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EXPLORATION OF PRIMARY METABOLITES FROM *LEMNA MINOR* AND DETERMINED ITS IMMUNOMODULATORY AND ANTIMICROBIAL ACTIVITY

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ABSTRACT

Objective- The objective of our study is to examine its antimicrobial and immunomodulatory activity of aqueous extract of whole plant i.e. *Lemna minor*. **Methods-** In this study, we evaluated its protein and amino acid content in aqueous extract and also determining its antimicrobial activity on four bacterial strains and one fungal strain. In addition, Elisa experiment was performed in order to determined its immunomodulatory potential against specific protein antigen i.e. ovalbumin. **Results-** The results showed its enhancement in protein content (Nanodrop method) at a very low concentration of aqueous extract and also showed its rich content in amino acids. In addition, aqueous extract at higher doses showed enhancement in antibody titre against ovalbumin and decline in proliferation rate of these four bacterial and one fungal strains at higher doses as compared to control. **Conclusion-** Overall data indicates its immunomodulatory and antimicrobial activity of *Lemna minor*.

KEYWORDS: Lemna minor; antimicrobial; immunomodulatory; bacteria; fungus.

INTRODUCTION

Proteins and amino acids that are present in aquatic plants that showed considerable attention for the last few years[1] and claimed its natural antimicrobial properties and also showed its ability to modulate (i.e. stimulatory or suppressive or immunoadjuvant) the immune responses of the host.[2, 3] These immunopharmacological properties will be studied because of primary (proteins and amino acids) and secondary metabolites that are present in every plant and served as the first line of defence against bacterial, fungal, and viral infections.[4] In this regard, our group focused on small aquatic plants and determined its antimicrobial and immunomodulatory activity.

One of the aquatic plants i.e. *Lemna minor* (Duckweed; family *Lemnaceae*) plant is very small and its size is around about 0.6 mm long.[5] Generally, this plant mostly reported in ponds and lakes that free floats on the surface of still or slow moving bodies of fresh water. As per the literature, distribution of *Lemna minor* in fresh water is generally influenced observed or by a number of factors i.e. nutrients availability; pH; light intensity; temperature; dissolved oxygen content and salinity.[6, 7] Lot of research work is already done with respect to its plant growth rate and it will mentioned that growth rate of *Lemna minor*, plant is inversely proportional to the increase in dissolved salt content of the water and their efficiency in water purification.[5-7] One of the most peculiar feature in *Lemna minor*, aquatic plant that generally absorb nutrients, heavy metals, phenols, pesticides, dioxins and pathogens from the water and it is often used as a remediation mechanism for basins with poor water quality.[6-9] In addition, this plant is also used in the manufacturing of many useful products because of its high protein and starch content.

In the literature, lemnan, pectic polysaccharide was earlier reported from freshly collected aquatic plant i.e. *Lemna minor* and revealed the presence of sugar residues i.e. D-galacturonic acid, galactose, arabinose, xylose and D-apiose, a branched chain sugar. These sugar moieties that are present in *Lemna minor* exhibit an immunomodulatory effect by activating the system of phagocytosis. In this regard, we focused on the whole plant in the form of aqueous extract i.e. *Lemna minor* and so one cannot judge on its amino acids, proteins and also studied about its immunological (against specific protein) and microbiological (bacterial and fungal strain) aspects.

MATERIALS AND METHODS

Plant Material: *Lemna minor*, aquatic plant were collected from aquarium of Vidya Pratishthan's School of Biotechnology (VSBT), Baramati, Maharashtra, India. These plants have been grown in VSBT for several months for fish feeding. Fresh stock of healthy plant were collected and dried in a shady area to prepare fine powder.

Estimation of protein and amino acid content

In order to determine its amino acid or protein content in aqueous extract of *Lemna minor* is needed. Several standard amino acids i.e. valine, leucine, serine, threonine and cysteine were used in order to determine its presence in aqueous extract.

Firstly, protein concentration was determined by Nanodrop method. Secondly, procedure required free amino acid estimation assay which was determined through UV visible spectrophotometric method. Bradford method can be used to measure free amino acid activity and its absorbance value was compared to standard amino acid (leucine, valine, serine, threonine and cysteine; Sisco research laboratory Private Limited, Mumbai, India) and then measured the amount of amino acid in aqueous extract.

