

# Duckweed as a Source of Antioxidants for Pharma Applications

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**Received date:** January 03, 2025; **Accepted date:** February 20, 2025; **Published date:** March 04, 2025

**Citation:** Ali Imran, Naseem Zahra, Muhammad K. Saeed, Imam Abidi SH, Ain Syed QU, (2025), Duckweed as a Source of Antioxidants for Pharma Applications, *J. Nutrition and Food Processing*, 8(3); DOI:10.31579/2637-8914/291

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## Abstract:

Duckweed is the smallest specie of flowering plants belonging to family Lemnaceae. Lemna Minor L. (LM) is a nearly universal aquatic perennial plant that is a member of the family Lemnaceae and genus Lemna. It is used in traditional medicine to cure a variety of conditions, including gout, rheumatism, allergies, asthma, vitiligo, jaundice, and glaucoma. It is a source enhanced with protein. These could yield more biomass that is rich in protein. In some impoverished parts of South Asia, such as Thailand, Myanmar, and Laos, duckweed is eaten by people. Their chemical makeup and strong growing capacity make them appropriate for use in applications related to human health. Reactive oxygen species (ROS) comprise free radicals such superoxide anion radicals (O<sub>2</sub><sup>-</sup>) as well as other radicals. Activated oxygen can take many different forms, including hydroxyl radicals (OH<sup>·</sup>) and non-free radical species like H<sub>2</sub>O<sub>2</sub> and singlet oxygen (O<sub>2</sub>). These chemicals are aggravating elements in aging and cellular damage. Antioxidants can slow the progression of many chronic diseases as well as lipid peroxidation. They can also shield the human body from the impacts of free radicals and ROS. The objective of this study is to check antioxidant activity of duckweed. The results of antioxidant studies of duckweed showed that all duckweed extracts have strong free radical scavenging ability, but the free radical scavenging activity of the water extract varied from (21.60±2.09-70.42±3.30%) than methanol extracts (10.27±1.40-37.73±2.60%) and methanol: water extracts (2.64±0.50-16.95±1.15%) at concentrations of 0.2–1.0 mg/ml. According to the findings, duckweed is cheap source of natural antioxidants that can be successfully used in pharmaceutical, food and other fields.

**Key words:** duckweed; antioxidants; nutritional aspects; pharma applications; DPPH

## Introduction

Duckweed (*Lemna minor*) has historically been used as a soporific, astringent, depurative, diuretic, antipruritic, and antiscorbutic. It was also used to treat oedema, measles, colds, and urinating difficulties. Along with many other ingredients, it included proteins, lipids, carbs, flavonoids, and trace minerals. The Pharmacological investigations demonstrated that it had cytotoxic, immunomodulatory, antioxidant, and antibacterial properties (Al-Snafi, 2019).

It has been evident in recent years that these chemicals make up duckweed chemical composition: vitamins, triterpene compounds, aliphatic and phenolic acids, proteins (up to 35%), vegetable fibers (up to 17%), lipids (up to 5%), polysaccharides, flavonoids, amino acids, and other things (Petrova-Tacheva et al., 2020). These compounds have a notable portion of antioxidant action. The most usually utilized cell reinforcements right now are BHT, BHA, propylgallate and tert-butyl hydroquinone (Gülçin et al., 2010). Be that as it may, their wellbeing has as of late been addressed because of poisonousness, liver harm and conceivable cancer-causing nature. In this manner, the improvement of more secure cell reinforcements from normal starting points has been of interest.

Duckweed has been proposed as a potential source of pharmaceuticals. Duckweed species including *L. minor*, *L. trisulca*, and *S. polyrrhiza* have reportedly been used extensively as folk medicine in China, Korea, and a few European countries, according to earlier investigations. Duckweed

doesn't have many negative effects on human health when utilized in pharma products (Bolotova, 2015; Doğan et al., 2022; Ahn et al., 2004).

Duckweeds have a variety of pharmacological effects, as recent studies have shown. *L. minor* possesses antibacterial properties against both gram-positive and gram-negative bacteria, including *Shigella flexneri*, *Escherichia coli*, *Salmonella typhi*, and *Pseudomonas fluorescens*. As such, it may serve as a substitute for antibacterial drugs in the management of a range of illnesses (González-Rentería et al., 2020; Mane et al., 2017). Additionally, *S. polyrrhiza* demonstrated antibiotic activity against two fungal infections, one gram-positive bacterium, and seven gram-negative bacilli (Das et al., 2012). Duckweed's flavonoids may provide metabolites that support antioxidant action (Pagliuso et al., 2020).

