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Full Length Research Article

Exploration of Bioactive Compounds, Antibacterial and Antibiofilm Activity of Marine Macroalgae (*Ulva rigida*) Isolated from Rameshwaram Coastal Region

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Abstract

Antibacterial resistance is a critical issue that has led to an increase in demand for new antibacterial compounds. In this point of view, present study was evaluating the antimicrobial and antibiofilm activity of algae extract against food isolates. Totally 7 types of bacterial genera were observed from meat samples and subjected to screening the biofilm producing isolates. Among the 18 isolates, 77.7% of were biofilm producers, which were confirmed with Congo red agar plate method. Among the 7 test pathogens, *K. pneumoniae and E. faecalis* were highly suppressed, 10mg of concentration was suppressed the all isolates. In case of antibiofilm process, highest activity was observed against above-mentioned isolates. The GC-MS result showed the presence of several important antimicrobial fatty acids like 9, 12-Octadecadienoic acid, Hexadecanedioic acid, 1,15-Pentadecanedioic acid. In this study, *Ulva rigida* was shown to have a wide spectrum of antimicrobial activity and be antibiofilm activity at low concentration; therefore, *Ulva rigida* extract could have important application in the field of antimicrobial production as alternative to antibiotics.

***Key words*:** *Ulva rigida*, Antimicrobial activity, Antibiofilm activity, Fatty acid, Hexadecanedioic acid

Antibacterial resistance among food born bacterial isolates can vary widely depending on the bacteria species, antibiotic used, and environmental factors. Studies have found that common food-borne bacteria like *Escherichia coli*, *Salmonella enterica*, and *Listeria monocytogenes* can be more resistant to certain antimicrobial drugs. For example, some strains of *E. coli* have been found to be resistant to commonly used antibacterial agents such as ampicillin and tetracycline. Additionally, some food-borne pathogens, such as *Campylobacter jejuni*, have been shown to have high levels of intrinsic resistance to certain antibiotics. This is due to the presence of specific genetic mutations that can reduce the effectiveness of the antibiotic. Furthermore, environmental factors such as the presence of other bacteria, presence of other antimicrobial agents, and presence of other drugs can also increase the level of antibiotic resistance in food-borne bacteria [1-3].

One of the major factors contributing to antibiotic resistance is biofilm production, a process which enables bacteria to form a highly structured and protective matrix around them to shield them from antibiotics. In India, there have been numerous studies on antibiotic resistance in biofilm-producing meat isolates. The researchers also found that these biofilm-producing isolates were significantly more resistant to antibiotics than non-biofilm producing isolates. In another study, conducted in 2017, researchers investigated the prevalence of antibiotic resistance in biofilm-producing meat isolates in India. The results showed that out of a total of 110 isolates, 81 were able to form biofilms, and of these, 57.4% were resistant to one or more antibiotics. The most common antibiotics to which the isolates were resistant were ampicillin, tetracycline, and ciprofloxacin.

In this situation, urgently need inhibition of biofilm and multidrug resistance isolates by using alternative way of inhibitory activity. Number of authors utilized the algae for treating the bacterial infection. Farina Mujeeb *et al*. [4] studied the antibacterial activity of algae-derived products against food isolates. The study isolated several species of algae including, *Chlorella vulgaris*, *Chaetomorpha linum* and *Ulva sp*. Zbakh *et al*. [5] were assessed the antibacterial activity of 21 species of marine algae against food isolates. The results revealed that all the 13 species of marine algae tested exhibited antibacterial activity against the food isolates. The most active species of marine algae were *Sargassum wightii*, *Padina tetrastromatica*, and *Caulerpa racemosa*. A study conducted by Trigui *et al*. [6] investigated the antibacterial activities of Ulva (*Ulva rigida*)

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against food isolates. The study tested the aqueous and ethanolic extracts of Ulva against a total of 30 bacterial species. However, antibacterial activity of *Ulva rigida* against biofilm producing isolates has not been extensively studied. Therefore, the present goal was determined the antibacterial activity of *Ulva rigida* against biofilm producing food isolates and inhibited the formation of biofilm.

MATERIALS AND METHODS

Collection of food samples

A total of five chicken samples were obtained from retail outlets in and around the Namakkal area. Samples are aseptically packaged and promptly shipped to the laboratory, where they are reviewed and assayed within 24 hours. All samples were pulverized with phosphate buffered saline using a mortar and pestle. Inoculate one loop of the minced specimen onto a sterile selective medium agar plate. Label the inoculated plate perfectly and incubate at 37 °C for 24 h. After incubation, colony identification was based on colony morphology in selective media, and confirmed isolates were stored in nutrient agar slant still further study.

