- Docosadienoic acid C 22:2	6.9525	2.7940	11.4030	15.3509
Nervonic acid C 24:1	ND	0.9413	3.0256	4.2409
Saturated fatty acids (SFA)	39.2790	26.8942	27.5568	26.9621
Unsaturated fatty acids (USFA)	60.7209	73.1057	72.4431	73.0378
Mono unsaturated fatty acids(MUFA)	3.1739	4.4477	6.5067	6.0936
Poly unsaturated fatty acids (PUFA)	57.5470	68.6580	65.9364	66.9441
U:S	1.5458	2.7182	2.6288	2.7089

ND not detected (Limit of Detection = 0.1 µg/mL)

Table 06 - Characterization of FTIR spectra of examined duckweed varieties

Wavenumber range (cm ⁻¹)	Functional Group	Mode of vibration	Functional group	References
3400	N-H	N-H stretching	Amide A	(Kong & Yu, 2007; Venkateshan & Wang, 2017)
2950	N-H	N-H stretching	Amide B	(Kong & Yu, 2007; Venkateshan & Wang, 2017)
1600-1700	C=O	C=O stretching	Amide I	(HU, 2010; Kong & Yu, 2007; Krimm & Bandekar, 1986; Venkateshan & Wang, 2017)
1500-1600	N-H C-N	CN stretching NH bending	Amide II	(HU, 2010; Kong & Yu, 2007; Krimm & Bandekar, 1986; Venkateshan & Wang, 2017)
1200-1300	N-H C-N	CN stretching NH bending	Amide III	(HU, 2010; Kong & Yu, 2007; Krimm & Bandekar, 1986; Venkateshan & Wang, 2017)
1000- 1100	C-O	C-O stretching	Carbohydrates	(Venkateshan & Wang, 2017)

Table 7: Anti-diabetic activity of samples in different extracts

Sample	α - amylase inhibition (IC ₅₀ (µg/mL)		
	Water	60% Ethanol	70% Ethanol
<i>Spirodela polyriza</i>	0.19 ±0.00 ^{b,A}	3.66 ±0.03 ^{b,C}	0.14 ± 0.00 ^{b,B}
<i>Lemna minor</i>	0.19 ± 0.00 ^{a,A}	0.71 ± 0.03 ^{a,C}	1.53 ± 0.07 ^{a,B}
<i>Lemna perpusilla</i>	0.54 ±0.05 ^{c,A}	3.37 ±0.26 ^{c,C}	2.16 ± 0.55 ^{c,B}
<i>Landoltia punctata</i>	1.66 ±0.47 ^{c,A}	4.56 ± 0.34 ^{c,C}	0.14 ±0.00 ^{c,B}
Acarbose	12.16 ±0.10		

Values are expressed as mean deviation (n=3). Means with simple letters for samples and capital letters for extractions within a column are significantly different (P<0.05) FW- Fresh weight, DW- Dry weight.

Table 8: Anti-obesity activity of samples in different extracts

Sample	Lipase inhibition (IC ₅₀ (µg/mL)		
	Water	60% Ethanol	70% Ethanol
<i>Spirodela polyriza</i>	6.53 ± 0.11 ^{d,C}	1.39 ± 0.02 ^{d,A}	10.62 ± 0.02 ^{d,B}
<i>Lemna minor</i>	4.29± 0.05 ^{a,C}	2.35 ± 0.01 ^{a,A}	1.75 ± 0.02 ^{a,B}
<i>Lemna perpusilla</i>	8.35 ± 0.01 ^{c,C}	4.18± 0.10 ^{c,A}	2.86± 0.02 ^{c,B}
<i>Landoltia punctata</i>	7.69 ± 0.10 ^{b,C}	3.58 ± 0.01 ^{b,A}	2.62 ± 0.12 ^{b,B}
Orlistat	1.33 ±0.44		

Values are expressed as mean deviation (n=3). Means with simple letters for samples and capital letters for extractions within a column are significantly different (P<0.05).

Table 9: Mean diameter of inhibition zones (mm) of crude extracts from different duckweed varieties against *E. coli* at various concentrations.

