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Antibacterial activity of seagrass species of *Cymodocea serrulata* against chosen bacterial fish pathogens

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ABSTRACT

The purpose of this study was to investigate antibacterial activity of seagrass *Cymodocea serrulata*. The 6th acetone fraction from *C. serrulata* root extract showed maximum activity [12mm, 12mm and 12mm] against three [*A. hydrophila*, *B. subtilis* and *Serratia* sp.,] of the five fish pathogens tested and minimum activity of 7mm, 8mm was noticed against *V. parahaemolyticus*, *V. harveyi* respectively. The 1st fraction of hexane from *C. serrulata* root extract showed antibacterial activity against five fish pathogens viz., *B. subtilis*, *A. hydrophila*, *V. parahaemolyticus*, *Serratia* sp., and *V. harveyi*. This showed maximum activity [10mm] against *V. parahaemolyticus* and minimum activity of 8mm, 7mm were observed against *B. subtilis*, *Serratia* sp., respectively.

Key words: *Cymodocea serrulata*, antibacterial activity, fish pathogens, seagrass.

INTRODUCTION

Natural products have been an important resource for the maintenance of life for ages. Several life-saving drugs have been developed from the plants. The plant kingdom has provided an endless source of medicinal plants first used in their crude forms as herbal teas, syrups, infusions, ointments, liniments and powders [Tsao and Zeltzer, 2005]. Herbal remedies and alternative medicines are used throughout the world and in the past herbs often represented the original sources of most drugs [Cooper, 2004]. Marine species are known to produce a large number of structurally diverse secondary metabolites [Faulkner, 2000 and Blunt *et al.*, 2004]. Sea grass

meadows enhance the biodiversity and habitat diversity of coastal water. It has been estimated that over 153 species of microalgae, 359 species of macro algae and 178 species of invertebrates are found on the sea grass blades as epiphytes and epizooties [Philips and McRoy, 1980]. A variety of medicines and chemicals are also prepared from sea grass and their associates. The main objective of the present study is to explore the cheapest and novel antibacterial compounds from seagrass [*C. serrulata*] of Palk Strait of India for alternative treatment against fish pathogens.

MATERIALS AND METHODS

Fresh roots from a Seagrass species viz., *Cymodocea serrulata* were collected from Thondi coastal area 99° 4' N and 79° 10' 45" E situated in Palk Strait region of TamilNadu. Collected samples were washed thrice in tap water to remove adhering soil particles and salts and once with sterile distilled water. The chopped air dried roots of *C. serrulata* [2kg] were taken separately in an air tight glass jars and required quantity of ethanol and water mixture [3:1] was added and kept for 7 days for the extraction of bioactive compounds. After 7 days, the contents were stirred well and then filtered by using muslin cloth. The root extract were concentrated to two third of the volume by distillation. The colloidal form of the root extract from *C. serrulata* [300g] was again kept for dryness under room temperature so as to enable to obtain solid form of extract and stored in a sterile glass container for further use. The percentage of extraction was calculated using the following formula: Percentage of extraction [%] = Weight of the extract [g]/ Weight of the plant material [g] X 100. The percentage of extraction for the *C. serrulata* is 15%.

The extract were further subjected for separation by column chromatography packed with 500 g of silica gel [230 – 400 mesh] [MERCK] with the maximum height of 50 cm and eluted successively with 50 ml of ethanol, chloroform, benzene, hexane, acetone and water. The corresponding fractions of 1-6 were collected from *C. serrulata*. The obtained fractions were subjected for evaporation so as to enable to obtain powder form of extract and stored at 4°C for further use.

