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Phytochemical and antibacterial characterization of *Cymodocea serrulata*

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Abstract

The present study was designed to evaluate the antibacterial and anti-inflammatory potential of three different extracts of *Cymodocea serrulata*. The extracts of the seagrass were tested against *Streptococcus bovis, Staphylococcus aureus, Escherichia coli, Salmonella typhi, Chlamydia pneumoniae and Helicobacter pylori* by agar method. The anti-inflammatory activity of *C. serrulata* was done by protein denaturation method. Phytochemical screening revealed the presence of carbohydrates, reducing sugars, alkaloids, saponins, phenolic compounds and flavonoids in aqueous seagrass extract. The results of the present study conclude that the studied plant possesses broad-spectrum antibacterial and antioxidant properties and may act as a potent antioxidant for biological systems susceptible to free radical-mediated reactions.

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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. Herbal remedies and alternative medicines are used throughout the world and in the past herbs often represented the original sources of most drugs. Marine species are known to produce a large number of structurally diverse secondary metabolites (Sundaram Ravikumar *et al.*, 2011).

Seagrasses, a group of marine flowering plants, inhabit the tidal and sub-tidal zones of shallow and sheltered localities of seas, gulfs, bays, backwaters, lagoons, and estuaries along temperate and tropical coastlines of the world (Green and Short, 2003; Short *et al.*, 2001). With only about 72 species and 13 genera, seagrasses play key ecological roles in fisheries production, sediment accumulation, and stabilization (Ronnback *et al.*, 2007) and have direct value to humanity as food, feed, green manure, and medicine (Newmaster *et al.*, 2011; Ragupathi *et al.*, 2013). Phytochemical analyses of seagrass species have shown that they are potential sources of antioxidants (Ragupathi *et al.*, 2010; Rengasamy *et al.*, 2011), antibacterial, antifungal and anti-inflammatory agents (Puglisi *et al.*, 2007; Yuvaraj *et al.*, 2012), and source of anticancer compounds (Folmer *et al.*, 2010). The present study investigates the anti-inflammatory properties of the important seagrass *Cymodocea serrulata* from Thanjavur, Tamil Nadu, India, along with an estimation of its phytochemical and antimicrobial activity.

Materials and methods

Sample collection

Algal samples will be collected from Thanjavur district, East costal region, Tamil Nadu. The w*et al*gal species were identified by standard according to their morphologies (Menez *et al.*, 1983 and Coles *et al.*, 2004). Wet algal species will be first washed with sea water to remove the debris like sand, sea shells, pieces of wood and tiny stones. It will be shade dried for 24 hours and then finally dried in a tray drier at 60°C to remove the water content. Dry algae obtained will be finely chopped into pieces and then ground into fine powder using mortar and pestle. Microwave drying makes the drying process faster without any degradation of cell components.

Preparation of extract

For extraction, different solvents such as methanol, ethanol and chloroform were added to 100 g of powdered leaves separately and placed in Soxhlet apparatus for 24 h. The extracts were filtered with Whatman 40 filter paper and then concentrated using a rotary evaporator to give rise to a semi-solid mass. Each solvent extraction method was repeated thrice for the purpose of accuracy. The residues obtained were stored in refrigerator for further analysis.

Phytochemical screening

Qualitative phytochemical screenings were performed using standard procedures (Sofowora, 1993; Trease and Evans, 1989). The occurrence of phytochemicals in the crude extracts of *Cymodocea serrulata* was determined.

Screening of antibacterial activity

Antibacterial activity of the *Cymodocea serrulata* was tested using the agar diffusion method described by Collin and Lyne (1970). The extracts were tested for the antibacterial activity against the six bacterial species such as *Streptococcus bovis, Staphylococcus aureus, Escherichia coli, Salmonella typhi, Chlamydia pneumoniae* and *Helicobacter pylori. Cymodocea serrulata* were prepared and tested using Nutrient agar medium. The plates were incubated at 37°C for 24 hours and the zones of inhibition measured.

