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Research Article

# FOURIER TRANSFORM INFRARED ANALYSIS OF *ULVA LACTUCA* AND *GRACILARIA CORTICATA* AND THEIR EFFECT ON ANTIBACTERIAL ACTIVITY

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**ABSTRACT**

In the present work, we used two seaweeds *Ulva lactuca* and *Gracilaria corticata,* which were collected from Hare Island in the Gulf of Mannar of Tuticorin coast. Ethanol was taken as the solvent for extraction. The crude extract was purified using column chromatography. Antibacterial activity of crude and column purified fractions were tested against *Klebsiella, Aeromonas, Staphylococcus, Escherichia* and *Pseudomonas* using well-diffusion method. Maximum zone of inhibition (9 mm) was found in the crude extract of *G. corticata* against *Pseudomonas sp*. Minimum zone of inhibition (4 mm) was found in *U. lactuca* fraction1 against *Escherichia coli*. Highest antibacterial activity was obtained in red seaweed, whereas, green seaweed showed less antibacterial activity. From this study, we can conclude that red seaweeds have more active than green seaweeds. The seaweed powder was analyzed in Fourier transform infrared spectrometer. It was observed that both seaweed contained phenol and alcohol compounds, which were responsible for the antibacterial activity.

**Keywords:** Antibacterial activity, Fourier transform infrared, Seaweeds, *Ulva lactuca, Gracilaria corticata.*

## INTRODUCTION

Seaweeds are multicellular macroalgae used as potential renewable resource in the field of medical and commercial environment, which are also used as food, feed and fertilizer in many parts of the world. Biostimulant properties of seaweeds are explored for use in the development of novel antibiotics. Many metabolites isolated from marine algae have bioactive efforts [1]. Bioactive natural products are widely distributed in the plant kingdom and extract from different plants, as well as red, green and brown macro, and micro algae can be used as natural products [2]. Marine algae represent an inexhaustible reservoir of raw materials used in pharmaceutical, medicine, food industries and cosmetics [3]. In recent years, research on the chemistry of seaweeds has experienced a tremendous increase due to the need for compounds possessing bioactivities of possible pharmaceutical applications or other potential economic properties [4]. Marine algae serve as an important source of bioactive natural substances [5]. Special attention has been paid to antibacterial activities related to marine algae against several pathogens [6]. The extracts and active constituents of various marine algae have been shown to have antibacterial activity against Gram-positive and Gram-negative bacteria [7]. The antimicrobial compounds derived from the marine algae consist of a diverse group of chemical compounds [8]. In the present study, antibacterial activities of *Ulva lactuca* was investigated against some fish pathogenic bacteria.

## METHODS

### Collection of seaweed

Live samples of the seaweed *U. lactuca* and *Gracilaria corticata* was collected by handpicking during low tide from Hare Island in the Gulf of Mannar of Tuticorin coast (08°46' 2.15"N latitude; 78°11' 16.05"E longitude). Shade-dried seaweeds were ground to a fine powder using an electric mixture grinder. The powdered samples were then stored in refrigerator for further use.

### Preparation of extracts by soxhlet extraction method

The powdered samples were extracted by using soxhlet apparatus. Ethanol was taken as the solvent for extraction. A total of 25 g of the sample and 250 ml of the solvent were taken for extraction the apparatus was run for 4 hrs and syrupy extracts were collected. The extracts obtained were concentrated by evaporation. Then, the extract was stored in cold storage for further use.

### Column chromatography

Column chromatography is one of the most useful methods for the separation and purification of crude extract. The ethanolic crude extracts were applied in a silica gel column (230-400 mesh), packed with chloroform and eluted with a mixture of chloroform and methanol in the following proportions 9:1, 8:2 and 7:3. After purification, the fractions were stored at a temperature of −20°C until the determination of antibacterial activity.

### Well diffusion method

The antibacterial activity of fraction and crude extracts of *U. lactuca* and *G. corticata* against five species of bacteria namely *Klebsiella pneumonia, Staphylococcus aureus, Aeromonas hydrophilla, Escherichia coli* and *Pseudomonas sp*. were determined by well diffusion method. The circular well of 3 mm diameter and 25 µl holding capacity was prepared using well cutter. Streak plate method was performed to seed pathogenic bacterial culture on the agar plates. Using the loop which had been flamed, cooled and dipped in the inoculums, continuous horizontal streaks were made in the solid agar plates. Different fractions of the seaweed extracts were then added to the wells. The clear labels of sample were marked on the plate. The plates were then incubated at 37°C for 24 hrs.