Bacterial and fungal strains

Four bacterial species (*Pseudomonas fluorescens, Salmonella typhi, E. coli* and *Bacillus subtilis*) were used in the study, which includes Gram-negative bacteria (*Pseudomonas fluorescens, E. coli, Salmonella typhi*) and Gram-positive bacteria (*Bacillus subtilis*). Both gram positive and negative samples were collected from VSBT garden (soil sample) and confirmation of these samples through gram staining method. All these studies related to bacterial suspension following CLSI guidelines. Four bacterial strains were gown in nutritive media having glucose, yeast extract, sodium chloride, agar and other trace elements with pH 7.4 and maintained at 37ºC for 24 h in incubator. After incubation, bacterial colonies were appeared and identified through Bergeys manual of determinative bacteriology. Collect one colonies of each bacteria is diluted in phosphate buffered saline (PBS, pH 7.2) to reach the concentration of 10^7CFU/ml. In addition, prior to use, fungal isolates and prepared aqueous extract and was characterized on the basis of morphological, cultural, and biochemical characteristics as per standard methods

Antimicrobial activity

For these studies, bacterial strains (10^5 CFU/ml; 50 µl) and fungal extract (50 µl) strain were exposed to lysed human whole blood (100 µl) in two different sets and incubated with variable concentration of aqueous extract was taken and diluted it ranging from 100 mg/ml to 0.01 mg/ml (50 µl). Plates were placed at 37°C for 24 h. At the end of incubation, media in the wells was removed and replaced with fresh medium containing MTT solution (2.5 mg/ml; 10 µl) and incubated at 37°C for 4h. Thereafter, media-containing MTT was removed and formazan crystals were dissolved in DMSO and then its absorbance was recorded in microplate reader at 570 nm.[10, 11]

ELISA

Indirect ELISA was performed using Ovalbumin (i.e. 1 mg/ml; 100 µl) as coating antigen in 96 well high protein binding plate. Incubate 96 well plate overnight at 4ºC and then add blocking buffer (100 µl) after washing the plate with phosphate buffered saline (PBS, pH 7.4). Incubate plate for 1h at room temperature and then add variable concentration of aqueous extract of *Lemna minor*. Incubate the plate for another 4-5 h at carbon dioxide incubator. After incubation, add secondary antibody (horse anti-serum; 1:10000 dilution; 100 µl) in 96 well plate. Again, incubate plate for another 1h at carbon dioxide incubator. Finally, TMB substrate solution was added after washing the plate with PBS and then add stop solution. The optical density was measured at 450 nm.[12]

RESULTS

Estimation of protein and amino acids

In aqueous extract of *Lemna minor*, protein concentration was determined through Nanodrop method and its concentration is 4.917 mg/ml (10 µl) (**Fig.1**). In addition, amino acids were also calculated using standard amino acids and determined its content through Bradford method. The results of these studies showed that *Lemna minor* showed enhancement in amino acid content at higher doses as shown in **Fig.2**.

Antimicrobial activity

The results of these studies represents that *Lemna minor* showed maximum inhibition in case of bacteria (gram positive and gram negative) and fungi at higher concentration as compared to control. In other words, *Lemna minor* showed its antimicrobial activity against these bacterial and fungal pathogens (**Fig.3**).

ELISA

In this study, our results showed its enhancement in case of IgG titre of *Lemna minor* against weak antigen ovalbumin at higher doses as compared to standard alum and control. Overall, data represents its immunogenic potential of *Lemna minor* against specific protein antigen **(Fig.4).** Alum used as standard and showed its enhancement in IgG titre against ovalbumin as compared to control. In other words, *Lemna minor* showed its immunomodulatory potential against specific protein antigen i.e. ovalbumin.

