Antimicrobial Studies on the Crude Extracts of Some Selected Seaweeds against Cattle Pathogens

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ABSTRACT

As more than 70% of the world's surface is covered by oceans, the wide diversity of marine organisms offer a rich source of natural products. Marine environment contains a source of functional materials, including polyunsaturated fatty acids, polysaccharides, essential minerals and vitamins, antioxidant, enzymes and bioactive peptides. Among marine organisms, marine algae are rich sources of structurally diverse bioactive compounds with various biological activities. Fresh marine seaweeds *Gracilaria edulis* and *Sargassum sp*., were collected from Thondi coast, Tamil Nadu, South East coast of India and were screened for antibacterial activity in the present investigation. Crude Extracts were prepared using three different solvents such as, chloroform, methanol and distilled water from the dried and powdered seaweeds. The extracts were tested against cattle pathogens of *Staphylococcus aureus* **(MTTC No. 96)**, *Escherichia coli* **(MTTC No.** 1687**)**, *Proteus vulgaris* and *Klebsiella kneumoniae using* micro-litre assay. The rate of growth inhibition of bacterial cultures have been estimated by measuring the extend of zone formation. The methanol extracts of *G.edulis* showed highest growth inhibitory activity (18mm) against *Staphylococcus aureus* culture and which was followed by the methanolic extract (16mm) and Ethyl acetate extracts of *Sargassum sp.,* (14mm) against *Escherichia coli.* The methanol extract showed the broader and highest spectrum of antibacterial activity when compared with Ethyl acetate extract in the present investigation against the bacterial pathogens of cattles. Seaweeds contain various bioactive components, especially Phenolic compound present in seaweeds. These phenolic compounds have a good antimicrobial property. The results of the study clearly showed that, seaweed is an interesting source for biologically active compounds that may be applied for prophylaxis and therapy of cattle diseases caused by bacterial pathogens.

KEYWORDS: Seaweeds, *Gracilaria edulis, Sargassum sp. e*xtracts. –Cattle Pathogens – Antibacterial Activity

I. INTRODUCTION

Seaweeds are used for millennia as food source and in medicine by several eastern peoples. They also provide byproducts for several applications in Biotechnology [1], [2], [3], [5], [4]. Selective utilization of marine algae as potential source of pharmaceutical agents has been increasing in recent years. The algal extracts were used as a curative and preventive agent for various diseases such as antibiotics, antihelminthics, cough remedies, antihypertensive, antitumor and antidiarrhoea. Recently we have embarked on the chemical investigation of marine algae with a special accent on their bioactive properties [5]. Most of the bioactive substances isolated from marine algae are chemically classified as brominated, aromatics, nitrogen-heterocyclic, nitrosulphuric-heterocyclic, sterols, dibutanoids, proteins, peptides and sulphated polysaccharides. The crude extract thus obtained is subjected to broad based biological screening for antifungal, antiviral, antibacterial, antimalarial, antifilarial, hypoglycaemic and antifertility activity [6]. On other hand, the algae are also used as food stuff, animal fodder, fertilizer, industrial material such as agar and minor medicines [7]. There are reports of macroalgae derived compounds that have a broad range of biological activities, such as antibiotic, antiviral, antineoplastic, antifouling, anti-inflammatory, cytotoxic and antimitotic [8], [9], [10]. However, until the 1970s that large-scale screening of antimicrobial activity was not carried out [11] and in the past few decades, macroalgae are attracting increasing attention as a new source for bioactive compounds [12].

Nowadays, infectious diseases are responsible for a high morbidity and mortality rate and are consider as a public health problem because of their frequency and their severity. For the treatment of these diseases, people often use synthetic drug. But, bacteria developed a resistance mechanism to fight against most of the synthetic family of antibiotics. The resistant of microbes is due to indiscriminate utilization of commercial antimicrobial medicines supported by many scientists investigation for modern antimicrobial substances from several medicinal plants and seaweeds [13]. There are several bioactive compounds which are produced by seaweeds and they also possess the ability to prevent the disease caused by some gram negative and gram positive pathogenic bacteria [14]. In the present study, antibacterial efficacy of various organic solvent extracts of the seaweeds *Gracilaria edulis* (Rhodophyta), *Sargassum sps.,* (Phaeophyta) against some clinically important gram-positive and gram-negative cattle pathogenic bacteria species is reported.

