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SCREENING OF MEDICINAL ALGAL EXTRACTS FOR THEIR ANTIOXIDANT POTENTIAL

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Abstract
Reactive oxygen species are linked to the ageing process, exacerbating a variety of ailments such as Alzheimer's, Parkinson's, cancer, atherosclerosis, and arthritis, among others. These damaging impacts of reactive oxygen species can be mitigated to some level by the use of antioxidants found in algae. Algae are known to obtain a variety of bioactive secondary metabolites, and various chemicals have been produced from them for the drug industry's future development of innovative medications. In this regard, *Rhizoclonium* species and *Hydrodictyon reticulatum* (L.) Lagerheim isolated from Prayagraj district Ganga water were tested for antioxidant activities. Extracts of the algae selected for the study were prepared using different organic solvents (methanol, ethyl acetate and hexane). The results revealed that methanolic extracts of *Rhizoclonium* species had the maximum antioxidant activity (93.6%) and minimum with hexane (65.05%). *Hydrodictyon reticulatum* (L.) Lagerheim showed good inhibition activity with Ethyl Acetate (79.1%) and less activity with hexane (75.8%). All the extracts for the antioxidant activity have been compared with gallic acid. The main objective of the study was to evaluate the antioxidant activity of particular Ganga water algae and their potential for usage as significant medications in the health industry to treat diseases caused by reactive oxygen species. The DPPH free radical scavenging technique was used to measure the extracts' antioxidant activity.

Keywords: Antioxidant activity, Algae, Reactive Oxygen Species, Pharmaceutical Industry, Organic Solvent

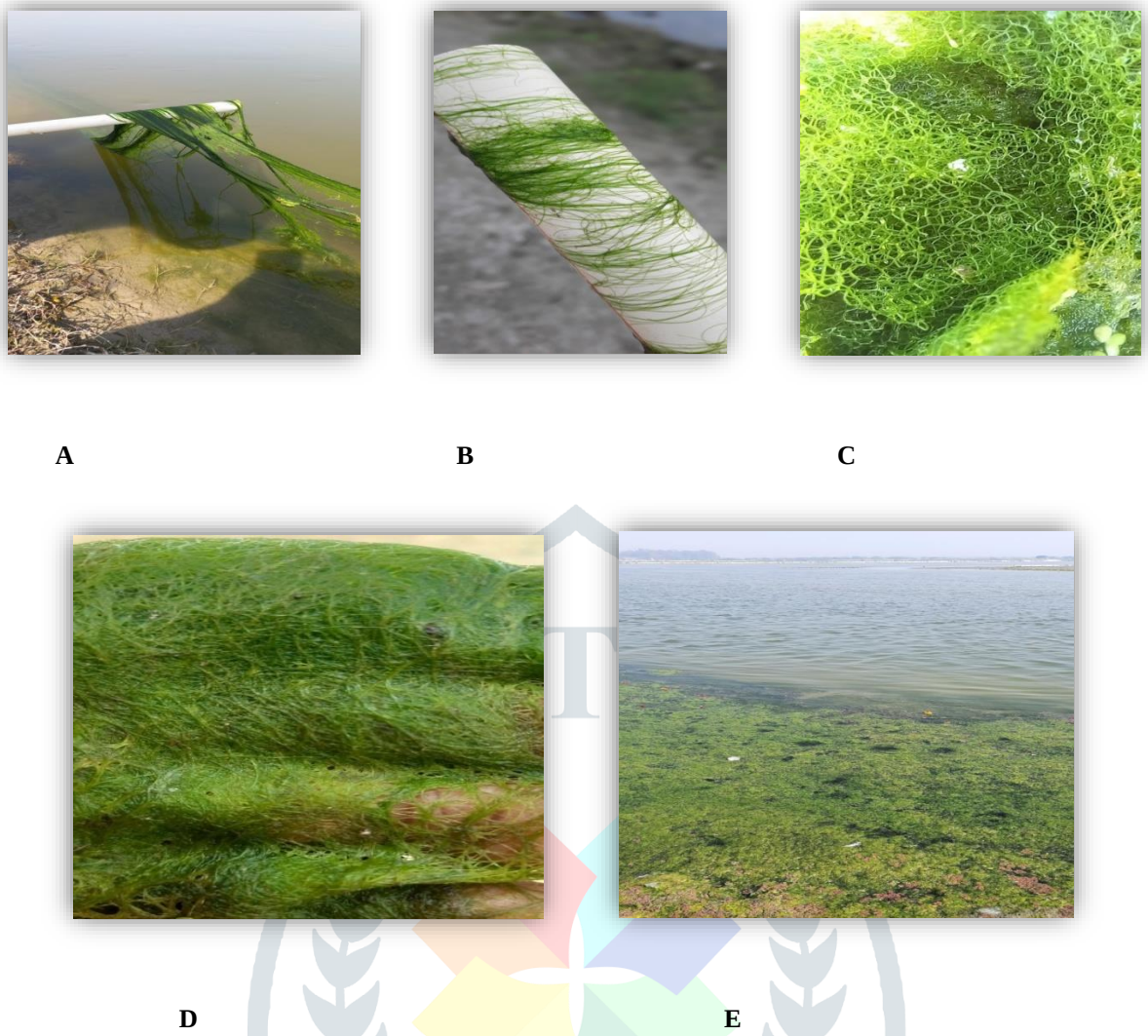
Introduction
It is well known that oxidative stress is one of the major triggering causes of several chronic and degenerative diseases, such as atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases, and others (Souri E. 2008). Oxidative stress is brought on by an unbalanced redox state of the body. Free radicals accumulate as a result of oxidative stress, which damages tissue. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) constitute the majority of free radicals. Enhanced metabolic activity, mitochondrial dysfunction, peroxisome activity, increased signaling via receptor mediation, activation of oncogenes, and increased activation of oxidase, cyclooxygenase, lipoxygenase, and thymidine phosphorylase are all causes of an increase in ROS in cancer cells (Czerwonka, A. 2018).
Antioxidants are the most efficient approach to getting rid of the free radicals that cause oxidative stress. Exogenous and endogenous, synthetic and natural antioxidants can be useful in reducing the development of free radicals by scavenging them or stimulating their breakdown and suppressing such illnesses (Souri E. 2008).

