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ANTIMICROBIAL ACTIVITY OF GREEN AND RED MACROALGAL EXTRACTS FROM PULICATE LAKE

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ABSTRACT:

Marine algae offer an alternative to chemical-based antibiotics due to their strong antibacterial activity and capacity to synthesise beneficial secondary metabolites. The green, brown, and red macroalgae have antibacterial activity because they contain a variety of phytochemicals and sulfated polysaccharides. We examined the antibacterial activity of extracts from Enteromorpha intestinalis, Ulva reticulata (green algae), Hypnea, and Gracilaria (red algae) against a few common human diseases in the current work.

KEYWORDS: *Macroalgae, Seaweeds, Liquid Fertilizer, Enteromorpha intestinalis, Ulva reticulata, and Hypnea and Gracilaria.*

INTRODUCTION

Algae are a group of marine plants that are becoming more prominent worldwide for both scientific and commercial purposes. According to Seenivasan et al. (2010), they are primitive, non-flowering plants lacking true stems and leaves. Macroscopic marine algae have been intimately linked to human life since ancient times. They are extensively utilized for a variety of purposes, including food, feed, fertilizer, medicine, and most notably, the production of economically significant phycocolloids (Levering et al., 1969; Chapman, 1970). Compared to terrestrial plants, marine algae have substantially greater concentrations of over 60 trace elements.

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Seaweeds, like other plants, provide a variety of organic and inorganic compounds that are beneficial for human health (Kuda et al., 2002). Seaweeds are considered to be a source of bioactive chemicals since they can produce a wide range of secondary metabolites with a wide range of biological activity. Seaweeds are a valuable natural source of bioactive compounds. Compounds having antioxidant, antiviral, antifungal, and antibacterial qualities have been identified in brown, red, and green algae, (Rajasulochana et al.2009 and Yang et al.2006).

Numerous reports exist on the therapeutic value of seaweeds from the families Chlorophyceae, Phaeophyceae, and Rhodophyceae distributed all over the world. The antibacterial activity of seaweeds has been consistently investigated by a number of scientists, both domestically and internationally.

The aim of this study was to evaluate the antibacterial activity of marine macroalgae from the families Chlorophyceae (*Ulva reticulate, Enteromorpha intestinalis*) and Rhodophyceae (*Hypnea* sp., *Gracilaria* sp.), which were collected from Pulicate Lake, Pazhaverkadu, Tiruvallur, Tamilnadu. Five different solvents were used in the study: ethanol, methanol, chloroform, ethyl acetate, and DMSO against human pathogenic bacteria (2-Gram Positive and 3-Gram Negative).

MATERIALS AND METHODS

SAMPLE COLLECTION

The seaweeds *Enteromorpha intestinalis*, *Ulva reticulate*, *Hypnea sp*., and *Gracillaria sp.* (Plate 1), were collected from Pulicate Lake, Thiruvallur. After being harvested, the algae were thoroughly washed using tap water to get rid of any unwanted elements. Samples were shade dried for 7 to 8 days, or until it becomes brittle. Subsequently, it was preserved for further analysis after being ground using a mixer grinder.

PREPARATION OF ALGAL EXTRACT

For crude extraction, organic solvents such as ethanol, methanol, chloroform, dimethyl sulfoxide (DMSO), and ethyl acetate were selected. Solvents were added to the samples in 2:20 w/v ratio to create crude extracts for each sample. The resulting crude extracts were filtered through A1 filter paper and then concentrated by evaporating. Using the agar disc diffusion method and agar well diffusion, the antibacterial activity of the algal extracts was further investigated.

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ANTIBACTERIAL ACTIVITY TEST

The agar well diffusion test and disc diffusion method (Heatley, 1944) were used to measure antibacterial activity. For this investigation, five human infections were selected.

Plate 1. Macroalgae used for antibacterial activity
(a) *Enteromorpha intestinalis*, (b) *Ulva reticulate* *(c) Hypnea sp. (d) Gracilaria sp.*

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RESULTS AND DISCUSSION

Extracts of green and red seaweed were evaluated for their ability to inhibit bacteria. In this study, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillis subtilis*, *Escherichia coli*, and *Klebsiella pneumonia* were the five test pathogens used to examine the inhibition of microbial growth by the five different solvents.