Duckweed varieties	Zone of Inhibition								
	W			60% EtOH			70% EtOH		
	20mg/ml	10mg/ml	5mg/ml	20mg/ml	10mg/ml	5mg/ml	20mg/ml	10mg/ml	5mg/ml
SP	17.33± 1.15 ^{a,A,a1}	15.67± 1.15 ^{a,A,b1}	11.67± 1.15 ^{a,A,c1}	13.33± 0.57 ^{a,C, a1}	11.00± 1.00 ^{a,C, b1}	5.00± 0.00 ^{a,C, c1}	16.67 ±0.57 ^{a,B, a1}	8.00 ±1.00 ^{a, B, b1}	5.00 ±0.00 ^{a, B, c1}
LaP	14.67±0.57 ^{c,A, a1}	13.67±1.15 ^{c,A, b1}	5.67±0.57 ^{c,A, c1}	5.33± 0.57 ^{c,C, a1}	5.67±0.57 ^{c, C, b1}	5.00±0.00 ^{c, C, c1}	15.3± 0.57 ^{c,B, a1}	14.67 ± 0.57 ^{c,B, b1}	5.00± 0.00 ^{c, B, c1}
LP	16.00 ±1.00 ^{b,A, a1}	13.33 ± 1.15 ^{b,A, b1}	9.67± 1.15 ^{b,A, c1}	10.33± 0.57 ^{b,C, a1}	5.00± 0.00 ^{b,C, b1}	5.00± 0.00 ^{b,C, c1}	11.00± 1.00 ^{b,B, a1}	13.00 ±1.00 ^{b, B, b1}	9.67±1.15 ^{b,B, c1}
LM	11.00 ± 1.00 ^{b,A, a1}	8.67 ± 0.57 ^{b,A, b1}	9.33± 0.57 ^{b,A, c1}	11.67 ±0.57 ^{b,C, a1}	7.67 ±0.57 ^{b,C, b1}	5.33 ± 0.57 ^{b,C, c1}	12.67 ± 0.57 ^{b,B, a1}	15.33± 0.57 ^{b,B, b1}	10.67± 1.15 ^{b,B, c1}

Values are expressed as mean ± standard deviation (n=3). Means with simple letters for samples, capital letters for extractions and italic letters for concentrations. Different letters within the column are significantly different (P<0.05)

Table 10: Mean diameter of inhibition zones (mm) of crude extracts from different duckweed varieties against *S. aureus* at various concentrations.

Duckweed varieties	Zone of Inhibition								
	W			60% EtOH			70% EtOH		
	20mg/ml	10mg/ml	5mg/ml	20mg/ml	10mg/ml	5mg/ml	20mg/ml	10mg/ml	5mg/ml
SP	15.33 ± 0.57 ^{b,C,a1}	15.00± 1.00 ^{b,C,b1}	11.00± 1.00 ^{b,C,c1}	16.67 ±0.57 ^{b,A, a1}	16.00 ±1.00 ^{b, A, b1}	11.67 ± 2.89 ^{b,A, c1}	19.33± 0.57 ^{b,B, a1}	14.33 ± 0.57 ^{b,B, b1}	9.67± 0.57 ^{b,B, c1}
LaP	21.00 ± 1.00 ^{a,C, a1}	15.67 ± 1.15 ^{a,C, b1}	13.33 ± 0.57 ^{a,C, c1}	20.33± 0.57 ^{a,A, a1}	18.00± 1.00 ^{a,A, b1}	16.00± 1.00 ^{a,A, c1}	23.00± 1.00 ^{a, B, a1}	19.33 ± 0.57 ^{a, B, b1}	10.67± 0.57 ^{a, B, c1}
LP	9.67 ± 1.15 ^{b,C, a1}	10.00 ± 1.00 ^{b,C, b1}	9.33 ± 0.57 ^{b,C, c1}	21.33± 1.15 ^{b,A, a1}	16.00± 1.00 ^{b, A, b1}	9.33 ± 0.57 ^{b,A, c1}	21.00 ± 1.00 ^{b, B, a1}	20.33± 0.57 ^{b,B, b1}	13.33 ±1.15 ^{b,B, c1}
LM	13.3 ± 1.15 ^{b,C, a1}	9.33±0.57 ^{b,C, b1}	9.67± 0.57 ^{b,C, c1}	21.33± 0.57 ^{b,A, a1}	19.33±0.57 ^{b,A, b1}	18.00±1.00 ^{b,A, c1}	18.67± 0.57 ^{b,B, a1}	9.67±0.57 ^{b, B, b1}	9.33± 0.57 ^{b,B, c1}

Values are expressed as mean ± standard deviation (n=3). Means with simple letters for samples, capital letters for extractions and italic letters for concentrations. Different letters within the column are significantly different (P<0.05)

Table 11: Mean diameter of inhibition zones (mm) of crude extracts from different duckweed varieties against *A.niger* at various concentrations.

