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Antibacterial Properties of Selected Green Seaweeds from Vedalai Coastal Waters; Gulf of Mannar Marine Biosphere Reserve

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Abstract: Some commonly occurring green algae *Codium adherens, Ulva reticulata* and *Halimeda tuna* have been collected from the coast of Vedalai, Gulf of Mannar, Tamilnadu and were evaluated for antibacterial activity by agar diffusion method. Seven different solvents namely Acetone, Methanol, Chloroform, Diethyl ether, Ethyl acetate, Ethanol and Petroleum ether were used for extraction. The zone of inhibition were compared. The ethanol extract shows the better result for the other extracts. Some extracts found more effective than the commercial medicine. The maximum antibacterial activity was noted in ethanol extracts showed activity against *Staphylococcus* sp. (13 mm) and the minimum was recorded in methanol extracts against *Escherichia coli*, *Staphylococcus* sp. and *proteus* sp. (2 mm), *Streptococcus* sp. (2 mm), *Enterococci* sp. (3mm).

Key words: Seaweed · Antibacterial activity · Vedalai coast · Agar diffusion method

INTRODUCTION

Several compounds from the ocean show pharmacological activities and discovery of bioactive compounds, primarily for deadly diseases like cancer, Acquired Immuno Deficiency Syndrome (AIDS) Arthritis etc., while other compounds have been developed an analysis or to treat inflammation etc., marine algae are widely distributed in the coastal regions of many continents. Several works have been carried out on the extracts from marine algae. Extracts of marine algae were reported to exhibit antibacterial activity. [1, 2]. Sreenivasa Rao and Parekh [3] showed that crude extracts of Indian seaweeds are active only against gram positive bacteria. Antimicrobial activities on bacteria and fungi were reported by Hellio *et al.,* [4]. The present study deals with antibacterial activity of different extracts of 3 green algae collected from Vedalai coastal waters (Gulf of Mannar Coast).

MATERIALS AND METHODS

The samples of *Codium adherens*, *Ulva lactuca* and *Halimeda tuna* were collected by handpicking at Vedalai coastal waters (Gulf of Mannar Coast). The collected samples were cleaned well with seawater to remove all the extraneous matter such as epiphytes, sand particles, pebbles and shells and brought to the laboratory in plastic bags. The samples were then thoroughly washed with freshwater, blotted and spread out room temperature for drying. Shade dried samples were grounded fine powder.

Preparation of Extracts

Collection of Samples: The dried plant materials blended into a coarse powder before extraction portions of the powdered samples (5gm) were soaked in about 40ml of the solvents (methanol, acetone, petroleum ether, ethanol, ethyl acetate, choloroform and diethyl ether) for 72 hours [5]. The resultant crude extracts were weighed and deep frozen (-20°C) until tested.

Bacterial Strains Used for Assay: *Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Enteroccoci* sp., *Proteus* sp., *Streptococcus* sp., *Pseudomonas aeruginosa, Vibrio parahaemolyticus, Salmonella* sp., *Shewanella* sp., *Vibrio flurialis* and *V. splendidus.* Microbial strains were obtained from the Muthaiah Medical College, Annamalai University, Annamalai Nagar. The bacterial stock cultures were maintained at 4° C.

Antibacterial Assay: The bioassay was carried out using the agar diffusion method [5]. with paper disc of 6 mm

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diameter prepared from whatman No: 1 filter paper. The antibacterial assay using gram +ve and gram −ve bacteria, were carried out using the agar plate method. The bacterial inocula was grown in nutrient broth overnight and a fixed volume inoculated into 10ml aliquots nutrient agar, mixed and then poured over a nutrient agar base in sterile petridishes; this formed the bacterial lawn. Initially both paper discs and well were used for testing the crude extracts. The paper disc of 6mm diameter soaked in 6µl of crude extract and placed on to the bacterial lawn after it had solidified, standard antibiotic disc used for control. The plates were incubated at 37°C overnight. The zones of inhibition were measured after the 24 hours incubation.

