Chemical study of the macrophyte duckweed (*Lemna minor* L.)

Estudio químico de la macrófita lenteja de agua (*Lemna minor* L.)

Estudio químico da macrófita lentilha-d`água (*Lemna minor* L.)

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Abstract

Duckweed (*Lemna minor* L.) has attracted considerable attention in the scientific field due to its nutritional contribution and capacity to phytoremediate waters. Therefore, the objective of the study was to analyze the chemical composition of the macrophyte (*Lemna minor*) from natural environments. Chemical compounds and Weende composition were determined from the plant, and the fresh weight gain was observed in different types of water (deep well and bovine slurry), waters that were subjected to physical-chemical analysis. The chemical analysis of the macrophyte resulted in the presence of 1.42 $mg.g^{-1}$ of total chlorophyll; 2.35 $mg.kg^{-1}$ of ascorbic acid; tannin content less than 2.50 $mg.kg^{-1}$; 45.34 $mg.kg^{-1}$ of phenols; also the presence of alkaloids, phenols and reducing sugars in the chemical screening. The Weende analysis indicates a composition of: 89 % of dry matter, 30 % of crude protein, 4 % of gross energy, 3.2 % of ether extract, 15 % of ashes, 32 % of nitrogen-free extract and 10 % of fiber. The fresh weight gain of *Lemna minor* obtained in water contaminated with bovine slurry increased significantly 13 $g.day.m^3$ and 5 $g.day.m^3$ in deep well water. The physical-chemical properties of the water improve their quality 16 days after treatment with this aquatic plant with respect to the initial analysis. This macrophyte exhibits remarkable phytoremediation properties to absorb, metabolize and stabilize various pollutants effective in the purification of contaminated waters.

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Resumen

La lenteja de agua (*Lemna minor* L.) ha provocado una considerable atención en el ámbito científico por su aporte nutricional y capacidad de fitorremediar aguas. Por tanto, el objetivo del estudio fue analizar la composición química de la macrófita (*Lemna minor*) proveniente de ambientes naturales. De la planta se determinaron compuestos químicos, composición Weende, además se observó la ganancia de peso fresco en diferentes tipos de agua (pozo profundo y purín bovino), aguas que fueron sometidas a análisis físico químico. El análisis químico de la macrófita resultó con presencia de 1,42 $mg.g^{-1}$ de clorofila total; 2,35 $mg.kg^{-1}$ de ácido ascórbico; contenido de taninos menor a 2,50 $mg.kg^{-1}$; 45,34 $mg.kg^{-1}$ de fenoles; además con presencia de alcaloides, fenoles y azucares reductores al screening químico. El análisis de Weende indica una composición de: 89 % materia seca, 30 % proteína cruda, 4 % energía bruta, 3,2 % extracto etéreo, 15 % cenizas, 32 % extracto libre de nitrógeno y 10 % fibra. La ganancia de peso fresco de *Lemna minor* obtenida en agua contaminada con purín bovino aumentó significativamente 13 $g.día.m^3$ y 5 $g.día.m^3$ en agua pozo profundo. Las propiedades físico-químicas del agua mejoran su calidad a los 16 días de tratadas con esta planta acuática con respecto al análisis inicial. Esta macrófita exhibe notables propiedades de fitorremediación para absorber, metabolizar y estabilizar diversos contaminantes eficaces en la depuración de aguas contaminadas.

Palabras clave: aguas residuales, fitorremediación, fitoquímica, tratamiento.

