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Studies on *Ulva fasciata* and *Chaetomorpha antennina* from Tenneti Park, Visakhapatnam Coastal Area, India

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Seaweed is considered as herbal medicine and food source utilized by the coastal community. Seaweed is commonly consumed because it is an important source of iodine. *Ulva fasciata* and *Chaetomorpha antennina* are green seaweed wildly grow in marine environment. Green algae are

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involved in photosynthetic reaction. These are observed worldwide. These algal blooms are a consequence of human activities. Phytochemical compounds are secondary metabolite groups in living organisms that have a certain function for humans. Algae are known to contain a huge variety of bioactive compounds having high potentiality towards medical field. They are seen mainly in shallow waters with high degree of salinity. Now-a-days they have commercially important with their valuable components in the form of as bioactive compounds. The objective of this study was to analyse the potential anti-bacterial activity and antifungal activity, the presence of phytochemical and biomolecules in *U. fasciata* and *C. antennina*. The samples of *U. fasciata* and *C. antennina* was collected aseptically from Tenneti park, Visakhapatnam coastal zone. The research phase includes solvent extraction, phytochemical screening, and antibacterial activity. The zone of inhibition ranges from 0.5nm to 0.7nm by the *U. fasciata* extracts whereas the extracts from *C. antennina* ranges from 0.2nm to 0.3 nm. The presence of phytochemicals was observed in both the green seaweeds. The present study is biofuel extraction from the 2 seaweed extracts. The findings gave a result that *C. antennina* have high potentiality of biofuel production.

Keywords: Sea weeds; Ulva fasciata; Chaetomorpha antennina; anti-bacterial activity; anti-fungal activity; biofuel.

1. INTRODUCTION

"Algae are defined as a group of predominantly aquatic, photosynthetic, and nucleus-bearing organisms that lack the true roots, stems, leaves and specialized multicellular reproductive structures of plants. Algae are an ideal source of nutrients as they are rich in protein, lipids, vitamins, minerals, and essential fatty acids. As a matter of fact, extracts from organisms (plants and animals) and microorganisms (bacteria, algae, fungi) are well known sources of compounds with interesting biological and therapeutic properties" [1,2]. "For example, more than 75% of drugs utilized to treat infectious diseases are derived from natural sources" [3]. "Algae are known to produce secondary metabolites other than those produced by terrestrial organisms" [4]. "Therefore, they have been indicated to be a source of compounds of biomedical interest" [5-7]. "Green algae represent the largest algal group found on earth and inhabit different ecosystems, including fresh and marine habitats" [2]. "They range from unicellular to multi-cellular, microscopic to macroscopic forms. Their thallus varies from free filaments to shaped forms" [2]. "Green algae are characterized by the production of a wide range of metabolites, including polysaccharides, polyphenols, terpenes, and carotenoids which play many different biological activities such as antimicrobial, antioxidant, and antitumor activities" [8]. "Ulva is one of the most widely distributed green algal genera known as sea lettuce" [9]. "Ulva is known to be a good source of food, development of novel drugs and functional foods, and pharmaceutical, in addition to different agricultural applications" [10]. "It has proven to be a rich source of structurally diverse bioactive compounds with valuable biomedical potential" [11]. "The famous ulva product produced exclusively by the *Ulva* genera is a water-soluble polysaccharide with many biological activities, including anticancer and antimicrobial" [12]. "Algae biofuels may provide a viable alternative to fossil fuels; however, this technology must overcome a number of hurdles before it can compete in the fuel market and be broadly deployed. In recent years, biomass has been recognized as a prospective renewable energy source to address these challenges, among which algae is attracting increasing attention due to its fast growth rate, high photosynthetic efficiency and global distribution" [13-15]

2. MATERIALS AND METHODS

The algal samples were collected from Tenneti park with latitude 17.7477° N and longitude 83.3506° E with a vast rocky shore with algal growth.

2.1 Sample Collection

The samples are collected from tenneti beach Visakhapatnam. Two types of algal samples were collected in the month of May during summer season aseptically by using gloves and fore-ceps in to clean and grease free bottles. The algal sample analyzed at the spot by using google lens to identify the genes. Collected samples were carried to the microbiology lab, St. Ann's college for women **(**Fig. 1).

Fig.1. Fresh washed Ulva fasciata algal sample

2.2 Microscopic Observation

Fresh *Ulva fasciata* and *Chaetomorpha antennina* green algal samples were observed under electrical microscope by using sterile glass slide and coverslip (Figs. 2 &3).

2.3 Weighing

The *Ulva fasciata* and *Chaetomorpha antennina* algal samples were washed under running tap water to remove the sand particles and water by using blotting papers. The dried algal samples were weighed by using digital weighing balance. The samples were dried under shadow for three days to determine the dry weight. The dried algal samples were made into fine powder by using motor and pestle and stored it for further studies. (Figs. 4 & 5).

