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LEMNA GIBBA FOR PHYTOREMEDIATION OF POLLUTANTS AND VALORIZATION AS BIOACTIVE BIOMASS: A MULTIFUNCTIONAL RESOURCE

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ABSTRACT

Many plants with therapeutic and environmental benefits can be found in Morocco's varied aquatic and terrestrial flora. The floating duckweed Lemna gibba L. is attracting more interest because of its potential for phytoremediation and biotechnological applications. This study supports a circular economic approach that aims to sustainably valorise biomass sources from wastewater treatment plants. *Lemna gibba*, sourced from a wastewater treatment lagooning station, demonstrated strong phytoremediation potential alongside rich bioactive profiles. Phytochemical characterization via gas chromatography-mass spectrometry (GC-MS) revealed high protein content (27.57%), chlorophylls, carotenoids, and flavonoids, with major constituents including phthalic acid ester and siloxane. Antioxidant assays showed significant activity (IC_{50}= 13.77 ± 0.00064 mg/mL; EC_{50} = 8.51 ± 0.00028 mg/mL), while extracts exhibited antibacterial efficacy against Bacillus subtilis, Proteus mirabilis, and Pseudomonas aeruginosa. These results highlight L. gibba's dual role as a natural depollutant for wastewater and a source of bioactive molecules. This work opens pathways for integrating aquatic plant valorisation into sustainable resource management, contributing to environmental biotechnology by enabling low-cost, circular economy solutions for pollution control and pharmacological applications in Morocco and beyond.

Keywords: *Lemna gibba*, Protein, Antioxidant, Antibacterial, Gas Chromatography, Valorisation.

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INTRODUCTION

Biological systems using plants act as life-support systems, eliminating waste through phytoremediation and producing food. Aquatic plants in particular are highly efficient at purifying water by recovering nutrients, while their edible biomass can provide a food supplement for aquatic animals.[1] Duckweed (family Lemnaceae) is renowned worldwide as a powerful and environmentally sustainable plant for removing nutrients and other pollutants from wastewater, growing in a variety of environments (usually stagnant water) with very high concentrations of nutrients. Despite its role in bioaccumulation, this plant is also known for its important nutritional aspects. Duckweed is a prospective source of protein for both humans and animals.[2] Recent research has also revealed its potential antioxidant and antibacterial properties[3,4], emphasising the importance of phenolic compounds and flavonoids in medicinal applications.[5] Phenolic compounds present in plants are an important part of the human and animal diet, preserving nutritional quality thanks to their antioxidant properties and their beneficial effects in reducing health disorders and delaying the formation of toxic oxidation products.[6] This study is based on a circular economic strategy. Consequently, *Lemna gibba* could be exploited, for example, as a preservative for

foodstuffs, due to the abundance of these various bioactive compounds[7], Alternatively, the ethanolic extract of *Lemna gibba* can be added to interleaved papers to cure historical papers[8], while *Lemna minor* extracts can be used to polymer systems for food packaging.[9] This plant is a wise alternative, not only for its high purification potential, but also for its long-term beneficial biomass valorisation, contributing to sustainable and inclusive wastewater management, hence the aim of this phytochemical study.

EXPERIMENTAL

Plant Sample and Extraction

Duckweed samples were collected from lagoons at the pilot wastewater treatment plant of the Bouregreg complex located at (33°56'33.6 "N 6°48'00.5" W) of the International Water and Sanitation Institute (ONEE-branche eau), Rabat (Morocco). The plant was cleaned several times with tap water and then dried in the fresh air for a few days. Soxhlet extraction was carried out using three different solvents in the following yields: ethanolic extract (R= 36.06%), methanolic extract (R= 30.60%) and aqueous extract (R= 12%), in order to determine the protein and phenolic compounds and evaluate the antioxidant and antibacterial activities**.**

Total Polyphenols and Flavonoids Assay

The Folin-Ciocalteu colourimetric method, employing gallic acid as a standard, is used to measure polyphenol concentration. The samples' absorbance at 765 nm was measured with a UV spectrophotometer after 30 minutes of incubation in a water bath at 45°C. The total phenolic compound concentration was measured in milligrams of gallic acid equivalents per gram of dry extract. The total flavonoid concentration was assessed using the aluminium chloride colourimetric method, with absorbance measured at 510 nm against a blank, and reported as milligrams of rutin equivalents per gram of extract.[10]

