Antimicrobial Activity of Seagrss (*Cymodocea serrulata*) from South West Coast of Sri Lanka

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Introduction

Sri Lanka has rich oceanic vegetation along its coastal water bodies. However, utilization of them is limited when compared to terrestrial plants which are used as natural alternatives especially in Ayurveda remedy. It is expected that marine vegetation also ensure the potential bioactivity. Marine plants derived natural products are known as secondary metabolites which are bioactive compounds responsible for antimicrobial activities. Documented results from most of the Atlantic, Pacific, and Indian Ocean resultant macro algae exhibits broad range of biological activities. Some of these are antibacterial, antifungal, antiviral *etc.* On the other hand, few literature are available on the therapeutic values of seagrasses in Sri Lanka. Therefore, the objective of this study was to test the antimicrobial activity of some selected seagrass species collected from the Beruwela beach rocky platforms and Hikkaduwa coast of Sri Lanka.

Methodology

Collection and preparation of samples **-** The fresh seagrass species (*Cymodocea serrulata*) was collected by hand picking during the low tidal conditions from the submerged rocky platforms of Barberrian reef and in Hikkaduwa coast. The collected vegetation was cleaned well with tap water and distilled water. Then the samples were drained and spread on the filter paper to remove excess water. Samples were chopped into nearly 1cm length pieces prior to grinding using liquid nitrogen.

Solvent extraction - Coarsely powdered samples were subjected to solvent extraction by using chloroform, methanol and water solvents. The powdered form of samples and solvents were taken (1:10 w/v) and kept for 24 hours at room temperature (27 °C) in the orbital shaker at 150 rpm. Later, the extracts were filtered through a Buchner funnel with muslin cloth followed by Whatman number 1 filter paper. The resulting filtrates were concentrated by using rotary evaporator.

Test microorganism - Human pathogenic Gram positive bacteria *Staphylococcus aureus*, Gram negative bacteria- *Escherichia coli*, and a fungal species *Candida albicans* were used to defeat the antimicrobial activity of *C. serrulata.*

Antimicrobial susceptibility test **-** Antimicrobial activity of extracts was performed by using the disc diffusion method and agar well diffusion method. The stock solution was prepared with extract of 100 mg/ ml concentration of respective solvents. Sterile discs of 6 mm diameter were prepared in three different quantities (1 mg, 2 mg, and 5 mg). Each plate contained discs with three different quantities and negative control. Agar well diffusion method was carried with all extracts in same concentration as 100 mg/ ml in three different quantities (5 mg, 10 mg and 20 mg). In positive control Kanamycin 10 µl (3µg/ µl) was used for bacterial species and Flucanozole (1.25 µg/ µl) was used as antifungal agent. The plates were incubated overnight.

Results and Discussion

Methanol and chloroform extracts of *C. serrulata* showed inhibitory activity against Gram positive pathogenic bacteria, *S. aureus* in both paper disc and well diffusion methods (Figure 1 and Table 1). However, they gave higher inhibitory activity when the quantity of extracts was increased up to five milligram to twenty milligram. In addition, *C. serrulata* extracts obtained from the chloroform was active against *C. albicans* when the amount was more than five milligram (Figure 2 and Table 2). Nevertheless, choloroform extracts did not work much in the lower concentrations. In addition, there was similar antimicrobial activity observed in seagrass samples which were collected from above mentioned two sites.

