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Macrophytes: biomass with high transformation potential and a promising source of bioactive compounds

Macrófitas: biomassa com alto potencial de transformação e uma fonte promissora de compostos bioativos

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RESUMO

As macrófitas possuem um grande potencial de transformação. São plantas aquáticas que auxiliam no equilíbrio do ecossistema, como a lentilha-d'água. Assim, o estudo teve como objetivo avaliar o crescimento de macrófitas cultivadas em águas residuárias, bem como analisar o perfil químico e a eficiência de suas biomoléculas para aplicação biotecnológica. As macrófitas foram coletadas em Três Lagoas (Lemna minor) e Dourados (Landoltia punctata) e transportadas para o laboratório, onde as amostras foram higienizadas em água corrente e destilada, separadas e preparadas para cultivo em recipiente plástico por 15 dias. em diferentes tratamentos. As amostras foram lavadas em água destilada, maceradas e o sobrenadante separado por centrifugação por 10 min a 4.000 rpm, e utilizado para análise molecular com espectrofotômetro (UV-vis) com varredura entre 200 e 800 nm, a atividade antioxidante pelo método de DPPH, composição nutricional e uso por pesquisa exploratória em banco de dados. A análise dos espectros UV-vis sugeriu a presença de aminoácidos e clorofila. L. punctata apresentou maior sequestro de radicais livres quando cultivada em vinhaça, enquanto L. minor apresentou a menor atividade antioxidante em água de peixe. Essas plantas são consideradas uma fonte promissora de biomoléculas que podem ser utilizadas em diferentes processos biotecnológicos.

Palavras-chave: Lemna minor; Landoltia punctata; Biomoléculas, Processos Biotecnológicos

ABSTRACT

Macrophytes have a great potential for transformation. They are aquatic plants that help balance the ecosystem, such as duckweed. Thus, the study aimed to evaluate the growth of macrophytes grown in wastewater, as well as to analyze the chemical profile and efficiency of their biomolecules for biotechnological application. The macrophytes were collected in Três Lagoas (Lemna minor) and Dourados (Landoltia punctata) and transported to the laboratory, where the samples were cleaned in running and distilled water, separated and prepared for cultivation in a plastic container for 15 days. in different treatments. The samples were washed in distilled water, macerated and the supernatant separated by centrifugation for 10 min at 4,000 rpm, and used for molecular analysis with a spectrophotometer (UV-vis) with scanning between 200 and 800 nm, the antioxidant activity by the DPPH method, nutritional composition and use by exploratory database research. Analysis of UV-vis spectra suggested the presence of amino acids and chlorophyll. L. punctata showed the highest free radical scavenging when grown in vinasse, while L. minor showed the lowest antioxidant activity in fish water. These plants are considered a promising source of biomolecules that can be used in different biotechnological processes.

Keywords: Lemna minor; Landoltia punctata; Biomolecules, Biotechnological Processes

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1. INTRODUCTION

The use of renewable sources has contributed effectively to minimizing the action of the greenhouse effect and the preservation of the natural environment. It is a fact that biomasses are the main protagonists in the search for natural sources to expand the energy matrix to meet the growing world demand for energy. In the environmental context, the increase in the use of biomass has reduced the use of oil and contributed to the advancement of a sustainable industrial society with concepts of cleaner production. It is already proven that the use of biomass as an energy source brings promising results, such as the possibility of reducing the emission of gases in the atmosphere that is responsible for climate change (Huang et al 2018). The energy produced through the use of plant biomass can be used directly in different processes requiring low investment.

Brazil emerges as a promising country that aggregates great potential for biomass production, taking advantage of its territorial availability and favourable climate. This country has already consolidated itself with the production of ethanol from sugarcane, highly efficient biomass when it comes to bioenergy conversion. However, other renewable sources can be used both for production and for transformation into energy or other products depending solely on their availability as raw materials (Perea-Moreno et al., 2019).