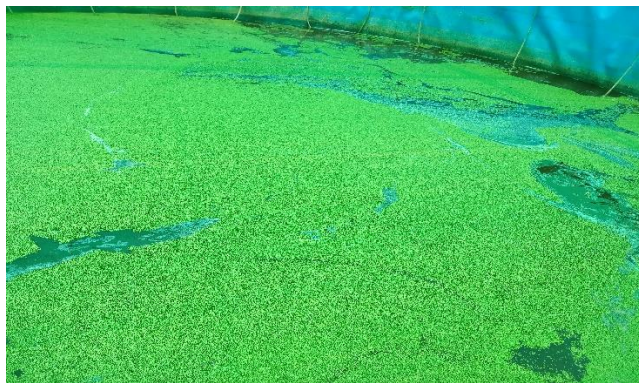
Several epidemiological studies show the advantages of consuming antioxidants, which reduce the risk of oxidative stress-related diseases like diabetes, cancer, and heart disease. Dietary antioxidants can help prevent and treat a number of ailments by scavenging free radicals and lowering oxidative stress (Adin et al., 2023). Dietary antioxidants are a hidden alternative for synthetic antioxidants, as their usage is restricted due to possible health risks. Plant biowaste is produced in large and inexpensive quantities, which makes it suitable for use in the food industry to create novel and beneficial foods like antioxidants (Saeed et al., 2022).

Figure 1 and Figure 2 showed the development of duckweed in pond installed at PCSIR Laboratories Complex, Lahore, Pakistan. The current

study aims to detect antioxidant activity of duckweed cultivated in the pond installed at PCSIR Laboratories Complex, Lahore Pakistan.



**Figure 1:** Augmentation of duckweed in pond at PCSIR Laboratories Complex, Lahore Pakistan



**Figure 2.** Growth of duckweed in pond

## Methodology

### Chemicals and Reagents

Methanol, chloroform, citric acid, DPPH, and Folin's phenol reagent are analytical grade chemicals obtained from Sigma, Aldrich, and Fluka Chemical Co. (St. Ouis, Mo., USA). The distilled water was made using equipment for distillation.

### Material preparation

Duckweed was taken from pond and was properly cleaned and washed with distilled water. The duckweed was dried in a hot air oven at 45°C. By using milling blender, the dried duckweed was grinded to homogeneous powder that was passed through a mesh screen sieve. The duckweed powder was kept stored in polyethylene air tight bad at 4±2 °C in refrigerator for further analysis (Saeed et al, 2024).

### pH of duckweed powder

A suspension of duckweed powder (5% w/v) was prepared and agitated for 5 minutes and then let to stand for 30 minutes before filtration. The filtrate's pH value was determined by using a pH meter (InoLab pH Level-1, Germany) (Saeed et al, 2024).

### Preparation of extract for antioxidant study

5g of duckweed powder was taken and was added to 100ml methanol and methanol: water (1:1). In case of aqueous solution extraction 5g of duckweed powder was added to 100ml distilled water and heated for 2

hours. The supernatant was collected and filtered with filter paper for antioxidant study (Velumani, 2016).

### Antioxidant activity by DPPH assay

The DPPH free radical scavenging activity was measured using duckweed powder methanolic, aqueous, and chloroform extracts, slightly modified according to Brand-Williams (1995) method (Brand-Williams et al., 1995; Saeed et al., 2021). The ability of stable 2, 2-diphenyl-1-picrylhydrazyl to scavenge free radicals was used to assess the antioxidant activity of extracts from duckweed. Three milliliters of a 0.004% DPPH in methanol solution were applied to samples containing one to five milligrams per milliliter. The optical densities of various solution combinations were determined at 517 nm using a spectrophotometer (UV-Vis-1700, Shimadzu, Japan) after 30 minutes in the dark. Antioxidant activity was measured using 100 µl of methanol and 3 ml of DPPH solution as a blank. Following formula was used to calculate antioxidant activity.

$$\text{Antioxidant activity \%} = 1 - [A_{\text{sample}}/A_{\text{control}}] \times 100$$

