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RESEARCH ARTICLE

ANTIMICROBIAL ACTIVITIES OF CERTAIN SEAWEEDS FROM MUDDY SHORE PLACES OF KERALA

Selvaraju Raja*, Karuppiah Kannan, Kalamani Velmurugan, Samynathan, M. and Ephsy K. Davis
Department of Zoology, Kongunadu Arts and Science College (Autonomous), Coimbatore – 641029, Tamil Nadu, India

ABSTRACT

A study was carried out to reveal the growth inhibitory effect of methanol crude extract (MCE) and methanol supernatant extract (MSE) of sea weeds: 1) *Gracillaria corticata,* 2) *Hypnea musciforms,* 3) *Gelidium micropterum* and 4) *Hypnea valentiae* against six bacterial pathogens, 1) *Pseudomonas aeruginosa,* 2) *Bacillus licheniformis,* 3) *Serratia marcescens,* 4*) Aeromonas hydrophila,* 5) *Acinetobacter baumanii,* 6) *Escherichia coli* and two fungal strains, 1) *Aspergillus niger* and 2) *Candida albicans* respectively. Well diffusion method using zone of inhibition as indicator for growth inhibition was adopted. The results showed that methanol extracts of seaweeds *viz.*, *Gracillaria corticata, Hypnea musciforms,* and *Hypnea valentiae* prevented the growth of pathogenic bacteria and fungi. The effect on growth was observed as zone of inhibition, the diameter of which was indicated in the units of a millimeter. The growth of the bacterium, *Serratia marcescens* was affected by methanol supernatant extract of the three types of seaweeds, *Gracillaria corticata, Hypnea musciforms,* and *Hypnea valentiae,* and by the methanolic crude extract of *Hypnea musciforms* and *Hypnea valentiae.* However, the growths of other species of bacteria were not controlled by either of the extracts of the seaweeds except, *Bacillus licheniformis* which was controlled by only *Gracillaria corticata.* The growth of fungi: *Aspergillus niger* and *Candida albicans* were inhibited by the methanol extracts of *Gracillaria corticata.* Between the two forms of methanolic extracts i.e., supernatant and crude, the efficiency of the supernatant extract was greater than that of crude one. Further, among the three types of seaweeds which showed an effect on the growth of microbes, the level of the zone of inhibition caused by *Gracillaria corticata* was statistically higher than that of the other two, *Hypnea musciforms,* and *Hypnea valentiae.*

Keywords: *Gracillaria corticata, Hypnea musciforms, Gelidium micropterum, Hypnea valentiae,* Antimicrobial activity**.**

1. INTRODUCTION

Various natural antimicrobial compounds have been recorded in a marine environment more than those in the terrestrial one. Marine organisms such as marine algae are source materials for structurally unique natural products with pharmacological and biological activities. Among the marine organisms, the macroalgae (seaweeds) occupy a special site as a source of biomedical compounds [1]. Seaweeds have been recognized as potential sources of antibiotic substances. Synthesis of different metabolites from seaweeds is an indicator of the presence of antimicrobial active compounds [2].

Algae are part of a heterogeneous group of photosynthetic organisms and the division includes multicellular organisms, macroalgae or seaweed (reaching sizes of up to 60 m in length), and unicellular organisms, also known as microalgae (measuring from 1 mm to several cm). One way to classify macroalgae is on the basis of their pigmentation: (i) brown seaweed- Phaeophyceae, (ii) red seaweed-Rhodophyceae and (iii) green seaweed-Chlorophyceae [3]. Global utilization of macro-algae is a multi-billion dollar industry. In recent years pharmaceutical firms have started looking towards marine organisms, including seaweeds, in their search for new drugs from natural products [4]. Compounds with biological activities or pharmacological properties (bioactivities) have been discovered in marine bacteria, invertebrates, and algae [5]. Seaweeds contain many different secondary metabolites which have a wide spectrum of biological activities. It was observed, the presence

