REVIEW ARTICLE

PHARMACOLOGICAL AND THERAPEUTIC POTENTIAL OF *LEMNA MINOR* (COMMON DUCKWEED): A COMPREHENSIVE REVIEW

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Abstract:

Traditional medicine encompasses ancient knowledge, skills, and practices used to maintain health and treat diseases, with systems like Ayurveda and Traditional Chinese Medicine still widely practiced. *Lemna minor* (common duckweed), a small free-floating aquatic plant in the family Araceae, is globally distributed in freshwater habitats and valued for its rapid growth, nutrient uptake efficiency, and diverse bioapplications. This study compiles its botanical profile, distribution, and pharmacological properties. Taxonomically, *L. minor* consists of small green fronds with a single root, reproducing mainly asexually, and disperses via waterfowl. Phytochemical constituents include phenolics, flavonoids, tannins, alkaloids, saponins, and terpenoids, contributing to its bioactivities. Antimicrobial evaluations revealed activity against various Gram-positive and Gram-negative bacteria, *Candida* spp., and phytopathogenic fungi, with efficacy varying by solvent extract and concentration. Methanolic extracts showed the strongest antibacterial effect, while aqueous and ethanolic extracts demonstrated notable antifungal inhibition, particularly against *Aspergillus niger*. Immunosuppressive assays indicated a dose-dependent reduction in lymphocyte proliferation in virally infected human blood samples. The plant also exhibited significant antileishmanial activity against both promastigote and amastigote forms of *Leishmania tropica*. Acute toxicity testing in Wistar rats indicated no adverse effects up to 2000 mg/kg, confirming a wide safety margin. These findings support *L. minor* as a promising source of bioactive compounds with potential therapeutic applications, warranting further pharmacological and mechanistic studies.

Keyword: *Lemna minor*, Plant Profile, Pharmacological Activity.

1. Introduction:

Traditional medicine refers to the knowledge, practices, and skills developed over generations within various societies before the era of modern medicine. These practices are used to maintain health as well as to prevent, diagnose, and treat physical and mental illnesses. It includes herbal medicine, acupuncture, Ayurvedic medicine, Unani, traditional Chinese medicine (TCM), and many others. Traditional medicine systems are widely used in Africa, Asia, and Latin America and are being increasingly integrated with modern medical systems globally. Ayurveda, the traditional Indian medicine (TIM) and traditional Chinese medicine (TCM) remain the most ancient yet living traditions. These are the two 'great traditions' with sound philosophical, experiential and experimental basis. Increased side effects, lack of curative treatment for several chronic diseases, high cost of new drugs, microbial resistance and emerging diseases are some reasons for renewed public interest in complementary and alternative medicines. [1]

Lemna minor, commonly referred to as common duckweed, is a small free-floating aquatic plant that belongs to the family Araceae (formerly Lemnaceae). It is one of the simplest and fastest-growing angiosperms found in freshwater ecosystems across the globe. Due to its high growth rate, ease of cultivation, and nutrient uptake efficiency, *Lemna minor* is widely used in phytoremediation, bioassays, wastewater treatment, and as a protein-rich biomass for animal feed. This plant floats on the water surface and reproduces primarily by asexual budding, enabling rapid population growth under favourable conditions. It absorbs nutrients directly from water through its fronds, making it an excellent candidate for pollutant removal, including heavy metals, nitrates, phosphates, and organic compounds. [2]

2. Plant Profile:

2.1. Taxanomical Classification:

Kingdom: Plantae
Subkingdom: Viridiplantae
Superdivision: Embryophyta
Division: Tracheophyta
Subdivision: Spermatophytina
Class: Magnoliopsida
Superorder: Lilianae
Order: Alismatales
Family: Araceae
Genus: *Lemna*
Species: *minor* [3]

2.2. Distribution:

Lemna minor is widely distributed across the globe, particularly in Africa, Asia, Europe, and North America, where it naturally inhabits freshwater bodies such as ponds and slow-flowing streams. However, it is generally absent from arctic and subarctic regions due to unsuitable climatic conditions.

While not originally native to Australasia and South America, it has been introduced and become naturalised in those areas.