II. MATERIALS AND METHODS

A. Collection of algae

The seaweeds, such as, Brown alga *Sargassum sp.* (Phaeophyceae) and red alga *Gracilaria edulis* (Rhodophyceae) were collected using snorkeling and skin diving techniques from Balk Bay waters of Thondi coast, Tamil Nadu, India. The seaweed species were identified using standard references and also compared with the preserved specimen available in the Museum of Central Marine Research Institute, Mandapam camp.

B. Extract preparation

The methanolic and ethyl acetate extracts of *Sargassum sp.* and *Gracilaria edulis* were prepared separately (1:50 w/ v) using Soxhlet Apparatus. Each extraction was carried out in a soxhlet apparatus for a duration of 24 hours and after evaporation in vacuum the extracts were stored at -20 °C until use [15].

C. Test organisms

The antimicrobial activity of the seaweeds extracts were tested against the following cattle pathogens such as, Gram-positive bacteria *Staphylococcus aureus* (MTCC No: 96) and the three Gram-negative bacterial species such as, *Escherichia coli* (MTCC No: 1687), Institute of Microbial Technology (IMTECH), Chandigarh, India. The other strains of such as, *Proteus vulgaris* and *Klebsiella pneumonia* were obtained from King's Institute and Veterinary College, Chennai.

D. Antibacterial Assay

The sterilized Petri plates were poured with Nutrient agar medium and labeled. The 30µl of identified test organisms viz., *Staphylococcus aureus (MTTC) Escherichia coli (MTTC), Proteus vulgaris* and *Klebsiella pneumoniae* were inoculated and spreaded on the agar medium using sterilized "L" rod so as to make lawn. The agar surface was allowed to dry for five minutes. Then the prepared algal extract impregnated discs were placed over the agar using sterilized forceps. After 24 hours of incubation at 37°C the zone of inhibition was measured and tabulated.

III. RESULT AND DISCUSSION

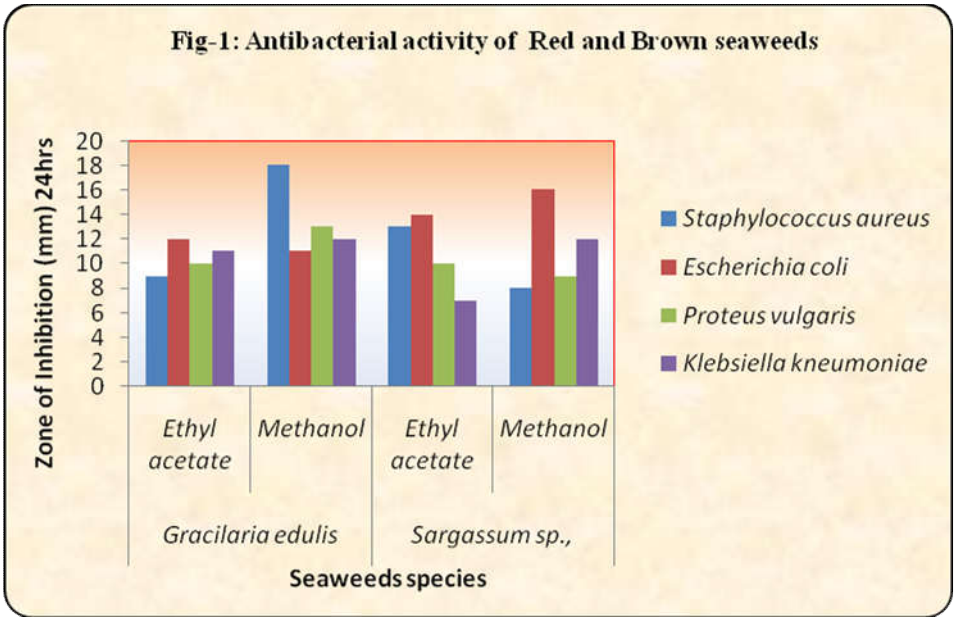
The antimicrobial activity of methanol and ethyl acetate extracts of *G.edulis* and *Sargassum sp.,* against bacterial pathogens and the result presented in the Table- 1 and Figure-1. The methanol extracts of *G.edulis* showed highest growth inhibitory activity (18mm) against *Staphylococcus aureus* culture and which was followed by the methanolic extract (16mm) and Ethyl acetate extracts of *Sargassum sp.,* (14mm) against *Escherichia coli.* The methanolic extract showed the broader and highest spectrum of antibacterial activity when compared with Ethyl acetate extract in the present investigation against the bacterial pathogens of cattles. The minimum zone of inhibition was observed in the ethyl acetate extract (7mm) of *Sargassum sp.,* against *Klebsiella pneumoniae* culture.