*Correspondence: Selvaraju Raja, Department of Zoology, Kongunadu Arts and Science College (Autonomous), Coimbatore - 641029, Tamil Nadu, India. E.mail: rajaselvaraju12@kongunaducollege.ac.in.

of cytostatic, antiviral, anthelmintic, antifungal, and antibacterial activities compounds in green, brown, and red algae with cytostatic, antiviral, anthelmintic, antifungal, and antibacterial activities [6]. Seaweeds are considered to be the main source of bioactive compounds with a wide range of biological activities, such as antibiotics, antioxidants, and anti-inflammatory [7]. Some macroalgae have bio-active components which affected the germination of some pathogenic bacteria, it contains different substances which incorporated medicine and pharmacotherapy, whereas some of the isolated substances have bacteriostatic and bactericidal properties. Different diseases were treated with antibiotics, extracted from terrestrial sources that were used as therapeutic agents; new compounds were present in oceans and have commercial value [8].

Several researchers attempted to identify organisms that produce bioactive substances, antioxidant and antimicrobial activity of seaweeds have been reported previously [9]. A good number of reports show that seaweeds are also a rich source of antioxidant compounds and screened extensively to isolate lifesaving drugs or biologically active substances all over the world. Marine algae are a potential source of new secondary metabolites [9]. Numerous chemical compounds were identified as antimicrobial agents including phenolic compounds, phlorotannins, terpenoids, fatty acids, chlorellin, and steroids [10].

However, the quantitative ranges of these bioactive molecules can fluctuate widely according to season, environmental conditions, geographical location, and reproductive stage [11]. It has been reported that among the algae groups, Phaeophyceae exhibited the highest antibacterial activity and 75% of the most secondary metabolites were derived from brown algae [12]. Currently, a large number of structurally unique bioactive agents derived from seaweeds have been identified and some of them are under experimentation or are being developed as new pharmaceutical drugs [13]. Most of the compounds of the marine algae show anti-bacterial activities and also metabolites isolated from marine algae have been shown to possess bioactive effects [14]. Seaweed *Ulva fasciata* have shown antimicrobial activities against *Staphylococcus aureus* and *Pseudomonas aeruginosa* that are commonly found among human infections [15].

In recent years there has been found an increase in the resistance of microorganisms to antibiotics that are usually used in the treatment of some diseases. To overcome this problem, new therapeutic drugs from natural products have been explored [16]. Marine algae have been extensively documented for their capacity to provide a rich source of primary and secondary metabolites [17]. There are several substances obtained from algae that are already in use in traditional medicine for a long time [18].

Although there are numerous publications on antimicrobial activity of algal extracts, they usually report results of algae collected from their natural habitats. In fact, to the best of my knowledge, there is no data concerning the antimicrobial potential of algae from aquaculture systems. The objectives of the present study are therefore to evaluate the antimicrobial activity of methanol extracts of four seaweeds *viz*., 1) *Gracillaria corticata,* 2) *Hypnea musciforms*, 3) *Gelidium micropterum* and 4) *Hypnea valentiae* against six bacterial pathogens, *viz.*, 1) *Pseudomonas aeruginos,* 2) *Bacillus licheniformis,* 3) *Serratia marcescens,* 4) *Aeromonas hydrophila* 5) *Acinetobacter baumanii,* 6) *Escherichia coli* and two fungal strains *viz*., 1) *Aspergillus niger* and 2) *Candida albicans.* In the present study, we report the efficacy of seaweeds collected from the West coast of India against multi resistant pathogens.

2. MATERIALS AND METHODS

2.1. Sample collection

The seaweeds, *Gracillaria corticata, Hypnea musciforms, Gelidium micropterum* and *Hypnea valentiae* were collected from Manjeshwar coast, Kerala. The collected samples were thoroughly washed with seawater to remove all the extraneous matter such as epiphytes, sand particles, pebbles, and shells, and samples were brought to the laboratory species-wise in separate plastic bags. The samples were again cleaned with fresh water and distilled water in the laboratory. The samples were then air-dried under the shadow. Each type was separately powdered and stored at room temperature until extraction.