Resumo

A lentilha-d’água (*Lemna minor* L.) tem causado considerável atenção no meio científico por sua contribuição nutricional e capacidade de fitorremediar águas. Portanto, o objetivo do estudo foi analisar a composição química da macrófita (*Lemna minor*) de ambientes naturais. Os compostos químicos e a composição Weende foram determinados a partir da planta, e o ganho de massa fresca foi observado em diferentes tipos de água (poço fundo e esterco bovino), águas que foram submetidas a análises físico-químicas. A análise química da macrófita resultou na presença de 1,42 $mg.g^{-1}$ de clorofila total; 2,35 $mg.kg^{-1}$ de ácido ascórbico; teor de taninos inferior a 2,50 $mg.kg^{-1}$; 45,34 $mg.kg^{-1}$ de fenóis; também com a presença de alcalóides, fenóis e açúcares redutores na triagem química. A análise de Weende indica composição de: 89 % de matéria seca, 30 % de proteína bruta, 4 % de energia bruta, 3,2 % de extrato etéreo, 15 % de cinzas, 32 % de extrato isento de nitrogênio e 10 % de fibra. O ganho de massa fresca de *Lemna minor* obtido em água contaminada com dejeto bovino aumentou significativamente em 13 $g.dia.m^3$ e 5 $g.dia.m^3$ em água de poço profundo. As propriedades físico-químicas da água melhoram sua qualidade 16 dias após o tratamento com esta planta aquática em comparação com a análise inicial. Esta macrófita apresenta notáveis propriedades de fitorremediação para absorver, metabolizar e estabilizar vários contaminantes eficazes na purificação de águas contaminadas.

Palavras-chave: águas residuais, fitorremediação, fitoquímica, tratamento.

Introduction

Duckweed (*Lemna minor* L.) is a macrophyte that has attracted increasing scientific interest due to its chemical characteristics and versatility in the phytoremediation of polluted waters and in human and animal food (Ávila *et al*., 2020). It is capable of assimilating water pollutants and has a high nutritional value, which positions it as a promising resource to address current environmental and food challenges (Salas *et al*., 2016). This dual potential highlights its importance in ecological sustainability and food security.

In the context of phytoremediation, duckweed has demonstrated its effectiveness in removing heavy metals (Intriago *et al*., 2024), agrochemicals (Jaimes Prada *et al*., 2024) and other organic pollutants (Benavides *et al*., 2021), offering a sustainable and economical option for cleaning up aquatic ecosystems (Garaboto, 2015). Its capacity for bioaccumulation and tolerance to adverse conditions positions it as an invaluable tool in the restoration of water bodies affected by human activity and industry (Vargas & Barajas, 2016).

Duckweed also stands out for its chemical composition rich in protein, vitamins, minerals and bioactive compounds (Blanco, 2018). This nutritional profile makes it a key resource for improving food availability for both humans and animals (Mercado-Albarrán *et al*., 2019). In addition, its rapid growth and ability to adapt to diverse conditions make it a sustainable alternative for food production (Bello-Armenta & Marín, 2023).

L. minor has demonstrated a remarkable ability to absorb toxic metals such as lead (Pb), cadmium (Cd), mercury (Hg) and chromium (Cr) from water, an ability that results from its ability to accumulate metals in its tissues (Irin & Hasanuzzaman, 2024). Furthermore, duckweed not only absorbs heavy metals, but can also transform heavy metals into a less toxic form for the ecosystem through metabolic processes (Liebers *et al*., 2023).

The identification and characterisation of metabolic compounds in *L. minor* is crucial to understand its potential as a phytoremediation plant. A study by Miras-Moreno *et al*. (2022) suggests that secondary metabolites, such as flavonoids and phenolic compounds, may increase the tolerance of *L. minor* to heavy metals, acting as chelating agents that reduce the toxicity of these metals at the cellular level. Chlorophyll indicates the photosynthetic capacity of the plant, affecting its growth and pollutant uptake. Ascorbic acid acts as an antioxidant, protecting the plant from environmental stress and improving its survival. In addition, tannins and phenols help to sequester heavy metals and reduce the toxicity of pollutants, thus increasing their ability to purify water (Vámos *et al*., 2023).

The objective was to analyse the chemical composition of the macrophyte (*Lemna minor* L.) . This study aims to contribute to the understanding of its role in the detoxification of aquatic ecosystems, its nutritional use and to promote its sustainable use.

