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# Phytochemical Screening and Antimicrobial Activity of *Ulva lactuca* L.

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Seaweeds are significant source of bioactive compounds. Present investigation is aimed to evaluate and assess the phytochemicals and antimicrobial efficiency of the seaweed, *Ulva lactuca* collected from Mahim Beach, Palghar, Maharashtara, India. Crude solvent extracts of *U. lactuca* were obtained by soxhlet (Hot Extraction) and maceration (Cold Extraction) using ethanol. Phytochemical screening of the extracts was carried out as per standard methods. The extracts were tested against human bacterial pathogen *E. coli.* The extracts exhibited presence of phytochemical constituents like flavonoids, tannins, cardiac glycoside, sterols, terpenoids and saponins. The results revealed that ethanolic extracts of well diffusion method exhibited higher degree of antibacterial activities than the paper disc method. Present study suggests that the phytochemical constituent of the seaweed *Ulva lactuca* has potential antibacterial activity. Further purification of active compounds can be used as a source of antibiotics for the treatment of disease causing pathogens.

**Keywords :** *Ulva lactuca*, Seaweed, Ethanolic extract.

## INTRODUCTION

India has a long coastline of more than 7500 km. Its marine resources are spread over in the Indian Ocean, Arabian Sea, and Bay of Bengal. The exclusive economic zone (EEZ) of the country has an area of 2.02 million sq. km comprising 0.86 million sq. km on the west coast, 0.56 million sq. km on the east coast and 0.6 million sq. km around the Andaman and Nicobar islands. The east coast supports activities such as agriculture and aquaculture while a number of industries are supported on the west coast.

Seaweeds form one of the important living resources grouped under three divisions namely, Chlorophyceae (green algae), Phaeophyceae (brown algae) and Rhodophyceae (red algae). Marine organisms are rich source of structurally novel and biologically active metabolites (Ely *et al.*, 2004;Laport *et al.*, 2009). Many of these marine compounds, which exhibit a range of different activities have been isolated and some of them have been tested for potential use as new pharmaceuticals (Laport *et al.*, 2009; Molinski *et al.*, 2009). Seaweeds are the only source of phytochemicals namely agaragar, carrageenan and algin, which are extensively used in various industries such as food, confectionary, textiles, pharmaceuticals, dairy and paper industries mostly as gelling, stabilizing and thickening agents. The culture of seaweed is a growing worldwide industry, producing 14.5 million tons (wet weight) worth US$7.54 billion in 2007. There have been number of reports of antibacterial activity from marine plants and special attention has been reported for antibacterial and antifungal activities related to marine algae against several pathogens (Rangaiah *et al.*, 2010). The antibacterial activity of seaweeds is generally assayed using extracts in various organic solvent for example acetone, methanol-toluene, ether and chloroform-methanol (Kolanjinathan and Stella, 2009). Use of different organic solvents provides a higher efficiency in extracting compounds for antimicrobial activity (Cordeiro *et al.*, 2006). *Ulva* is a genus of marine and brackish water green macroalgae. It is edible and often called 'Sea Lettuce'. *Ulva* species have a relatively high growth rate compared to other algae in nature as well as in cultivation facilities. In nature, growth rates of up to 35% have been reported (Pedersen and Borum 1996). *Ulva lactuca* has long heen used as food and as a traditional medical agent to treat various infections and diseases (Kim *et al.*, 2007). Many of the seaweeds possess bio-active components which inhibit the growth of some of the gram positive and negative bacterial pathogens. Present study was aimed to investigate the phytochemicals and antibacterial properties of *U. lactuca* extract of ethanol as solvent against human pathogenic bacteria. Phytochemical analysis and antibacterial activity of *Ulva lactuca* from Mahim beach (Dist.Palghar) has not been studied for biotechnological applications and biopharmaceuticals.

## MATERIAL AND METHODS

Fresh seaweed *Ulva lactuca* was collected from intertidal regions of Mahim Coast, Palghar during the month of December. The collected sample was cleaned with the

seawater until unwanted impurities, adhering sand particles and extraneous matter like epiphytes, pebbles, shells were removed and it was immediately brought to the laboratory in sterile plastic bags containing sea water in order to prevent evaporation. It was then washed thoroughly with tap water to remove the surface salty materials.