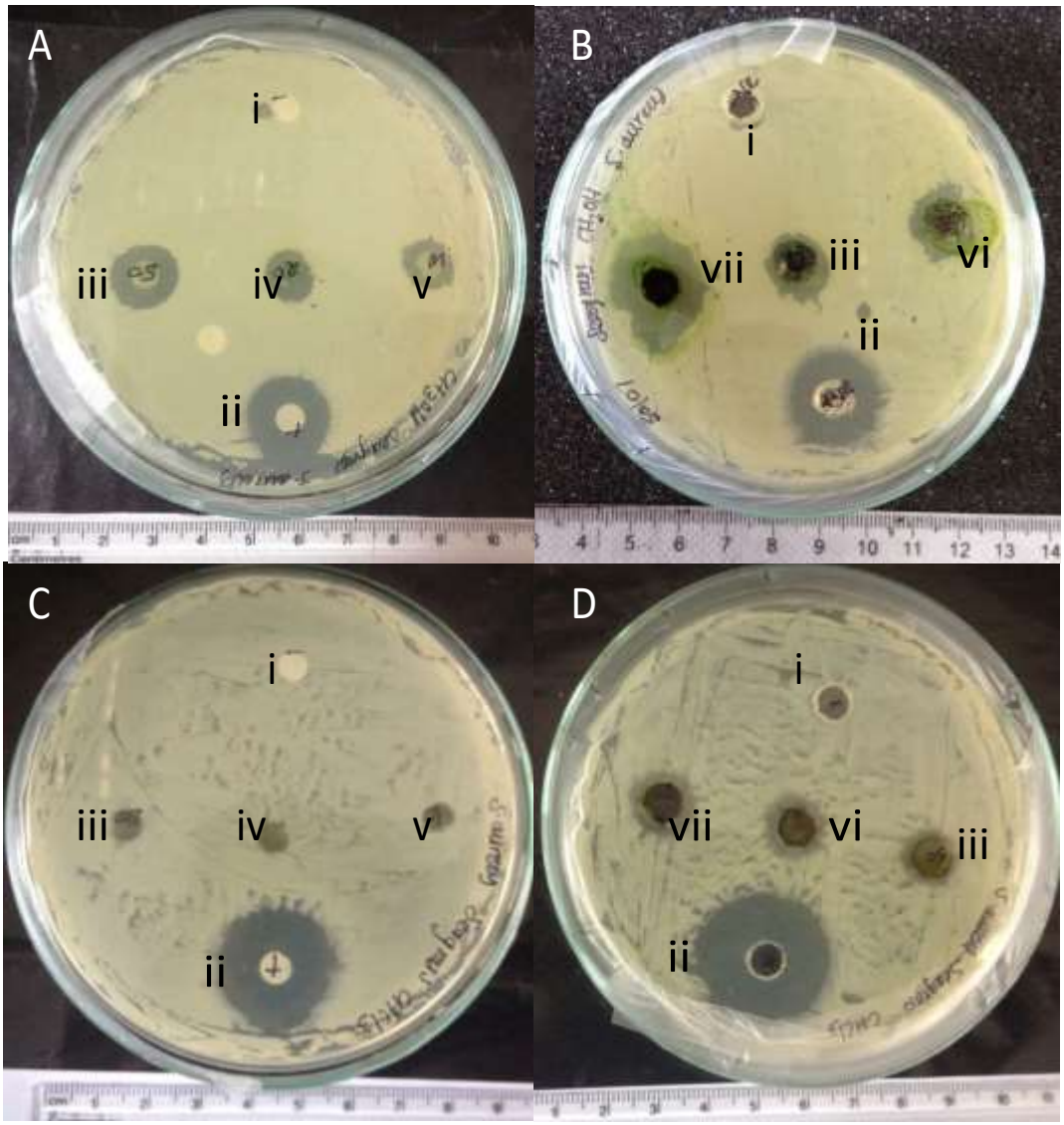

Figure 1. A & B methanol, C & D are chloroform extracts of *C. serrulata against S. aureus*.

i-negative & ii- positive controls, iii –five milligram, iv –two milligram, v –one milligram, vi – ten milligram and vii- twenty milligram extracts.

The inhibition zones increased with the concentrations used for loading. Further, none of the water extracts have given positive results with any of the pathogens. Moreover, none of the extracts have shown activity against *E. coli.*

Table 1. Antimicrobial activity of Chloroform and methanol extracts of *Cymodocea serrulata* against *Staphylococcus aureus* (IZ- Inhibition Zone).

	Loaded amounts of chloroform extract & controls							
	paper disc diffusion			well diffusion			chloroform	kanamycin
Quantity of samples	1 mg	2 mg	5mg	5 mg	10 mg	20 mg	50 µl	30 µg
Readings of Inhibitory Zone (mm)	0	0	2	4	5	7	0	13
	0	0	0	3	4	6	0	16
	0	0	3	7	10	13	0	15
mean values of Inhibitory Zone (mm)	0	0	1.67	4.67	6.33	8.67	0	14.67
	Loaded amounts of methanol extract & controls							
	paper disc diffusion			well diffusion			methanol	kanamycin
Quantity of samples	1 mg	2 mg	5 mg	5 mg	10 mg	20 mg	50 µl	30 µg
Readings of IZ (mm)	1	3	6	7	10	13	0	14
	2	4	5	4	8	12	0	16
	4	5	7	4	6	8	0	14
mean values of IZ (mm)	2.33	4	6	5	8	11	0	14.67