RESULTS

Codium adherens: The extract obtained using ethanol showed a maximum activity against pathogens like *Staphylococcus aureus* (11 mm), *Salmonella* sp., (6 mm) and *Enterococci* sp., (6mm). minimum activity against *proteus* sp., (3 mm), *Klebsiella pneumoniae* (2 mm), *Escherichia coli* (2 mm), *Streptococcus* sp., (2 mm), *Pseudomonas aeruginosa* (2 mm), *Vibrio parahaemolyticus* (2 mm) and *V. splendidus* (2 mm). Where it has no activity against *Shewanella* sp. and *V. flurialis*.

Observation made from methanol extract showed a maximum activity against *Klebsiella pneumoniae*, (6 mm) and *Enterococci* sp. (6 mm) and the minimum activity against *Pseudomonas aeruginosa* (4 mm), *Escherichia coli* (2mm), *Streptococcus* sp., (2 mm), *Salmonella* sp., (2 mm), *Shewanella* sp., (2 mm), *Staphylococcus aureus* (2 mm) and *Vibrio flurialis* (2 mm). It has no activity against pathogens like *Vibrio splendidus* and *Proteus* sp.

The extracts obtained using chloroform showed a maximum activity against pathogen like *Vibrio splendidus* (4 mm), *Escherichia coli* (3mm), *Staphylococcus aureus* (3 mm), *Enterococci* sp., (3 mm), *Streptococcus* sp., (3 mm), *Vibrio splendidus* (3 mm) and *Proteus* sp., (2 mm). Showed no activity against pathogen like *Klebsiella pneumoniae*, *Vibrio parahaemolyticus*, *Salmonella* sp. and *Shewanella* sp.

The diethyl ether observed the maximum activity against pathogen *Vibrio splendidus* (7 mm). Showed the minimum activity against pathogens like *Escherichia coli* (4 mm), *Shewanella* sp., (4 mm), *Vibrio flurialis* (4 mm), *Proteus* sp., (3 mm), *Proteus sp*., (2 mm), *Streptococcus* sp., (2 mm), *Pseudomonas aeruginosa* (2 mm), *Vibrio parahaemolyticus* (2mm) and *Vibrio splendidus* (2mm). Where as no activity pathogen like *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Salmonella* sp.

The petroleum ether proved the minimum activity against pathogen like *Klebsiella pneumoniae* (3 mm), *Salmonella* sp., (3 mm), *Enterococci* sp., (2 mm), *Streptococcus* sp., (2 mm) and *Vibrio splendidus* (2 mm). Where as no activity pathogen like *Escherichia coli, Staphylococcus aureus* and *Salmonella* sp., *Proteus* sp., *Pseudomonas aeruginosa* and *Shewanella* sp.

Ethyl acetate extract observed minimum activity against pathogen like *Staphylococcus aureus* (3 mm), *Pseudomonas aeruginosa* (3 mm), *Enterococci* sp., (2 mm), *Salmonella* sp., (2 mm) and *Vibrio flurialis* (2 mm). It has no activity against pathogens like *Klebsiella pneumoniae, Escherichia coli, Proteus* sp., *Streptococcus* sp., *Vibrio parahaemolyticus, Shewanella* sp. and *Vibrio splendidus*.