Among these renewable sources, we highlight the macrophytes that have a great potential for transformation and that are little explored. They are aquatic plants that influence the context of their habitat, constantly maintaining and balancing the ecosystem, conserving the food supply for aquatic beings, having an effective action in the accumulation of nutrients and heavy metals and playing an important role in the maintenance of diversity. other aquatic organisms (Haroon et al., 2020; Othman and Haroon, 2020). However, when they grow in a disorderly way, they cause environmental problems. However, they are excellent biofactories of bioactive molecules that can be used as a promising source for the production of various antibiotics, therapeutic, bioplastic, biofuel, biogas, animal feed and paper products (Haroon, 2020, Haroon and Abdel- Aal, 2016; El -Sheekh et al., 2017).

These plants develop and colonize different types of aquatic environments such as swamps or different water bodies, they have great growth potential in addition to good adaptive capacity in the face of habitat changes (Oliveira Junior et al., 2020). In the Brazilian territory, there are numerous species of macrophytes that have a high phytoremediation capacity, being used to remove contaminants present in water bodies (Swain et al., 2014). Some characteristics of these plants can be highlighted, such as *Lemna minor* belonging to the family of vascular aquatic plants that float and are fast-growing even in adverse conditions. This plant produces, on average, 20 individuals in its vegetative reproductive cycle (Kutschera; Niklas, 2015). This propagation characteristic makes these plants a possible raw material for different industrial processes.

Macrophytes have in their chemical composition sources of carbon such as glucose, on average 31%, crude protein ranging from 36 to 46%, in addition to other compounds such as lignin, cellulose, fibre, lipids and starch. However, the availability of these compounds may vary according to the species (Matos et al., 2014). A good example was reported in the studies by Verma and Suthar (2015), who cultivated Lemna and observed an expressive accumulation of proteins, amino acids and fibres, demonstrating the potential for using this aquatic plant as a raw material to obtain industrial products and even as a nutritional supplement for animal feed. This condition is a result of the high capacity that these plants have for the absorption and conversion of nutrients from the medium into biomass (Zhao et al., 2012).

Landoltia punctata is another macrophyte species that can be found in different parts of the world. It is a small floating plant, with leaves that produce fine roots and that prefers shallow, nutrient-rich waters such as ponds, ditches and swamps (Lalau et.al 2015). This macrophyte is also known as duckweed and is reproduced by vegetative leaf budding (Xiao et.al 2013), forming an interconnected complex that acts as a single large system. *L. punctata* is widely used for wastewater treatment to minimize the load of contaminants. This plant has fast growth and with that, there is an accumulation of biomass, this characteristic can be used to use this plant as a raw material for the production of energy with bioethanol (Tao et al., 2013).

Wastewater presents an ideal niche for the cultivation of macrophytes, as these aquatic plants need nutrients and organic matter. According to Xiao et al. (2013), these waters are an ideal environment for the development of macrophytes, in addition, these plants have a high growth rate. The annual average growth and accumulation of biomass is 7.26 g in dry weight m2 /day, with a production of 26.50 tons per hectare, thus presenting a higher biomass yield compared to terrestrial plants (Zhao et al, 2014). Given the above, this study aims to evaluate the growth of two species of macrophytes, *Landoltia* *punctata* and *Lemna minor*, cultivated in wastewater, as well as to analyze the chemical profile and the efficiency of their biomolecules for biotechnological application.

2. MATERIAL AND METHODS

2.1 Collection and cultivation of samples

Macrophytes were collected in the municipality of Três Lagoas (*Lemna minor*) and Dourados (*Landoltia punctata*). The plants were collected and transported in plastic containers to the laboratory for the respective analyses. The samples were washed in running and distilled water, remaining at rest for 10 minutes, then separated and prepared for cultivation. Macrophytes were cultivated in a plastic container for 15 days in different treatments (Table 1). After this period, the samples were washed in distilled water and macerated in a porcelain crucible and centrifuged for 10 min at 4,000 rpm, and the supernatant was used for analysis.