Pigments Content

Determination of pigment concentration in 100mg of dried plant was homogenised in 80% Acetone, and the homogenate was filtered. The pigment concentration (chlorophyll a, chlorophyll b, and carotenoids) was measured following the Arnon equations and extracted with ethanol. The pigment content was measured by spectrophotometer measurements (Ultraspec 3100 pro-Amersham Biosciences) at 480 nm, 645 nm and 663 nm wavelengths.[11]

Proteins Content Determination

The protein content determination was performed after protein extraction using a lysis buffer (tris-HCl 100mM, pH 8,5, containing 14mM du ß-mercaptoéthanol). 50 mg of the plant biomass was grinded using Liquid Nitrogen by Mortar and then suspended for 20 minutes in 5 mL of lysis buffer. The hydrolysate biomass was centrifuged at 12,000 rpm for 20 min at +4°C. The procedure was performed twice, and the supernatants were collected and mixed. Protein content was estimated by Bradford assay.[12]

Antioxidant Activity Test

Antioxidant activity was evaluated using two complementary techniques to account for the complex nature of oxidation metabolism. Antioxidant activity has been measured using the DPPH test, which quantifies extracts' ability to trap free radicals, and the FRAP test, which quantifies ferric reduction capacity. The combination of these two procedures allows for a more detailed evaluation of the antioxidant potential of the extracts.

DPPH and FRAP Tests

The antioxidant activity of *Lemna gibba* extract has been examined using the DPPH test, as described by Sahin.[13] The absorbance of the reaction mixture was measured at 517 nm, and the same technique was followed for the control (DPPH without extract). Quercetin (0.38-6.09 mg/mL) served as the reference antioxidant. The antioxidant capacity with the FRAP assay using the Oyaizu method[14], which assesses the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}). The absorbance of the reaction mixture was measured at 700 nm, Catechin (0.65–21.39 µg/mL). and the findings were represented in terms of standard antioxidants.

Bacterial Strains

The antibacterial activity of ethanolic and aqueous extracts of *Lemna gibba* was investigated against five pathogenic bacteria (*Staphylococcus aureus, Bacillus subtilis, Proteus mirabilis, Escherichia coli, and Pseudomonas aeruginosa*). The extracts were made by macerating dried plants in appropriate solvents. The disk diffusion method was employed, with paper disks impregnated with extract placed on Petri dishes seeded with Mueller-Hinton agar. After a 24-hour incubation at 37°C, the inhibition zones were measured and compared to negative and positive controls. Results are presented as the mean ± SD of three independent replicates.[5]

Extract Characterisation using GC/MS

Determining the phytochemical composition is crucially important for establishing the link between the bioactive components and the functional characteristics of the extract. Against this background, a detailed analysis of the *Lemna gibba L.* ethanolic extract was carried out using gas chromatography combined with mass spectrometry (GC-MS) on a Perkin-Elmer Auto System XL (Waltham, MA, USA). This approach helps to highlight both the variety and the proportion of the chemicals present.

RESULTS AND DISCUSSION

Protein and Pigment Content

Table-1 presents the pigment and protein content of the ethanolic extract of the duckweed L*emna gibba*.

Table-1: Chlorophyll, Carotenoid and Protein Content

Total Chlorophyll (mg/g)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Carotenoid (mg/g)	Protein (mg/g)
1.711±0 .101	1.173±0.048	0.506±0.097	0.313±0.040	274.33±1.35

The following pigment and protein concentrations were determined from the analysis of the ethanolic extract of *Lemna gibba*. These results show the biochemical diversity of *Lemna gibba*, making it an interesting aquatic plant for use in both phytoremediation and natural pharmacology. Our *Lemna gibba* extract contains 274.33±1.35 mg/g protein, i.e. 27.57% protein, which is below the average known protein content of *Lemna gibba*, which is generally between 29.3 and 38.5%.[15,16] This is explained by the relationship between protein content and the concentration of nutrients in the culture medium, particularly the concentration of nitrogen.[41] In our growth lagoon, the concentration of nitrogen by the Kjeldah method in the incoming wastewater was 12.63 mg N/L, which fell to 6.39 mg N/L after purification. Our study supports the suggestion of Leng[15], contrary to some recent results, which show that the source of nitrogen has no significant impact on protein content. The chlorophyll and carotenoid content in the ethanolic extract, although relatively modest, is significant and confirms recent studies on the chemical composition of duckweed, which provides an interesting mixture of carotenoids, flavonoids and polyphenols.