Acetone extract obtained maximum activity against *Vibrio parahaemolyticus* (7 mm), *Salmonella* sp., (7 mm) and *Proteus* sp., (6 mm) minimum activity against *Staphylococcus aureus* (4 mm), *Vibrio flurialis* (4 mm), *Shewanella* sp., (3 mm), *Klebsiella pneumoniae* (2 mm) and *Vibrio splendidus* (2 mm). Where as no activity *pathogen like Escherichia coli, Enterococci sp. and Pseudomonas aeruginosa.*

Ulva reticulata: The investigation made on ethanol extracts maximum activity activity against *Staphylococcus aureus* (13 mm), *Proteus* sp., (9 mm) minimum activity against *Streptococcus* sp., (8 mm), *Pseudomonas aeruginosa* (8 mm), *Vibrio flurialis* (8 mm), *Enterococci* sp., (5 mm), *Vibrio splendidus* (5 mm), *Klebsiella pneumoniae* (4 mm), *Salmonella* sp., (3 mm), *Vibrio parahaemolyticus* (3 mm) and *Escherichia coli* (2 mm). Where as no activity pathogen like *Shewanella* sp.

Methanol extract obtained maximum activity against *Escherichia coli* (6 mm), *Enterococci* sp., (5 mm) and the minimum activity against *Vibrio parahaemolyticus* (4 mm), *Salmonella* sp., (3 mm) and *Proteus* sp., (3 mm). Where as no activity pathogen like *Klebsiella pneumoniae*, *Staphylococcus aureus, Streptococcus* sp., *Pseudomonas aeruginosa, Shewanella* sp., *Vibrio splendidus* and *Vibrio flurialis*.

The extracts using chloroform showed maximum activity against *Vibrio splendidus* (8 mm), *Streptococcus* sp., (8 mm) and the minimum activity against *Vibrio parahaemolyticus* (6 mm), *Escherichia coli* (5 mm), *Enterococci* sp., (4 mm), *Proteus* sp., (4 mm), *Pseudomonas aeruginosa* (3 mm) and *Salmonella* sp., (3 mm). Where as no activity pathogen like *Staphylococcus aureus, Shewanella* sp. and *Vibrio flurialis*.

The extraction by way of diethyl ether observed minimum activity against *Vibrio flurialis* (5 mm), *Escherichia coli* (4 mm), *Streptococcus* sp., (4 mm), *Vibrio parahaemolyticus* (4 mm), *Vibrio splendidus* (4 mm), *Klebsiella pneumoniae* (3 mm), *Staphylococcus aureus* (3 mm), *Enterococci* sp., (3 mm), *Proteus* sp., (2 mm) and *Pseudomonas aeruginosa* (2 mm) showed no activity against *Salmonella* sp. and *Shewanella* sp.

The petroleum ether extract proved minimum activity against *Enterococci* sp.,

(3 mm), *Proteus* sp., (2 mm) and *Vibrio parahaemolyticus* (2 mm). Where as no activity pathogen like *Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Streptococcus* sp., *Pseudomonas aeruginosa* and *Vibrio splendidus*.

Ethyl acetate extract observed minimum activity against pathogen like *Vibrio splendidus* (4 mm), *Salmonella* sp., (4 mm), *Enterococci* sp., (3 mm), *Proteus* sp., (3 mm), *Staphylococcus aureus* (2 mm), *Streptococcus* sp., (2 mm) *Vibrio parahaemolyticus* (2 mm) and *Shewanella* sp., (2 mm). Showed no activity against *Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa* and *Vibrio flurialis.*

Acetone extract pointed out maximum activity against pathogens *Enterococci* sp., (9 mm) and *Proteus* sp., (6 mm) minimum against *Salmonella* sp., (5 mm), *Klebsiella pneumoniae* (4 mm), *Staphylococcus aureus* (3 mm) and *Streptococcus* sp., (3 mm). Where as no activity pathogen like *Escherichia coli, Pseudomonas aeruginosa*, *Shewanella* sp., *Vibrio flurialis* and *Vibrio splendidus*.

Halimeda tuna: The extract obtained using ethanol showed a maximum activity against pathogens like *Proteus* sp., (11 mm), *Streptococcus* sp., (10 mm) and *Klebsiella pneumoniae* (10 mm) and the minimum activity against *Enterococci* sp., (5 mm), *Escherichia coli* (4 mm), *Staphylococcus aureus* (4 mm), *Pseudomonas aeruginosa* (2 mm), *Vibrio parahaemolyticus* (2 mm), *Salmonella* sp., (2 mm), *Vibrio flurialis* (2 mm) and *Vibrio splendidus* (2 mm). Showed no activity against *Shewanella* sp.