2.2. Preparation of seaweed methanol extract

The powdered sample (10 g) was extracted in Soxhlet apparatus using methanol (250 ml) as a solvent for 8h at 60˚C. Extracts are filtered using a muslin cloth, followed by Whatman No.1 filter paper and the solvents were allowed to evaporate in Hot air oven under 40˚C. The extracts obtained were stored in a refrigerator. The extracts were carried out following the standard procedure dried extracts are used for assay [19]. Each concentrated filtrate

was made into different concentrations (20 µg/ml, 40 µg/ml, 60 µg/ml and 80 µg/ml) using methanol.

2.3. Test pathogens

The pathogens used in the present study viz. *Pseudomonas aeruginosa, Bacillus licheniformis, Serratia marcescens, Aeromonas hydrophila, Acinetobacter baumanii, Escherichia coli, Aspergillus niger,* and *Candida albicans* were obtained from the Research Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore, Tamil Nadu, India.

2.4. Assay of antimicrobial activity

All the bacterial and fungal cultures were subjected to test their susceptibility/ resistance pattern to the methanol extract of the seaweeds by well diffusion method as described by Bauer *et al*. [20] using Mueller Hinton agar (HiMedia, India). Sterilized media were dispensed into sterile petri dishes aseptically. Broth cultures of the test organisms were swabbed over the surface of the separate agar plate using sterile cotton swab aseptically. In each of these plates, wells (10mm) were cut out using a sterile cork borer.

Different concentrations (20, 40, 60, and 80 µg/ml) of dried seaweed extracts were prepared in methanol and loaded into the respective labelled wells using sterile pipettes. In each plate, one of the wells was used as a control (solvent alone). The plates were incubated at 37^{o}C for 24hrs for bacteria and 5 days for fungi.

After the incubation, the diameters of inhibition zones were measured using a ruler and expressed in millimeters. Based on the level of inhibition zone the response of microbes to the extract was compared and classified as resistant, intermediate, and sensitive according to Johnson and case [21]. If the diameter of the zone of inhibition was 10 mm or less, the microbe concerned was considered resistant to the seaweed extract. If the diameter was 16mm and above, the microbe was marked susceptible. If it was in the range between 11and 5mm, the microbe was noted as intermediate in response to the type of seaweed used.

3. RESULTS

3.1. Antimicrobial activity of the seaweed, Gracillaria corticata

The control well showed no zone of inhibition and the bacterial, as well as fungal growth was found around the well which was incubated with solvent, methanol only. There was a zone of inhibition in the medium of *Bacillus licheniformis* of 11 mm around the well provided with crude methanol extract at a concentration of 80 µg/ml. In the experiment with methanol extract (supernatant) the zone of inhibition for *B. licheniformis* extended to the level of 15mm (Figures 3 and 4). The growth of bacterium *Serratia marcescens* was well controlled by this species of seaweed as evidenced by a gradual increase in the diameter of zone of inhibition from 13 mm to 20 mm for the concentrations from 20 µg/ml to 80 µg/ml of methanol extracts supernatant (Figure 5).

The species *Gracillaria corticata* the seaweed also showed its effect on the growth of fungus, *Aspergillus niger* as indicated from the zone of inhibition as 10mm, 12 mm, and 30 mm for concentrations 40 µg/ml, 60 µg/ml, and 80 µg/ml, respectively (Figure 6). The fungus, *C. albicans* showed a susceptible level of the zone of inhibition (16 mm) to the crude methanol extract of the seaweed, *G. coticata*. Thus, both the bacterial species and the fungal species were found as susceptible to the methanol extract supernatant of this seaweed.