Marine halophytes provide good source of medicinal as well as natural health products or compounds by synthesis or secretion. These groups of plants provides large amount of antioxidants, phenol compounds, enzymes, biomolecules like carbohydrate content and some other biochemical compounds like free amino acids, phytochemical set in terms of reducing the saline stress. Phenolics compounds are synthesized via phenyl propanoid pathway and play a defence mechanism against biotic and abiotic stress. The antibacterial activity of seaweeds may be influenced by some factors such as the habitat and the season of algal collection, different growth stages of plants, experimental methods etc. But variation in antibacterial activity may be due to the method of extraction and solvent used in the extraction preparation [16]. The present study reported that the methanol extracts of *G.edulis* showed maximum activity of (18mm) against *Staphylococcus aureus* culture and which was followed by

the methanolic extract (16mm) and Ethyl acetate extracts of *Sargassum sp.,* (14mm) against *Escherichia coli.* The methanol extract showed the broader and highest spectrum of antibacterial activity when compared with Ethyl acetate extract in the present investigation against the bacterial pathogens of cattles. This result proved the presence of antimicrobial activity red algae *G.edulis* and brown algae *Sargassum sp*.,. Because various bioactive components are presents this seaweeds such as, o-Ethyl S-3-(dimethylamino) propyl methylphosphonothiolate, Trimethyl [4-(2-methyl-4-oxo-2-pentyl) phenoxy]silane, Bisphenol, bis (tert-butyldimethylsilyl) ether, Benzo[h]quinoline, 2,4-dimethyl- compounds were also identified by GC-MS analysis as significant antimicrobial activity [17].

Table-1: Antibacterial activity of Red and Brown seaweeds

S.No	Test organisms	Zone of Inhibition (mm) 24hrs			
		Gracilaria edulis		*Sargassum sp.,*	
		Ethyl acetate	Methanol	Ethyl acetate	Methanol
		30µl	30µl	30µl	30µl
1	*Staphylococcus aureus*	9	18	13	8
2	*Escherichia coli*	12	11	14	16
3	*Proteus vulgaris*	10	13	10	9
4	*Klebsiella kneumoniae*	11	12	7	12



IV. CONCLUSION

The present study provides valuable information regarding the potential of seaweed as natural sources for Antimicrobial activities. The results emerged from the present study validate the potential use of renewable sources like these candidate seaweed to offer excellent nutritional and health package for use in food supplements in nutraceutical formulation and as health food for human consumption. The results of the present study demonstrated that, the *G.edulis* and *Sargassum sp.,* respectively possessed antimicrobial activities against the clinically isolated species of bacteria. The antimicrobial activities suggest the possibility of therapeutic value of seaweed against the bacterial infection. The present study concluded that the marine red algae *G.edulis* and Brown algae *Sargassum sp.,* have variety of biologically active molecules which can be used as a source of antibiotics. Further study needs the purification of active compounds and structural elucidation can be used for drug discovery.

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