Materials and methods

The macrophyte sample was taken from a natural aquifer in Ciudadela San Fernando 2, El Triunfo, Guayas province, Ecuador, located at 2º31’41‘‘ S - 79º41’50‘‘ W at 44 m.a.s.l. 1 kg fresh weight of the duckweed was taken to the chemistry laboratory of the biotechnology department of the State University of Milagro, Ecuador.

Quantification of Chlorophyll content

Chlorophyll was determined using the spectrophotometric method of Hiscox & Israelstam (1979). 0.5 g of fresh plant sample was ground in a mortar and pestle and mixed with 10 mL of 80 % acetone to create a homogeneous suspension, which was then immediately filtered and collected in a test tube. To avoid degradation of the chlorophyll, the test tube was wrapped in aluminium foil. The absorbance of the solution was measured at 663 nm and 645 nm using a spectrophotometer.

Determination of ascorbic acid

(a) Mobile phase: 15.6 g disodium phosphate and 12.2 g dipotassium phosphate were dissolved in 2000 mL water, adjusting the pH to 2.5 ± 0.05 with phosphoric acid. (b) Chromatographic system: A Perkin Elmer 200 Series liquid chromatograph at 245 nm with a 6 x 150 mm column and a flow rate of 0.60 mL.min^{-1} was used. The standard preparation was introduced into the chromatograph and the reading was taken. c) Sample extraction: For sample extraction, 5:100 metaphosphoric acid was used and dilutions of the sample were made to 0.5 mg.mL^{-1} to adjust the method (Gutierrez *et al*., 2007).

Determination of total tannins

It was established under the Folin Ciocalteu method, 0.1 g of dry sample was weighed and mixed with 10 mL of distilled water, shaking using a vortex for 1 minute and centrifuging at 3000 rpm for 10 minutes. The supernatant was collected to determine tannins. A standard solution of tannic acid (0 to 100 mg.L^{-1}) was prepared. 0.5 mL of each standard solution was added to a test tube, followed by the addition of 2.5 mL of Folin-Ciocalteu's reagent. After 5 minutes, 2 mL of 20 % sodium carbonate was added, mixed and the volume adjusted to 10 mL. Finally, absorbance was measured at 765 nm and a calibration curve was constructed (Kasay *et al*., 2013).

Determination of total phenols

The phenolic concentration of the hydroalcoholic extract of duckweed was measured spectrophotometrically using the Folin Ciocalteu chemist. The calibration curve was adjusted with gallic acid and four concentrations (1, 3, 6 and 9 mg.L^{-1} of distilled water) were prepared. To the solutions 250 µL of Folin Ciocalteu 1N and 1250 μL of 20 % sodium carbonate were added and allowed to stand for several hours. Finally, absorbance was recorded at 760 nm (Blainski *et al*., 2013).

Determination of secondary metabolites

Secondary metabolites were identified from the 50/50 hydroethanolic extract. To 1 mL of the extract 3 drops of Mayer's Reagent was added, the formation of a white coloured precipitate is indicative of the presence of alkaloids, while, to 1 mL of the extract 3 drops of Wagner's Reagent was added, the formation of a brownish red/dark brown coloured precipitate indicates the presence of alkaloids. Tannins were detected with 45 % iron chloride. 1 mL of sol A and 1 mL of sol B is added to 0.5 mL of the extract and heated in a water bath for 10 min, the appearance of a dark red cuprous precipitate is reported as positive for reducing sugars (Díaz Solares *et al*., 2015).

Weende analysis

Moisture was measured by oven-drying at 105 °C for 24 hours, crude protein was analysed by the Kjeldahl method, and ash was obtained by ashing at 500 °C. Ether extract was extracted using Soxhlet, while crude fibre was determined by acid and base digestion (Grenfield & Southgate, 2003).

Description of physicochemical analysis of water treated with (*Lemna minor* L.)