By producing secondary metabolites, marine algae employ targeted antimicrobial chemical defence mechanisms. The ecological interactions that take place between marine macro- and microorganisms depend on these compounds. Numerous marine algae species' extracts prevent both Gram-positive and Gram-negative bacteria from growth (Rao and Parekh, 1981). The antibacterial activity of the seaweed *Gracilaria edlis*, which is linked to epiphytic bacteria, was examined by Naqvi et al. (1981) in relation to human bacterial infections from Indian waters and the west coast of India. Using methanol, ethyl acetate, and aqueous extracts, he investigated the antibacterial activity of marine macroalgae *Hypnea cervicornis* against eight human pathogenic bacteria.

CHLOROPHYCEAE

ENTEROMORPHA SP. EFFECTS

In *Enteromorpha*, the DMSO extract was the most effective against all five bacteria, followed by methanol and ethyl acetate. They exhibited activity against four bacteria. *Staphylococcus aureus* was determined to have the maximum zone of inhibition (26 mm). *Bacillus subtilis* (17 mm) showed the highest zone of inhibition in the methanol extract, followed by *Staphylococcus aureus* (12 mm). In extracts of *Enteromorpha intestinalis* containing ethanol, methanol, and chloroform, *Pseudomonas aeruginosa* did not show any activity (Table 1).

ULVA RETICULATA EFFECTS

In *Ulva reticulate*, the DMSO extract also showed activity against all five pathogens tested, followed by ethyl acetate which showed activity against four pathogenic bacteria excluding *Escherichia coli*. Gram negative bacteria showed no activity in any of the extracts, however *Bacillus subtilis* showed the highest zone of inhibition (14 mm) in methanol, followed by *Staphylococcus aureus* (10 mm).

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TABLE 1. ANTIBACTERIAL ACTIVITY OF CHLOROPHYCEAE.

CLINCAL PATHOGENS	GRAM +VE/-VE	*ENTEROMORPHA INTESTAINALIS*					*ULVA RETICULATE*				
		ET	M	C	EA	DMSO	ET	M	C	EA	DMSO
Bacillus subtilis	+ve	15	17	11	-	7	12	14	9	8	5
Staphylococcus aureus	+ve	11	12	-	26	8	10	10	-	11	12
Klebsiella pneumonia	-ve	12	8	10	8	12	9	-	9	9	9
Pseudomonas aeroginosa	-ve	-	-	-	8	9	-	-	-	8	10
Escherichia coli	-ve	-	11	-	9	8	-	-	-	-	9

The *Ulva reticulate* ethanolic extract had the highest efficacy against *Bacillus subtilis* (12 mm). The highest activity against *Staphylococcus aureus* (12 mm) and *Pseudomonas aeruginosa* (10 mm) was demonstrated by the DMSO extract. *Enteromorpha intestinalis* exhibited highest activity against the studied pathogens among all the Chlorophyceae.

Latha and Hemalatha (2011) found that the *Enteromorpha* chloroform extract exhibited good antibacterial activity when compared to the *Chaetomorpha* chloroform extract. When compared to Mohamed Elanwar et al. (2010), our results were distinct. They claimed that *Ulva faciata* methanol extract showed the highest inhibition zone while *Ulva lactuca* ethanol extracts showed the lowest activity (Table 1).

RHODOPHYCEAE

HYPNEA SP. EFFECTS

Again, DMSO showed efficacy against all five pathogens and proved to be a good extract. Ethyl acetate, on the other hand, showed activity against four pathogens, with the exception of *Pseudomonas aeruginosa*. Of all the extracts ethyl acetate exhibited the maximum zone of inhibition against *Staphylococcus aureus* (17 mm).

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TABLE 2. ANTIBACTERIAL ACTIVITY OF RHODOPHYCEAE.

CLINCAL PATHOGENS	GRAM +VE /-VE	*HYPNEA SP.*					*GRACILARIA SP.*				
		ET	M	C	EA	DMSO	ET	M	C	EA	DMSO
Bacillus subtilis	+ve	10	10	10	7	8	16	11	11	7	8
Stphylococcus aureus	+ve	9	11	-	17	11	-	11	10	-	9
Klebsiella pneumonia	-ve	-	-	15	8	7	-	-	22	10	12
Pseudomonas aeruginosa	-ve	-	-	9	-	10	-	-	9	-	8
Escherichia coli	-ve	-	-	-	9	10	-	-	-	9	-

Subsequently, the chloroform extract showed the maximum zone of inhibition for *Klebsiella pneumonia* (15mm). There was no activity of the ethanol and methanol extracts against gram negative bacteria (Table 2).