Table. 1. Antimicrobial sensitivity of column chromatographic fractions of *cymodocea serrulata* against chosen fish pathogens

Name of the fish pathogens	Zone of inhibition in mm in diameter											
	n-C ₆ H ₁₄ (n-Hexane)						CH ₃ COCH ₃ (Acetone)					
	1	2	3	4	5	6	1	2	3	4	5	6
<i>Bacillus subtilis</i>	-	8	-	-	-	7	-	-	-	-	-	-
<i>Aeromonas hydrophila</i>	7	--	-	-	-	-	8	8	7	7	8	7
<i>Vibrio parahaemolyticus</i>	12	7	7	7	-	7	8	8	7	9	8	7
<i>Serratia sp.</i>	7	-	-	-	-	-	10	8	10	7	10	12
<i>Vibrio harveyi</i>	-	-	-	-	-	-	8	8	8	9	9	7

Antimicrobial sensitivity assay

Filter paper disc method was used for screening of seagrass root extract against five fish pathogens viz., *Bacillus subtilis*, *Aeromonas hydrophila*, *Vibrio parahaemolyticus*, *Serratia sp.*, and *V. harveyi*. Whatmann No.1 filter paper disc [5mm diameter] impregnated with different fractions [5mg. disc-1] was placed on Muller Hinton Agar [HIMEDIA, MUMBAI] which was previously inoculated with test organisms. Control disc was maintained without the extracts. All the plates were incubated overnight at 37⁰ C under static conditions. After that, the zone of inhibition appearing around the discs were measured and recorded in millimeter in diameter. Triplicate samples were maintained for each bacterial strain.

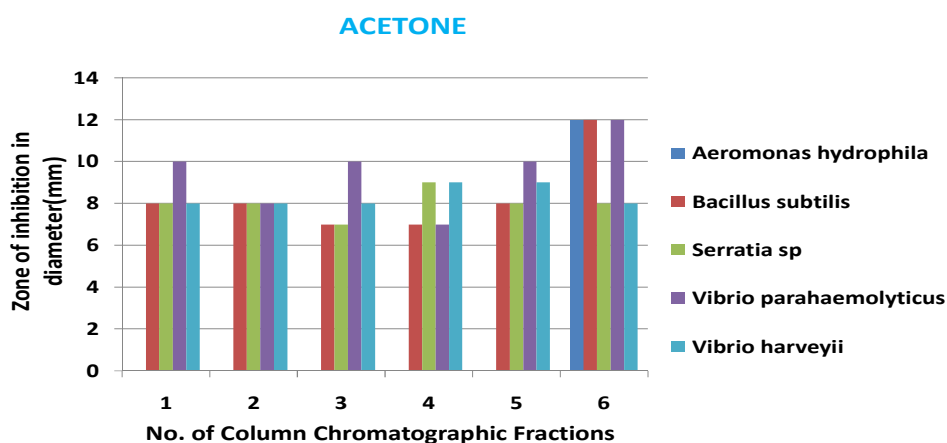


Fig 2. Pattern of average zone of inhibition by column chromatographic fractions of Acetone against fish pathogens

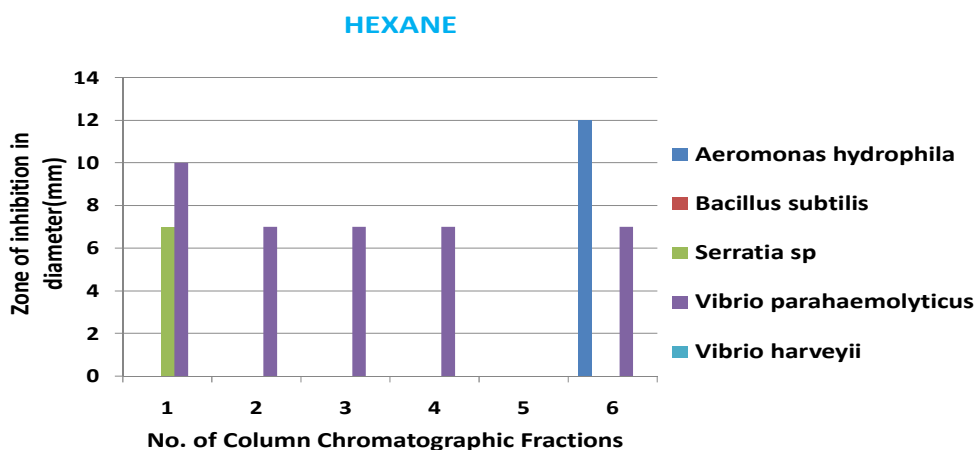


Fig 3. Pattern of average zone of inhibition by column chromatographic fractions of Acetone against fish pathogens