Because of its high quantities of carbs, proteins, lipids, and polyunsaturated fatty acids, microalgal biomass has historically been used as a food and food additive. Microalgal biomass contains pigments, phenolic antioxidants, vitamins, and minerals that may have health advantages (Koyande, A. K. 2019). Algal biomass has been shown to have the ability to produce high-value commodities including bioactive molecules, biostimulants, and biologically active compounds (Khoo, C. G. 2019). In *Chlorella pyrenoidosa* and *Spirulina platensis*, for example, phenolic compounds with antioxidant activity were discovered at total levels of around 18 and 43 mg gallic acid eq. per gm biomass, respectively (Machu, L. 2015). To function as antioxidants, phenolic compounds can transfer a single electron or a hydrogen atom (Goiris, K. 2012). They are thought to form the cornerstone of an antioxidant defence system that shields against degenerative disorders linked to an excess of free radicals or reactive oxygen species, including cancer, cardiovascular disease, diabetes, osteoporosis, and neurodegeneration (Pandey, K. B. 2009). Through oxidative/antioxidant bleaching, they can serve as skin-whitening agents for cosmeceutical purposes (Almendinger, M. 2020). In addition to phenolic chemicals, pigments also exhibit some antioxidant action. Because of their conjugated double bonds, carotenoids, a broad family of fat-soluble pigments, can absorb short-wave light and give plants and animals their distinctive color tones. According to research, carotenoids can physically extinguish singlet oxygen, eliminate radicals by transferring hydrogen atoms, or receive electrons from radicals (Martínez, A. 2008). Depending on the algal strain and growing conditions, the carotenoid concentration of biomass can range from around 1 to 30 mg g-1 (Banskota, A. H. 2019). Chlorophyll, carotenoids, and other colors that are linked with fat accumulate in many animal tissues when utilized as feed ingredients, as is well known (Almendinger, M. 2021). The antioxidant activity of biomass and extracts isn't reliant on a single component, but rather a blend of many antioxidants' aromatic chemicals (like phenolic compounds and aromatic amino acids), carotenoids, and other unclassified substances (Goiris, K. 2012). Because of these combinatorial, cumulative, or hostile effects, even when the amounts of individual bioactive compounds are known, the final antioxidant capacity may still be greater or lower (Wang, S. 2011), necessitating a study of the biomass and derived extracts. However, as phenolic chemicals are more commonly found in plants, the debate over their presence in microalgae and cyanobacteria is still quite contentious (Almendinger, M. 2021).