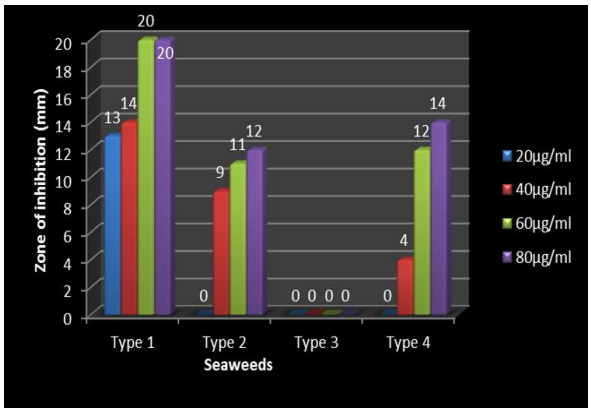

Fig. 1. Diameter of Zone of inhibition (mm) as observed in culture media of pathogenic bacteria, *Serratia marcescens* treated with methanol extract supernatant of seaweeds, 1) *Gracillaria corticata, 2) Hypnea musciforms, 3)Gelidium micropterum* and 4) *Hypnea valentiae*

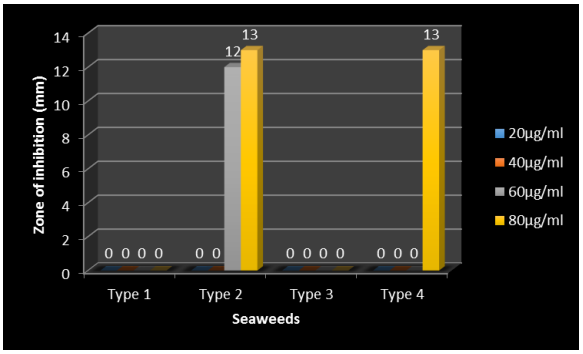

Fig. 2. Diameter of Zone of inhibition (mm) as observed in culture media of pathogenic bacteria, *Serratia marcescens* treated with crude methanol extract of seaweeds, 1) *Gracillaria corticata, 2) Hypnea musciforms, 3) Gelidium micropterum* and 4) *Hypnea valentiae*

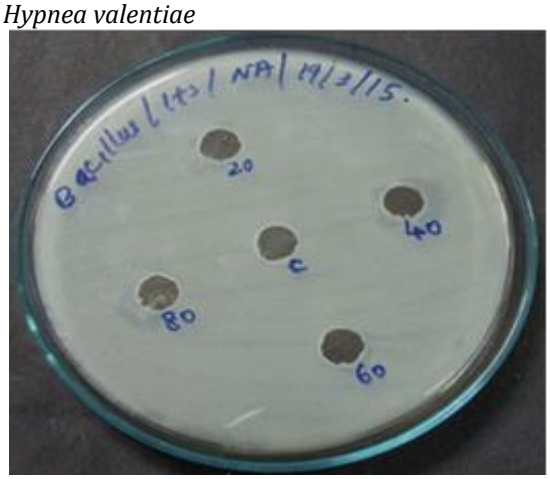

Fig. 3. The photograph shows zone of inhibition on well diffusion medium between *Bacillus licheniformis* and *the* methanol extract (supernatant) of seaweed, *Gracillaria corticata* at concentrations, 20 µg/ml, 40 µg/ml, 80 µg/ml and control.

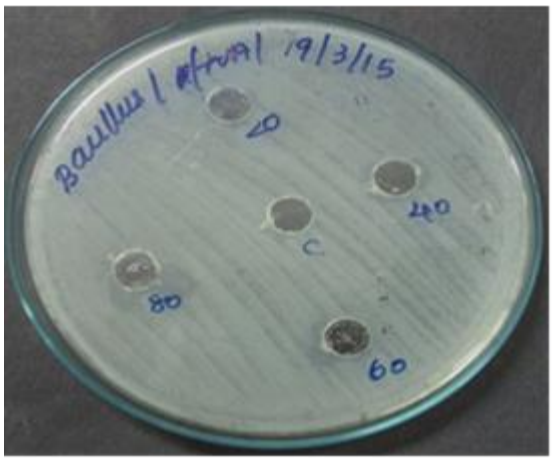

Fig. 4. Photograph shows zone of inhibition on medium between *Bacillus licheniformis* and methanol extract (crude) of seaweed, *Gracillaria corticata* at concentrations, 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and control.