Anti-inflammatory activity

Anti-inflammatory activity of the seagrass extract was determined by *in vitro* method such as protein denaturation (Mizushima *et al.,* 1968, Elias and Rao, 1988).

Results and discussion

Preliminary phytochemical screening

The phytochemicals were analysed qualitatively by using standard protocols in different solvent extract of sea grass. The protein, reducing sugar, phenol, tannins, amino acid and steroids were found in all the extracts. The flavonoids, anthraquinones and terpenoids were present in methanol and chloroform extracts. Tanins, alkaloids, amino acids, steroids and phenol were present in the ethanol extract of *C. serrulata*. The saponins, resins and glycosides were present only in the methanol extracts of sea grass *C. serrulata* (Table 1).

Table 1. Qualitative phytochemical analysis for the extracts of *C. serrulata*

SL	Phytochemicals	Solvents		
		Methanol	Ethanol	Chloroform
1	Proteins	+	+	+
2	Resins	+	-	-
3	Tannins	+	+	+
4	Saponins	+	+	+
5	Flavonoids	+	+	+
6	Alkaloids	+	+	+
7	Amino acids	+	+	+
8	Steroids	+	+	+
9	Reducing sugar	+	+	+
10	Glycosides	+	+	+
11	Anthraquinones	+	-	+
12	Terpenoids	+	+	+
13	Phenol	+	+	+

This is consistent with the findings of Ragupathi *et al.* (2013a) who had reported the qualitative analysis of the above phytoconstituents in the methanolic extracts of five seagrasses like *Enhalus acoroides*, *Thalassia hemprichii, Halodule pinifolia, Cymodocea serrulata* and *Cymodocea rotundata* from Chinnapallam coast of Tamil Nadu. Athiperumalsami *et al.* (2008) screened four seagrasses such as *Halophila ovalis, S. isoetifolium, C. serrulata and H. pinifolia* and reported 15 phytochemicals from benzene and petroleum ether extract of *S. isoetifolium* collected from Gulf of Mannar. The results of the present study is also in line with the results of Girija *et al.* (2013a) who reported the presence of ten phytoconstituents in the methanol extracts of *C. serrulata* collected from the study site.

Antibacterial analysis

The zone of inhibition measured for bacteria using well diffusion method. The antibacterial analysis (Table 2) showed a remarkable activity against the bacterial pathogens with different extract of *C. serrulata*. The maximum activity compared to the control shows the potential of the seagrass and is an indicator for determining the significance of the activity against the pathogens. The overall antibacterial analysis reveals maximum against the *H. pylori* and minimum activity was noted against the *E. coli*. Overall observation reveals that the seagrass has inhibitory activity against all the pathogens studied. *C. serrulata* is a potential source of broad-spectrum antibacterial agents due to the presence of flavonoids, which have been reported to be involved in inhibition of nucleic acid biosynthesis and other metabolic processes (Cushnie and Lamb, 2005).

Some of the seagrasses have been used in traditional medicine for example in India for malaria, skin diseases and the early stage of leprosy (Kumar *et al.*, 2008). Some extracts also have antibacterial activity (Engel *et al.*, 2006; Ross *et al.*, 2008; Ravikumar *et al.*, 2011). During the long period of co-evolution, a cooperative relationship has been formed between each endophyte and its host plant. Some endophytes have the ability to produce similar bioactive compounds to those that originate from their terrestrial host plants (Zhao *et al.*, 2011).

Anti-inflammatory activity

The percentage of protein denaturation for extract and standard drug diclofenac was done at 100µg/ml, 200µg/ml, 300µg/ml, 400µg/ml and 500µg/ml respectively as given in Table 3. The *C. serrulata* exhibits minimum stabilization 21.8% at 100µg/ml and maximum stabilization 35.3% at 500µg/ml which was presented in Table 3. % inhibition of protein denaturation activity was exhibited on the basis of concentration dependent manner. Sodium diclofenac was used as a standard. During inflammation the lysosomal enzymes is released which produced a variety of disorders and these enzymes is said to be related to acute or chronic inflammation.

