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Anticancer activity of *Cymodocea serrulata* against different cell lines

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Abstract

Seagrasses have a long history of being used for a variety of remedial purposes, such as treatment of fever, skin diseases, muscle pains, wounds and stomach problems. Cancer is a debilitating disease resulting from uncontrolled proliferation. One major treatment strategy for cancer is the application of chemotherapeutic drugs which kill cancer cells. In this study the anticancer potentials of plants was investigated against MCF7 cell line (Human breast adenocarcinoma cell line) and SHP-77 (human lung adenocarcinoma epithelial cell line). Cytotoxicity of seagrass extract was determined by MTT assay. The results showed that the methanolic extract of *Cymodocea serrulata* possessed a moderate amount of anticancer activity and the IC_{50} value was recorded. The most potent anticancer activity was observed with the methanolic extract of *C. serrulata* with IC_{50} values of 52.5 µg/ml and 51.7µg/ml on MCF7 and SHP-77 cells respectively. The ethanolic extract would be studied further for isolation and characterization of active components for lead optimization studies.

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Introduction

Cancer is one of the major human diseases and causes large suffering and economic loss world-wide. Chemotherapy is one of the methods of treating cancer. However the chemotherapeutic drugs are highly toxic and have devastating side effects. Various new strategies are being developed to control and treat several human cancers (Modha and Modha, 2007). Over 60% of anticancer drugs available in the market are of natural origin. Natural products are also the lead molecules for many of the drugs that are in use (Cragg *et al*., 1997). Therefore, the phytochemicals present in several herbal products and plants may have the potential to act as preventive or therapeutic agents against various human cancers (Modha and Modha, 2007). The increased popularity of herbal remedies for cancer therapy perhaps can be attributed to the belief that herbal drugs provide benefit over that of allopathy medicines while being less toxic. Since the conventional therapies have devastating side effects, there is a continuous need for search of new herbal cures of cancer (Aquil *et al*., 2006).

Apoptosis, or programmed cell death, is one of the most finely coordinated regulatory functions for maintenance of the homeostasis in the living organism. It involves the continuous checking of the cellular integrity and cascade-like events of self-destruction when the integrity of the organism is endangered. Morphological hallmarks of apoptosis are nuclear condensation, cell shrinkage, membrane blebbing and the formation of apoptotic bodies. These changes are accompanied by biochemical features, including DNA fragmentation and the proteolytic cleavage of a variety of intracellular substrates.

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*., 2013a). 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 and Yuvaraj *et al*., 2012), and source of anticancer compounds. The present investigation was taken up for evaluating the antiproliferative potential possessed by the seagrass methanolic extract of *Cymodocea serrulata* against different human cancer cell lines.

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 and Calumpong, 1983). Wet algal species will be first washed with sea water to remove the debris like sand, sea shells, and 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

The preliminary phytochemical evaluation of seagrass was carried on extract prepared by successive extraction method in Soxhlet. The previously dried

powdered (50 gm) were extracted in a Soxhlet apparatus with ethanol successively. The resultant extracts were evaporated to dryness under vacuum. These extract were subjected to chemical test for different phytoconstituents viz. alkaloids, carbohydrates, phenolics, flavonoids, proteins, amino acids, saponins, mucilage and resins etc. Chemical tests were identifying the phytochemicals as described (Sofowora, 1993; Trease and Evans, 1983; Harborne, 1973). Alkaloids, carbohydrates, tannins and phenols, flavonoides, gums and mucilage, fixed oils and fats and saponins were qualitatively analyzed.

Tumour cell lines

Cell lines of different tissue origin such as SHP-77 (human lung adenocarcinoma epithelial cell line) and MCF7 cell line (Human breast adenocarcinoma cell line) were used. Cells were cultured in MEM (Minimum Essential Media) supplemented with Sodium Bicarbonate, EDTA, FCS (Foetal Calf Serum) and incubated in humidified atmosphere of 5% CO_2 and 37°C. The culture medium was changed every two days. All cell lines used were of human origin in order to more closely mimic how plant extracts would affect human cancer cells. Cells were generally cultured in 10 mL of appropriate medium in 75 cm^2 tissue culture (T-75) flasks at 37˚C in a humidified atmosphere of 5% CO_2/ 95% air. Cells were passages weekly and medium replaced fortnightly.