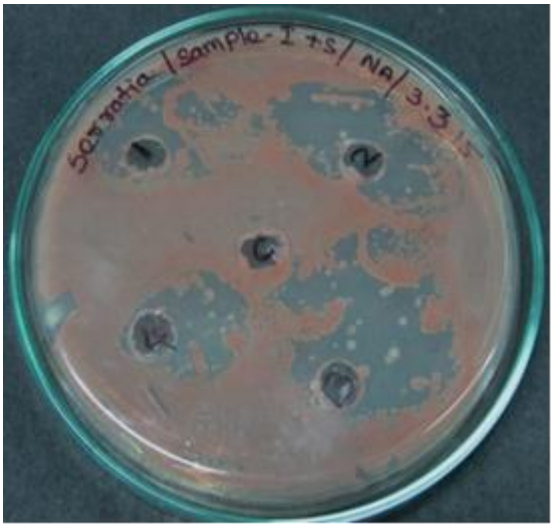

Fig. 5. Photograph shows zone of inhibition on medium between *Serratia marcescens* and methanol extract (Supernatant) of seaweed, *Gracillaria corticata* at concentrations, 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and control.

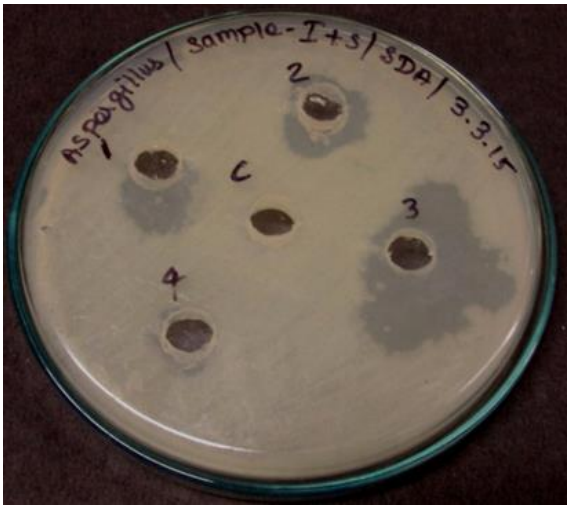

Fig. 6. Photograph shows zone of inhibition on medium between *Aspergillus niger* and methanol extract (Supernatant) of seaweed, *Gracillaria corticata* at concentrations, 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and control.

3.2. Antimicrobial activity of the seaweed, Hypnea musciforms

The controlling effect was observed for both crude methanol extract as well as methanol extract supernatant of this seaweed over the bacterium, *Serratia marcescens*. The crude extract at concentrations 60 µg/ml and 80 µg/ml prevented the growth of this species of the bacterium as evidenced from the diameter of zone of inhibition, 12mm and 13mm, respectively. The zone of inhibition of 9 mm, 11 mm, and 12mm was recorded for methanol extract supernatant concentrations 40 µg/ml, 60 µg/ml and 80 µg/ml, respectively. The growth of other bacterial and fungal types *Pseudomonas aeruginos, Bacillus licheniformis, Aeromonas hydrophila, Acinetobacter baumanii, Escherichia coli, Aspergillus niger* and *Candida albicans* were not affected by this seaweed.

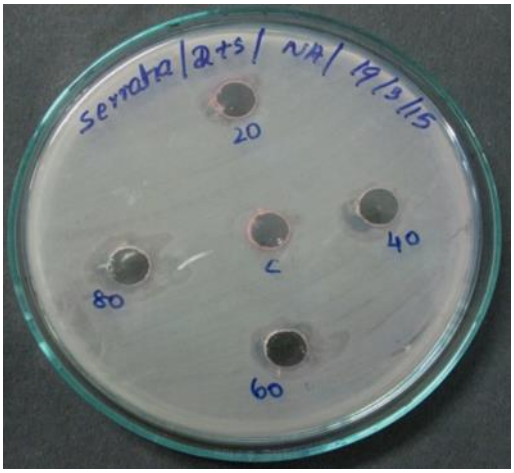

Fig. 7. Photograph shows zone of inhibition on medium between *Serratia marcescens* and methanol extract (supernatant) of seaweed, *Hypnea musciforms* at concentrations, 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and control.