Usually, antioxidants are compounds that either prevent or efficiently limit the harmful activity of reactive oxygen variants. Since herbs and spices are now a natural source of antioxidants, extensive research has been done on their antioxidant qualities (Premkumar, K. 2001). Phycocyanin, chlorophyll, myxoxanthophyll, beta-carotene, zeaxanthin, and xanthophyll are just a few of the granule compounds found in *Arthrospira platensis* which is a well-known blue-green alga and a rich source of biological products (Al-Qahtani, W. H. 2019). There has been a lot of interest in the use of extracts and medicinal algae for the treatment of cancer, and it would appear that these alternatives to chemical treatments are acceptable (Tajvidi, E. 2021).

Material and methods

Algal samples were collected from Ganga River Phaphamau Prayagraj, UP (25.505º N, 81.868º E) in March 2021. Chemicals such as Methanol were purchased from Merck life sciences Pvt. Ltd. Mumbai India; Ethyl acetate was purchased from Thermo Fisher Scientific India Pvt. Ltd. Mumbai; Hexane LOBA CHEMIE Pvt. Ltd Mumbai, India; Gallic acid was purchased from CDH Pvt. Ltd. New Delhi, India. 1, 1- diphenyl-2- picrylhydrazyl (DPPH*) was purchased from HiMedia Pvt. Ltd. Mumbai, India. The absorbance of all samples was recorded by UV- Visible double-beam spectrophotometer (SYSTRONICS AU-2701). The experiments were performed in the Department of Botany and Department of Chemistry, CMP Degree College (Constituent College of University of Allahabad) Prayagraj, Uttar Pradesh, India in October 2021.



A **B** **C**

D **E**

Fig. A- B Algal sample *Rhizoclonium* sp., **C- D** Algal sample *Hydrodictyon* sp., **E** Ganga River

1. Sample Preparation

The algal biomass was collected in the month of October 2021 from the River Ganga. Systematic authentication of the algal samples was carried out at Botany Department CMP Degree College Prayagraj and the status assigned to the samples are *Rhizoclonium* species i.e., sample-1 and *Hydrodictyon reticulatum* (L.) Lagerheim i.e., sample-2. The wet algal biomass collected from River Ganga Prayagraj was manually washed several times with water to remove its impurities like sand, leaves etc. After washing the biomass was dried in natural sunlight for 2-4 days followed by drying in an oven at 45^0C for 24 hrs after which the milling of the samples was done and the fraction with 200-500 µm particle size was then used for further experiment.

2. Preparation of sample extract

Crushed samples of algae (2g) were refluxed separately with 20 ml of methanol, ethyl acetate and hexane in the water bath for 30 minutes. The extracts were filtered and solvents were evaporated in a rotary evaporator under pressure to obtain powder form. Now the antioxidant potential of different solvent extracts of the above medicinal algae and their active principals were evaluated by DPPH* assay.