Duckweed varieties	Zone of Inhibition								
	W			60% EtOH			70% EtOH		
	20mg/ml	10mg/ml	5mg/ml	20mg/ml	10mg/ml	5mg/ml	20mg/ml	10mg/ml	5mg/ml
SP	8.67 ± 0.57 <i>a, A, a₁</i>	9.67±1.15 ^a , <i>A, a₁</i>	9.33± 0.57 <i>a, A, a₁</i>	6.67±0.57 ^a , <i>B, a₁</i>	9.00±0.00 ^a , <i>B, a₁</i>	9.67±1.15 <i>a, B, a₁</i>	10.67±0.57 ^a , <i>A, a₁</i>	8.67±0.57 <i>a, A, a₁</i>	6.33±0.57 ^a , <i>A, a₁</i>
LaP	9.33± 0.57 ^a , <i>A, a₁</i>	10.00 ± 1.00 <i>a, A, a₁</i>	10.33± 0.57 <i>a, A, a₁</i>	8.33 ± 0.57 <i>a, B, a₁</i>	9.00 ± 0.00 <i>a, B, a₁</i>	8.67 ± 0.57 <i>a, B, a₁</i>	8.33± 0.57 ^a , <i>A, a₁</i>	6.33± 0.57 <i>a, A, a₁</i>	10.00± 1.73 ^a , <i>A, a₁</i>
LP	7.00± 0.00 ^b , <i>A, a₁</i>	8.00± 1.00 ^b , <i>A, a₁</i>	6.00± 0.00 ^b , <i>A, a₁</i>	7.33± 0.57 <i>b, B, a₁</i>	6.67 ± 0.57 <i>b, B, a₁</i>	6.67± 0.57 <i>b, B, a₁</i>	9.00± 0.00 ^b , <i>A, a₁</i>	10.33± 0.57 ^b , <i>A, a₁</i>	7.00± 1.73 <i>b, A, a₁</i>
LM	9.33± 1.15 <i>b, A, a₁</i>	6.67± 0.57 ^b , <i>A, a₁</i>	8.67± 0.5 <i>b, A, a₁</i>	8.33 ± 0.57 ^b , <i>B, a₁</i>	6.33± 1.15 ^b , <i>B, a₁</i>	6.67± 0.57 ^b , <i>B, a₁</i>	8.33± 0.57 <i>b, A, a₁</i>	8.33± 0.57 ^b , <i>A, a₁</i>	7.33± 0.57 ^b , <i>A, a₁</i>

Values are expressed as mean ± standard deviation (n=3). Means with simple letters for samples, capital letters for extractions and italic letters for concentrations. Different letters within the column are significantly different (P<0.05)

Table 12: Mean diameter of inhibition zones (mm) of crude extracts from different duckweed varieties against *C. albicans* at various concentrations.

