Studies on *Ulva fasciata* and *Chaetomorpha antennina* from Tenneti park, Visakhapatnam coastal area, India

Abstract

Seaweed ~~has~~ is considered as herbal medicine and food source~~s~~ utilized by the coastal community. Seaweed is commonly consumed because it is an important source of iodine. *Ulva* ~~*fasciata* and~~*fasciata* and *Chaetomorpha antennina* are green seaweed wildly grow in marine ~~water resources~~ environment. The objective of this study was to ~~analysis~~ analyse the potential anti-bacterial and antifungi ~~activity~~ activities ~~and anti fungal~~, the presence of phytochemical and biomolecules ~~in *U.*~~in U. *fasciata* and *C. antennina*. The samples ~~of *U.*~~of U. *fasciata* and *C.* ~~*antennina* was~~*antennina* was collected aseptically from Tenneti park, Visakhapatnam coastal zone. The research phase ~~including~~ includes solvent extraction, phytochemical screening, and antibacterial activity. The zone of inhibition ranges from 0.5nm to 0.7~~nm by~~nm by the *U. fasciata* extracts whereas the extracts from *C. antennina* ranges from 0.2nm to 0.3 nm. The presence of phytochemicals ~~were~~was observed in both the green seaweeds. The key focus of the present study is biofuel extraction from the seaweed extracts. The findings gave a result that *C. antennina* have high potentiality of biofuel production.

Comment [EU1]: This contradicts your earlier aim.

Key Points: Sea weeds, *Ulva fasciata*, *Chaetomorpha antennina*, Anti-bacterial activity, Anti- fungal activity and 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. ~~The utilization of natural bioactive compounds in pharmacology is known to be an efficacious procedure.~~ As a matter of fact, extracts from organisms (plants and animals) and microorganisms (bacteria, algae, fungi) are well known sources of compounds ~~provided~~ with interesting biological and therapeutic properties [10,12]. For example, more than 75% of drugs utilized to treat infectious diseases are derived from natural sources [11]. ~~From this point of view,~~ Algae ~~have been demonstrated~~ are known to produce secondary metabolites other than those produced by terrestrial organisms [5]. Therefore, they have been indicated to be a source of compounds of biomedical interest [6,14 ,15]. Green algae represent the largest algal group found on earth and inhabit different ecosystems, including fresh and marine habitats [16]. They range from unicellular to multi-cellular, microscopic to macroscopic forms. Their thallus ~~vary~~varies from free filaments to shaped forms [16]. 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 [9]. Ulva is one of the most widely distributed green algal genera known as sea lettuce [7]. 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 [8]. It has proven to be a rich source of structurally diverse bioactive compounds with valuable biomedical potential [2]. The famous ulva product produced exclusively by the *Ulva* genera is a water-soluble polysaccharide with many

biological activities, including anticancer and antimicrobial [1]. 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.

2. MATERIALS AND METHODS:

~~2.1 SAMPLING~~2.1 SAMPLING ~~STATION :~~STATION: 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.

2.2 SAMPLE COLLLECTION:

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-capes 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

2.3 Microscopic observation:

Fresh *Ulva fasciata* and *Chaetomorpha antennina* green algal samples were observed under electrical microscope by using sterile glass slide and coverslip **(Fig2 &3).**

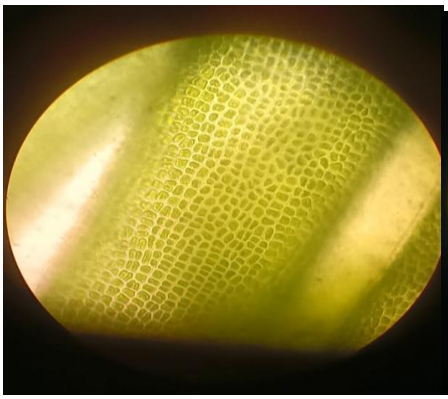

Fig:2- *Chaetomorpha antennina*

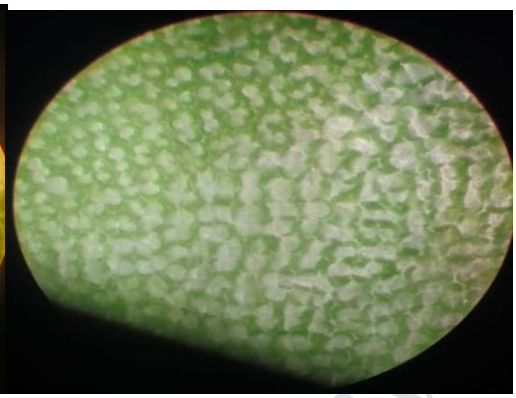

Fig:3- *Ulva fasciata*

2.4 Weighing:

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

Fig:4- Ulva fasciata

Fig:5-Chaetomorpha antennina

2.5 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.