3. 1, 1-Diphenyl-2-picrylhydrazyl assay (DPPH* Assay)

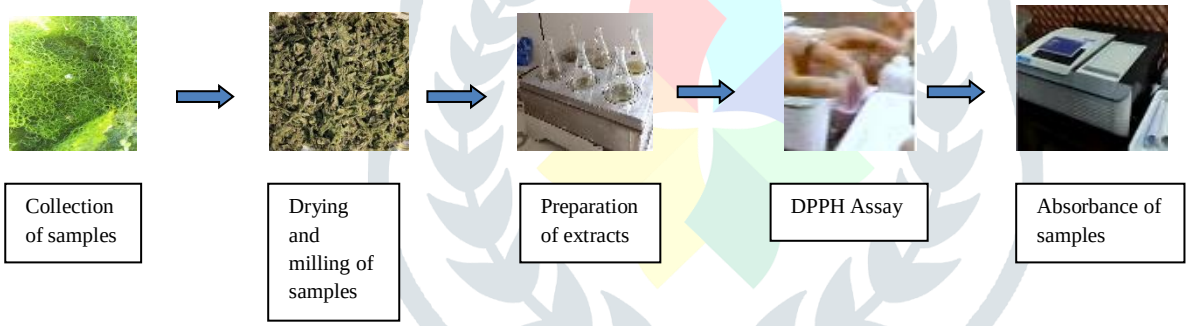
The capacity of the algal extract to donate hydrogen atoms or electrons was assessed by bleaching a purple-coloured methanol, ethyl acetate and hexane solutions of DPPH*; gallic acid was employed as the reference. The stable radical DPPH* is used as the reagent in this spectrophotometric test. The process entails measuring the decline in DPPH*(0.002%) absorbance at 517 nm absorption maximum.
The stock solutions of the extracts were prepared in methanol, ethyl acetate and hexane (1 mg/10 ml). Different volume (2.0, 1.5, 1.0, 0.5, 0.25 and 0.125 ml) of extracts was taken in separate test tubes and volumes were made up to 2 ml with different solvents. Now, 2ml of DPPH* solution was added to each test tube and kept in dark for 30 minutes. The same procedure was followed for gallic acid as well. Later optical absorbance was recorded at 517 nm using UV- Visible spectrophotometer.

Methanol with DPPH* was used as a control. All the samples were tested in triplicate.

The formula used for the calculation is:

$$\% \text{ Inhibition of DPPH* activity} = (A - B / A) \times 100$$

Where A = absorbance of control; B = absorbance of the sample.



Results

The antioxidant value of algal extracts of different solvents and their bioactive constituents were evaluated by DPPH* assay.

Calculation of % Radical Scavenging from DPPH Assay

Table 1: Antioxidant Activity of *Rhizoclonium* species

Concentration (µg/ml)	Sample A with methanol (%)	Sample A with Ethyl Acetate (%)	Sample A with Hexane (%)	Gallic Acid (%)
12.5	87.06	74.9	45.9	89.3
25	88.4	75.4	48.8	93.93
50	89.9	75.8	50.57	94.21
100	91.3	77.7	55.2	94.79
150	92.27	79.3	62.9	95.08
200	93.6	82.8	65.05	95.66

Table 2: Antioxidant Activity of *Hydrodictyon* species

Concentration (µg/ml)	Sample B with methanol (%)	Sample B with Ethyl Acetate (%)	Sample B with Hexane (%)	Gallic Acid (%)
12.5	66.2	60.23	70.07	89.3
25	67.5	63.12	71.81	93.93
50	69.1	71	73.16	94.21
100	69.8	77.6	73.9	94.79
150	74.5	77.9	75.4	95.08
200	77.2	79.1	75.8	95.66