RESULTS AND DISCUSSION

Natural products are considered as an important source of new antibacterial agents. Drugs derived from natural products or drugs semi-synthetically obtained from natural sources corresponded to 78% of the new drugs [Cragg *et al.*, 1997]. Marine forms comprising approximately a half of the total global biodiversity, large-scale screening will continue to play an important role in development of new drugs [Xu *et al.*, 2004]. Marine organisms are a rich source of structurally novel and biologically active metabolites. Many chemically unique compounds of marine origin with different biological activity have been isolated and a number of them are under investigation and are being developed as new pharmaceuticals [Faulkner, 2000a, b; Rocha *et al.*, 2001; Schwartsmann *et al.*, 2001]. Several species of seagrass produce antimicrobial compounds that may act to reduce or control microbial growth. Marine organisms collected from the South East coast of India have been shown to possess a number of biological activities [Ely *et al.*, 2004].

Microbial disease is the most significant problem in aqua industries. Prevention and treatment of microbial disease have been practiced by the application of chemical, antibiotics, which needs continuous and large volume of water exchange, and it also leads to the development of resistance strains of pathogenic microorganisms. Sparks [1981] explained that, the drug resistance may be due to pre-existing factor in the microorganisms, or it may be due to some acquired factors. Resistance became major problem as the widespread use of antibiotics led to the elimination of sensitive organisms from the population accompanying the resistant organisms [Pelczer *et al.*, 1993]. Antibiotic resistance of bacterial pathogens is reported from all areas of aquaculture, ranging from warm to cold water. Decreased efficacy has been documented in antibiotics regardless of their mechanism of action. Microbial resistance has been shown in different classes of antibiotics including those affecting protein synthesis such as tetracycline and erythromycin, aminoglycosides like neomycin, antimetabolites such as the sulfa drugs and potentiated sulfonamides and quinolones like oxolinic acid [Dixon, 1991].

Though most of the clinical antibiotics are produced by soil microorganisms, higher plants have also been a source of antibiotics. Marine halophytes known to produce their novel secondary metabolites are not reported in the terrestrial plants. Hence, the present study has been taken effort to find out the variety of metabolites present in the seagrass species of *C. serrulata* and the antimicrobial sensitivity assay against the fish pathogens viz., *B. subtilis*, *A. hydrophila*, *V. parahaemolyticus*, *Serratia* sp., and *V. harveyi* by disc diffusion assay. The seven acetone fractions and six hexane fractions of *Cymodocea serrulata* root extracts were collected and tested against fish pathogens. Among the seven fraction of acetone, the 6th fraction of acetone from *C. serrulata* root extract showed maximum antibacterial activity [12mm, 12mm and 12mm] against three [*A. hydrophila*, *B. subtilis*, *Serratia* sp.,] of the five fish pathogens and minimum activity of 7mm, 8mm against *V. parahaemolyticus*, *V. harveyi* respectively.

The 1st fraction of hexane from *C. serrulata* root extract were showed activity against five fish pathogens viz., *B. subtilis*, *A. hydrophila*, *V. parahaemolyticus*, *Serratia* sp., and *V. harveyi*. This showed maximum activity [10mm] against *V. parahaemolyticus* and minimum activity of 8mm, 7mm were recorded against *B. subtilis* and *Serratia* sp., respectively. Harrison and Chan [1980] reported that the *Zostera marina* showed effective sensitivity against *S. aureus*, which

was also showed sensitivity in the recent study. Bhosale *et al.*, [2002] reported that the extracts from *C.rotundata* were effective against several *Bacillus* species [Bernard and Pesando, 1989]. The estuarine submerged aquatic plants *Potamogeton pectinatus*, *P. perfoliatus* and *Ruppia maritima* inhibited the growth of gram-positive bacteria *Bacillus* and *Mycobacterium species*.

The above results showed that the antibacterial activity and bioactive chemical characterization of seagrass *Cymodocea serrulata* have their own characteristic spectra and spectral parameters. This report also demonstrating that the antimicrobial activity of the seagrass is an encouraging trend unraveling the potential of the Indian coastline as a source of marine organisms worthy of further investigation. These organisms are currently being investigated in detail with the objective of isolating biologically active molecules which would prove to be lead chemicals for drug discovery.

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