Duckweed varieties	Zone of Inhibition								
	W			60% EtOH			70% EtOH		
	20mg/ml	10mg/ml	5mg/ml	20mg/ml	10mg/ml	5mg/ml	20mg/ml	10mg/ml	5mg/ml
SP	5.67± 0.57 ^b , <i>B, b₁</i>	9.00± 1.00 ^b , <i>B, c₁</i>	8.33± 1.15 ^b , <i>B, a₁</i>	6.33± 0.57 ^b , <i>C, b₁</i>	6.67± 1.15 ^b , <i>C, c₁</i>	6.33 ± 0.57 ^b , <i>C, a₁</i>	8.67 ± 0.57 ^b , <i>A, b₁</i>	26.67± 0.57 ^b , <i>A, c₁</i>	7.67 ± 1.15 ^b , <i>A, a₁</i>
LaP	5.67± 0.57 ^a , <i>B, b₁</i>	10.00± 1.00 ^a , <i>B, c₁</i>	30.67± 1.15 ^a , <i>B, a₁</i>	15.67± 0.57 ^a , <i>C, b₁</i>	10.67± 2.31 ^a , <i>C, c₁</i>	5.33± 0.57 <i>a, C, a₁</i>	31.00± 1.00 <i>a, A, b₁</i>	7.33 ± 0.57 <i>a, A, c₁</i>	31.00± 1.00 <i>a, A, a₁</i>
LP	12.33 ± 0.57 ^c , <i>B, b₁</i>	5.67 ± 0.57 <i>c, B, c₁</i>	7.00 ± 0.00 <i>c, B, a₁</i>	5.67± 0.57 <i>c, C, b₁</i>	7.67± 0.57 <i>c, C, c₁</i>	6.67 ± 1.15 <i>c, C, a₁</i>	7.00 ± 1.00 <i>c, A, b₁</i>	8.67± 1.15 ^c , <i>A, c₁</i>	9.67 ± 1.15 ^c , <i>A, a₁</i>
LM	9.33 ± 0.57 ^c , <i>B, b₁</i>	7.00 ± 0.00 <i>c, B, c₁</i>	12.67± 0.57 <i>c, B, a₁</i>	7.67± 0.57 <i>c, C, b₁</i>	7.00 ± 0.00 <i>c, C, c₁</i>	5.33 ± 0.57 <i>c, C, a₁</i>	6.33 ± 0.57 <i>c, A, b₁</i>	5.33 ± 0.57 <i>c, A, c₁</i>	5.67± 0.57 <i>c, A, a₁</i>

Values are expressed as mean ± standard deviation (n=3). Means with simple letters for samples, capital letters for extractions and italic letters for concentrations. Different letters within the column are significantly different (P<0.05)

Table 13: Content of polyphenolic compounds in methanol extract of four duckweed varieties

Phenolic Compound	Amount of phenolic compounds in Duckweed Varieties ($\mu\text{g}/\text{mg DM}$)			
	LM	LP	LaP	SP
Vanilin acid	0.00165	0.45825	ND	0.00005
Sinapic acid	ND	0.00285	0.00615	0.00665
Rutin acid	3.0588	2.9881	2.9612	2.9958
P-Coumaric acid	ND	0.0009	0.03885	0.02615
Gallic acid	ND	ND	0.2634	0.3996
Ferrulic acid	ND	0.00505	0.003	0.00065
Chlorogenic acid	0.002	ND	0.0002	0.00665
Catechin acid	0.0085	0.00285	0.0001	0.0002
Caffeic acid	0.00145	ND	0.01205	0.01765

ND not detected (Limit of Detection = 0.1 $\mu\text{g}/\text{mL}$)

Figures

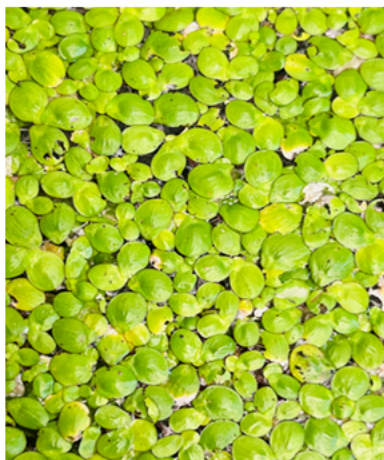


Fig. 1a



Fig. 1b



Fig. 1c



Fig. 1d

Figure 1

Examined duckweed varieties, *Spirodela polyrhiza* (1a) *Lemna minor* (1b) *Lemna perpusilla* (1c) *Landoltia punctata*(1d)