### Statistical Evaluation

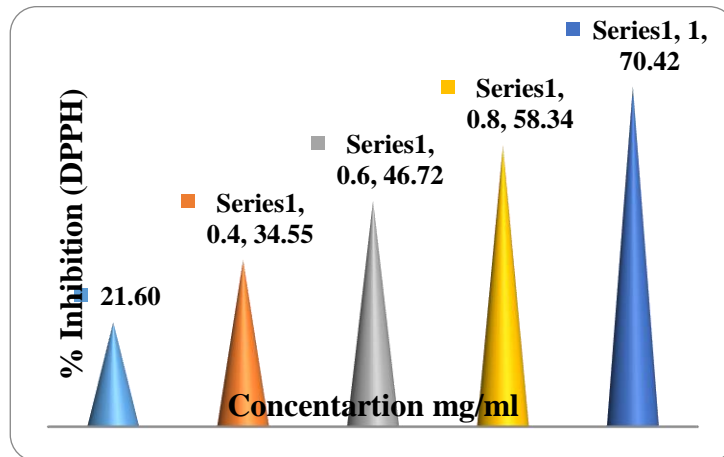
The results were displayed as mean standard deviation (SD). The utilization of one-way investigation of variance to genuinely assessed the information (Sharoba et al., 2013). The Tukey test was done to see whether there was any progression between sample means that were significant at p=0.05.

**Results and Discussion**

pH of the duckweed powder was determined and it was 6.5. The test DPPH (2,2-diphenyl-1-picrylhydrazyl) is frequently used to evaluate a ingredient's ability to scavenge free radicals (Saeed et al., 2022, Tawfeeq et al., 2023). The antioxidant properties of different powdered duckweed extracts demonstrated that the water extract had the best ability to scavenge free radicals compared to the methanol extract, with methanol: water extracts coming in second. Duckweed' percentage inhibition of DPPH in of the water extract (Figure 3) varied from (21.60±2.09-70.42±3.30%) than methanol extracts ((Figure 4) (10.27±1.40-37.73±2.60%) and methanol: water (1:1) extracts (Figure 5) (2.64±0.50-16.95±1.15%) at concentrations of 0.2–1.0 mg/ml. The outcomes align

with the specified body of literature (Hu et al., 2022). Increasing the concentration of the sample, decrease the absorbance values which increase the % inhibition value that is capable of scavenging DPPH free radicals.

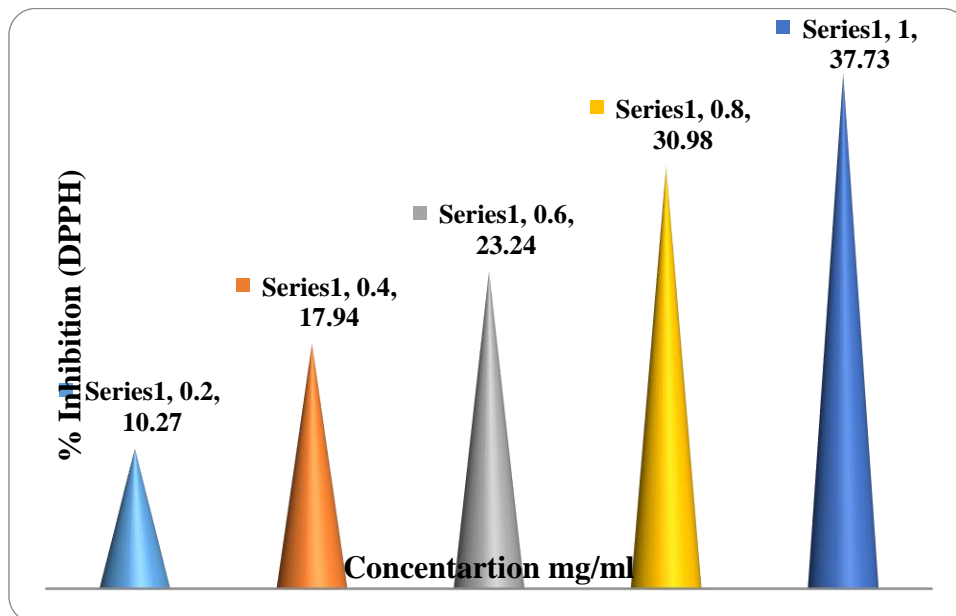
The lipid peroxidation of linoleic acid emulsion was completely inhibited at 45 mug mL<sup>-1</sup> concentrations of lyophilized water extract (WELM) and ethanol extract (EELM) of duckweed, with 100% and 94.2% inhibition, respectively. Conversely, at the same concentration, BHA, BHT, alpha-tocopherol, and trolox showed inhibition of 92.2%, 99.6%, 84.6%, and 95.6%, respectively, on linoleic acid emulsion peroxidation in comparison<sup>3</sup>. The L. minor that we utilize has been shown to be a potent antioxidant (Doğan et al., 2022).



**Figure 3:** % Inhibition (DPPH) of water extract of duckweed powder

In a study conducted by Islam et al. (2021), it was shown that DPPH scavenging activity of methanol extract of duckweed at 250µg/ml was 85.29±0.42 %. However, in the current study the methanol extract showed 37.73±2.60% inhibition at mg/ml by only soaking duckweed powder in methanol overnight.