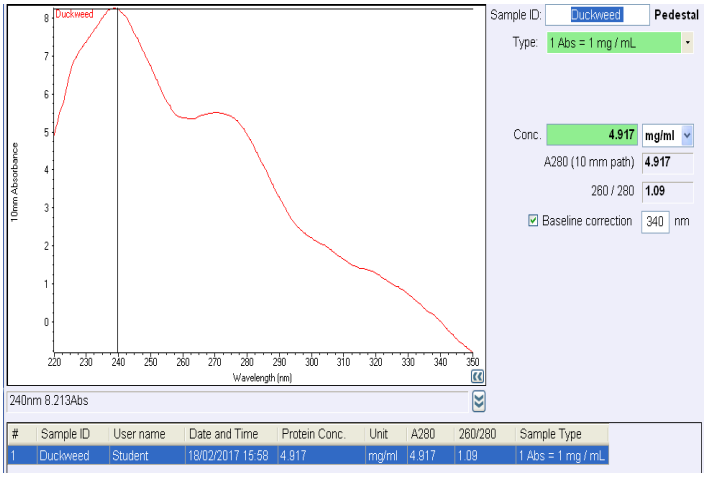

Fig.1. Estimation of protein content in aqueous extract of *Lemna minor* using Nanodrop method.

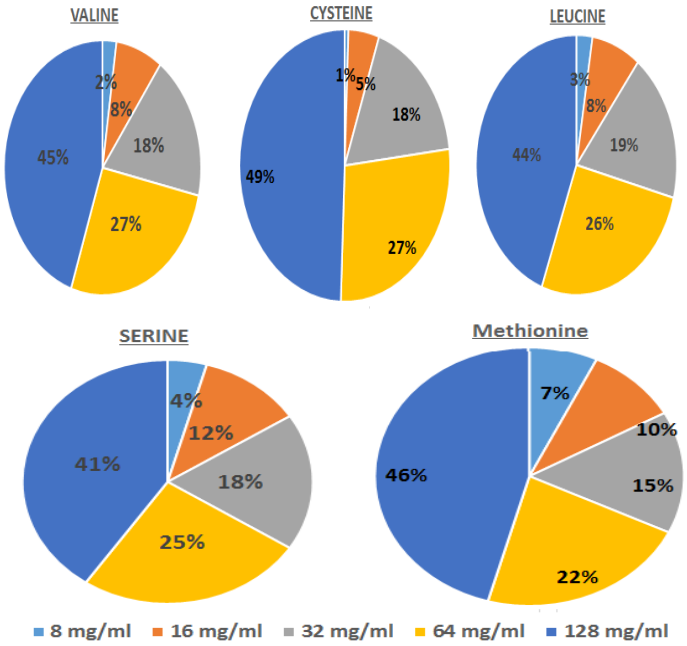

Fig.2. Amino acid percentage content in aqueous extract of *Lemna minor*.

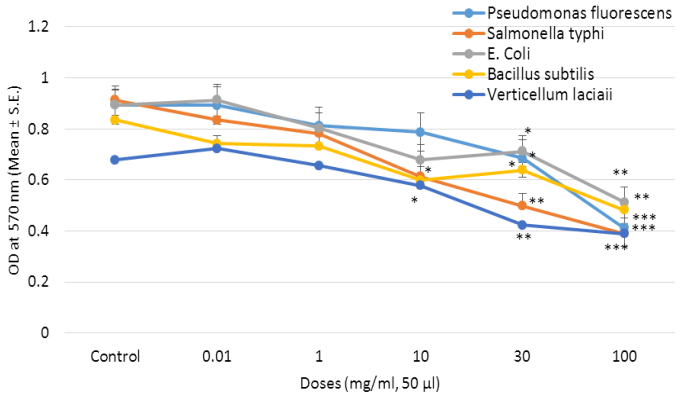

Fig. 3: Antimicrobial activity. Bacterial strains (105 CFU/ml; 50 µl) and fungal extract (50 µl) strain were treated with variable concentration (0.01 – 100 mg/ml, 100 µl) of aqueous extract of Lemna minor in lysed human whole blood. After incubation, add MTT solution and then incubated at 37°C for 4h. Thereafter, media-containing MTT was removed and formazan crystals were dissolved in DMSO and then its absorbance was recorded in microplate reader at 570 nm. The difference between the control and variable doses of aqueous extract is determined by one way ANOVA test.

