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Charophyceae is Promising for Valuable Phytochemical and Antibacterial Properties-A Study

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Abstract
Charophyceae Freshwater algae of genus or macroalgae have been studied for a long time and are ecologically significant. They have recently attracted attention as reservoirs of bioactive compounds with pharmaceutical potential. However, their taxonomical identification is often complicated by morphological plasticity, requiring molecular approaches for reliable authentication. This study aims to genetically identify using conserved domain DNA sequences using PCR and sequencing. Photo-chemicals profiling of ethanolic extract indicate presence of bioactive compound. Antibacterial activity was evaluated against E.coli using standard disk diffusion test.

Keywords: Chara sp., antibacterial, genetic identification, phytochemical analysis, Anti-Microbial-Resistance.

Introduction

The emergence of antimicrobial and multi drug resistance in recent times has created an urgent need for novel therapeutic agents sourced from natural origins. Algae, particularly freshwater macroalgae, represent an underexplored resource for bioactive compounds with potential pharmaceutical relevance. Members of the genus Chara (Charophyceae), commonly known as stonewarts, play vital ecological role in freshwater ecosystems, yet their phytochemical richness and possible biomedical applications remain insufficiently studied (Alsunbul et al., 2025; Khongsai et al., 2023).

Accurate taxonomic identification of macroalgae is challenging due to pronounced morphological plasticity influenced by the environmental conditions. To accurately identify the species, molecular approach like ribosomal (16S rRNA) or the plastidial (rbcl) gene sequence analysis are best of the time (Schneider et al., 2015; Yong et al., 2024).

Preliminary studies have indicated that these macroalgae species contain a variety of secondary metabolites, which include phenols, flavonoids, tannins etc., (Al-Moula et al., 2017; Chankaew et al., 2020) which are associated with antimicrobial and antioxidant properties. Extracts of Chara vulgaris and Chara corallina have been found to be inhibitory in nature against bacterial and fungal pathogens, highlighting the potential of these macroalgae as source of bioactive compounds (Chowdhury et al., 2015; Asmaa et al., 2021; Muhammad et al., 2001; Rajan et al., 2019). However, most existing studies are limited in scope, often lacking molecular authentication.

The current study is designed to fill these gaps by integrating three key components: (i) molecular identification of macroalgae species using barcoding markers, (ii) phytochemical screening of crude extracts to characterize major classes of phytochemicals, and (iii) evaluation of antimicrobial activity against E.coli bacteria.

Materials and Methods

Ribulose - 1, 5 - bisphosphate carboxylase (rbcl) gene based identification, Genomic DNA of macroalgae sample collected from Ratu Lake, Ranchi (Location: 23.414377, 85.216785) was purified using Algal DNA Isolation Kit from Bunshi Bioscience Pvt. Ltd. PCR was done using GoTaq Master Mix (Promega). Ribulose - 1, 5 - bisphosphate carboxylase (rbcl) gene was amplified using universal primer set; forward primer 5'- AGAGTTTGWTCNTGGCTCAG – 3' and reverse primer 5' – GGTTACNTTGTNACGACTT – 3' (Srinivasan et al., 2015). PCR cycling and parameters: PCR cycling parameters were set as follows: initial denaturation at 94°C for 5 minutes, denaturation at 94°C for 30 seconds, primer annealing at 50°C for 45 seconds, extension at 72°C for 1 minute and final extension at 72°C for 5 minutes.

Phytochemical analysis: 5 grams of macroalgae sample was crushed in absolute ethanol and debris was separated using centrifugation. The extract was tested for phytochemical presence (Banu and Catherin, 2015): (a) Alkaloid test: Wagner's Test - macroalgae extract was treated with a few drops of Wagner's Reagent to test the presence of alkaloid. (b) Phenol test: Ferric Chloride Test - macroalgae extract was treated with a few drops of Ferric Chloride to test the presence of phenols (c) Flavonoids test: Alkaline Reagent test - macroalgae extract was treated with a few drops of Sodium Hydroxide and dilute Hydrochloric Acid to test the presence of flavonoids.