The biological activity of marine plant crude extracts may vary with the presence of biochemical compounds which change due to the habitat and the season of sample collection, different growth stages of plants, experimental approaches such as extraction methods, using of solvents *etc.* (Karthikaidevi *et al.,* 2012). Further, many findings with algal crude extacts are active against Gram positive bacteria than the Gram negative bacteria (Rao and Parekh, 1981). It may be due to the complex cell wall structure in Gram nagative bacteria. According to Ncube *et al.*(2008), polyphenolic compounds such as flavonols and other reported bioactive compounds are generally soluble in methanol like polar solvents. According to our study, methanol extracts of seagrass show the antibacterial activity due to the flavonoids. Also, the environmental conditions, plants growth stages, season of sample collection, species selection, extraction methods, antimicrobial test methods and test microbes also determine the antimicrobial activity (Nostro *et al.,* 2001 and Hammer *et al.,* 1999). None of the water extracts showed antimicrobial activity. Antimicrobial active compounds were insoluble in water. However, organic solvents have the potential to extract biologically active substances (Parekh et al., 2006).

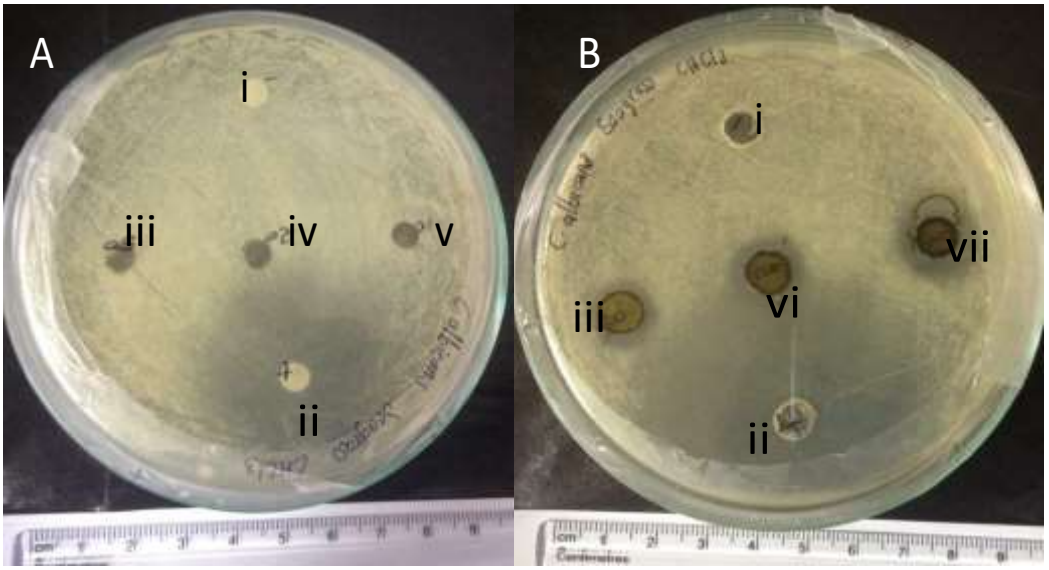

Figure 2. A & B *C. serrulata* are chloroform extracts against *C. albicans.*

i- negative & ii- positive controls, iii –five milligram, iv –two milligram, v –one milligram, vi – ten milligram and vii- twenty milligram extracts.

Table 2. Antimicrobial activity of Chloroform extracts of Cymodocea *serrulata* against *Candida albicans.*

	Loaded amounts of extract &controls							
	paper disc diffusion			well diffusion			chloroform	Flucanozole
Quantity of samples	1 mg	2 mg	5 mg	5 mg	10 mg	20 mg	50 µl	60 µg
Readings of IZ (mm)	0	0	2	3	5	7	0	14
	0	0	0	3	4	5	0	15
	0	0	2	2	4	6	0	13
mean values of IZ (mm)	0	0	1.33	2.67	4.33	6	0	14

Conclusions

In conclusion, seagrass *C. Serrulata* in South West coast of Sri Lanka have the potential of producing natural products for antimicrobial activity against pathogenic *S. aureus* and *C. albicans.* Further studies can identify the biologically active compounds having the relevant antimicrobial properties.

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