Biofilm assay

Biofilm procedure was performed using the method of Freeman *et al*. [7]. The isolated bacterial food isolates were inoculated onto sterile brain-heart infusion agar medium supplemented with sucrose (5%) and Congo red (0.08 g/L). All plates were incubated at 37°C for 24 hours. Black colonies with dry crystalline consistency indicate strong biofilm formation.

Collection of algae samples

During the winter months of December to March (2018-2019), samples of *Ulva rigida* were collected from surface water at a depth of approximately 50 m in a rocky area at the coast of Rameshwaram. The samples were then aseptically transferred to the laboratory in sterilized plastic bags filled with seawater to prevent the dryness of the collected samples. Then rinse gently with fresh water to remove surface salt. After washing, let it dry for about 2-3 days (48-72 hours) in the shade [8].

Identification of algae

The algae were identified based on their taxonomical characteristics which were published by [9]. The identification procedures were carried out at Department of botany, Arignar Anna Government Arts College, Namakkal, Tamil Nadu, and India.

Preparation of extract

The extraction procedure was carryout withHuda Sheikh *et al*. [10]. The *Ulva rigida* extract was prepared by boiling 10 gm of the algae in 100 mL of ethanol for 8 hrs. The extract was filtered using Whattman number 1 filter paper, and obtained filtrate was dried by evaporation under reduced pressure in a rotary evaporator and residue was dissolved in respective solvents.

Antimicrobial activity of Ulva rigida extract

The agar well method was used for the determination of antimicrobial activity of *Ulva rigida* extracts. The overnight grown culture of biofilm positive isolates was spread over on the sterile Muller Hinton agar plates. The wells were made with metallic borer and wells were filled with different concentrations of extract and negative controls were maintained where pure solvents were used instead of the algal extract. The Ampicillin (10µg) was filled in one well and which consider as positive control. All plates were incubated at 37 °C for 24 hours. The zones were measured and recorded [11].

Antibiofilm activity of Ulva rigida extract

A 96-well plate (flat bottom, polystyrene) was used to determine the anti-biofilm activity of the *Ulva rigida* extract [12]. The percentage of inhibition of biofilm formation was calculated using following equation:

Percentage biofilm inhibition= [1-(OD620 of cells treated with Ag NPs or plant extracts / OD620 of the non-treated control) × 100]

RESULTS AND DISCUSSION

Biofilms and drug resistant bacteria isolates pose a significant threat to public health. They can cause a variety of illnesses caused by antibiotic-resistant bacteria. These bacteria can spread quickly and can be difficult to treat. As such, it is important to battle against these bacteria in order to protect the public health and to ensure that treatments are effective. One alternative way to battle biofilm and drug resistance food bacterial isolates is by using algae-based compounds. Algae-based compounds contain a variety of compounds, such as polysaccharides, proteins, lipids, and other compounds, which have antimicrobial activities. Additionally, these compounds are also known to possess antifungal, antiviral, and anti-inflammatory activities. Therefore, the use of algae-based compounds can be an effective way to battle biofilm formation and drug resistance in food bacterial isolates [13]. In the present study, seven genera of 18 bacterial isolates were observed from meat samples, which were subjected to determinate the biofilm producers.77.7% of isolates as biofilm producers, all isolates were showed positively, especially *E. coli* and *S. aureus* showed hundred percentage of positives of biofilm producers.

Biofilm production can increase antibiotic resistance by providing physical protection to bacteria from antibiotics, as well as by promoting genetic exchange among bacteria, which can lead to the spread of antibiotic resistance genes. Biofilms can also reduce the effectiveness of antibiotics by limiting their access to the bacteria and by providing an environment that reduces their efficacy. Biofilms can also reduce the ability of the immune system to detect and destroy bacteria [14-15]. There is an urgent need for new methods in the cure of biofilm-associated infections. Algae extract has been shown to be effective in inhibiting biofilm formation in a range of food isolates.