Antioxidant Activity and Flavonoid and Polyphenol Contents

In this study, we chose to determine the antioxidant properties of the ethanolic extract of *Lemna gibba* by two different tests, respectively, the DPPH and the FRAP test. Quercetin and Catechin were used as standards. Antioxidant in Table-2. Table-3 indicates the contents of polyphenols and flavonoids of the same extract.

Table-2: Antioxidant Activity of the Ethanolic Extract of *Lemna gibba*

Extract and standards	DPPH IC50 (µg/mL)	FRAP EC50 (µg/mL)
EtOH extract	13766.96 ±0.641	8506.21±0.28
Quercetin	5.49 ±0.02	
Catechin		13.0±0.03

Table-3: Polyphenol and Flavonoid Content of the Ethanolic Extract of *Lemna gibba*

TPC (mg Eq AG/g Extract)	52.73±0.035
TFC (mg Eq Q/g Extract)	20.71±0.01

In our research, the content of polyphenols and flavonoids, as well as DPPH scavenging activity, and the carotenoid and chlorophyll content obtained, make *Lemna gibba* a perfect new candidate for nutritional and medicinal use. The results obtained (Table-2) demonstrate the antioxidant potential of *Lemna gibba*, which is rarely studied as a species, and match the results achieved on the much-studied Lemna minor. The fact that *Lemna* in general can have significant antioxidant properties is explained by the chemical compounds they contain, such as phenolic compounds like gallic acid, tannins, flavonoids, sterols, carotenoids and amino acids. [17]

Antibacterial Activity

Table-4 lists the results of the antibacterial activity that we evaluated of the ethanolic and aqueous extract of *Lemna gibba* against: *Bacillus subtilis, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli*.

Table-4: Antibacterial Activity of Ethanolic and Aqueous Extracts of *Lemna gibba*

	Bacillus subtilis	*Proteus mirabilis*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Escherichia coli*
Fraction. Aq	n. a	n. a	n. a	n. a	n. a
Fraction EtOH/Aq	10.6±0.3	10.3±0.6	9.6±0.3	n. a	n. a
Fraction entie Aq	n. a	n. a	n. a	n. a	n. a
Fraction entie EtOH/Aq	8.6±0.3	8.6±0.3	n. a	n. a	n.a

*With: n. a= not active; results are inhibitor diameters in mm

As Table-4 illustrates, the diameters of the zones of inhibition evaluated varied between 8 and 10 mm, and only the ethanolic/aqueous extract fraction was able to show activity against *Bacillus subtilis*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. And becomes inactive against *Pseudomonas aeruginosa* in the case of the entie fraction. EtOH/Aq. In our case, our *Lemna gibba* extracts showed no activity against Staphylococcus aureus, contrary to the study conducted by Jani.[3] As regards Lemnaceas in general, information is limited. The authors did not find any inhibitory effect in the disk diffusion test, so they are in disagreement, but a few studies carried out by different researchers have shown that the bactericidal effect of methanolic extract, in particular, is mainly attributed to phenolic compounds and flavonoids, which have antimicrobial properties.[18]

GC-MS Analysis

Thirteen compounds were identified through GC-MS spectrum analysis (Fig.-1 and 2). The main peak identified at 25.65 min refers to a phthalic acid ester[19,20], an aromatic substance commonly used to modify the physicochemical characteristics of various products. The second most abundant component was identified as a modified siloxane, known for its thermal and chemical stability. The identification of these compounds in the *Lemna gibba L.* extract can be attributed to its bioaccumulation property.

CONCLUSION

Phytochemical analysis carried out *on Lemna gibba L.* reveals the potential of this aquatic plant, not only for phytoremediation, but also as a potential source of natural antioxidant compounds. The studies quantified important biomolecules such as proteins, chlorophyll, carotenoids, flavonoids and polyphenols, which exhibit considerable antioxidant activity, especially in the ethanol-based extract. These findings indicate that *Lemna gibba* could be extremely important in the development of value-added products in the pharmaceutical, cosmetics and agri-food sectors.