2.4 Photosynthetic Activity

The *Ulva fasciata* and *Chaetomorpha antennina* algal samples was kept in the dark room and observed for its photosynthesis reaction by its green coloration.

2.5 Algal Powder as Culture Media

Culture media was prepared by add 3gms of algal powder of the both the samples separately in 100ml nutrient media to know whether it is supporting the growth of bacteria and fungi. For this study we inoculated 3 bacterial and 2 fungal cells and incubated for 14days.

2.6 Extract Preparation

Solvents used for extraction are methanol, ethanol and chloroform for extraction. 10ml of each solvent were taken into 50ml conical flasks separately and added 2 algal dry powder in a separate conical flask and kept for three days under optimum conditions.

2.7 Biomolecule

2.7.1 Carbohydrates

The presence of carbohydrates analyzed by the using standard Molish test

2.7.2 Protein

The presence of protein was analyzed by biuret test

2.7.3 Amino-acids

Ninhydrin test was conducted to know about the presence of amino acids in the extracts.

2.8 Phytochemicals Analysis

2.8.1 Test for flavonoids

The stock solution (1 mL) of ethanol extract of algal was taken in a test tube and added few drops of dilute 2 % NaOH solution. An intense yellow colour was appeared in the test tube. It became colourless when on addition of a few drops of dilute acid.

2.8.2 Test for alkaloids

One gram of algal dry pellet was taken in a conical flask and added 100ml distilled water and 20ml acetic acid. Hagar's reagent was added to the prepared crude solution and allow it for 8-10hours.

2.8.3 Test for terpenoids

The dry crude algal extract (5 mg) was dissolved in chloroform (2 mL) and then acetic anhydride (1 mL) was added to it. Concentrated sulphuric acid (1 mL) was added to the solution.

2.9 Antimicrobial Activity of the Algal Extracts

2.9.1 Bacterial and fungal strains

The antimicrobial potency of each extract was evaluated using three bacterial strains and two fungal strains. One strains of

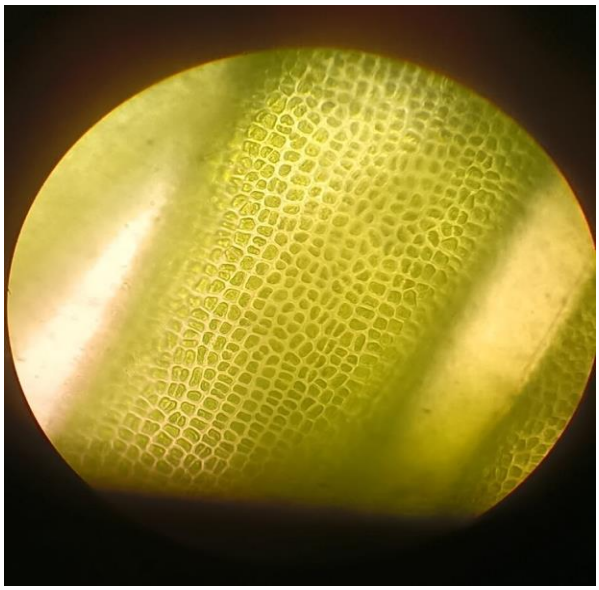

Fig. 2. *Chaetomorpha antennina*

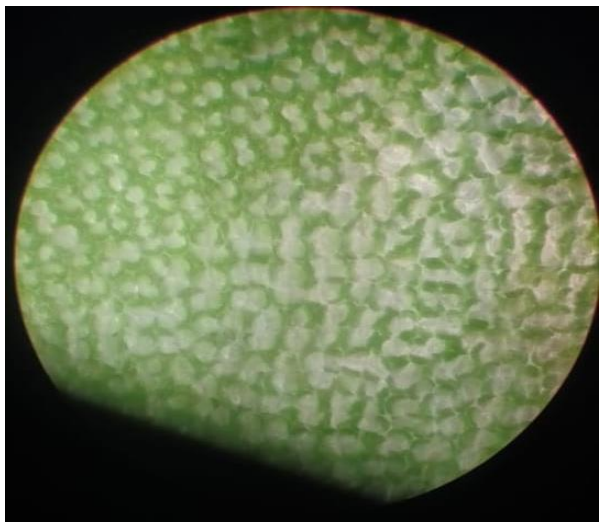

Fig. 3. *Ulva fasciata*

Gram-positive *Staphylococcus aureus* and two strains of Gram-negative *Escherichia coli* and *Klebsiella pneumoniae* bacteria. Two fungal strains used for antifungal activity was *Aspergillus niger* and *Trichoderma harizianum*. The bacterial and fungal strains were provided from the Microbial type culture collection (MTCC), Chandigarh, India.

2.9.2 Inoculum preparation

Each bacterial and fungal strains was sub-cultured overnight at 35^{o}C in Mueller-Hinton agar slants and PDA slants. The microbial growth was harvested using 5 ml of sterile broth kept overnight in orbital shaker at 37oC for 24 hours. Separate bacterial and fungal lawn plates were prepared by inoculating fresh broth by using spread plate technique on solidified Mueller-Hinton (Bacterial Media) and PDA agar plate (Fungal media).