2.3. Botanical Description:

Lemna minor is a small, free-floating aquatic plant commonly found in freshwater environments. Each plant typically consists of one to four oval-shaped fronds, each attached to a single root that dangles in the water, usually measuring between 1 and 2 cm in length. The fronds are light green, ranging from 1–8 mm long and 0.6–5 mm wide, and contain three (occasionally five) veins along with internal air spaces that aid in buoyancy. The plant primarily propagates through asexual division, where new fronds bud off and become independent. Although flowering is rare, when it does occur, the flowers are about 1 mm in diameter, enclosed in a membranous, cup-shaped structure that houses a single ovule and two stamens. The resulting seed is small (around 1 mm long) and marked with 8 to 15 ridges. Birds play a key role in the plant's dispersal, as its sticky root can cling to their feathers or feet, allowing the plant to be transported to and colonize new aquatic habitats. [4]

3. Pharmacological Activities:

3.1. Anti-Microbial Activity:

3.1.1. The antibacterial and antifungal activities of the water extract (WELM) and ethanol extract (EELM) of *Lemna minor* were evaluated using the disk diffusion method. For inoculum preparation, 3 to 5 similar bacterial colonies were transferred into 5 mL of Tryptone Soya Broth (Oxoid CM129, UK), a nutrient-rich medium suitable for growing aerobic and facultative anaerobic microorganisms, as well as some fungi. These cultures were incubated for 24 hours at 37 °C. To test antimicrobial activity, sterile 6-mm filter paper disks were soaked with 45 µg of either the water or ethanol extract (prepared at 1 µg/mL concentration). Both extracts were dissolved in sterile water with the help of a magnetic stirrer. Solvent-only controls and standard antibiotic disks were also prepared using the same solvents. The disks were then placed on Mueller Hinton Agar (Oxoid CM337), and bacterial inoculums were adjusted to a concentration of 1×10^8 CFU/mL. Following incubation, the zones of inhibition surrounding the disks were measured to assess antimicrobial activity. Clear halos around the disks indicated effective microbial inhibition. The results are based on the average of three replicates. To benchmark antimicrobial sensitivity, standard antibiotics were used as positive controls: ampicillin (10 µg/disk), amoxicillin (25 µg/disk), and cefuroxime (30 µg/disk) for bacteria, and miconazole nitrate (40 µg/disk) for fungi, as per Clinical and Laboratory Standards Institute guidelines. The study examined 21 bacterial strains and 4 Candida species for their response to both extracts (see Table 3). While most Gram-positive and Gram-negative bacteria, as well as Candida spp., showed susceptibility to the extracts, neither WELM nor EELM exhibited inhibitory effects against *Staphylococcus aureus, Escherichia coli, Staphylococcus xylosus, Enterobacter aerogenes, Proteus vulgaris, Proteus mirabilis, Pseudomonas fluorescens, Klebsiella pneumoniae*, or *Klebsiella oxytoca*. However, both extracts were effective against *Candida parapsilosis* and *Candida glabrata*. [5]

3.1.2. The antibacterial potential of the extracts was assessed at six concentrations (5000, 500, 50, 5, 0.5, and 0.05 µg mL^{-1}) using the microdilution technique, which are suitable for testing both polar and non-polar antimicrobial agents. The assay employed a *Pseudomonas fluorescens* inoculum standardized

to 1.5×10^7 CFU mL^{-1} in Muller–Hinton broth (Difco®). Minimum inhibitory concentration (MIC) values were determined after 24 h of incubation at 28 °C. Following incubation, Petri plates were prepared in triplicate, and colony-forming units (CFU mL^{-1}) were counted to quantify viable cells. The extracts of *Lemna minor* demonstrated clear antibacterial activity against *P. fluorescens*. Statistical analysis revealed significant differences between treatments and the control ($p = 0.001$), as well as between methanolic and chloroform extracts ($p = 0.002$) and between methanolic and hexane extracts ($p < 0.001$). Significant variation ($p = 0.001$) was also observed in bacterial inhibition across different exposure times (24, 48, and 72 h) for all three extracts, and between the concentrations tested. The chloroform and hexane extracts showed the greatest effect between 24 and 48 h, but their bactericidal activity declined by 72 h. Conversely, the methanolic extract's effect became more pronounced at 72 h, with statistically significant differences between concentrations ($p = 0.001$). The MIC for all three extracts at 24 h was 0.05 µg mL^{-1}. In this work, extracts with differing polarity profiles all exhibited antimicrobial effects, though with statistically significant variation in potency. These differences likely arise from the distinct classes of bioactive molecules solubilized by each solvent. The methanolic extract displayed the strongest activity, suggesting that the majority of the active constituents are polar compounds. *Lemna* species are known to contain phenolics such as gallic acid, tannins, flavonoids, anthocyanins, quercetin, and other bioactives including thiols and terpene-based steroids, all of which have documented antimicrobial properties. The antimicrobial activity observed in the hexane extract is likely attributable to non-polar terpenes, particularly steroidal terpenes. [6]

3.2. Immunosuppressive Effect:

Lymphocyte Proliferation Assay:

Human blood samples treated with anticoagulant EDTA and confirmed to be virally infected were obtained from Mangal Pathology Laboratory, Baramati, Maharashtra, India. For this experiment, 100 µL of lysed whole blood was cultured with varying concentrations (1–30 mg/mL) of flavonoids extracted from duckweed powder, in the presence or absence of ovalbumin (OVA) at 1 mg/mL (50 µL). The mixtures were placed into 96-well plates and incubated at 37°C for 48 hours. Ovalbumin (OVA) served as the standard antigen in this immunological assay. After the initial incubation, the plates were centrifuged at 2500 rpm for 10 minutes at 4°C, the supernatant was removed, and fresh complete medium was added. The plates were then subjected to a second incubation for 4 hours, during which MTT solution (5 mg/mL, 10 µL) was added to assess cell viability and proliferation. Following incubation, the contents were centrifuged again, the supernatant was discarded, and the resulting pellet was dissolved in dimethyl sulfoxide (DMSO). Absorbance was measured at 570 nm to determine cellular metabolic activity. Increasing concentrations of flavonoids from duckweed led to a dose-dependent reduction in the OVA-specific immune response in the virally infected human blood samples. The findings indicated a suppression of lymphocyte proliferation at higher flavonoid and terpenoid concentrations, suggesting a potential immunosuppressive effect of the extracts. [7]

3.3. Anti-Bacterial Activity:

The antibacterial properties of *Lemna minor* extracts were assessed using the Kirby-Bauer well diffusion method against four bacterial strains: *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia*

coli, and *Pseudomonas aeruginosa*. These bacterial cultures were procured from the Government College Type Culture Collection. Potato Dextrose Agar (PDA) was utilized as the growth medium and was poured into sterile Petri dishes, allowing it to solidify. Bacterial lawns were created by uniformly spreading the cultures over the agar surface using sterile swabs. Sterile metal borers were used to create 10 mm diameter wells in the agar. The *L. minor* extract stock solution was prepared at a concentration of 3 mg/mL in dimethyl sulfoxide (DMSO). Three different volumes—25 µL, 50 µL, and 75 µL—of this stock solution were introduced into separate wells. Pure DMSO served as the negative control, while ceftriaxone (a broad-spectrum antibiotic; CAS No. 73384-59-5) was used as the positive control. The inoculated plates were incubated at 37 °C for 24 hours, after which the zones of inhibition around each well were measured in millimeters using a ruler. Each test was conducted in triplicate. To quantify antibacterial efficacy, the percentage of growth inhibition (I) was calculated using the formula:

$$\textbf{Inhibition (\%)} = 100 \times (C - T) / C$$

where *C* is the growth observed in the control, and *T* represents the growth in the presence of the extract. For evaluating antibacterial activity, an aqueous extract of *L. minor* was also prepared at 4 mg/mL, from which 25 µL, 50 µL, and 75 µL doses were tested. The same bacterial strains were used: *P. aeruginosa*, *E. coli*, *S. typhi*, and *S. aureus*. All tested concentrations exhibited notable antibacterial activity against the selected organisms. At the highest concentration (75 µL), the extract showed the greatest inhibition against *E. coli* (21.4 mm), followed by *S. aureus* (19.1 mm), *P. aeruginosa* (18.1 mm), and *S. typhi* (15.4 mm). At 50 µL, the zones of inhibition were 19.2 mm for *E. coli*, 17.3 mm for *S. aureus*, 16.1 mm for *P. aeruginosa*, and 13.8 mm for *S. typhi*. With the lowest concentration (25 µL), inhibition zones were 16.1 mm for *E. coli*, 14.2 mm for *S. aureus*, 12.8 mm for *P. aeruginosa*, and 11.1 mm for *S. typhi*. These findings indicate a dose-dependent antibacterial effect of the *L. minor* extracts, with E. coli showing the highest sensitivity across all concentrations. [8]

3.4. Anti-Fungal Activity:

To evaluate the antifungal properties of *Lemna minor* extracts, four fungal strains—*Aspergillus niger, Ascochyta rabiei, Fusarium oxysporum*, and *Alternaria alternata*—were obtained from the same type culture collection. The Kirby-Bauer well diffusion method was employed for the analysis. Potato Dextrose Agar (PDA) was used as the growth medium and poured into sterile Petri dishes. After the medium solidified, fungal lawns were created, and 10 mm wells were made using a sterile borer. Stock solutions of the plant extract were prepared at a concentration of 4 mg/mL in dimethyl sulfoxide (DMSO). From this stock, 25 µL, 50 µL, and 75 µL volumes were introduced into individual wells. Pure DMSO served as the negative control, while fluconazole was used as the positive control. The plates were incubated at 26 °C for 24 to 48 hours, after which the zones of inhibition around each well were measured in millimeters using a ruler. Each assay was performed in triplicate. To assess the extent of fungal growth suppression, the following formula was used:

$$\textbf{Growth Inhibition (\%)} = [(C - T) / C] \times 100$$

where *C* represents fungal growth in the control plate and *T* indicates growth in the test sample. The antifungal assay revealed that all tested concentrations of *L. minor* extract demonstrated significant inhibitory effects on the growth of the selected fungal strains. At the highest dose (75 µL), the greatest

zone of inhibition was recorded against *A. niger* (21 mm), followed by *Ascochyta rabiei* (19 mm), *Fusarium oxysporum* (17.1 mm), and *Alternaria alternata* (16.3 mm). At the 50 µL dose, the inhibition zones were 17.7 mm for *A. niger*, 16.3 mm for *Ascochyta rabiei*, 15.2 mm for *F. oxysporum*, and 14.1 mm for *A. alternata*. For the lowest concentration (25 µL), the extract still showed notable activity: *A. niger* (16.1 mm), *Ascochyta rabiei* (15.2 mm), *F. oxysporum* (14.1 mm), and *A. alternata* (12.4 mm). These findings confirm that the antifungal efficacy increased with extract concentration, with *A. niger* being the most sensitive species. [8]

3.5. Antileishmanial Activity:

The antileishmanial potential of *Lemna minor* extracts was assessed against both promastigote and amastigote forms of *Leishmania tropica.* Aqueous extracts of *L. minor* were prepared at concentrations of 25, 50, 100, and 200 mg/mL, and their lethal effects on the parasite were evaluated using linear regression analysis to determine the effective dose. The percentage of haemolysis was calculated using the formula:

% Haemolysis = [(Sample – Control) / Control] × 100

Leishmaniasis is a severe and contagious parasitic disease caused by protozoans of the genus *Leishmania*. According to the World Health Organization, the disease remains endemic in approximately 85 countries, with 1.5 to 2 million new cases reported globally each year. Its continued spread is attributed to ineffective control of vectors and the limited availability of affordable treatments. In this study, the cytotoxic activity of the plant extracts was tested against L. tropica using MTT assays, targeting both promastigote and amastigote stages. The extracts, applied in concentrations ranging from 25 to 120 µg/mL, showed significant parasiticidal activity. At 150 µg/mL, the extracts induced 75.40 ± 1.16% mortality in promastigotes and 60.15 ± 0.12% mortality in amastigotes, demonstrating a dose-dependent effect. The observed antileishmanial activity is attributed to the presence of bioactive phytochemicals such as alkaloids, flavonoids, saponins, and terpenoids in *L. minor*. These compounds disrupt critical metabolic functions of the parasite, including energy generation, protein synthesis, and DNA replication, ultimately leading to the inhibition or death of the parasite. [8]

3.6. Acute Toxicity Study:

For the acute toxicity assessment, healthy albino Wistar rats weighing between 180–200 g were selected and housed under standard laboratory conditions in accordance with OECD guideline 425. The ethanolic extract of *Lemna minor* (EELM) was administered orally at a dose of up to 2000 mg/kg. The animals were closely monitored for any signs of toxicity during the first 4 hours' post-treatment and at regular intervals over the initial 24-hour period. During this time, food was withheld, but water was provided ad libitum for the first 3–4 hours after dosing. Observations included behavioural and physiological changes such as grooming, sedation, alterations in sleep, tremors, diarrhoea, and mortality. Monitoring continued daily for 14 days. No signs of toxicity or mortality were recorded at the administered dose, indicating that EELM was well tolerated up to 2000 mg/kg. Based on these findings, two safe dose levels—200 mg/kg and 400 mg/kg—were selected for subsequent evaluation in the paracetamol-induced bioactivation model. [9]

CONCLUSION:

Lemna minor, a fast-growing aquatic plant with a simple morphology, demonstrates significant pharmacological potential supported by diverse bioactive constituents such as flavonoids, phenolics, terpenoids, saponins, and alkaloids. The reviewed studies confirm its broad-spectrum antimicrobial efficacy, showing activity against several bacterial and fungal strains, with notable potency in methanolic extracts due to the abundance of polar compounds. Additionally, L. minor exhibits promising immunosuppressive effects, dose-dependent antileishmanial activity, and measurable efficacy in inhibiting microbial growth in both aqueous and organic solvent extracts. Its high sensitivity against certain pathogens, particularly *Escherichia coli* and *Aspergillus niger*, underscores its potential as a natural antimicrobial agent. Acute toxicity studies reveal a wide safety margin, with no adverse effects observed at doses up to 2000 mg/kg in animal models. Collectively, these findings highlight L. minor as a valuable candidate for further research and development in phytopharmaceuticals, especially for combating microbial resistance, parasitic infections, and in immunomodulatory applications, while its non-toxic nature supports its potential integration into complementary and alternative medicine systems.

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Declaration of Competing Interest:

The authors declare that they have no competing interests.

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