2.6 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.

2.7 EXTRACT PREPARATION:

~~We have taken three~~ Ssolvents used for extraction are ~~like~~ 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.

2.8 BIOMOLECULE:

Carbohydrates: The presence of carbohydrates analyzed by the using standard Molish test

Protein: The presence of protein was analyzed by biuret test

Amino-acids: Ninhydrin test was conducted to know about the presence of anino acids in the extracts

2.9 PHYTOCHEMICALS ANALYSIS:

Test for ~~Flavonoids :~~Flavonoids:

The stock solution (1 mL) of ethanol extract of mushroom was taken in a test tube and added few ~~drop~~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 ~~drop~~drops of dilute acid.

Test for alkaloids

One gram of mushroom dry pellet ~~were~~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.

Test for terpenoids: The dry crude mushroom 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.

2.10 ANTIMICROBIAL ACTIVITY OF THE ALGAL EXTRACTS:

Bacterial and fungal strains

The antimicrobial potency of each extract was evaluated using three bacterial strains and two fungal strains. One strains of ~~Gram positive~~Gram-positive *Staphylococcus aureus* and two strains of ~~Gram negative~~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.

Inoculum preparation

Each bacterial and fungal strains was sub-cultured overnight at 35^{o}C in Mueller-Hilton 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-Hilton (Bacterial Media) and PDA agar plate (Fungal media).

Antibacterial Activity

The well diffusion method is used to evaluate antibacterial activity of ~~the each~~each mushroom extract. The extract residues (50 mg) were then loaded in the well on lawn of bacterial culture plates (Figure 4). The plates were kept in incubator at 37ºC for 24 h. The presence of inhibition zones ~~were~~was measured by using Hi-Media zone scale, recorded and considered as indication for antibacterial activity.

Antifungal Activity

The well diffusion method is used to evaluate antifungal activity of ~~the each~~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^{0}C for 24 h. The presence of inhibition zones ~~were~~was measured by using Hi-Media zone scale, recorded and considered as indication for antifungal activity.

2.11 BIOFUEL PRODUCTION:

Extracts are kept for Refluxed process **(Fig:6)**

Fig:6- Biofuel production after processing and evaporation

3. RESULTS:

The dry weight of the *Ulva fasciata* and *Chaetomorpha antennian* wereas 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 ~~reported~~ in ~~the~~ both ~~the~~ algal samples. The results were ~~mentioned~~ shown in ~~the~~ **Table1&2**. The maximum zone of inhibition for *K. pneumonia* ~~ranges from~~ are 0.6nm to 0.2nm ~~reported against *K. pneumonia* and~~ .T~~t~~here 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 quality of biofuels production was more in *Chaetomorpha antennian* when compared with *Ulva fasciata*. 3.3ml 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 ~~bacteria~~bacterial and fungal cell where they remained fresh and normal for about two months without any contamination.

Comment [EU2]: Quality or quantity?

Table:1- Phytochemical screening result of *Chaetomorpha antennina* crude extract

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

Table:2- Phytochemical screening result of *Ulva fasciata* crude extract

S. No	Phytochemical	Result

1	Flavonoid	Positive
2	Terpenoid	Positive
3	Alkaloid	positive

4. DISCUSSION:

Green algae are involved ~~in photosynthetic~~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. 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~~of as bioactive compounds. The present study was focused on the comparative parameters between two green ~~algal samples~~algal **samples** of *Ulva fasciata* and *Chaetomorpha antennina* ~~which are growing in~~ obtained in ~~the~~ same environment. The presence of biomolecules ~~are~~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 [13]. 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 [3]. Algae can also be used as a good source of ~~energy ,~~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 ~~having~~ contain antimicrobial ~~peptides in it~~compounds. ~~Further studies to be done to know the exact composition of AMP peptides and their interactions in *Ulva fasciata* and *Chaetomorpha antennina*~~. It is therefore probable that efforts to have a competitive source of organic fuels. 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.[4]

Comment [EU3]: Can be part of introduction

Comment [EU4]: inconclusive

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