Table 1 – Treatments used for the development of the study

Treatments	Composition (mL)
Pond water + Vinasse	2900/1001
Pond water + Fish water	1900/100
Pond water + Distilled water	1900/100

Source: Authorship (2022)

2.2 Analysis of the molecular profile of macrophytes

For the analysis of the compounds present in the samples, the supernatant obtained was added to 1 mm quartz cuvettes and the reading was carried out with a spectrophotometer (UV-vis Global Trade Technology model) and consisted of a scan between 200 and 800 nm.

2.3 Evaluation of antioxidant activity by DPPH

Antioxidant activity was evaluated using the stable free radical scavenging activity 1,1-diphenyl-2-picrylhydrazyl (DPPH). For this purpose, 100µl aliquots of the sample were added to 3 mL of the DPPH solution and kept in the dark for 30 min. After the reaction period, the samples were analyzed by spectrophotometer at 517nm, according to the method described by Deng et al. (2011).

2.4 Nutritional composition and use of lentils

Exploratory research was carried out in a database published on the composition of duckweed and the potential for use as a raw material for biotechnological processes.

3. RESULTS AND DISCUSSION

The UV-VIS spectrum profile showed similarities in the absorption peaks of macrophytes grown on different substrates. Peaks were observed in the bands corresponding to 240 to 310 nm, these peaks may be pertinent to chemical compounds which present an aromatic ring in molecular structure, such as the presence of the amino acids tyrosine, and tryptophan and phenylalanine (Figures 1A and 1B).

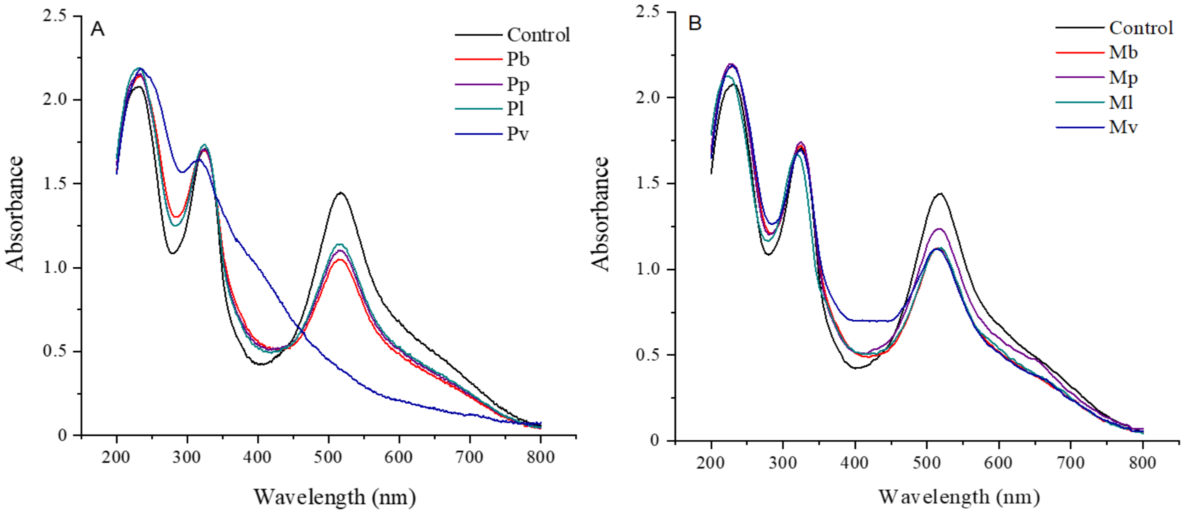

Figure 1 – Analysis of the UV-vis spectra of samples of macrophytes *L. punctata* (A) and *L. minor* (B) cultivated on different substrates. **Source:** Authorship (2022)

The evaluation of the antioxidant activity concerning the treatments carried out in the samples of macrophytes showed that there was a capacity to scavenge the DPPH radical. It can be observed that *L. punctata* showed greater scavenging of free radicals when cultivated with vinasse. On the other hand, *L. minor* showed the lowest antioxidant activity in the water-based treatment of fish (Figure 2).