Methanolic extract of *Rhizoclonium* species shows different antioxidant activity 93.6, 92.27, 91.3, 89.9, 88.4, 87.06 with different concentrations of 200, 150, 100, 50, 25 and 12.5 respectively **(fig 1)** whereas methanolic extract of *Hydrodictyon* species shows different antioxidant activity 77.2, 74.5, 69.8, 69.1, 67.5 and 66.2 with different concentrations 200, 150, 100, 50, 25 and 12.5 respectively **(fig 2)**. Ethyl acetate extract of *Rhizoclonium* species shows different antioxidant activity 82.8, 79.3, 77.7, 75.8, 75.4 and 74.9 with 200, 150, 100, 50, 25 and 12.5 respectively **(fig 1)** whereas ethyl acetate extract of *Hydrodictyon* species shows 79.1, 77.9, 77.6, 71.0, 63.12 and 60.23 with different concentrations 200, 150, 100, 50, 25 and 12.5 respectively **(fig 2)**. Hexane extract of *Rhizoclonium* species shows different antioxidant activity 65.05, 62.9, 55.2, 50.57, 48.8 and 45.9 with different concentrations of 200, 150, 100, 50, 25 and 12.5 respectively **(fig 1)** whereas hexane extract of *Hydrodictyon* species shows 75.8, 75.4, 73.9, 73.16, 71.81 and 70.07 with different concentrations 200, 150, 100, 50, 25 and 12.5 respectively **(fig 2)**. Gallic acid is used as a standard that shows maximum antioxidant activity of 95.66.

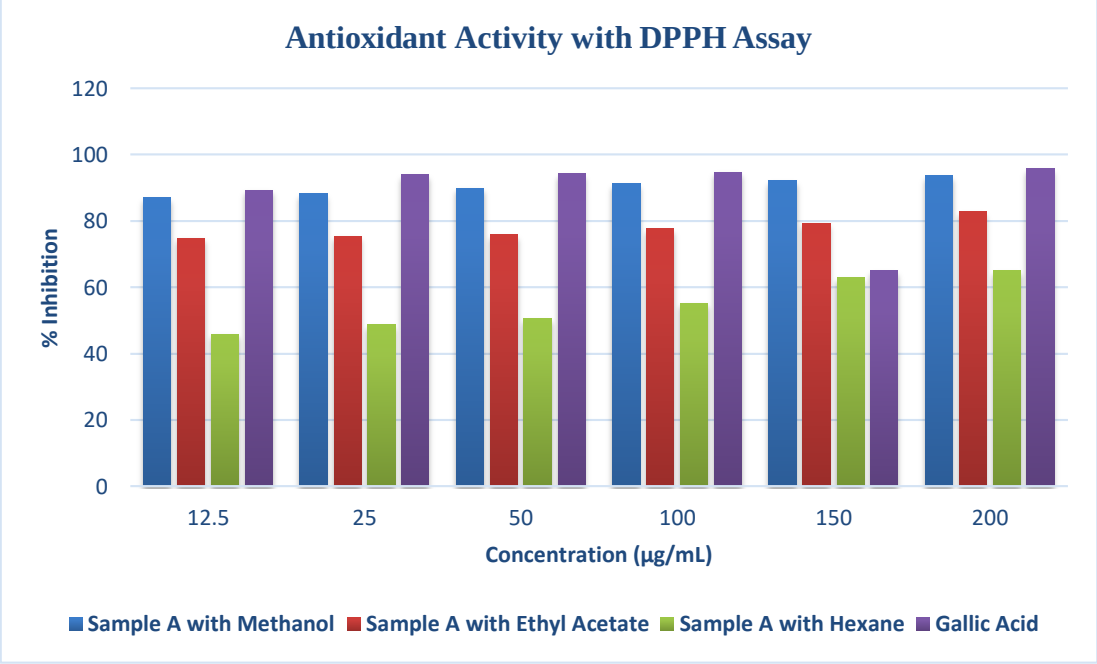


Figure 1: *Rhizoclonium* species showing antioxidant activity with three different organic solvents