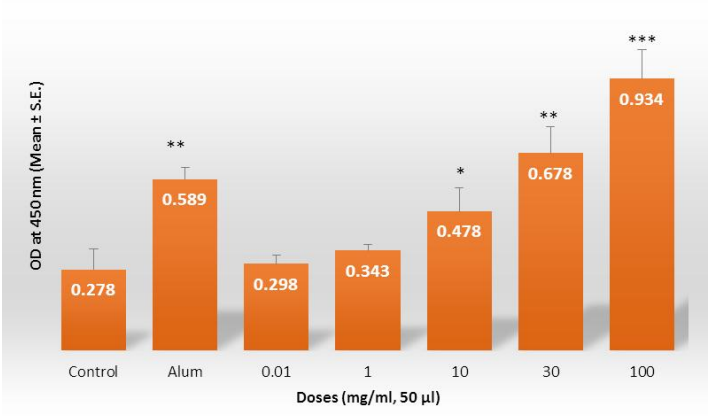

Fig. 4: ELISA assay. Indirect Elisa was performed using standard Ovalbumin (1 mg/ml; 100 µg/well) as coating antigen. Aqueous extract of Lemna minor were used for the estimation of anti-OVA antibody titre. Horse antiserum used as secondary antibody and optical density measured at 450 nm. The difference between the control and standard is determined by one way ANOVA test.

DISCUSSION

Lemna minor, aquatic plant that are present abundantly in lakes, streams and ponds. This aquatic plant is generally floats on the water surface and is very sensitive with respect to xenobiotic substances.[13,14] Lot of research articles that are published related to heavy metal toxicity test which is directly correlated with growth parameters and physiological and biochemical indicators, including changes in carbohydrate, protein and chlorophyll content.[15] Due to its small size and high multiplication rates of *Lemna minor* and included as one of the most used aquatic plants in toxicity testing procedures of various inorganic and organic chemicals.[16] Recently, none of these studies will be done related to aqueous extract of *Lemna minor* and studied its immunological and microbiological aspects. In this regard, we focused on its antimicrobial along with immunomodulatory effects of aqueous extract of *Lemna minor*.

In the present study, we identified various biologically active compounds in the form of protein including amino acids that are present in *Lemna minor* and determined its immunomodulatory potential against specific protein antigen i.e. ovalbumin and also measured its antimicrobial activity against these bacterial and fungal strains. Bradford method was applied and used to measure various amino acids (valine, leucine, serine, threonine and cysteine) that are present in aqueous extract, spectrophotometric method was applied and compared its activity with standard amino acid. In addition, protein estimation was determined through Nanodrop method. The results showed that *Lemna minor* showed rich amount of amino acids that are present including protein content. In this regard, biologically active substances in *Lemna minor* are of interest for further investigation of its immunopharmacological properties with the aim of seeking new medicinal properties.

In this study, ovalbumin used as coating antigen for estimating antibody production against variable doses of aqueous extract of *Lemna minor* pertaining to determined its immunological activity. This antigen was strongly recognized by aqueous extract containing primary (protein and amino acids) and secondary metabolites. In other words, there was good correlation between aqueous extract of *Lemna minor* and conventional specific protein (ovalbumin) antigen both by Elisa assay. The conclusion was that aqueous extract showed immunostimulatory potential at higher doses against ovalbumin. From this data it may confirm that at higher doses showed immunostimulatory activity. This study suggest that aqueous extract of *Lemna minor* may stimulate antigen (ovalbumin) specific immune response and this study will have a way for future investigators to elucidate a suitable component for immunomodulation in order to make an individual recovered from infectious diseases.

In this study, fresh whole plant of *Lemna minor* was tested for its antimicrobial activity against four bacterial and one fungal strains. The results showed that aqueous extract at higher concentration produced dose dependent zone of inhibition after 24 h incubation against both Gram-positive and Gram-negative bacteria including fungal strain in lysed human whole blood. From these studies, it is confirmed that aqueous extract are reported to have a better antimicrobial activity against different bacterial pathogens and fungal strain with significant inhibition of growth of these pathogens as compared to the aqueous extracts. However, further studies related to aqueous extract of *Lemna minor* are continued in order to explore or examined their effectiveness to inhibit the growth of parasites, viruses and fungi. Further studies are still is in progress in order to isolate secondary metabolites from this aqueous extract and hopefully it may showed better antimicrobial activity. Future epidemiological studies are necessary to understand the effectiveness/impact of use of these aqueous extracts from such medicinal plants in population.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between four authors. Dr Gupta designed the study, wrote the protocol and interpreted the data. Dr Gupta and Vishakha Mane anchored the field study, gathered the initial data and performed preliminary data analysis. Dr Gupta and Vishakha Mane, Dr Nilima and Dr Bharat Shinde managed the literature searches and produced the initial draft. All the authors read and approved the final manuscript.