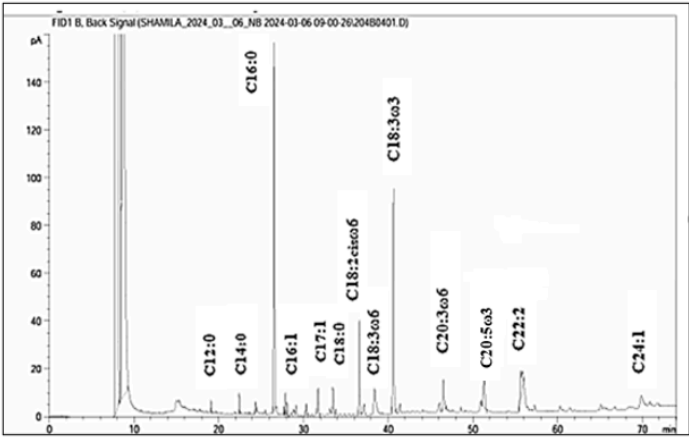


Fig. 2a

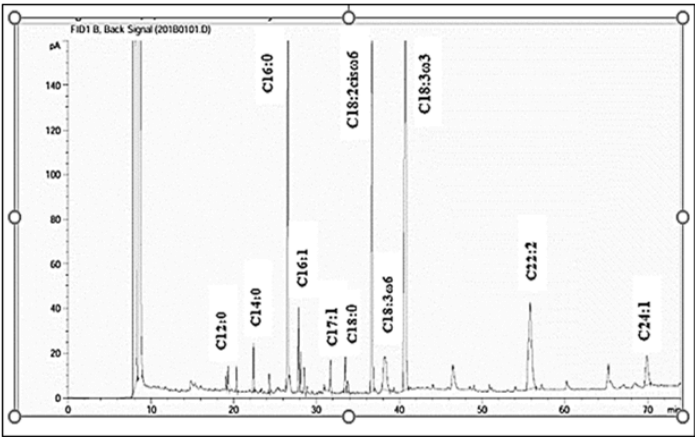


Fig. 2c

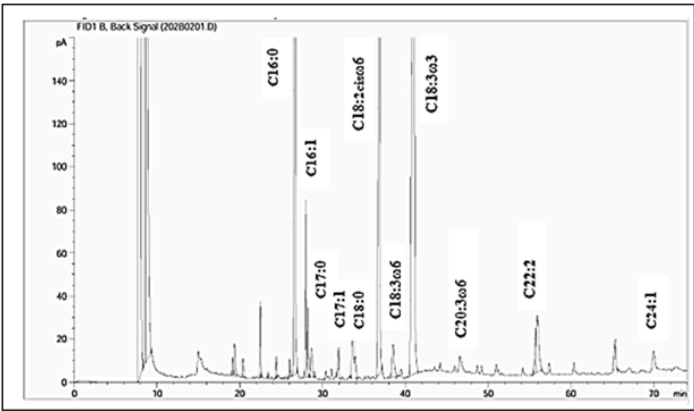


Fig. 2b

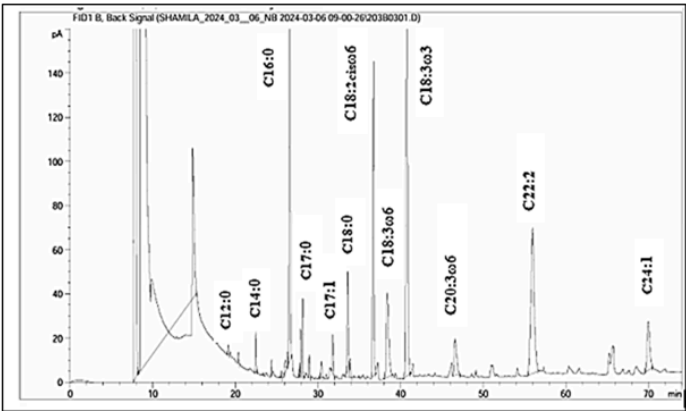


Fig. 2d

Figure 2

Fatty Acid Profile of Duckweed Varieties Analyzed by GC-FID, *Spirodela polyrhiza*(2a) *Lemna minor* (2b) *Lemna perpusilla* (2c) *Landoltia punctata*(2d)