Table 2. Antibacterial activity of *C. serrulata* extract against pathogens

Pathogens	Crude extracts (Zone of inhibition-mm)			Standard
	Methanol	Chloroform	Ethanol	
Staphylococcus aureus	17.5 ± 0.22	11.2 ± 0.18	13.5 ± 0.21	17.9 ± 0.62
Streptococcus bovis	15.7 ± 0.38	10.6 ± 0.21	12.2 ± 0.14	16.2 ± 0.18
Escherichia coli	12.9 ± 0.12	09.2 ± 0.12	10.6 ± 0.25	13.8 ± 0.25
Salmonella typhi,	17.1 ± 0.32	11.5 ± 0.17	13.9 ± 0.32	15.2 ± 0.15
Chlamydia pneumoniae	15.6 ± 0.25	10.3 ± 0.24	12.1 ± 0.13	16.8 ± 0.22
Helicobacter pylori	19.5 ± 0.18	14.4 ± 0.15	16.9 ± 0.25	21.7 ± 0.29

Each value is the Mean ± SD of three replicates

Table 3. Anti-inflammatory activity of *C. serrulata*

SL	Concentration (µg/ml)	% inhibition of protein denaturation		
		Methanol	Ethanol	Chloroform
1.	100	20.5 ± 0.03	19.2 ± 0.14	18.3 ± 0.16
2.	200	22.4 ± 0.15	21.7 ± 0.23	20.5 ± 0.32
3.	300	26.1 ± 0.21	24.3 ± 0.12	23.1 ± 0.18
4.	400	30.5 ± 0.18	28.6 ± 0.26	27.2 ± 0.25
5.	500	34.8 ± 0.39	32.4 ± 0.18	30.9 ± 0.12
6.	Standard	74.2 ± 0.16	70.8 ± 0.25	68.5 ± 0.22

The diclofenac drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane. The seagrass extracts of *C. serrulata* showed biphasic effects on protein denaturation method.

In the present study, the results show that methanolic extracts of *C. serrulata* have well anti-inflammatory properties *in vitro* in several models such as the inhibition of denaturation of proteins, 5-LOX, COX and ROS. Inflammation, which is a very complex physiopathological response, involves the production of free radicals derived from neutrophils, NO, ROS, cytokines, and prostaglandins during its process (Mannan *et al.*, 2008). Protein denaturation is the process by which proteins lose their tertiary structure and secondary structure. Proteins denaturation is a well-documented cause of inflammation (Anoop and Bindu, 2015). In various inflammatory and allergic disorders, COX and 5-LOX are the main enzymes in the synthesis of prostanoids and eicosanoids from polyunsaturated fatty acids. The effective reduction of chronic inflammatory conditions is important by double inhibition of LOX and COX (De Gaetano *et al.*, 2003). Substances capable of producing double inhibition of COX and 5-LOX with consequent substantial reduction in leukotriene and prostaglandin production produce a broad spectrum of anti-inflammatory activity and can be considered to have an excellent profile of pharmacological safety in clinical practice (Frey and Meyers, 2010). The *C. serrulata* exhibits minimum stabilization 21.8% at 100µg/ml and maximum stabilization 35.3% at 500µg/ml which was presented in Table 3. % inhibition of protein denaturation activity was exhibited on the basis of concentration dependent manner. Sodium diclofenac was used as a standard. During inflammation the lysosomal enzymes is released which produced a variety of disorders and these enzymes is said to be related to acute or chronic inflammation. The diclofenac drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane. These results are justified by the fact that compounds such as betulinic acid isolated from *D. thollonii* possess an in vitro inhibition property of cyclooxygenase (COX-1 and COX-2) and leukotriene B4 formation mediated by 5-LOX (Wenzig *et al.*, 2008).

On the basis of the results obtained in the present study, it is concluded that methanol extract of *C. serrulata* has potent anti-inflammatory and antibacterial activities. Thus the *C. serrulata* extract may be attributed to the presence of phenolic compounds and flavonoids etc., therefore, further investigation is needed to isolate and

identify the active compounds present in the plant extract and its efficacy.

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