2.9.3 Antibacterial activity

The well diffusion method is used to evaluate antibacterial activity of each algal extract. The extract residues (50 mg) were then loaded in the well on lawn of bacterial culture plates (Fig. 4). The plates were kept in incubator at 37^{0}C for 24 h. The presence of inhibition zones

was measured by using Hi-Media zone scale, recorded and considered as indication for antibacterial activity.

2.9.4 Antifungal activity

The well diffusion method is used to evaluate antifungal activity of each mushroom extract. The extract residues (50 mg) were then loaded in the well on lawn of fungal culture plates. The plates were kept in incubator at 27^0C for 24 h. The presence of inhibition zones was measured by using Hi-Media zone scale, recorded and considered as indication for antifungal activity.

2.10 Biofuel Production

In the ratio of 2:1 chloroform, methanol is added in sample of Chaetomorpha (2 gm) and ulva (8 gm).

Extracts are kept for Refluxed process (Fig. 6).

After 2hrs , samples are taken in a separated funnels by the addition of ether then the organic layer i.e. biofuel which was separated.

Fig. 4. *Ulva fasciata*

Fig. 5. *Chaetomorpha antennina*

Fig. 6. Biofuel production after processing and evaporation

3. RESULTS AND DISCUSSION

The dry weight of the *Ulva fasciata* and *Chaetomorpha antennian* were as 18.08 gms & 4.39 gms respectively. The photosynthetic reaction was not observed in the absence of light source. The phytochemicals like flavonoids, terpenoid and alkaloids were present in the both the algal samples. The results were shown in the Tables 1 & 2. The maximum zone of inhibition for *K. pneumonia* are 0.6nm to 0.2nm. There is no zone of inhibition against the other two bacterial cells of E. coli and *S. aureus*. There is no antifungal activity by the algal samples. The quantity of biofuels production was more in *Chaetomorpha antennian* when compared with *Ulva fasciata*. 3.3 ml of biofuel was extracted from 10gms of *Chaetomorpha antennian* and 0.6ml of biofuel was extracted from *Ulva fasciata*. The culture media prepared by using the algal powders are not supporting any kind of bacterial and fungal cell where they remained fresh and normal for about two months without any contamination.

Table 1. Phytochemical screening result of *Chaetomorpha antennina* crude extract

S. No	Phytochemical	Result
1	Flavonoid	Positive
2	Terpenoid	Positive
3	Alkaloid	Positive

The present study was focused on the comparative parameters between two green algal samples of *Ulva fasciata* and *Chaetomorpha antennina* obtained in same environment. “The presence of biomolecules is similar in the both the algal samples. The high carbohydrate fraction includes a large variety of easily-soluble polysaccharides, such as laminarin, alginate, mannitol or fucoidan in brown types; starch, mannans and sulphated galactans in red types and Ulva in green types” [16]. “They can be a source of essential amino acids where they involve in protein synthesis. The production of biofuels is attracting attention regarding three aspects: bioremediation for the ecosystem, a renewable energy source and economic savings” [17]. Algae can also be used as a good source of energy, boost up the immune system to fight against the pathogenic microorganisms. The inhibition of microbial growth in our studies made a sign that these algal not only rich in proteins and nutrients but also contain antimicrobial compounds. It is therefore probable that efforts to have a competitive source of organic fuels. “Liquid biofuels are the sum of bio gasoline, biodiesels, bio-jet kerosene and other liquid biofuels in the energy statistics” [18]. “The findings gave a result that *C. antennina* have high potentiality of biofuel production. New compounds useful for the pharmaceutical industry that could be isolated from *Ulva* will have to be chemically produced to be commercialized”.[19]. The use of algal biofuel is expected to play an important role in securing energy for the next decades, the processing of algae directly related to biofuels production decrease the pollution. “Seaweed (Macroalgae) are considered superior compared with terrestrial plants – in terms of solar energy

storage, nutrient assimilation and potential for biofuel production – due to their higher photosynthetic efficiency, higher biomass yield and rates" [20].

Table 2. Phytochemical screening result of *Ulva fasciata* crude extract

S. No	Phytochemical	Result
1	Flavonoid	Positive
2	Terpenoid	Positive
3	Alkaloid	positive

4. CONCLUSIONS

The current study was about the valuable by-products and biodiesel production by using local algae. This study demonstrates the composition and antimicrobial activity of extracts of *Ulva fasciata* and *Chaetomorpha antennian*. These natural resources are very beneficial for human health and also boost up the immunity. The research work carried to analyse and to know the natural potentiality of algae sample available at Visakhapatnam coast. There was a huge benefits of these algal cell. Further analysis to be done to know the interaction of antimicrobial compounds and their interactions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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