Research Paper Medical Science

# Culturing, Bioactivity And Chemical Analysis of Microalga *Chlamydomonas* Sp.

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**ABSTRACT**

An attempt was made to isolate and culture a microalgal species of Chlamydomonas from a freshwater pond. Bristol (BBM) medium was found to be an efficient growth medium of culture. A crude extract of the cultured alga was prepared in ethyl acetate and studies for its antimicrobial activity. Results were promising against a few bacteria under study. The active extract when analyzed using TLC and FTIR showed presence of several compounds mainly related to peptides.

**KEYWORDS** *Chlamydomonas*, algae, antimicrobial activity, FT-IR, TLC

## INTRODUCTION

Algae are very simple chlorophyll-containing organisms, found in diverse habitats of the earth. Algae are important as a natural source of bioactive molecules with a broad range of biological activities, such as antibiotics, antiviral, antitumoral, antioxidant and anti-inflammatory. They are also known as a source of amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, halogenated ketones and alkanes and cyclic polysulphides (Annamalai and Nallamuthu, 2014).

In last few years, microalgae metabolites are attracting enormous attention, and there are many reports highlighting the novelty of the microalgae (Choudhary et al., 2000; Kamble and Chavan, 2010; Pradhan et al., 2014). In view of this, the present study was undertaken to culture a freshwater microalga *Chlamydomonas*. Extract prepared from this alga was also studied for its antimicrobial activity and chemical characterization

## MATERIALS AND METHODS

*The Chlamydomonas sp. was collected and cultured from* a pond in Ratnagiri (16° 59.5′ N, 73° 16.5′ E), Maharashtra state during morning *by following the procedure of Jaiswar and Mehta (2014).*

### Preparation of Seed and Mass Culture

Sterilized Bristol medium was used to culture *Chlamydomonas sp.* because it is a well-known growth medium for many freshwater algal cultures. The flask was incubated at stationary condition for 10 days with 12 hrs light and dark condition respectively. After 10 days, the flask was opened and the culture media was drained out and the biofilm formed at the bottom of the flask was washed with sterile sea water (physical washing). After washing biofilm was scraped using sterile paint brush and transferred to 2 sterile centrifuge tubes (100 ml), 60ml of sterile distilled water was added to the centrifuge tubes and centrifuged at 3000 rpm for 10 min process is repeated for 2-3 times, obtained cell mass was treated with antibiotics (Penicillin (1mg/ml), Streptomycin (0.5 mg/ml) and Chloramphenicol (0.2mg/ml)) and incubated for 24 hrs at room temperature. After the antibiotic treatment the culture was inoculated to 5L flask containing 3L BBM media and incubated at stationary condition for 15 days with 12 hrs light and dark condition respectively, temperature 25°C.

### Preparation of crude extract

Ethyl acetate (1L) was added to 3L of culture media and kept on shaker for 4hrs and then fractionated with ethyl acetate (EA) in a separating funnel where it forms two layers; the aqueous layer and the clear organic (EA) layer. The aqueous layer was removed from the bottom and EA layer was removed from the top. The organic ethyl acetate layer was collected separately and the same extraction procedure was repeated three times. After extraction with ethyl acetate, n-Butanol was added to aqueous layer and shaken vigorously, same extraction procedure repeated three times. Respective organic solvents were then pooled together and concentrated in a separate Round Bottom Flask (RBF) on a rotary evaporator at 32°C, 150rpm to obtain crude ethyl acetate and Butanol extract.

### Anti-microbial assay

For this assay, Muller Hilton media (MHA) with 2% agar (basal layer) and 1% agar (seed layer) was used. First the plates were poured with 2% MHA medium (basal layer) and allowed to solidify. The pathogens were sub cultured in tubes each containing 5 ml of Nutrient broth, incubated and stored in refrigerator until use. 25µl of each culture were added to 15ml of 1% MHA (seed layer) medium and were poured onto the basal MHA medium. Wells having a diameter of 6mm were bored using a cork-borer. The crude extract obtained from microalgae was diluted to a concentration of 10mg/ml. Different concentrations of extract were loaded in the wells. The solvent ethyl acetate was also tested as positive control. The plates were incubated overnight at 30°C and the zones of inhibition were measured in millimeters.

### Chemical analysis

Chemical analysis of the ethyl acetate algal extract was carried out using thin layer Chromatography (TLC) and Fourier Transform Infra Red spectroscopy (FTIR). The TLC plate used was made up of aluminum sheet on to which silica gel was used as an absorbent. Sample of 2-3 µl was applied on the TLC plate with the help of capillary tube. The plate was run by using petroleum ether: ethyl acetate system (50:50 vol/vol) as a solvent system. TLC plate was developed by spraying 5% $H_2SO_4$ and ninhydrin for detection of various well separated bands. For FTIR, crude ethyl acetate extract (5 mg) was mixed with potassium bromide (KBR) and crushed to a fine powder using mortar and pestle. It was then placed in the moulds and pressed. Analysis was done using Shimadzu FTIR system. The scanning was done at frequency wavelength 400-4000 $cm^{-1}$ with resolution of 4 $cm^{-1}$.