Methanol extract obtained maximum activity against *Escherichia coli* (8 mm), *Vibrio splendidus* (8 mm) and the minimum activity against *Shewanella* sp., (4 mm), *Staphylococcus aureus* (4 mm), *Vibrio parahaemolyticus* (3 mm), *Pseudomonas aeruginosa* (3 mm), *Proteus* sp., (3 mm) and *Enterococci* sp., (2 mm) Where as no activity against *Klebsiella pneumoniae, Staphylococcus aureus, Salmonella* sp. and *Vibrio flurialis.*

The extracts using chloroform showed maximum activity against *Proteus* sp.,

(11 mm), *Vibrio splendidus* (9 mm) and *Pseudomonas aeruginosa* (3 mm) and the minimum activity against *Vibrio parahaemolyticus* (7 mm), *Shewanella* sp., (5 mm), *Klebsiella pneumoniae* (4 mm), *Enterococci* sp., (4 mm), *Vibrio flurialis* (4 mm), *Streptococcus* sp., (3 mm) and *Escherichia coli* (2 mm), Where as no activity against *Staphylococcus aureus* and *Salmonella* sp.

The extraction by way of diethyl ether observed minimum activity against *Pseudomonas aeruginosa* (10 mm) and *Vibrio parahaemolyticus* (6 mm) and the minimum activity against *Proteus* sp., (4 mm), *Enterococci* sp., (4 mm), *Salmonella* sp. (3 mm), *Klebsiella pneumoniae* (2 mm), *Staphylococcus aureus*

Table 1: Shows the antibacterial activity of *Codium adherens* against human pathogens

		Solvents used zone of inhibition mm in diameter							
S. No	Pathogens	Methanol	Chloroform	Ethanol	Diethyl ether	Petroleum ether	Ethyl acetate	Acetone	Control
1.	*Klebsiella pneumoniae*	6	-	2	-	3	-	2	-
2.	*Escherichia coli*	2	3	2	4	-	-	-	3
3.	*Staphylococcus aureus*	1	3	11	-	-	3	4	2
4.	*Enterococci* sp.	6	3	6	2	2	2	-	3
5.	*Proteus* sp.	-	2	3	3	-	-	6	1
6.	*Streptococcus* sp.	2	3	2	2	2	-	3	3
7.	*Pseudomonas aeruginosa*	4	6	2	2	-	3	-	4
8.	*Vibrio parahaemolyticus*	-	-	2	2	-	-	7	2
9.	*Salmonella* sp.	2	-	6	-	3	2	7	3
10.	*Shewanella* sp.	2	-	-	4	-	-	3	3
11.	*Vibrio flurialis*	2	3	-	4	3	2	4	4
12.	*Vibrio splendidus*	-	4	2	7	2	-	2	2

- - No sensitivity mm-Millimeter

Table 2: Shows the antibacterial activity of *Ulva reticulata* against human pathogens

S. No	Pathogens	Solvents used zone of inhibition mm in diameter							
		Methanol	Chloroform	Ethanol	Diethyl	Petroleum ether	Ethyl ether	Acetone acetate	Control
1.	*Klebsiella pneumoniae*	-	2	4	3	-	-	4	-
2.	*Escherichia coli*	6	5	2	4	-	-	-	3
3.	*Staphylococcus aureus*	-	-	13	3	-	2	3	2
4.	*Enterococci* sp.	5	4	5	3	3	3	9	3
5.	*Proteus* sp.	3	4	9	2	2	3	6	1
6.	*Streptococcus* sp.	-	8	8	4	-	2	3	4
7.	*Pseudomonas aeruginosa*	-	3	8	2	-	-	-	3
8.	*Vibrio parahaemolyticus*	4	6	3	4	2	2	-	2
9.	*Salmonella* sp.	3	3	3	-	-	4	5	2
10.	*Shewanella* sp.	-	-	-	-	-	2	-	1
11.	*Vibrio flurialis*	-	-	8	5	-	-	-	-
12.	*Vibrio splendidus*	-	8	5	4	-	4	-	-