In a study by Rima *et al.* [16] were showed that three algae extract significantly reduced the production of biofilm by the food isolates. The greatest inhibition was observed in the cases of *Bacillus cereus*, *Salmonella enterica*, and *Listeria monocytogenes*. The Ulva extract also reduced the adhesion of the isolates to surfaces and decreased their ability to form a mature biofilm. Fournière *et al*. [17] were concluded that Ulva extract could be an effective natural alternative for controlling biofilm formation by food isolates. Presently *Ulva rigida* was utilized to inhibiting the biofilm producing isolates. Among the7 test pathogen tested, *Klebsiella pneumoniae* and *Enterococcus faecalis* were highly suppressed and which exhibiting the zone of inhibition was ranged from 10±0.81mm to 17 ± 1.24. The inhibition activity was started with 2.5mg concentration of Ulva extract, which was against *Klebsiella pneumoniae* and *Enterococcus faecalis.* The *Proteus sp, Pseudomonas aeruginosa* and *Salmonella sp* were slightly inhibited with *Ulva rigida* extract, 10mg of concentration

extract was exhibited the 13±0.81mm of zone of inhibition. While using the standard antibiotic of ampicillin, the zone of inhibition was ranged from 12±0.81mm to 15±1.24mm (Table 1, Plate 1).

Table 1 Antibacterial activity of *Ulva rigida* against biofilm producing isolates

S. No	Isolates name	Concentration of extracts (mg) Zone of inhibition in mm				Ethanol	Ampicillin
		2.5	5	7.5	10		
1.	*E. coli*	-	10±1.24	13±0.81	16±0.81	-	15±1.24
2.	*S. aureus*	-	10±1.24	11±0.81	17±1.24	-	12±0.81
3.	*Proteus sp*	-	-	-	13±0.81	-	12±0.81
4.	*P. aeruginosa*	-	-	-	13±0.81	-	14±1.24
5.	*E. faecalis*	10±0.81	12±0.81	14±1.24	16.±1.24	-	14±1.24
6.	*K. pneumoniae*	10±1.24	11±0.81	14±1.24	16±0.81	-	-
7.	*Salmonella sp*	-	-	-	11±0.81		11±0.81