Equations are inserted in the body of the text, being numbered in the right side within parenthesis, as shown below: The macrophyte *L. minor* has been highlighted as a phytoremediator in numerous chemical pollutants present in wastewater. This plant has shown efficient results in the absorption of heavy metals, agrochemicals, organic and pharmacological compounds, and a tenuous behaviour about petrochemical, radioactive and nanomaterial residues (Ekperus et al., 2019).

Figure 2 – Evaluation of DPPH reduction in samples of macrophytes *L. punctata* and *L. minor*.

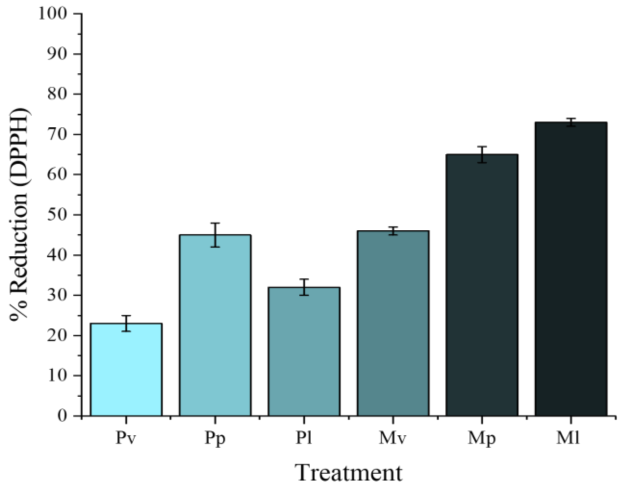

Figure 2 – Evaluation of DPPH reduction in samples of macrophytes *L. punctata* and *L. minor*. **Source:** Authorship (2022). For *L. punctata* the indication (P) was used followed by the cultivation treatment, as follows: (Pv) pond water + vinasse; (Pp) pond water + fish water; (Pl) pond water. For *L.minor* the indication (M) was used followed by the cultivation treatment, being: (Mv) pond water + vinasse; (Mp) pond water + fish water; (Ml) pond water.

Studies show that the association of *L. minor* and *L. punctata* used for bioremediation of pharmaceutical and personal care products, these plants were able to efficiently remove compounds such as ibuprofen, fluoxetine, triclosan and 2,4D (2,2,4-dichlorophenoxyacetic acid) in a period of 9 days, demonstrating that the associated macrophytes acted efficiently to extract about half of the pollutants in the water body (Main et al., 2017). Studies focused on the use of macrophytes for the bioremediation of aquatic pollutants are essential, mainly, to ensure the maintenance of these ecosystems, in this sense, understanding the mechanisms of these plants that promote the assimilation and degradation of these compounds is of paramount importance for the balance of environments water.

According to Tsolmon et al. (2021), *L. punctata* has a significant ability to scavenge free radicals against DPPH, depending solely on the culture medium. Aquatic plants have numerous active chemical compounds that can be used in other processes and even in the composition of pharmaceuticals, the food and cosmetics industry, some are rich in phenolic compounds and antioxidants, which can replace synthetic substances that may present certain toxicity (Islam et al., 2021).

Duckweeds are plants with a high potential for use, so their characterization in terms of composition is important, as there is an imminent possibility of using these plants for different purposes. Within this classification are *L. punctata* and *L. minor* (Ullah et al., 2021). These are plants that can be directed to biotechnological processes depending solely on the use of new technologies. Macrophytes are rich in phytochemicals such as phenolic compounds that are beneficial to health. Březinová and Vymazal (2018), analyzed the aquatic plants *Typha latifolia*, *Phragmites australis*, *Scirpus sylvaticus*, *Phalaris arundinaca*, *Carex nigra*, *Max Lyceria* and *Juncus effuses*, and found concentrations of total phenolic compounds ranging from 9.02 to 28.39 g kg-1 weight of biomass and inferred that the composition of macrophytes in terms of chemical compounds is directly related to the management conditions used.