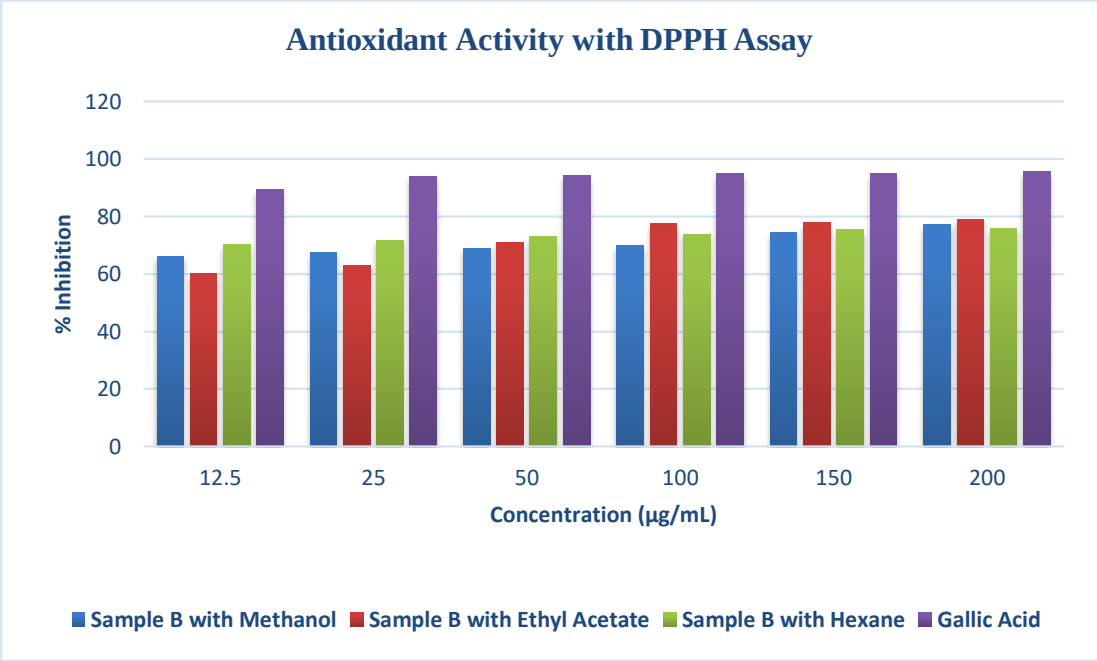


Figure 2: *Hydrodictyon reticulatum* (L.) Lagerheim showing antioxidant activity with three different organic solvents

Discussion

The methanolic extract of *Rhizoclonium* species showed higher antioxidant activity with 95.91% inhibition, in comparison to the methanolic extract of *Hydrodictyon* species which showed antioxidant activity with 77.2% inhibition. The ethyl acetate extract of *Rhizoclonium* species showed higher antioxidant activity with 82.8% inhibition while ethyl acetate extract of *Hydrodictyon* species showed antioxidant activity with 79.1% inhibition i.e. less than *Rhizoclonium* species. The hexane extract of *Rhizoclonium* species showed 65.05% inhibition and hexane extract of *Hydrodictyon* species showed antioxidant activity with 75.8% inhibition which is higher than *Rhizoclonium* species. All the extracts were compared with gallic acid that showed highest antioxidant activity with 95.66% inhibition.

Maximum antioxidant activity has been seen in *Rhizoclonium* species with methanol (a polar solvent) indicates that this species contains mostly polar compounds that have excellent inhibition capacity. *Rhizoclonium* species showed good inhibition capacity with ethyl acetate (less polar than methanol) means this species also contains some less polar compounds. This species with hexane (non-polar) have also some non-polar compounds which showed inhibition activity but less than methanol and ethyl acetate. The *Hydrodictyon* species with ethyl acetate showed maximum inhibition capacity than other two solvents. *Hydrodictyon* species with methanol showed good inhibition capacity but less than ethyl acetate means this species have polar compounds in less quantity. This species showed good inhibition capacity with hexane that is greater than *Rhizoclonium* species with hexane means *Hydrodictyon* species has more non polar compounds than *Rhizoclonium* species and now we can analyze that *Hydrodictyon* species have maximum less polar compounds with good inhibition capacity than polar and non-polar compounds.

Conclusion

It has been observed that all the extracts and bioactive constituents have good antioxidant potential. The most prominent antioxidant activity has been observed in the methanolic extract of *Rhizoclonium* species as compared to all other samples. These species of algae have the potential to be employed as important drugs in the pharmaceutical sector to cure disorders brought on by reactive oxygen species, according to the aforementioned experimental research.

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