Antibacterial activity: Ethanol extract of macroalgae sample was tested against E.coli for antibacterial activity. 100µl of E.coli culture was spread on LB Agar plate and macroalgae extract was soaked on filter paper and placed on the spread E.coli lawn and incubated at 37°C overnight.

Results: rbcl gene was amplified (Fig 1). PCR product Sequence obtained was analyzed using BLAST tool of NCBI Database. The genetic identification using rbcl gene sequence revealed the macroalgae taken for the study was Chara corallina (Fig 2). The ethanol extract of the Chara corallina extract showed significant inhibitory effect of E.coli bacteria with approximately 2.3 mm zone of inhibition (Fig 3). Phytochemicals like alkaloids, phenols, and flavonoids were also present in Chara corallina (Table – 1).

Table – 1: Phytochemical analysis of Chara sample.

Serial No.	Phytochemicals	Test	Present/Absent
1.	Alkaloids	Wagner's Test	+
2.	Flavonoids	Alkaline Reagent Test	+
3.	Phenols	Ferric Chloride Test	+

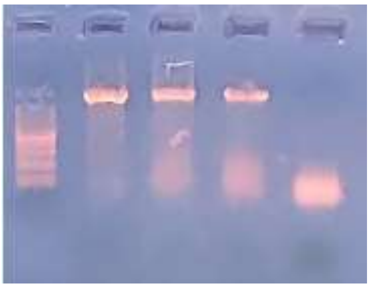

Fig 1: PCR amplification of *Chara corallina*

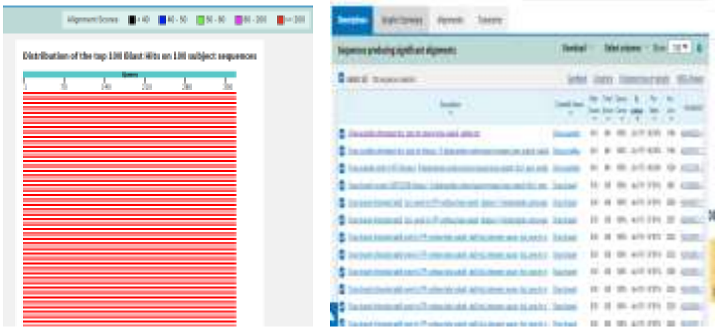

Fig 2: NCBI blast result confirmation of *Chara corallina*

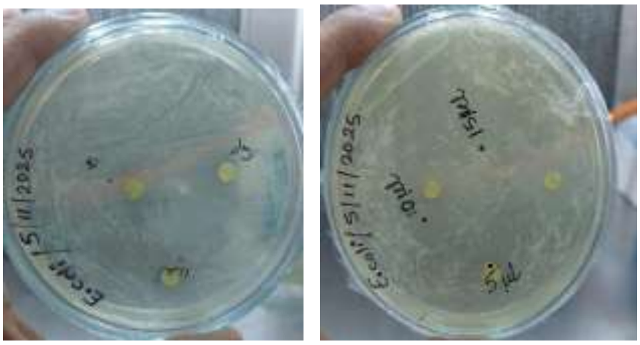

Fig 3: Antibacterial activity of *Chara corallina* against *E.coli*

Discussion:

The identified Chara corallina has been found to significantly inhibit the growth of E.coli bacteria indicating that it can be used for the development of new antibacterial agents and a potent drug for Anti-Microbial Resistance (AMR). Investigation of various sources for antibacterial effects needs to be done so that instances of frequent resistant microbial infections can be minimized.

Conclusion:

Through this study it can be concluded that water-bodies of Ranchi, Jharkhand have abundant presence of various natural bioresources like Charophyta Chara corallina that posses antibacterial activity, against E.coli as investigated in this study. These macroalgae must further be investigated against various other pathogens to assess its potential as a promising antimicrobial source.

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