## RESULTS

### Antimicrobial activity

The crude ethyl acetate algal extract showed promising activity against *E. coli* at 100 µg/well concentrations. The extract was also effective against *Pseudomonas aeroginosa* and *Vibrio cholera*. No activity was seen against *Staphylococcus aureus* and *Salmonella typhi* (Table 1).

**Table 1 Antimicrobial activity of crude ethyl acetate extract of alga *Chlamydomonas* sp.**

| Bacterial strains | Test concentrations (µg/well) | | | | | |
|---|---|---|---|---|---|---|
| | 5 | 10 | 30 | 50 | 70 | 100 |
| Escherichia coli | - | - | - | 3 | 5 | 14 |
| Pseudomonas aeroginosa | - | - | - | 2 | 5 | 8 |
| Staphylococcus aureus | - | - | - | - | - | - |
| Vibrio cholera | - | - | - | - | - | 6 |
| Salmonella typhi | - | - | - | - | - | - |

**Thin Layer Chromatography**

TLC plate when developed showed distinct bands indicating the presence of several compounds in the crude ethyl acetate algal extract (Fig. 1).


**Figure 1: TLC of crude ethyl acetate extract obtained from *Chlamydomonas sp***

**FTIR Analysis**

FTIR spectra (Fig. 2) of crude ethyl acetate extract of microalga gave bunch of peaks at 3444 $cm^{-1}$, 3421 $cm^{-1}$, 3282 $cm^{-1}$, 3201 $cm^{-1}$ indicative of NHs. Peaks in the region 1672 $cm^{-1}$, 1662 $cm^{-1}$, 1654 $cm^{-1}$, 1647 $cm^{-1}$, 1635 $cm^{-1}$ suggested presence of amide carbonyls.

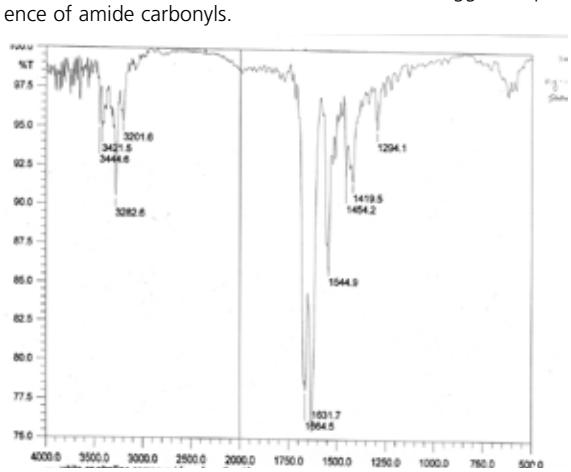


**Figure 2: FTIR analysis of crude extract of microalga *Chlamydomonas* sp.**

**DISCUSSION**

The present study indicates that the crude ethyl extract prepared from a freshwater microalga *Chlamydomonas* sp. has a promising antibacterial activity. This observation vindicates the studies made by Prakash et al., (2011) who reported that many freshwater microalgae from a river in South India possess antimicrobial activity. However, the researchers did not use the extracts of *Chlamydomonas* in their study.

Highest antimicrobial activity was observed by the ethyl acetate extracts of *Chlamydomonas* sp. against *E. coli*. It is a gram negative, straight, rod shaped bacterium arranged singly or in pairs. It causes mainly four types of clinical syndromes, urinary tract infections, diarrhoea or gastroenteritis, pyogenic infections and septicemia. The results of the present study suggest that the crude extracts of microalaga may be used to treat urinary tract infections, diarrhea, pyogenic infections and septicemia.

Renukadevi et al., (2011) reported antimicrobial and antioxidant of extracts of *Chlamydomonas reinhardtii* sp. a marine microalga. Researchers employed methanol, chloroform and water as solvents to prepare the extracts suggesting that more studies can be carried out using these solvents.

Chemical analysis indicated that the major molecules could be peptide and related compounds in the active crude extract suggesting that activity could be attributed due to the presence of these compounds which need more purification and characterization using sophisticated tools such as GC-MS and NMR.

**CONCLUSIONS**

The present investigation highlights the bioactive importance of fresh water microalga *Chlamydomonas sp. The crude extract showed antimicrobial activity against selected bacterial pathogens. The chemical analysis showed peptide or related molecules in crude bioactive extract obtained from microalga.*

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