MTT assay (Mossman, 1983)

Antiproliferative effects were measured in vitro by using MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]) assays. After treatment, the living cells were assayed by the addition of 20 µl of 5 mg/ml MTT solution. Finally, the reduced MTT was assayed at 545 nm wells with untreated cells were utilized as controls. Antiproliferative and cytotoxic effects were distinguished by cell number and the duration of treatment (72 h, 5000 cells/w, and 24 h, 25000 cells/w, respectively). Stock solutions of the tested materials were prepared with dimethyl sulfoxide (DMSO). The highest DMSO concentration (0.3%) of the medium did not have any significant effect on the cell proliferation. Extracts which demonstrated potent activity (growth inhibition > 50%) were selected for further in vitro testing (dose-response curve and cytotoxicity). To study the interactions between acridones and doxorubicin, a checkerboard method was applied. A series of 2-fold dilutions of the acridones was tested in combination with 2-fold dilutions of doxorubicin. The cell growth rate was determined with MTT staining drug interactions were evaluated according to the following system (fractional inhibitory index = FIX):

FIX < 0.5= Synergism
FIX = 0.51-1= Additive effect
1 < FIX < 2= Indifferent effect
FIX > 2= Antagonism

Results and discussion

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. Anticancer activity of *Cymodocea serrulata* was studied in different mammalian cell line. Anticancer activity of methanolic extract of *C. serrulata* as well as standard was determined through MTT cytotoxicity assay. In the preliminary study, the methanolic extract showed the good yielding capacity of phytocompounds activity. In this regards, the present investigation the methanolic extract of *C. serrulata* was studied in MCF7 and SHP-77 cell lines and its result labelled in the Table 2 and also made with standard drug tamoxifen.

Table 2. Survival analysis of cancer cells treated with extracts of *C. serrulata*

Concentrations (µg ml-1)	MCF7		SHP-77	
	Cell viability (%)	Cell inhibition (%)	Cell viability (%)	Cell inhibition (%)
25	78.5	21.5	75.7	24.3
50	69.3	30.7	63.4	36.6
75	58.6	41.4	54.3	45.7
100	49.8	50.2	46.7	53.3
125	38.6	61.4	41.3	58.7
150	30.5	69.5	27.2	72.8
175	22.2	77.8	23.3	76.7
200	15.3	84.7	16.5	83.5
Vehicle control (DMSO)	100	0	100	0

The minimum cell viability (15.3%) and maximum cell inhibition (84.7%) were noted in 200 µg/ml concentration of *C. serrulata* (Table 2). The IC_{50} value (65.8µg/ml and 67.6µg/ml) was calculated for anticancer activity of methanolic extract of *C. serrulata* against MCF7 and SHP-77 cell lines. The tamoxifen used as a standard for this study. In the standard, the minimum cell viability (19.6%) and maximum cell inhibition (80.4%) were observed in higher concentration. The percentage of cell inhibition was noted in the different concentrations of methanolic extract of *C. serrulata* ranges from 25 to 200 µg/ml. The lowest cell inhibition (21.5%) was recorded in the lowest concentration and highest cell inhibition (84.7%) was noted in the higher concentration of methanolic extract of *C. serrulata*. The above results are consistent with the findings of Prabhadevi *et al.* (1998) and Ragupathi Raja Kannan *et al.* (2013). Only a few studies have been done on the bioactivity of sea grass and showed that sea grasses such as *Thalassia testudinum, Posidoniaoceanic* and *Z. marina* had antibacterial (Harrison and Chan, 1980; Devi *et al.*, 1997; Bhosale *et al.*, 2002), antialgal (Harrison, 1982), antifungal (Jensen *et al.*, 1998), antiviral (Premanathan *et al.*, 1992), anti-inflammatory (Kontiza *et al.*, 2008) and antifouling (Bhosale *et al.*, 2002) activities.

Conclusion

Nowadays herbs are extensively used for the research purpose and it possesses more than one chemical entity so it has been widely used for the research investigations. Anticancer properties of many natural compounds isolated from different sea extracts have been reported. Research is being carried out throughout the world to find a lead compound which can block the development of cancer in humans. Nature has always been a great contributor towards this goal. Furthermore this study has to prove the cytotoxic effects of methanolic extract of *C. serrulata* may be conducted in clinical trials on patients suffering from cancer disease. To the best of our knowledge, the present study concluded that the *C. serrulata* have an anticancer activity against SHP-77

and MCF7 cell line. From this study, it is clear that *C. serrulata* extract have significant anti-cancer activity in cell line. The anti-cancer activity is probably due to the presence of phenolic compounds.

References

Aquil F, Ahmad I, Mehmood Z. 2006. Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. Truk J Biol **30**, 177–183.

Athiperumalsami T, Kumar V, Louis JL. 2008. Survey and phytochemical analysis of seagrasses in the Gulf of Mannar, southeast coast of India. Bot Mar **51**, 269–277.

Bhosale SH, Nagle VL, Jagtap TG. 2002. Antifouling potential of some marine organisms from India against species of Bacillus and Pseudomonas. Mar Biotechnol **4**, 111–118.

Cragg GM, Newman DJ, Snader KM. 1997. Natural products in drug discovery and development. J Nat Prod **60**, 52–60.