**Preparation of seaweed extract** : The collected *Ulva lactuca* sample was cleaned and washed with tap water to remove any associated debris. It was shade dried at room temperature (28 ± 2ºC) for 5 - 8 days or until they are brittle easily by hand. After completely drying, the seaweed material was ground to a fine powder using electrical blender. The extract was prepared using hot and cold extraction methods.

**Hot Extraction :** Ten gram powdered seaweed was extracted successively with 250 ml of ethanol in soxhlet extractor until the extract was clear. The extract was evaporated to dryness under reduced pressure using rotary vacuum evaporator. It was stored in a refrigerator at 4°C for further use.

**Cold Extraction :** Five gram of powdered seaweeds was extracted with 25 ml of ethanol in round bottom flask. It was kept on shaker for 24 hr. The crude extract was passed through Whatman No.1 filter paper and the filtrate was stored at 4°C for further use (Chandran *et al.*, 2006).

**Phytochemical screening :** The extracts was tested for steroids, alkaloids, phenolic compounds, saponins and glycosides. Phytochemical screening of the extract was carried out as per standard methods (Harbone 1998 and Kokate 2003).

**Disc preparation :**

**Preparation of algal disc for antibacterial activity :** 5 mm diameter disc was prepared using sterile Whatmann No.1 filter paper. The disc was impregnated with 20µl of ethanol extract of *Ulva lactuca* at four different concentrations ranging 1 mg/ml, 2 mg/ml, 3 mg/ml and 4 mg/ml to check their antibacterial activity.

**Bacterial inoculum preparation :** Bacterial inoculum was prepared by inoculating a loopful of test organisms in 5 ml of nutrient broth and incubated at 37°C for 3 - 5 hour till a moderate turbidity was developed.

**Disc diffusion method :** The antibacterial activity of *Ulva lactuca* extract was determined by disc diffusion method (Bauer *et al.*, 1966). Petri plates were prepared by pouring 20 ml of Mueller Hinton agar and allowed to solidify for the use in susceptibility test against bacteria, Plates were dried and 0.1 ml of standardized inoculum suspension was poured and uniformly spreaded. The excess inoculum was drained and the plates were allowed to dry for five minutes. After drying, the discs with extract were placed on the surface of the plate with sterile forceps and gently pressed to ensure contact with the agar surface. The plates were incubated at 37°C for 24 hour. The zone of inhibition was observed and measured in millimeter.

**Well-diffusion method :** *Ulva lactuca* was tested for antimicrobial activity by agar well-diffusion method against bacterial strain *Escherichia coli*. The pure cultures of bacterial strains were sub cultured on nutrient agar medium. Briefly, petriplates containing approximately 20- 25ml of Muller-Hinton agar medium were inoculated using a 0.1ml of each bacterial strain. Each strain was swabbed uniformly onto the individual plates using sterile cotton swabs. About 10 mm diameter of wells were made using gel puncture. Using a micropipette, different concentrations of the sample of seaweed extract (50 µl) was poured onto each well on all three plates. The levels of inhibition zone were measured after 24 hr incubation at 37°C.

# RESULTS AND DISCUSSION

**Phytochemical activity of *Ulva lactuca* :** The results of preliminary phytochemical analysis revealed the presence of saponins, taninns, terpenoids, Flavonoids-2, Flavonoids-3 and Cardiac Glycoside. Various workers have reported the presence of significant bioactive compounds in seaweeds (Vimalabai and Mary 2003, Mallikharjuna *et al.*, 2007, Prabha *et al.*, 2013).

**Antibacterial activity :**

Antibacterial activity of algal extracts was determined by paper disc method and well diffusion assay. The results are summarized in Table 2. The crude ethanolic extracts of the seaweed exhibited varying degree of antibacterial activity against the test organisms. On a general note, ethanolic extracts of well diffusion method exhibited higher degree of antibacterial activities than the paper disc method.

In present study the maximum activity (17mm) was recorded from 4 mg ethanolic extract of *Ulva lactuca* by well diffusion method (Plate 2) and minimum (6mm) from 1 mg by paper disc method (Plate 1). Different concentrations of the fractions isolated from the seaweeds revealed antibacterial activity against the test bacteria *Escherichia coli* (Parekh, 1984). According to present results, ethanolic extract of *Ulva lactuca* exhibited antibacterial activity against the gram negative bacteria *E.coli*. Most of the researchers reported many bioactive and pharmacologically important compounds from seaweeds used in medicine and pharmacy (Rao *et al.*, 1991., Siddhanta *et al.*, 1997).