*E. fluctuans* and *I. aquatica*, two aquatic plants ingested by humans, demonstrated DPPH scavenging activity of 84.80±0.70% and 79.52±0.87%, respectively, showed a scavenging pattern that was comparatively similar, as reported by Simlai, et al. (2014). The aquatic plants that were collected exhibited noteworthy DPPH radical scavenging capability due to the presence of secondary metabolites and polyphenols.



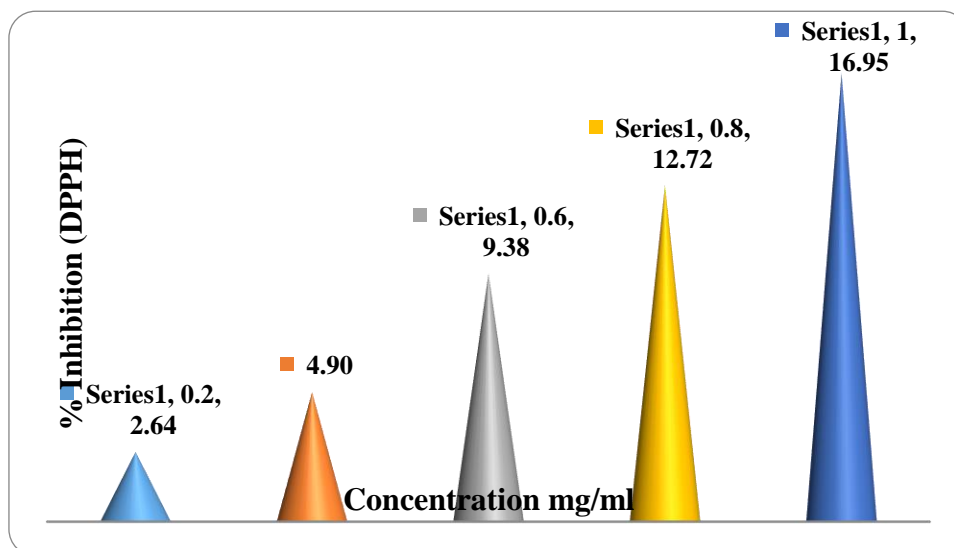
**Figure 4:** % Inhibition (DPPH) of methanol extract of duckweed powder

This aquatic plant (duckweed) is typically regarded as an unwanted species with little commercial value. However, this study examined its

potential medical benefits, including antioxidative capacity (Yahaya et al., 2022). Positive results from a variety of antioxidant assays suggest

that its phenolic components can take the role of synthetic, harmful antioxidants employed in the food, cosmetic, and pharmaceutical industries. Additional research is necessary to separate its phenolic

components and determine whether they have any potential uses as antioxidants in other fields.



**Figure 5:** % Inhibition (DPPH) of methanol: water (1:1) extract of duckweed powder

The oxidative stress is a major problem but the use of plant material can reduce ROS damage through several mechanisms (Masood et al., 2023). Duckweed powder can be used as a natural antioxidant and this free radical removal of duckweed powder was due to its polyphenolic content. It is believed that the total phenolic content of duckweed is higher in *L. minor* specie. To learn more about duckweed's potential as an affordable, sustainable source of health supplements with high antioxidant content (Masavang et al., 2022; Pagliuso et al., 2020; Xu et al., 2023; Appenroth et al., 2017, Dinev et al., 2021) more in-depth research should be done (Naseem et al., 2021).

## Conclusion

The present finding demonstrated that duckweed has excellent antioxidant potential and best for use in food, medication, and pharmaceuticals. % Inhibition (DPPH) assay of water extract of duckweed powder showed maximum antioxidants potential. In light of discussion above all the extracts have the potential to be used for the antioxidant properties of an aquatic plant: duckweed (*Lemna minor* L. Lemnaceae). Duckweed may also be used to reduce or eliminate lipid oxidation in food products, delay the development of harmful oxidation products, preserve nutritional value, and increase the shelf life of foods and medications.

## Statement Of Ethics

All the necessary ethical rules were followed by the researchers.

## Conflict Of Interest Statement

The authors declare that there is no conflict of interest.

## Authors Contribution

Dr. Naseem Zahra provided the idea of the research work and helped in compiling data and paper writing. Engr. Ali Imran performed experimental work on duckweed and improved duckweed growth. Dr. Muhammad Khalid Saeed and Dr. Naseem Zahra checked antioxidant activity. Dr. Syed Hussain Imam Abidi and Dr. Qurat-ul-Ain Syed reviewed all work.

## Funding Sources

No funding source was received for this study.

## Acknowledgement

The authors are thankful to all the reviewers contributing towards improving manuscript quality.

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