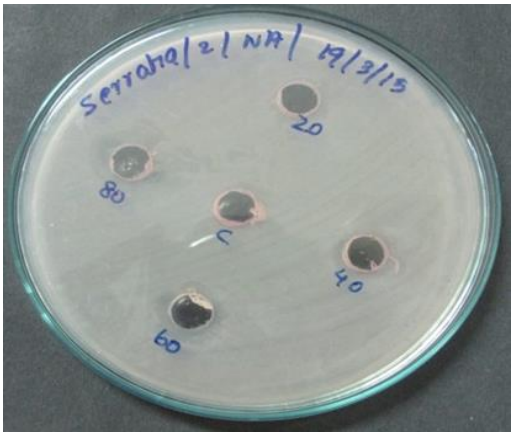

Fig. 8. Photograph shows zone of inhibition on medium between *Serratia marcescens* and methanol extract (crude) of seaweed, *Hypnea musciforms* at concentrations, 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and control.

3.3. Antimicrobial activity of the seaweed, Gelidium micropterum

Either crude methanol extracts or methanol extracts supernatant of this seaweed showed no effect on the growth of any of the bacterial and fungal types used in the present study. The culture medium inoculated with pathogens, *Pseudomonas aeruginos, Bacillus licheniformis, Serratia marcescens, Aeromonas hydrophila, Acinetobacter baumanii, Escherichia coli, Aspergillus niger,* and *Candida albicans* and different concentrations of the seaweed, *G. micropterum* was identical to that of the control in appearance. The growth of microbes was uniform throughout the area of culture irrespective of the wells supplied with different concentrations of methanol extracts of this seaweed.

3.4. Antimicrobial activity of the seaweed, Hypnea valentiae

The seaweed *H. valentiae* showed its effect on the growth of bacterium *Serratia marcescens* by crude methanolic concentration, 80 µg/ml as evidence that from a zone of inhibition of diameter 13mm. The extract supernatant was highly effective even at its low concentrations. The zone of inhibition at concentration 40 µg/ml was 4mm and at 60 µg/ml it was 12mm (Figures 9 and 10). The highest inhibition of growth was found around well provided with a concentration of 80µg/ml where the diameter of the zone of inhibition was 14mm. The gradual increase in the diameter of the zone of inhibition with methanol extract concentration of the seaweed indicates its antimicrobial activity against the bacterium. However, in either form of the extract, this seaweed did not show the effect on the growth of any other microbes selected for this study.

Fig. 9. Photograph shows zone of inhibition on medium between *Serratia marcescens* and methanol extract (Supernatant) of seaweed, *Hypnea valentiae* at concentrations, 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and control.

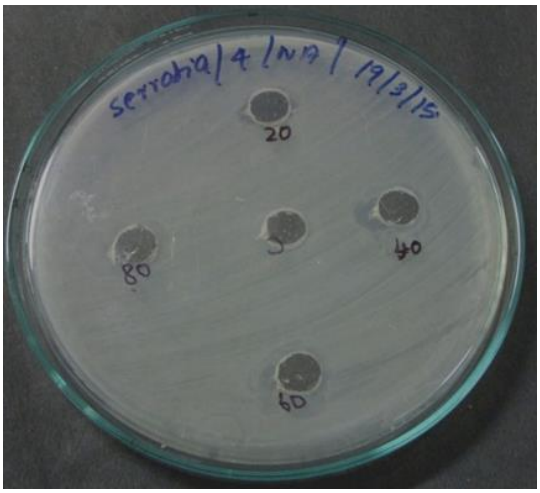

Fig. 10. Photograph shows zone of inhibition on medium between *Serratia marcescens* and methanol extract (Crude) of seaweed, *Hypnea valentiae* at concentrations, 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and control.