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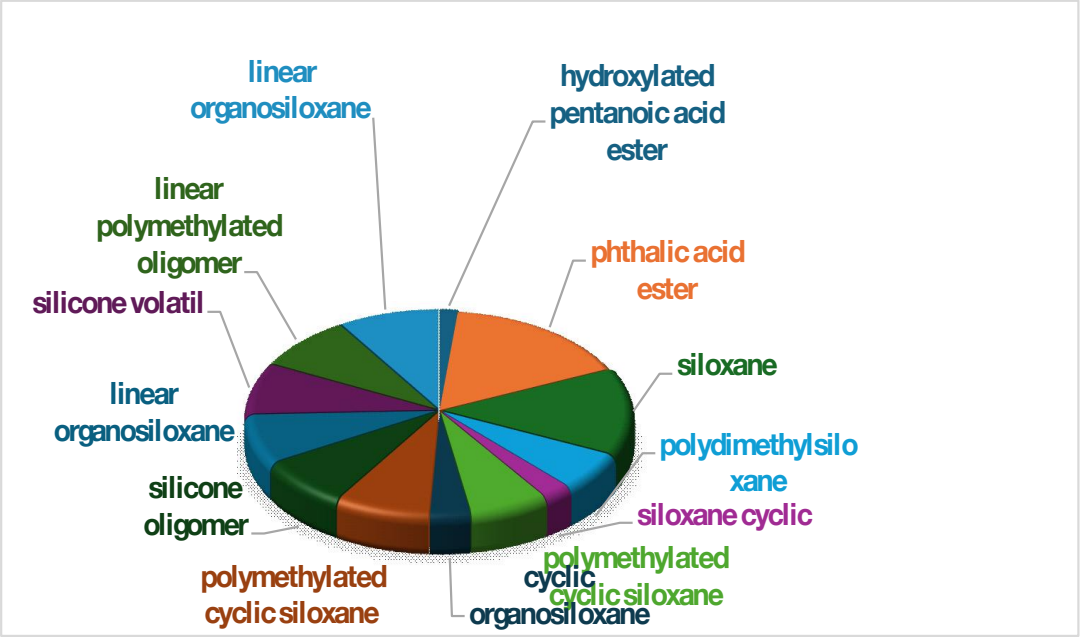

Fig.-1: GC/MS Analysis of *Lemna gibba*'s Extract

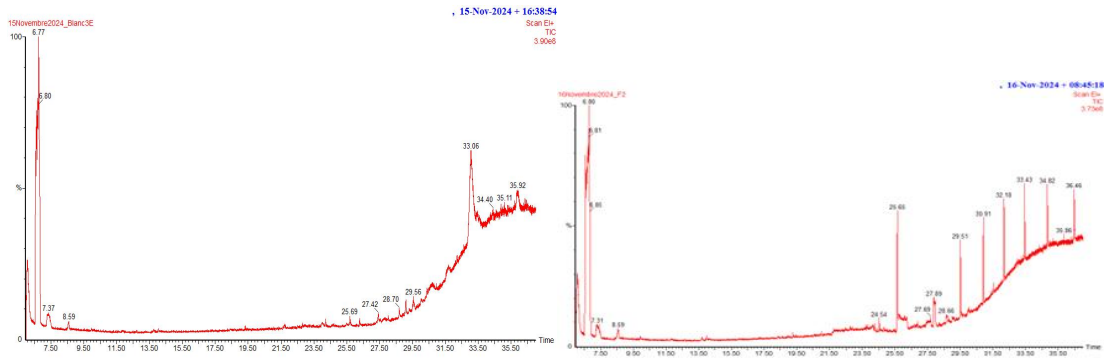

Fig.-2: Blank and Sample *Lemna gibba's* Extract GC/MS Spectra

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-6: Clean water and Sanitation.* All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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REFERENCES