Cultivation conditions are decisive for the concentration and variation of bioactive molecules in these plants, which can meet different demands of modern society through transformation processes. Macrophytes can be an option that can be used as raw material for industrial processes ranging from the production of clean energy, chemical compounds of natural origin and even new sources with high nutritional potential, as well as the reuse of water. residues in its cultivation. The versatility of these plants can also be observed by the biomass yield that according to Zhao et al. (2014), can be around 26 tons per hectare, with an average accumulation of 7.3 g in dry weight m2 per day. However, this yield may vary according to the type of cultivation and the conditions of the medium or substrate concerning nutrient availability. The biomass production of these plants can vary according to climate and temperature conditions, in tropical and subtropical climates the biomass production can exceed 28.4 t dry weight ha-1 year -1, with 19 t dry weight ha-1 (Ennabili et al., 2019).

Duckweeds are versatile plants that have high potential to be used as raw material for different processes, including large-scale bioenergy production. The composition of these plants can vary, however, their composition ranges from phytochemicals to essential nutrients such as carbohydrates, proteins and amino acids (Figure 3).

These plants play an important role in modern society, as the disorderly growth of the population over the years has increased residual pollutants that contaminate the environment. Water bodies are the most affected by the disposal of contaminants and it is estimated that around US$ 4.6 trillion per year is spent worldwide on the recovery of these areas (Vermeulen et al., 2020).

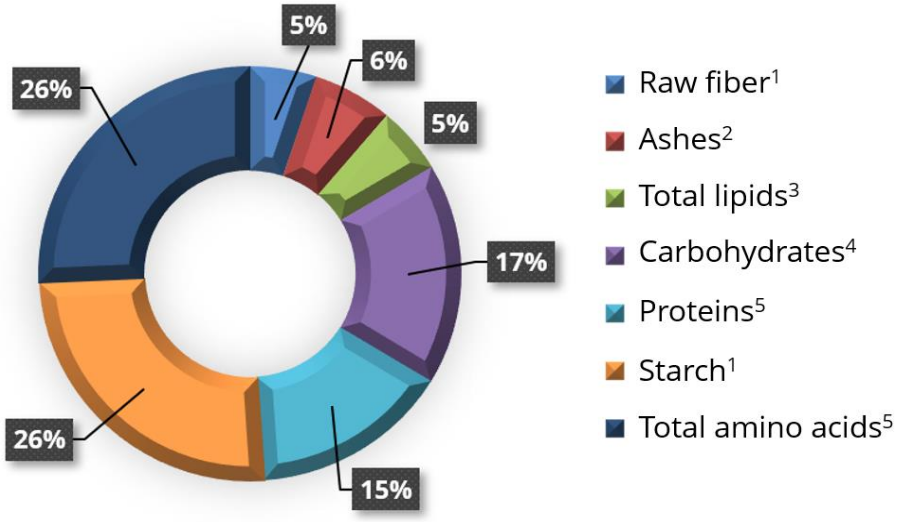

Figure 3 – Nutritional composition of duckweed. **Source:** Organized by the authors (2022) of [1]Verma e Suthar (2015); [2]Gaur e Suthar (2017); [3]Hemalatha e Mohan (2022); [4]Souto et al. (2019); [5]Appenroth et al. (2017).

Studies aimed at the growth of macrophytes cultivated in wastewater can efficiently contribute to the purification of water bodies, as well as enable the production of bioactive molecules in greater quantities, enabling the use of the great availability of this natural resource and its enormous biotechnological potential.

In this sense, as the literature shows, duckweed is a plant that has a high potential for adaptation and growth and is already used in environmental processes such as phytoremediation in natural flooded areas or even in artificial ponds (Tolibovich, 2021) and as biomass for the production of biofuels (Kaur et al., 2018; Liu et al., 2021). These authors point out that the hydrolysis process to obtain fermentable sugars is more efficient, these plants are rich in cellulose, hemicellulose, and starch and have a low lignin content, which makes the hydrolysis process faster and more economically viable when compared to other lignocellulosic biomasses.