### Fourier transform infrared (FT-IR) analysis

Infrared reflectance vibrational spectra were carried out on powdered samples using a spectrometer with instrument resolution of about (1/cm), in the wave number region (4000-400/cm) at room temperature [9].

## RESULT

When the fractions of *U. lactuca* were tested against different pathogens like *K. pneumonia, A. hydrophilla, S. aureus, E. coli* and *Pseudomonas sp*. highest zone of inhibition was found in *A. hydrophilla* in Fig. 2 fraction. Similarly, when the crude extract of *U. lactuca* was tested against the same bacterial pathogen highest zone of inhibition was found in *E. coli*. The fractions of *G. corticata* were tested against different pathogens like *K. pneumonia, A. hydrophilla, S. aureus, E. coli* and *Pseudomonas sp.*

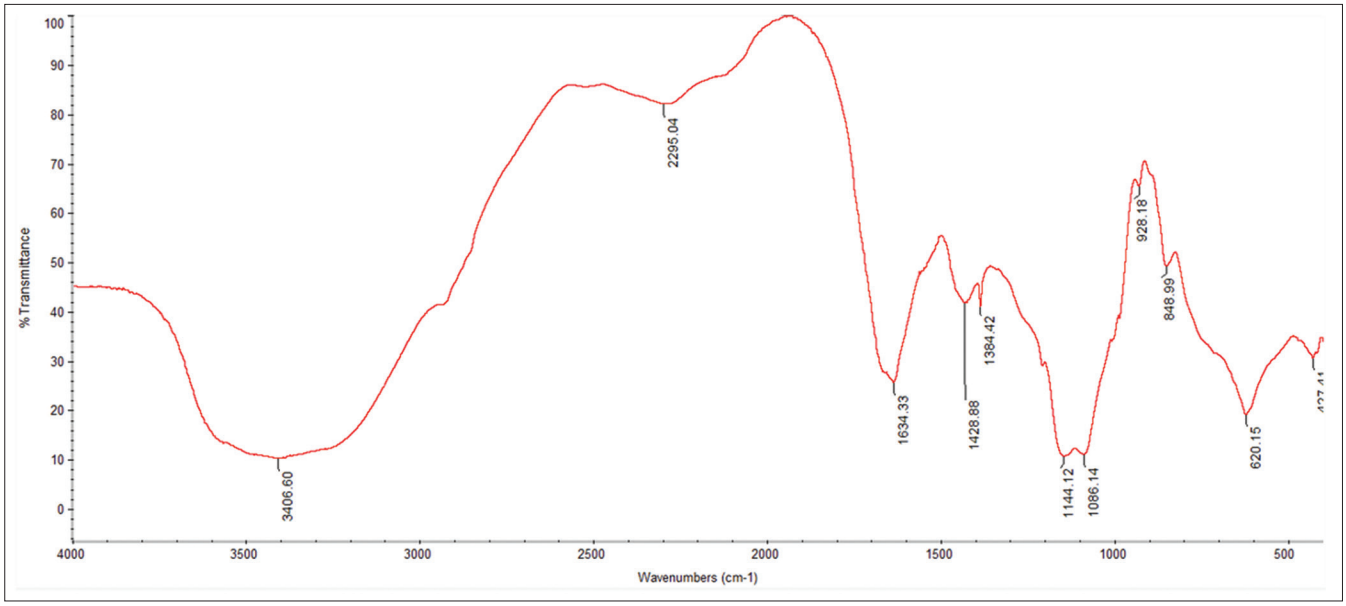


**Fig. 1: Fourier transform infrared spectrum of *Ulva lactuca***

**Table 1: The zone of inhibition (mm) of *Ulva lactuca* against different bacterial pathogens**

| Fractions | *K. pneumonia* | *A. hydrophilla* | *S. aureus* | *E. coli* | *Pseudomonas sp.* |
|---|---|---|---|---|---|
| F1 | 5±0.05 | 5±0.81 | 4±0.07 | 4±0 | 5±0.81 |
| F2 | 4±2.45 | 6±4.09 | 5±0.06 | 4±0.70 | 5±0.81 |
| F3 | 6±0.85 | 4±0.84 | 4±0.88 | 6±1.63 | 6±1.63 |
| Crude | 6±2.44 | 6±1.63 | 5±4.08 | 7±4.89 | 6±2.44 |