1. O. Obinna B, E. Joseph E, et A.I. Christina O, *International Advanced Research Journal in Science, Engineering and Technology*, **8**, 7(2021), https://doi.org/10.17148/IARJSET.2021.81202
2. M.S. Islam, M. S. Kabir, S. I. Khan, M. Ekramullah, G. B. Nair, R. B. Sack, D. A. Sack, *Canadian Journal of Microbiology*, **50**, 51(2004), https://doi.org/10.1139/w03-102
3. X. Wan, A. D. Steinman, X. Shu, Q. Cao, L. Yao, et L. Xie, *Ecotoxicology and Environmental Safety*, **185**, 109668(2019), https://doi.org/10.1016/j.ecoenv.2019.109668
4. S. K. Sil, S. Gupta, et F. A. Neela, *Bangladesh Journal of Botany*, **52**, 105(2023), https://doi.org/10.3329/bjb.v52i1.65241
5. S. H. Al-Mijalli, N. N. Mrabti, H. Ouassou, R. A. Sheikh, Assaggaf, S. Bakrim, E. M. Abdallah, M.M. Alshahrani, A. A .Al Awadh ,L. Lee ,Y. AlDhaheri ,A. Sahebkar ,G. Zengin ,A. A. Attar ,A. Bouyahya, H. N. Mrabti , *Molecules*, **27**, 7329(2022), https://doi.org/10.3390/molecules27217329
6. F. Shahidi, P.Ambigaipalan, *Journal of Functional Foods*, **18**, 820(2015), https://doi.org/10.1016/j.jff.2015.06.018
7. G.Rocchetti, A.Rebecchi , L.Zhang , M. Dallolio , D. Del Buono ,G.Freschi , L. Lucini, *Food Chemistry : X*, **20**, 101013(2023), https://doi.org/10.1016/j.fochx.2023.101013
8. W. A. Mohamed, M. M. A. Mansour, et M. Z. M. Salem, *Journal of Cleaner Production*, **219**, 846(2019), https://doi.org/10.1016/j.jclepro.2019.02.097
9. F.Luzi, D.Del Buono, B. Orfei, C. Moretti, R. Buonaurio , L. Torre, D. Puglia, *Food Packaging and Shelf Life*, **32**, 100822(2022), https://doi.org/10.1016/j.fpsl.2022.100822
10. T. Benali, H. Chtibi, M. E. Yamani, I. Marmouzi, A. Khabbach, et K. Hammani, *Moroccan Journal of Chemistry*, **8**, 552(2020), https://doi.org/10.48317/IMIST.PRSM/morjchem-v8i2.18788
11. D. I. Arnon, *Plant Physiology*, **24**, 1(1949), https://doi.org/10.1104/pp.24.1.1
12. J. kruger, *The Protein Protocols Handbook*, 17(2009), https://doi.org/10.1007/978-1-59745-198-7_4
13. F. Şahin, M. Güllüce , D. Daferera , A. Sökmen , M. Sökmen , M. Polissiou , G. Agar , H. Özer, *Food Control*, **15**, 549(2004), https://doi.org/10.1016/j.foodcont.2003.08.009
14. M. Oyaizu, *Japanese journal of nutrition*, **44**, 307(1986), http://dx.doi.org/10.5264/eiyogakuzashi.44.307
15. R. Chakrabarti, W. D. Clark, J. G. Sharma, R. K. Goswami, A. K. Shrivastav, et D. R. Tocher, *Frontiers in Chemistry*, **6**,479(2018), https://doi.org/10.3389/fchem.2018.00479
16. G. Yu, H. Liu, K. Venkateshan, S. Yan, J. Cheng, X. S. Sun, D. Wang., *American Society of Agricultural and Biological Engineers*, **54**, 555(2011), https://doi.org/10.13031/2013.36459
17. D. Pagliuso, A. Grandis, J. S. Fortirer, P. Camargo, E. I. S. Floh, et M. S. Buckeridge, *Agronomy*,**12**, 796(2022), https://doi.org/10.3390/agronomy12040796
18. M. González-Renteria, M. del Carmen Monroy-Dosta, X. Guzmán-García, I. Hernández-Calderas, et y. M. A. Ramos-Lopez, *Saudi Journal of Biological Sciences*, **27**,465(2020), https://doi.org/10.1016/j.sjbs.2020.09.043
19. L. Huang, X. Zhu, S.Zhou, Z.Cheng, K. Shi, C. Zhang, H. Shao, *Toxins*, **13**, 495(2021), https://doi.org/10.3390/toxins13070495
20. X. Wang, X. Sun, X.Wang , X. Qi , D. Wang , J. Jiang , J. Mao , F. Ma , L. Yu , L. Zhang , P.Li, *Food Chemistry: X*, **16**, 100520(2022), https://doi.org/10.1016/j.fochx.2022.100520

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