Two 1 m³ pools were set up, one with deep well water and one with bovine sewage water. 150 grams of fresh *L. minor* L. were introduced into each pool. The physico-chemical characteristics of the water before treatment and 16 days later (resistivity, salinity, total dissolved solids, conductivity, dissolved oxygen, temperature) were analysed with the help of a multi-parameter meter (HACH HQ Field Case, Case Model: HQ40D53000000). In addition, the fresh plant mass of *L. minor* was assessed after 16 days. The daily weight gain of duckweed was determined according to:

$$Daily\ fresh\ weight\ gain \rightarrow \frac{Final\ \ mass - Initial\ \ mass}{Days}.$$

Results and discussion

Table 1 presents the quantitative chemical characterisation of duckweed (*L. minor*) essential for understanding its properties and usefulness.

Table 1. Chemical characterisation of duckweed (*Lemna minor* L.).

Parameters	Results	Unit
Total chlorophyll	1.42	mg.g^{-1}
Ascorbic acid	2.35	mg.kg^{-1}
Tannins	<2.50	mg.kg^{-1}
Phenols	45.34	mg.kg^{-1}

Source: Own elaboration

Total chlorophyll in duckweed was 1.42 mg.g^{-1}, being an essential pigment for photosynthesis and used in animal feed (Krupka *et al*., 2023). *L. minor* presented 2.35 mg.kg^{-1} of ascorbic acid, essential for humans and animals, promoting growth and immune functions (Mora-Herrera *et al*., 2011). It was found <2.50 mg.kg^{-1} of tannins, within a safe and acceptable range for both human consumption and animal feed (Eck-Varanka *et al*., 2023; Inguanez *et al*., 2022; Utami *et al*., 2018). Finally, duckweed showed a high concentration of phenols 45.34 mg.kg^{-1}, which act as phytoalexins and antioxidants (Gómez *et al*., 2020; Radulović *et al*., 2020; Hernández-Moreno *et al*., 2022).

Table 2 shows the qualitative phytochemical screening of *L. minor* which was performed on the 50/50 % hydroethanolic extract obtained by maceration, extraction, colouring and foaming reactions.

Table 2. Phytochemical screening of *L. minor* L.

Parameters	Method	Metabolite	Results
Phytochemical screening	Qualitative colorimetric	Alkaloids	
		Mayer Reactant	Positive
		Wagner reactant	Positive
		Tannins	
		Ferric chloride assay	Positive
		Reducing sugars	
		Fehling test	Positive

Chemical screening of *L. minor* showed the presence of alkaloids, phenols and reducing sugars. Alkaloids act as defence mechanisms in plants and may have medicinal or hallucinogenic effects, depending on their interaction with the central nervous system (Cholich *et al*., 2021; Regni *et al*., 2024). Phenols are organic compounds that contribute

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to pigmentation and possess antioxidant, anti-inflammatory and anticarcinogenic properties, benefiting human health (Jiménez *et al*., 2022). Reducing sugars are essential for the transport and absorption of nutrients in plants and are a significant source of energy in human and animal diets, serving as an alternative to traditional protein sources (Chauca Espinoza *et al*., 2017).

Table 3 shows the analyses of deep well water and cattle slurry treated with *L. minor*.

Table 3. Analyses of deep well water and bovine slurry.

Parameter	Deep well			Cattle slurry		
	M¹	M²	σ	M¹	M²	σ
Resistivity (kΩ.cm^{-1})	3.36	3.85	0.25	3.04	3.18	0.07
Salinity (%)	0.14	0.12	0.01	0.16	0.15	0.01
Total Dissolved Solids (mg.L^{-1})	142.5	123.6	9.45	157.9	150.5	3.70
Conductivity ($\mu S.cm^{-1}$)	198	260	19	329	317	36
Dissolved oxygen (mg.L^{-1})	7.68	6.96	0.36	6.81	6.58	0.12
pH	8.32	7.80	0.26	8.32	7.50	0.41
Temperature (ºC)	26.5	26.7	0.1	27.2	27.7	0.25

M¹ Initial sample; M² Sample 16 days later; σ Standard deviation.