# CONCLUSION

Present study revealed that *U. lactuca* is rich source of secondary metabolites like, glycosides, flavonoids, saponins, terpenoides and tannins which are of great medicinal value. It possesses good antibacterial activity. Further purification of its active compounds can be used as a source of antibiotics for the treatment of disease causing pathogens.

**Table 1 : Phytochemical activity of *Ulva lactuca*.**

| Sr. No. | Compound | Chemical | Result/Appearance | Present/ Absent |
|---|---|---|---|---|
| 1. | Flavonoids-2 | Extract+conc $H_2SO_4$ (Adding dropwise) | Orange red colour | Present |
| 2. | Flavonoids-3 | Extract+$FeCl_3$ | Intense green colour | Present |
| 3. | Alkaloids | Extact+1% HCl + dropwise adding of drangendroffs Reagent | Orange to red ppt | Absent |
| 4. | Saponins | Extract+ D/W shake and warm in water bath | Broth persists present | Present |
| 5. | Tannins | Extract+10% Lead acetate | White ppt | Present |
| 6. | Steroids | Extract+2 ml acetic anhydride and dropwise addition of conc $H_2SO_4$ | Voilet to blue or green ppt | Absent |
| 7. | Terpenoids | Extract+2 ml choloroform+$H_2SO_4$ | Reddish Brown layer | Present |
| 8. | Cardiac Glycoside | Extract+Glacial acetic acid + dropwise $FeCl_3$+dropwise conc $H_2SO_4$ | Blue green colour | Present |

**Table 2 : Inhibition zone of Ethanolic seaweed extracts against test pathogens.**

| Seaweeds | Concentrations (mg) | Bacterial pathogens showing zone of inhibition (mm) | |
|---|---|---|---|
| | | *Escherichia coli* Paper disc method | *Escherichia coli* Well diffusion method |
| *Ulva lactuca* | 1 | 6 | 09 |
| | 2 | 7 | 12 |
| | 3 | 7 | 14 |
| | 4 | 10 | 17 |

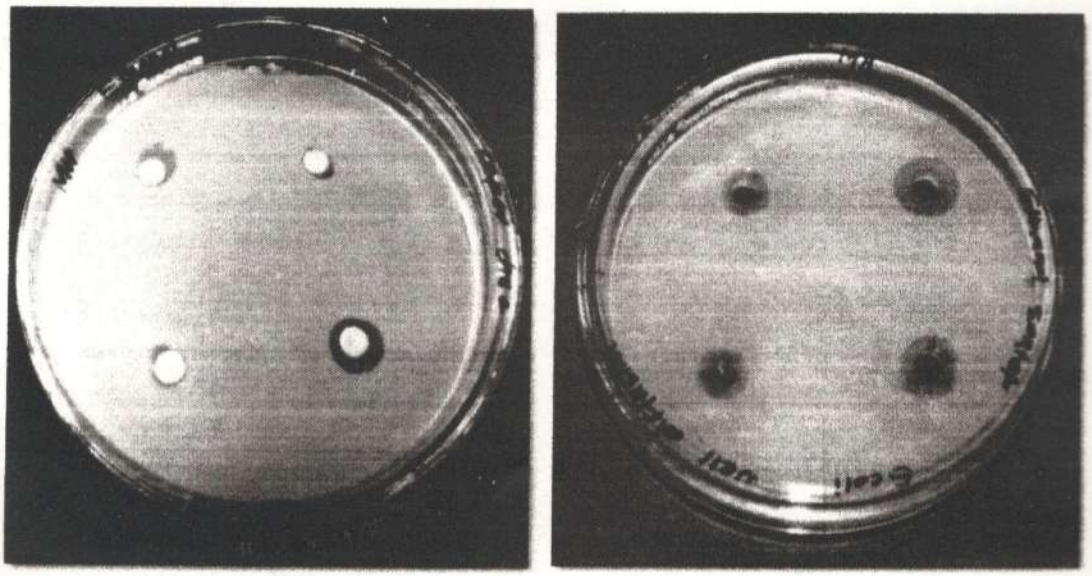

**Plate : 1 Paper disc method.** **Plate : 2 Well diffusion method.**

**Fig : Inhibition zones of *Ulva lactuca* ethanolic extract against *Escherichia coli* By using Paper disc method and Well diffusion method**

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