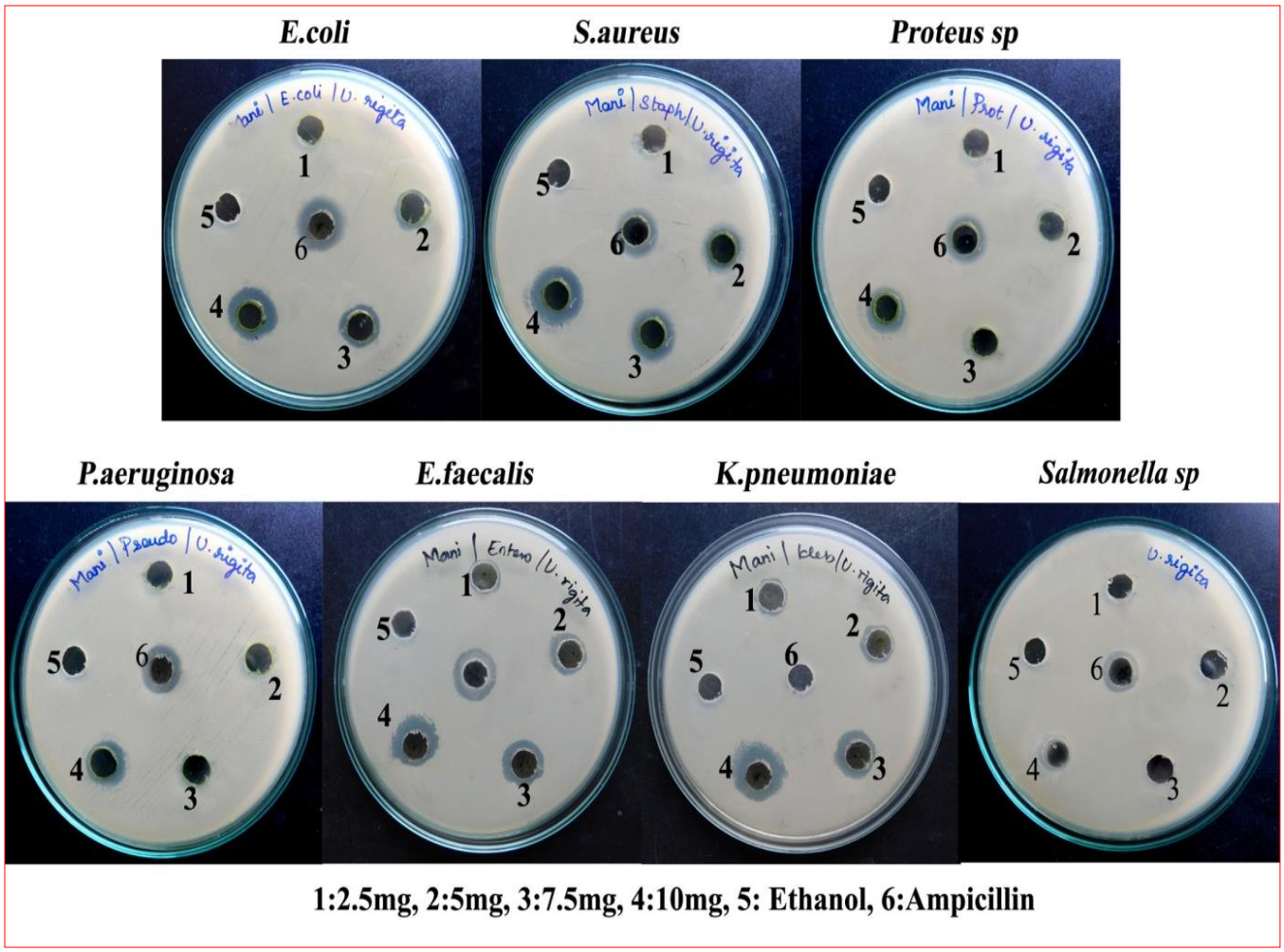

Plate 1 Antibacterial activity of *Ulva rigida* against biofilm producing isolates

In this study, no discrimination result was observed between the gram negative and gram-positive isolates. Furthermore, antibiofilm activity also observed against all isolates, the highest percentage of antibiofilm activity was observed against to *K. pneumoniae* (38%) and *E. faecalis* (34%), algae had poor antibiofilm activity against *P. aeruginosa* and *Proteus* sp. In our previous study, depicted the antibiofilm activity of *Ulva lactuca* against various bacterial isolates, however, according to literature study, no one was reported that antibiofilm activity of *Ulva rigida.*

In general, biofilm-producing isolates are more difficult to remove with antimicrobial agents. Some reports also found that inhibiting cell attachment was easier than removing established biofilms [18]. This proves that bacteria can persist on various biotic and abiotic surfaces and are more resistant to the effects of antimicrobials when they form biofilms [19]. This phenomenon was proved in the present study; as a pathogen such as Salmonella sp, *P. aeruginosa* and *Proteus*sp was not killed well by low concentration of *Ulva rigida*, may be those isolates were strongly producing the biofilm formation, however, this study observed that the inhibition activity was comparable to that of the standard antibiotic when used in high concentration of extract.

Factors that contribute to the development of resistance in biofilms include the presence of an extracellular polymeric matrix that leads to high attachment of microorganisms to surfaces, poor antibiotic permeability, or increased activity of efflux pumps that drive antimicrobials out of cells. The algal

extracts may have interfered with any of these factors. The plant extracts may have also interfered with the cell-to-cell communication strategies (quorum sensing) of the bacteria, thereby reducing biofilm formation [20].

The interaction of fatty acid compounds with cell adhesion receptors contributes to the anti-biofilm activity. The eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) families are widely discussed due to their nutritional and human health benefits. In addition, DHA and EPA are two major ω-3 PUFAs were act as antimicrobial and antibiofilm properties [21-22]. In the present study, found that fatty acids compounds of 9, 12-Octadecadienoic acid, Hexadecanedioic acid, 1,15-Pentadecanedioic acid that enhance this action (Table 2). Coraça-Huber *et al*. [23] were suppressed the Staphylococcus species producing biofilm formation with various fatty acids.

Table 2 Beneficial compounds of ethanol extract of *Ulva rigida*

S. No	Compound name	Molecular formula	Beneficial activity
1.	p-Cymene	$C_{10}H_{14}$	Antimicrobial activity
2.	Oxirane	C_2H_4O	Antimicrobial activity
3.	Azulene	$C_{10}H_8$	antiviral and antibacterial activity
4.	Anthracene	$C_{14}H_{10}$	Anticancer and antimicrobial, antioxidant activity
5.	9, 12-Octadecadienoic acid	$C_{18}H_{32}O_2$	Antimicrobial activity
6.	Hexadecanedioic acid	$C_{16}H_{30}O_4$	Antioxidant, antimicrobial, and anti-inflammatory activities

Limitation(s): The MICs were not performed, the present study can only be considered preliminary, and further study are needed to understand its potential use as an antimicrobial agent against food pathogens and food preservative.

CONCLUSION

Overall, these studies demonstrate that biofilm production is an important factor in the development of antibiotic resistance in meat isolates in India. The antibacterial and antibiofilm activity of algae extract is a promising area of research due to its potential to be used as an alternative to traditional antibiotics. This natural source of antimicrobial activity can be used to treat infections and reduce the spread of biofilm-forming bacteria in food. Further research is needed to understand the mechanisms behind these activities and to develop new strategies for utilizing algae-based Extracts in the treatment of bacterial infections.

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