*K. pneumonia: Klebsiella pneumonia, A. hydrophilla: Aeromonas hydrophilla, S. aureus: Staphylococcus aureus, E. coli: Escherichia coli, U. lactuca: Ulva lactuca*

**Table 2: FT-IR absorption frequencies ($cm^{-1}$), intensity estimation and functional group of seaweeds *U. lactuca***

| IR frequency ($cm^{-1}$) | Bond | Functional groups | *U. lactuca* IR frequency ($cm^{-1}$) |
|---|---|---|---|
| 3500-3200 | O–H stretch, H–bonded | Alcohols, phenols | 3406.60 |
| 2300-2200 | C≡N stretch | Nitriles | 2295.04 |
| 1670-1600 | C=O stretch | Amides | 1634.33 |
| 1500-1400 | C–C stretch | Aromatics | 1428.88 |
| 1400-1300 | N=O bend | Nitromethane | 1384.42 |
| 1250-1020 | C–N stretch | Aliphatic amines | 1144.12 |
| 1250-1020 | C–N stretch | Aliphatic amines | 1086.14 |
| 950-910 | O–H bend | Carboxylic acids | 928.18 |
| 850-550 | C–Cl stretch | Alkyl halides | 848.99 |
| 690-515 | C–Br stretch | Alkyl halides | 620.15 |

*U. lactuca: Ulva lactuca*, FT-IR: Fourier transform infrared

highest zone of inhibition was found in *K. pneumonia* in F2 fraction. Similarly, when the crude extract of *G. corticata* was tested against the same bacterial pathogen highest zone of inhibition was found in *Pseudomonas sp*.

**FT-IR analysis**

The FT-IR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The crude powder of *U. lactuca* was passed into the FT-IR and the functional groups of the components were separated based on its peak ratio. The results of FT-IR analysis showed different peaks at 620.15 and 848.99 functional group is alkyl halides, 928.18 functional group is carboxylic acids, 1086.14 and 1144.12 functional group are aliphatic amines, 1384.42 functional group is nitro methane, 1428.88 functional group is aromatics, 1634.33 functional group is amides, 2295.04 functional group is nitriles and 3406.60 functional group are alcohols, phenols were present in *U. lactuca sample* (Table 2). Similarly, the crude powder of *G. corticata* was passed into the FT-IR and the functional groups of the components were separated based on its peak ratio. The results of FT-IR analysis showed different peak value they are 3321.46 functional groups are alcohols, phenols, 2925.49 functional group is alkanes, 2084.83 functional group are allenes, ketenes, is ocyanates, isothiocyanates, 1647.84 functional group isamides, 1471.30 functional group isaromatics, 1116.62 functional group isaliphatic amines, 874.41 functional group are primary and secondary amines, 750.98, 712.56, 657.10 and 617.26 functional group is alkylhalides were present in *G. corticata* sample (Table 4).

## DISCUSSION

Seaweed extracts showed various bio potential activities such as antibacterial [10]. Antibacterial activity of red, brown and green algae against both Gram-positive and Gram-negative bacteria has been established by several scientists [11]. Marine environment contains a source of functional materials, including polyunsaturated fatty acids, polysaccharides, essential minerals, vitamins, antioxidants, enzymes and bioactive peptides [12]. FT-IR is a valuable tool for measuring many chemical constituents in plants and seaweeds and it is used to reveal some qualitative aspects regarding the organic compounds [13]. In the present study, FT-IR is used to identify the functional group in the seaweeds. Alcohols are commonly found to have antimicrobial properties against both Gram-positive and Gram-negative bacteria [14]. Phenolic compounds exhibited good antimicrobial activities [15,16]. In the present study both seaweed contained phenol and alcohol compounds, which were responsible for the antibacterial activity. In the present study, *U. lactuca* is compared to *G. corticata* the *G. corticata* is more active.