In the area of food production, these plants stand out for their nutritional components such as essential amino acids, protein and lipid content, and can be a raw material for nutraceutical compounds in human food and as a complement in animal feed, considering their energy value (Yahaya et al., 2022; Yahya et al., 2022).

For the pharmaceutical industry in the composition of antibacterials and antiseptics. Studies developed by González - Renteria et al. (2020), using extract of *L. minor* prepared with different solvents obtained bactericidal action against Pseudomonas fluorescens. Mane et al. (2017), using aqueous extracts of *L. minor* against Gram-positive and Gram-negative bacteria observed that there was antimicrobial activity. These studies reinforce the potential for employability of these plants in different applications (Figure 4). Thus demonstrating the versatility of macrophytes, especially duckweed as a promising raw material for biotechnological processes.

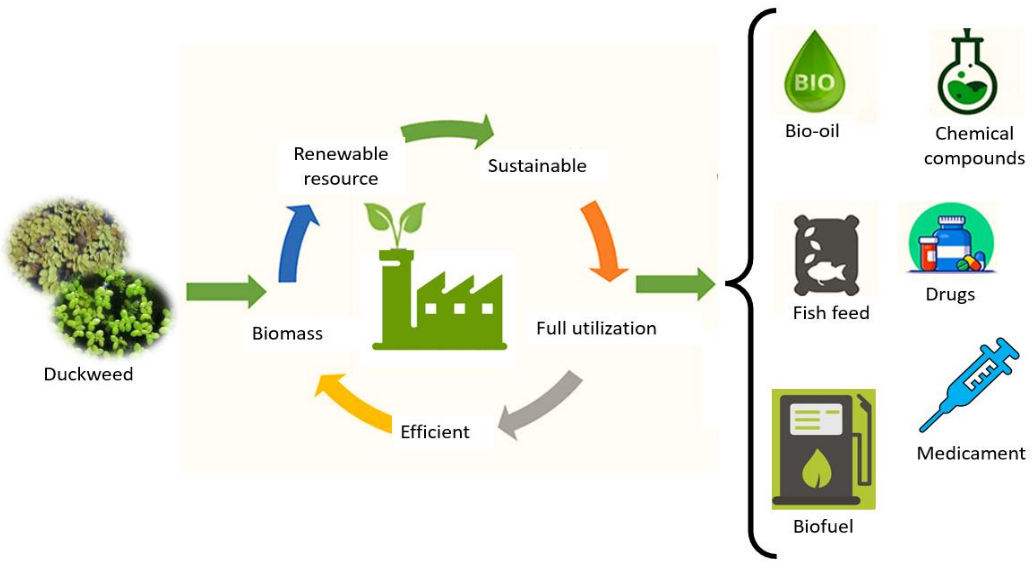

Figure 4 – Different products that can be obtained by transformation processes using macrophytes as raw material. Source: Authorship (2022).

4. CONCLUSIONS

Analysis of the UV-vis spectra of samples of macrophytes *L. punctata* and *L. minor* cultivated in wastewater, showed bands corresponding to compounds such as amino acids and chlorophyll.

In the evaluation of antioxidant activity, *L. punctata* showed the highest scavenging of free radicals when cultivated in vinasse while *L. minor* showed the lowest antioxidant activity in fish water. The treatments used possibly induced a greater production of these compounds, since the amount of certain molecules present in the macrophytes is related to the form of cultivation.

Macrophyte species have great potential to be used in transformation processes, as well as in biotechnological processes such as decontamination against numerous pharmaceutical contaminants, heavy metals, organic compounds and hydrocarbons due to their ability to bioaccumulate. These plants are considered a promising source of bioactive compounds and are considered an excellent alternative for new studies related to environmental issues, energy and the production of molecules of biotechnological interest.

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