The resistivity of well water increased to 0.49 ohms per centimetre after 16 days of duckweed application, while water contaminated by bovine slurry showed 0.14 ohms per centimetre, indicating an increase in charged particles. According to Sánchez-García (2021), resistivity varies according to the concentration of salts and minerals, highlighting the importance of dissolved ions in the electrical conductivity of water. Improvements in salinity, total dissolved solids and pH parameters were recorded in both well and slurry water, suggesting that *L. minor* optimises the physical parameters of water (Pérez *et al*., 2022). Conductivity also decreased, reaching 38 µS.cm^{-1} and 12 µS.cm^{-1}, indicating the lentil's ability to absorb nutrients and pollutants (Vargas & Barajas, 2016). However, a drop in dissolved oxygen was evidenced, reaching 6.96 mg.L^{-1} in deep well water and 6.58 mg.L^{-1} in slurry water, which may affect the health of the aquatic ecosystem (Diaz, 2002).

However, figure 1 shows the fresh weight of *L. minor* reached in 16 days in different types of water with an initial sowing of 150 grams.

After 16 days of study, a remarkable increase in the plant mass of *L. minor*, an aquatic plant commonly used as an indicator of water quality, was observed. On average, the mass of *L. minor* in the cattle slurry contaminated water increased significantly by 13 g.day.m^{-3}, compared to the modest increase of 5 g.day.m^{-3} observed in the deep well water. Canales-Gutiérrez, (2010) mention that *L. minor* has the ability to increase its biomass production based on the nutrient content of the water where it is found, tripling its biomass in 7 days when fertilisers are added. This explains its increase in fresh weight gain in water with bovine slurry.

The Weende analysis of *L. minor* was determined (table 4).

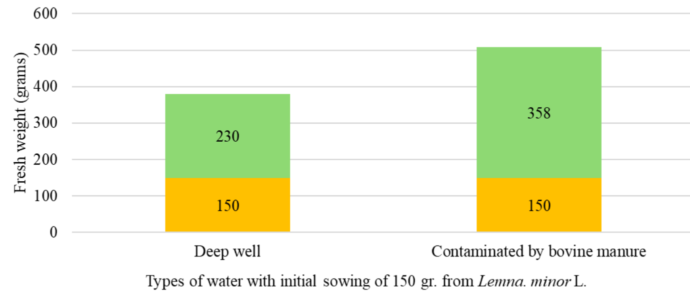

Figure 1. Fresh weight gain of *Lemna minor* L. 16 days after planting in different types of water.

Table 4. Weende analysis of *Lemna minor* L.

Parmeter	Content (%)
Dry matter	89
Crude protein	30
Crude energy	4.0
Ethereal extract	3.2
Ash	15
Free Nitrogen Extract	32
Fibre	10

Source: Own elaboration.

Duckweed is a sustainable source of protein, with up to 40 % of its dry biomass rich in minerals. Its rapid growth makes it ideal for animal, aquaculture and human food. Córdoba *et al*. (2010), Sońta *et al*. (2023) and Soria-Hernández *et al*. (2024) highlight that its inclusion in commercial diets can reduce production costs in tilapia and pig farms by up to 50 %, demonstrating its economic and environmental viability.

Conclusions

Lemna minor L. has been chemically characterised, highlighting the presence of chlorophyll, vitamin C and phenols, with antioxidant properties beneficial to human and animal health. In addition, alkaloids, phenols and reducing sugars, compounds that play crucial roles in the plant's defence mechanisms, were identified.

Water treatment with *L. minor* has shown significant improvement in physical and chemical parameters such as resistivity, salinity, pH and conductivity.

Its remarkable increase in biomass in a short time positions it as a sustainable and protein-rich source. These findings underline the nutritional and environmental value of *L. minor*, highlighting its importance in food security and the preservation of natural resources.

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