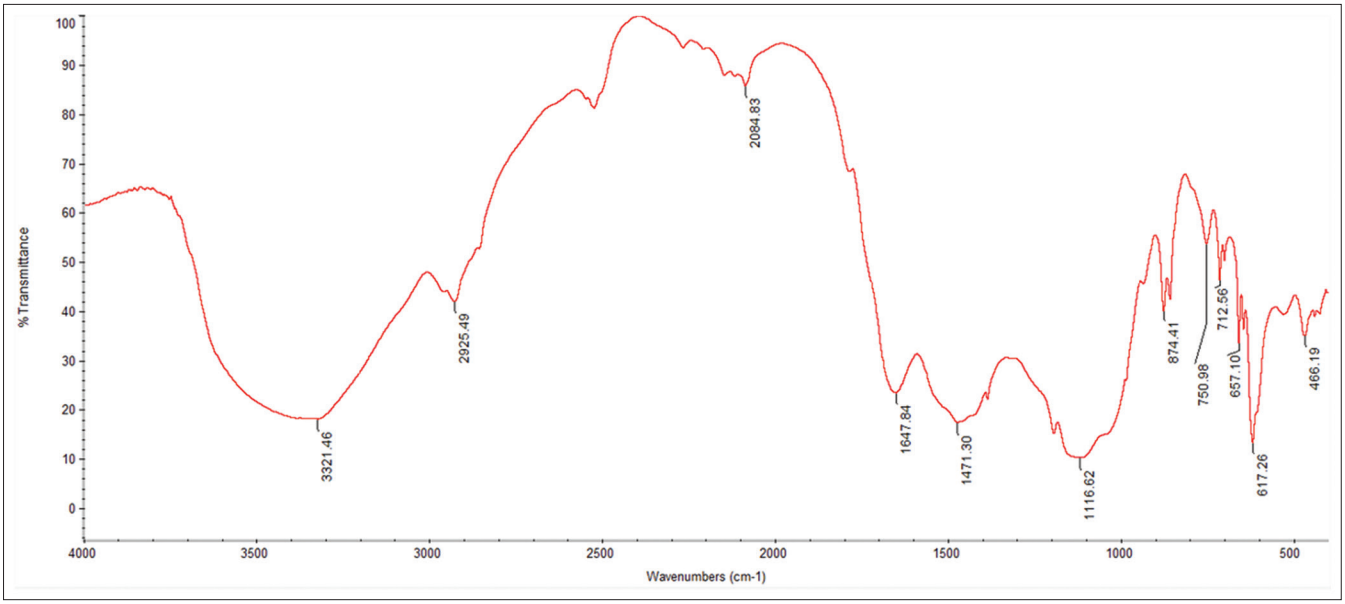


**Fig. 2: Fourier transform infrared spectrum of *Gracilaria corticata***

**Table 3: The zone of inhibition (mm) of *G. corticata* against different bacterial pathogens**

| Fractions | *K. pneumonia* | *A. hydrophilla* | *S. aureus* | *E. coli* | *Pseudomonas sp.* |
|---|---|---|---|---|---|
| F1 | 4±0.50 | 5±0.52 | 4±0.50 | 4±0.62 | 4±0.42 |
| F2 | 7±0.15 | 6±0.24 | 4±0.35 | 5±0.60 | 6±0.17 |
| F3 | 5±0.55 | 4±0.84 | 5±0.82 | 4±1.81 | 5±1.63 |
| Crude | 8±0.44 | 7±1.68 | 6±3.48 | 6±0.89 | 9±0 |

*K. pneumonia: Klebsiella pneumonia, A. hydrophilla: Aeromonas hydrophilla, S. aureus: Staphylococcus aureus, E. coli: Escherichia coli, G. corticata: Gracilaria corticata*

**Table 4: FT-IR absorption frequencies ($cm^{-1}$), intensity estimation and functional group of seaweeds *G. corticata***

| IR frequency ($cm^{-1}$) | Bond | Functional groups | *G. corticata* |
|---|---|---|---|
| 3500-3200 | O–H stretch, H–bonded | Alcohols, phenols | 3321.46 |
| 3000-2850 | C–H stretch | Alkanes | 2925.49 |
| 2270-1950 | X=C=Y | Allenes, ketenes, isocyanates, isothiocyanates | 2084.83 |
| 1670-1600 | C=O Stretch | Amides | 1647.84 |
| 1500-1400 | C–C stretch | Aromatics | 1471.30 |
| 1250-1020 | C–N stretch | Aliphatic amines | 1116.62 |
| 910-665 | N–H wag | Primary, secondary amines | 874.41 |
| 850-550 | C–Cl stretch | Alkyl halides | 750.98 |
| 850-550 | C–Cl stretch | Alkyl halides | 712.56 |
| 690-515 | C–Br stretch | Alkyl halides | 657.10 |
| 690-515 | C–Br stretch | Alkyl halides | 617.26 |

*G. corticata: Gracilaria corticata*, FT-IR: Fourier transform infrared

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