GRACILARIA SP. EFFECTS

In *Gracilaria sp*., the DMSO and ethyl acetate extracts were effective against four pathogens. Of all the extracts, Chloroform showed the maximum zone of inhibition against *Klebsiella pneumoniae* (22 mm). The maximum zone of inhibition (16 mm) against *Bacillus subtilis* was showed by the ethanolic extract; whereas, no activity was observed against the other four pathogens. In Gram-positive bacteria, the methanolic extract showed the maximum zone of inhibition. Gram-negative bacteria did not exhibit any activity. Among the Rhodophyceae the *Hypnea sp*. showed highest zone activity against the pathogens tested (Table 2).

Thus, the current investigation shows that the antibacterial activity of green and red algae against specific strains of human pathogenic bacteria differs upon the type of extraction solvent. *Enteromorpha intestinalis* extract had the best antibacterial activity. The DMSO extract of all the seaweeds inhibited most of the tested bacteria.

In a study conducted by Arun and Rengasamy (2000), evaluated the antibacterial capability of eleven seaweeds found in six regions along the Tamil Nadu coast and seven distinct locations in

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Muttukadu, Chennai. The highest antibacterial activity was shown by unsaponified fractions of red and green seaweeds, which were followed by brown algal methanol extracts. Rajasulochana et al. (2009) reported that the extract residues of brown algae (*Padina* sp.) and red algae (*Kappa* sp.) had good antibacterial activity compounds. Rao et al., (1991) reported that red and brown seaweeds had greater antibacterial activity in comparison to green algae.

According to Adaikalarajet al. (2012), the methanol extract of *E. prolifera* against *E. coli* had the lowest antibacterial activity, whereas the aqueous extract of *Gracilaria verrucosa* showed the highest activity against *Pseudomonas aeruginosa*. In the aqueous extract of all seaweeds, the microbial stains *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhi*, and *Candida albicans* did not exhibit any antibacterial activity. According to Elif Ayşe Erdoğan Eliuz et al. (2019), *Enteromorpha* sp. Methanolic Extract and Gelatin Film Solution showed the antimicrobial activity against several pathogens.

When compared to commercial antibiotics, Murugaboopathy et al. (2020) demonstrated that *Ulva lactuca* aqueous extracts had only moderate antimicrobial activity. Afzal et al. (2023) provided a brief overview of the phytochemicals that were isolated from algae and how they were used as dietary supplements for therapeutic purposes. Additionally, they attempted to determine whether metabolites generated from algae may be used as medicines to treat viral infections similar to SARS-CoV-2.

According to the current study findings, macroalgae taken from Pulicate Lake have the potential to contain bioactive compounds and serve as a source for antibacterial agent. The notable discrepancies between our findings and those of earlier research could be attributed to several factors, including seasonal fluctuations, intraspecific variability in the production of secondary metabolites, and the condition or state of the sample as well as the season in which the algae were collected. In addition, a number of factors, including the plant's age, duration of storage, temperature, media preparation, and pH, may have an indirect impact on the level of antibiotic activity (Rao, 1995; Fenical, 1975; Ragan and Glombitza, 1986). Furthermore, variations in the extraction methodologies capacity to retrieve the active metabolites and variations in the assay techniques could lead to differences in the target strains' susceptibilities. Because test materials contain trace contaminants, this is an unavoidable fact for any biochemical study (Incituney, et al., 2006). According to various reports, the seaweed extracts are more effective against gram-positive bacteria than gram-negative bacteria (Kannabiran, 2009; John et al., 2011; Pasando et al., 1984).

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CONCLUSION

The marine macroalgae *Enteromorpha intestinalis*, *Ulva reticulate*, *Hypnea sp*., and *Gracilaria sp.*, as well as their solvent extracts, showed notable antibacterial qualities against the examined human pathogenic bacteria, according to the study's findings. Among the four seaweeds examined for their antibacterial activity, the DMSO when used as a solvent, showed activity against all of the pathogens, but the Ethyl acetate extract of *Enteromorpha intestinalis* and *Hypnea* sp. showed highest zone of inhibition among the four seaweeds examined for their antibacterial activity.

The dissimilarities in morphology between gram-positive and gram-negative bacteria may be the cause of their varying susceptibilities. Gram-negative bacteria contain structural lipopolysaccharide components in their outer phospholipidic membrane. As a result, lipophilic solutes cannot pass through the cell membrane, while hydrophilic solutes are selectively blocked by porins, which have an exclusion limit of roughly 600 Da. Because gram-positive bacteria only have an exterior peptidoglycan layer—which is ineffective as a permeability barrier—they should thus be more susceptible. So, we derive the conclusion that, of the organisms employed in this study, *Enteromorpha intestinalis*, a green alga, exhibited the maximum antibacterial activity, suggesting that it may be used in natural medicine to treat a range of illnesses.

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