3.5. Comparative account of the effect of seaweeds on microbes

Among the four types of seaweeds, *Gracillaria corticata, Hypnea musciforms, Gelidium micropterum* and *Hypnea val entiae* used in the present study no preventive effect was shown by *G. micropterum* on the growth of all the types of microbes selected, *Pseudomonas aeruginos, Bacillus licheniformis, Serratia marcescens, Aeromonas hydrophila, Acinetobacter baumanii, Escherichia coli (*Bacteria) *Aspergillus niger* and *Candida albicans* (Fungi).

The growth of a bacterium, *Serratia marcescens* was affected by methanol extract supernatant of the three types of seaweeds, *Gracillaria corticata, Hypnea musciforms,* and *Hypnea valentiae,* and by the crude methanolic extract of *Hypnea musciforms* and *Hypnea valentiae* (Fig. 1&2). However, the growths of other species of bacteria were not controlled by either of the extracts of the seaweeds except, *Bacillus licheniformis* which was controlled by only *Gracillaria corticata.* The growth of fungus, *Aspergillus niger,* and *Candida albicans* was inhibited by the methanol extracts of *Gracillaria corticata.*

Between the two forms of methanolic extracts i.e., supernatant and crude, the efficiency of extract supernatant was found greater than that of crude one. Further, among the three types of seaweeds, significant effect on the growth of microbes, the level of zone of inhibition caused by *Gracillaria corticata* was statistically higher than that of the other two, *Hypnea musciforms,* and *Hypnea valentiae.*

4. DISCUSSION

Many researchers have reported on the antioxidant and antimicrobial activity of seaweeds [22,23]. The extraction of antimicrobials from different species of seaweeds was solvent dependent. Methanol was a good solvent for the extraction of antimicrobials from brown seaweeds whereas acetone was better for red and green species [24]. Extracts of marine algae were reported to exhibit antibacterial activity [25]. Several workers have reported that the seaweed extracts exhibit inhibitory activity against a number of gram positive and gram negative bacterial pathogens. A number of seaweeds have been studied for their antibacterial activity. Padma Sridhar *et al*. [26] screened the antibacterial activity of extracts of marine algae representing Chlorophyta and Rhododphyta collected from Vishakapatnam Coast against two pathogens and also tested their ability to inactivate the enzyme penicillinase under *in vitro*. Padmakumar and Ayyakkannu [27] revealed the antimicrobial activity of marine algae collected from Porto Novo and Pondicherry waters, against 6 bacterial and 2 fungal pathogens.

Rao and Parekh [28] showed that crude extracts of seaweeds are active only against gram positive bacteria. Vanitha *et al*. [29] reported the antibacterial action of nine seaweeds collected

from the Kanyakumari coast against human upper respiratory tract pathogens which include both Gram positive and Gram negative bacteria. Kandhasamy and Arunachalam [30] found out the *in vitro* antibacterial property of seaweeds viz. *Caulerpa racemosa, Ulva lactuca, Gracilaria folifera, Hypnea musciformis, Sargassum teneerimum, S. myriocystem,* and *Padina tetrastomatica* collected from Koodankullam village, Tirunelveli, Tamilnadu against gram negative and gram positive pathogenic bacteria. Some commonly occurring marine algae *Caulerpa scalpelliformis, Ulva lactuca, Pandina tetrastromatica, Stoecchospermum marginatum* and *Acanthophora spicifera* have been collected from the coast of Tuticorin, Tamilnadu and evaluated for antifungal and antibacterial activity by using four solvents such as petroleum ether, chloroform, methonal, and benzene [31]. Arul Senthil *et al*. [32] found out the antibacterial activity of the methanol, diethyl ether, acetone, and dichloromethane extracts of *Padina boergesnii* collected from Tuticorin Coast against 10 human pathogenic bacteria.

5. CONCLUSION

To conclude, among the extracts of seaweeds tested in the present study, *Gracillaria corticata* has been found as highly effective against bacterial and fungal pathogenic organisms and hence recommended to be used as antimicrobial agent. Further, the effect of methanol extract supernatant has shown greater effect rather than its counterpart crude methanol extract. Hence, suggested that further analysis is required to identify the active component which would be relatively showing higher effect in methanol extract supernatant.

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