Devi P, Solimabi W, D'Souza L, Sonak S, Kamat Y, Singbal SYS. 1997. Screening of some marine plants for activity against marine fouling bacteria. Bot Mar **40**, 87–91.

Girija K, Parthiban C, Hemalatha A, Saranya C, Anantharaman P. 2013a. Evaluation of antioxidant activities and preliminary phytochemical analysis of seagrass *Halodule pinifolia*, *Halophila ovalis* and *Syringodium isoetifolium*. The J Phytochem Photon **114**, 181–187.

Green EP, Short FT. 2003. World Atlas of Seagrasses. University of California Press, Berkeley.

Harborne JB. 1973. Phytochemical Methods, A Guide to Modern Techniques of Plant Analysis. Chapman and Hall, London, pp. 33–41.

Harrison PG, Chan AT. 1980. Inhibition of the growth of micro-algae and bacteria by extracts of eelgrass (*Zostera marina*). Mar Biol **61**, 21–26.

Harrison PG. 1982. Control of microbial growth and of amphipod grazing by water-soluble compounds from leaves of *Zostera marina*. Mar Biol **67**, 225–230.

Kontiza I, Stavri M, Zloh M, Vagias C, Gibbons S, Roussis V. 2008. New metabolites with antibacterial activity from the marine angiosperm *Cymodocea nodosa*. Tetrahedron **64,** 1696–1702. https://doi.org/10.1016/j.tet.2007.12.007.

Jensen PR, Jenkins KM, Porter D, Fenical W. 1998. Evidence that a new antibiotic flavone glycoside chemically defends the seagrass *Thalassia testudinum* against zoosporic fungi. Appl Environ Microbiol **64**, 1490–1496.

Menez E, Calumpong H. 1983. *Thalassodendron ciliatum*: An unreported seagrass from the Philippines. Micronesica **18**(2), 103–111.

Modha J, Modha N. 2007. Role of Ayurveda in the management of cancer. International Centre for Ayurveda Studies, Gujarat Ayurveda University, Jamnagar, Gujarat, India.

Mossman T. 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods **65**, 55–63.

Newmaster AF, Berg KJ, Ragupathy S, Palanisamy M, Sambandan K, Newmaster SG. 2011. Local knowledge and conservation of seagrasses in the Tamil Nadu state of India. J Ethnobiol Ethnomed **7**, 37.

Prabhadevi V, Sathish SS, Johnson M, Venkatramani B, Janakiraman N. 1998. Phytochemical studies on *Allamanda cathartica* L. using GC-MS. Asian Pacific J Trop Biomed, 550–554.

Premanathan M, Chandra K, Bajbai SK, Kathiresan K. 1992. A survey of some Indian medicinal plants for antiviral activity. Bot Mar **35**, 321–324.

Puglisi MP, Engel S, Jensen PR, Fenical W. 2007. Antimicrobial activities of extracts from Indo-Pacific marine plants against marine pathogens and saprophytes. Mar Biol **150**, 531–540.

Ragupathi Raja Kannan R, Arumugam R, Anantharaman P. 2010. Antibacterial potential of three seagrasses against human pathogens. Asian Pacific J Trop Med **11**, 890–893.

Ragupathi Raja Kannan R, Arumugam R, Iyapparaj P. 2013. In vitro antibacterial, cytotoxicity, and haemolytic activities and phytochemical analysis of seagrasses from the Gulf of Mannar, South India. Food Chem **136**(3–4), 1484–1489.

Ragupathi RKR, Arumugam R, Anantharaman P. 2013a. Phytochemical constituents, antioxidant properties, and p-coumaric acid analysis in some seagrasses. Food Res Int **12**, 25–28.

Rengasamy S, Miller A, Eimer BC. 2011. Evaluation of the filtration performance of NIOSH-approved N95 filtering facepiece respirators by photometric and number-based test methods. J Occup Environ Hyg **8**, 23–30.

Ronnback P, Crona B, Ingwall L. 2007. The return of ecosystem goods and services in replanted mangrove forests: Perspectives from local communities in Kenya. J Environ Conser **34**(4), 313–324.

Short FT, Coles RG, Pergent-Martini C. 2001. Global seagrass research methods. Elsevier Science B.V., Amsterdam.

Sofowora EA. 1993. Medicinal plants and traditional medicine in Africa. John Wiley and Sons Ltd, Nigeria, pp. 1–3.

Trease GE, Evans WC. 1983. Textbook of Pharmacognosy, 12th ed. Balliere, Tindall, London, pp. 57–59.

Yuvaraj N, Kanmani P, Satishkumar R, Paari A, Pattukumar V, Arul V. 2012. Seagrass as a potential source of natural antioxidant and anti-inflammatory agents. Pharmaceut Biol **50**(4), 458–467.