CONFLICT OF INTEREST

Authors have declared that no conflicts of interest exist.

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REFERENCES

1. Hancock RE, Sahl HG. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nat Biotechnol 2006; 24: 1551–1557.
2. Gupta A, Khajuria A, Singh J, Bedi KL, Satti NK, Dutt P, Suri KA, Suri OP, Qazi GN. Immunomodulatory activity of biopolymeric fraction RLJ-NE-205 from Picrorhiza kurroa. International Immunopharmacology 2006; 6(10): 1543 - 1549.
3. Khajuria A, Gupta A, Singh S, Suden P, Singh J, Gupta BD, Suri KA, Suden P, Srinivas VK, Ella E, Qazi GN. Immunomodulatory activity of biopolymeric fraction BOS 2000 from Boswellia serrata. Phytotherapy research 2007; 22(3): 340 – 348.
4. Gupta A, Khamkar PR, Chaphalkar SR. Applications and uses of active ingredients from medicinal plants. Indian Journal of novel drug delivery 2014; 6(2): 106 – 111.
5. Davis SM, Drake KD, Maier KJ. Toxicity of boron to the duckweed Spirodella polyrrhiza. Chemosphere 2002; 48: 615-620.
6. Driever SM, Nes EHV, Roijackers RMM. Growth limitation of Lemna minor due to high plant density. Aquat Bot 2005; 81: 245-251.
7. Prasad MNV, Malek P, Waloszek A, Bojko M, Strazalka K. Physiological responses of Lemna trisulca L. (duckweed) to cadmium and copper accumulation. Plant Science 2001; 161: 881-889.
8. Axtell NR, Sternberg SPK, Claussen, K. Lead and nickel removal using Microspora and Lemna minor. Bioresource Technol 2003; 89: 41-48.
9. Kamal M, Ghaly AE, Mahmoud N, Cote R. Phytoaccumulation of heavy metals by aquatic plants. Environ Int 2004; 29: 1029-1039.
10. Gupta A, Potdar J, Chaphalkar SR. Evaluation of antibacterial activity of medicinal plants against Pseudomonas fluorescens and Bacillus subtillis. World Journal of Pharmaceutical and medical research 2016; 2 (4): 142 -145.
11. Gupta A, Potdar J, Chaphalkar SR. Screening of medicinal plants through ELISA against Pacillomyces liacinus and Trichoderma viride. World Journal of Pharmacy and Pharmaceutical sciences 2016; 5 (8): 829-837.
12. Gupta A, Chaphalkar SR. Immunoadjuvant potential of Azadirachta indica against rabies, hepatitis and DPT vaccine antigen. International Journal of Medical and pharmaceutical sciences 2015; 5(7): 1 – 5.
13. Das P, Samantaray S, Rout GR. Studies on cadmium toxicity in plants: A review. Environmental Pollution 1997; 98: 29–36.
14. Garnczarska M, Ratajczak. Metabolic responses of Lemna minor to lead ions I. Growth, chlorophyll level and activity of fermentative enzymes. Acta Physiologia Plantarum 2000; 22: 423–427.
15. Maltby L, Clayton SA, Yu H, Mcloighlin N, Wood RM, Yin D. Using single-species toxicity tests, community-level responses, and toxicity identification evaluations to investigate effluent impacts, Environmental Toxicology and Chemistry 2000; 19: 151–157.
16. Manusadzianas L, Balkelyte L, Sadauskas K, Blinova I, Põllumaa L, Kahru A. Ecotoxicological study of Lithuanian and Estonian wastewaters: selection of the biotests, and correspondence between toxicity and chemical-based indices, Aquatic Toxicology 2003; 63: 27–41.