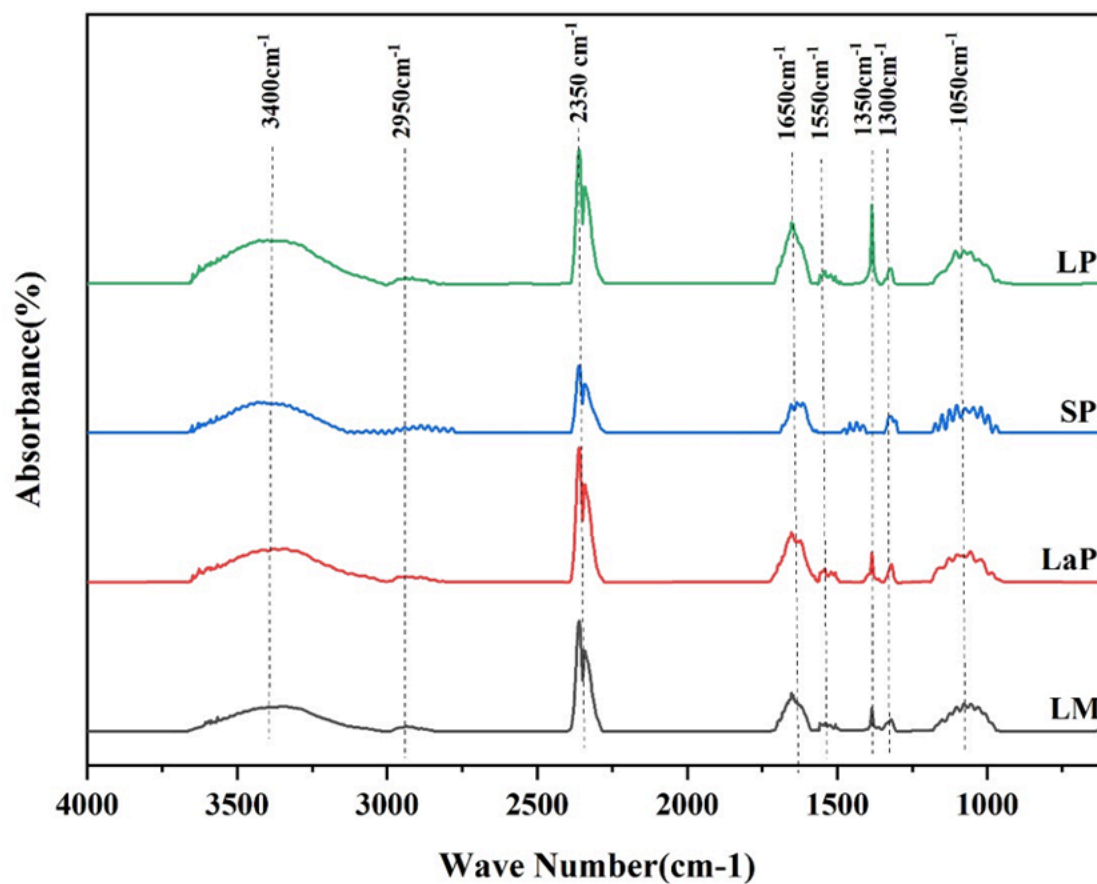


Figure 3

Fourier transform infrared spectra of examined duckweed varieties

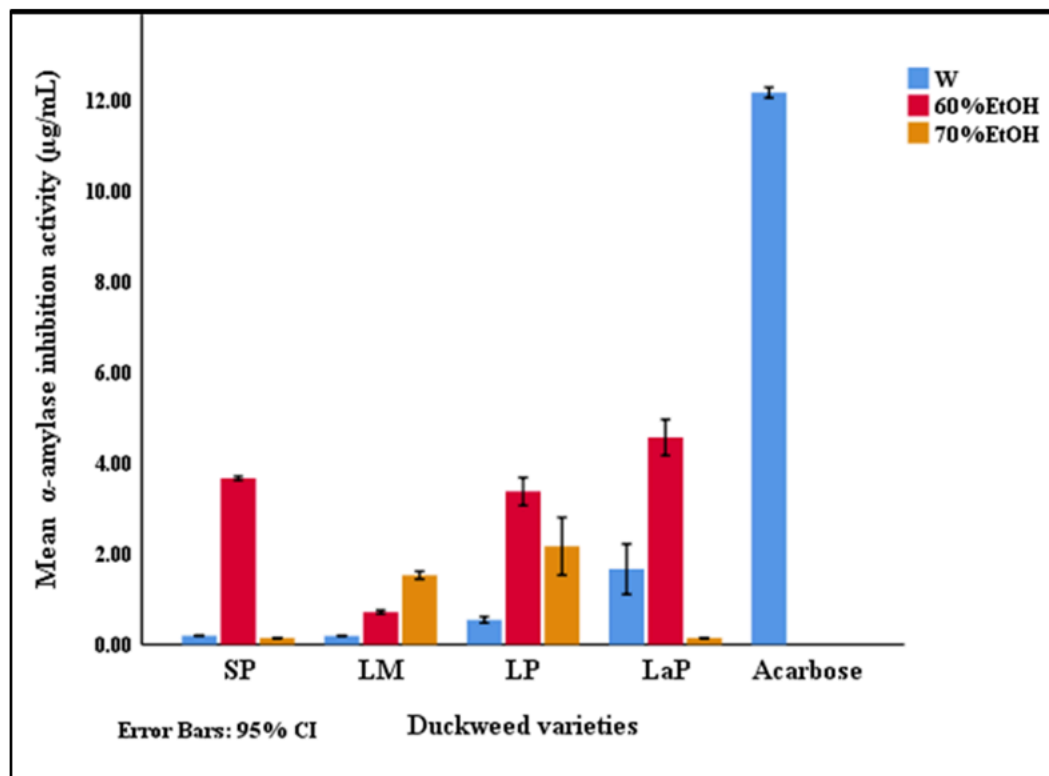


Figure 4

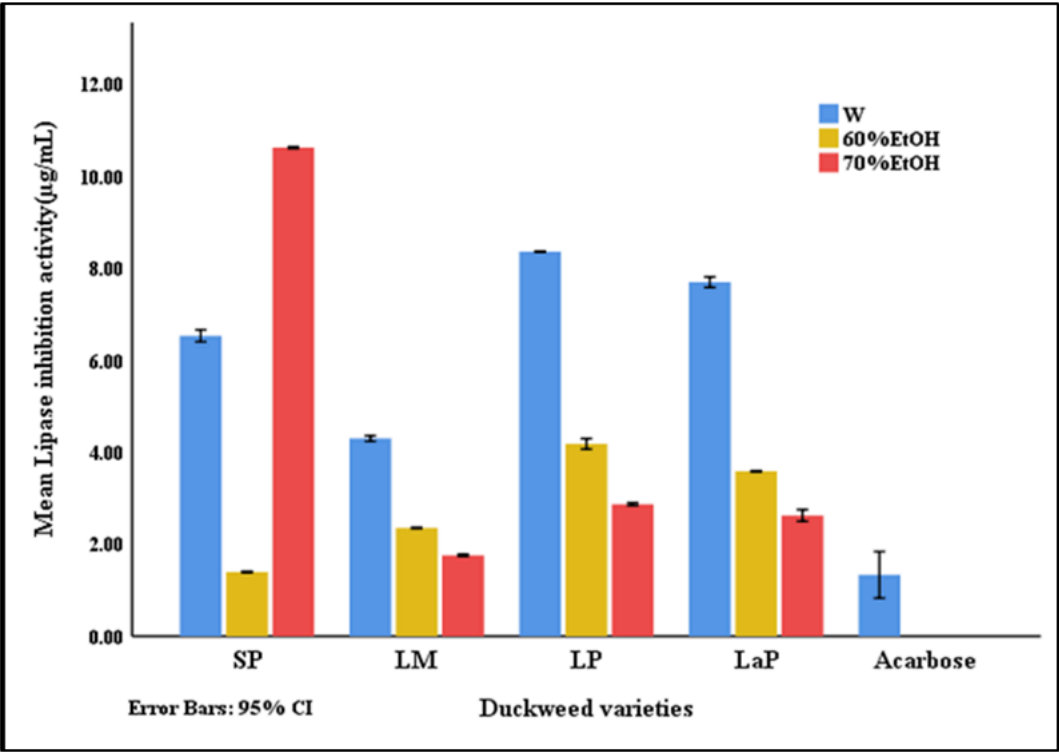


Fig 5

Figure 5

Evaluation of α Lipase inhibition in duckweed varieties using solvent extracts

Supplementary Files

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- [GraphicalAbstract.docx](#)