- - No sensitivity mm-Millimeter

Table 3: Shows the antibacterial activity of *Halimeda tuna* against human pathogens

S. No	Pathogens	Solvents used zone of inhibition mm in diameter							
		Methanol	Chloroform	Ethanol	Diethyl ether	Petroleum ether	Ethyl acetate	Acetone	Control
1.	*Klebsiella pneumoniae*	-	4	10	2	-	2	7	-
2.	*Escherichia coli*	8	2	4	-	-	2	6	2
3.	*Staphylococcus aureus*	4	-	3	2	3	-	7	-
4.	*Enterococci* sp.	3	4	5	3	-	-	7	4
5.	*Proteus* sp.	3	11	11	4	-	-	11	2
6.	*Streptococcus* sp.	-	3	10	-	3	-	5	3
7.	*Pseudomonas aeruginosa*	3	8	2	10	3	2	2	-
8.	*Vibrio parahaemolyticus*	3	7	2	6	3	-	-	-
9.	*Salmonella* sp.	-	-	2	3	-	-	-	2
10.	*Shewanella* sp.	4	5	-	2	5	2	-	-
11.	*Vibrio flurialis*	-	4	2	-	2	-	-	2
12.	*Vibrio splendidus*	8	9	2	2	-	-	-	2

- - No sensitivity mm-Millimeter

(2 mm), *Shewanella* sp., (2 mm) and *Vibrio splendidus* (2 mm). Where as no activity against *Escherichia coli, Streptococcus* sp. and *Vibrio flurialis*.

The petroleum ether extract proved minimum activity against *Shewanella* sp., (5 mm), *Staphylococcus aureus* (3 mm), *Pseudomonas aeruginosa* (3 mm), *Vibrio parahaemolyticus* (3 mm) and *Vibrio splendidus* (2 mm). Where as no activity against *Klebsiella pneumoniae, Enterococci* sp., *Proteus* sp. and *Vibrio flurialis*.

Ethyl acetate extract observed minimum activity against pathogen like *Klebsiella pneumoniae* (2 mm), *Pseudomonas aeruginosa* (2 mm), *Shewanella* sp., (2 mm) and *Vibrio splendidus* (2 mm). Where as no activity against *Staphylococcus aureus, Enterococci* sp., *Proteus* sp., *Streptococcus* sp. and *Vibrio flurialis*.

Acetone extract pointed out maximum activity against *Proteus* sp., (11 mm), *Klebsiella pneumoniae* (7 mm), *Staphylococcus aureus* (7 mm), *Enterococci* sp. (7 mm) and the minimum activity against *Escherichia coli* (6 mm), *Streptococcus* sp., (5 mm) and *Pseudomonas aeruginosa* (2 mm). Where as no activity against *Vibrio parahaemolyticus, Salmonella* sp., *Shewanella* sp., *Vibrio flurialis* and *Vibrio splendidus.*

DISCUSSION

The antimicrobial activity of seaweeds may be influenced by some factors such as the habitat and the season of algal collection, different growth stages of plant, experimental methods, etc., Although a variety of solvents have been employed in screening seaweeds for antimicrobial activity, it is still uncertain what kinds of solvent is the most effective and suitable for extraction of seaweeds. A few workers tried using different solvents for screening the antimicrobial activity of seaweeds and made comparisons.

Martinez-Nadal *et al.,* [6] mentioned that benzene and diethyl ether were suitable solvents for extracting the antibiotic principle. Parekh *et al.,* [7] reported that extracts obtained with acetone, ethyl alcohol and ether showed higher antibacterial activity than that of extracts obtained with chloroform. Rosell and Srivastava [8] found similar antibacterial activity when they screened brown algae from Canada with acetone, chloroform, ethyl-ether, methanol and acetic acid.

De compos-Takaki *et al.,* [9] studied the chlorophyceae off Brazilian coast and have high antibacterial activity. Based on these results, it is felt that due to the presence of high water content in fresh samples the antibiotic principle is diluted leading to dissipated activity, while dried samples accumulated or concentrated the antibiotic principle. They also indicate that degradation and active metabolites may not occur during shade drying.

Sastry and Rao [10] carried out a successive extraction using benzene, chloroform and methanol reported the chloroform extract exhibited the strongest activity. It can be seen from the above reports that the efficiency of chloroform in the extraction of seaweeds remains uncertain.

The results from the present screening revealed that the strongest antibacterial activity was exhibited by the ethanol extract and the least by the ethyl acetate and petroleum ether. In some species (such as *Gelidium amansii*) the inhibitory activity was only observed in the extract obtained with one kind of solvent but not in extracts obtained in other solvents, which may suggest that a particular solvent is required to extract some antimicrobial substances within the algal plant and therefore the percentage of inhibitory activity will go up when several solvents are used in the screening.

Antibacterial activities of seaweeds also varied with the species division. Rao and Parekh [11] Padmakumar and Ayyakannu [12] reported that the species of Rhodophyta showed the highest antibacterial activity, whereas Caccamese and Azzolina [13] and Pesando and Caram [14] found out the highest antibacterial activity was exhibited by the species of Phaeophyta. The reason for this was not explained by these workers but it was suggested that more species have to be screened before coming to definite conclusion. In the present study, the species of chlorophyta showed the strongest activities against the test bacteria and fungi, which was in agreement with the findings of Padmakumar and Ayyakannu [12]. It may probably due to the tested seaweeds vertical distribution. Green algae mostly occur in the intertidal zone lower region, which may be advantage for the protection of the active compounds within the algal plant from degradation.

The active compounds in the species that the strong antibacterial activities our study remain to be identified. Hornsey and Hide [15] used acetone as a solvent for extracting antimicrobial compounds from British marine algae. Selvi *et al*., [16] screened around 20 algae using methanol and ethanol along Idinthakarai coast and they reported that *Bacillus subtilis* and *Staphylococcus* sp. were highly susceptible to most of the algal extracts. In the present investigation the ethanol extract showed less activity against *Staphylococcus* sp.

Thirumaran and Anantharaman [17] reported that antibacterial activity of marine macro alga *Caulerpa scalpeliformis* from Gulf of Mannar coast, the maximum activity was noted in methanol extracts against *Salmonella typhii, Micrococcus* sp. and *Shigella bodii.*

Umamaheswari *et al.,* [18] observed the antibacterial activity of marine macro alga *Chaetomorpha aerea* collected from Vellar estuary the maximum antibacterial potential was recorded from ethanol extract against *Pseudomonas aeruginosa* and the minimum was noted in methanol extracts against *Micrococcus* sp. and *Salmonella typhii*.

Thirumaran and Anantharaman [19] screened the antimicrobial activity of *Enteromorpha compressa* using ethanol and methanol along the Gulf of Mannar Coast and they reported that *Shigella sonii* and *Mucor* sp. were highly susceptible than the other extracts. In the present study of seaweed *ulva reticulata* shows promising, results against antibacterial pathogens. This finding leads support to that of Naqvi *et al.,* [20] who demonstrated that some marine plants showed antibacterial activity against three bacterial strains.

CONCLUSION

The solvent extracts of three different seaweeds used in the present study showed significant bacterial action. The interest information is that the product in the form natural good for health and fails to cause side effects. From these preliminary investigations the algal members of both the estuaries merit further investigation. Further, a detailed study to correlate the physiological stages of the algae. With their antibacterial activity is essential.

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