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Research Article

Invitro Anticancer Activity of Seagrass 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 the 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 *Cymodocea serrulata* seagrass were investigated against the 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 ethanolic extract of *Cymodocea serrulata* has moderate anticancer activity, and the IC_{50} value was recorded. The most potent anticancer activity was observed with the ethanolic extract of *C. serrulate* 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 to isolate and characterize active components for lead optimization studies.

Keywords: Anticancer, Cytotoxicity, Seagrass Extract, *Cymodocea serrulata*, SHP-77 and MCF7 Cells, MTT Assay

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1. INTRODUCTION

Cancer is one of the major human diseases and causes immense suffering and economic loss worldwide. Chemotherapy is one of the methods of treating cancer. However, chemotherapeutic drugs are highly toxic and have devastating side effects. Therefore, various new strategies are being developed to control and treat several human cancers [1]. 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 [2]. Therefore, the phytochemicals present in several herbal products and plants may act as preventive or therapeutic agents against various human cancers [1]. The increased popularity of herbal remedies for cancer therapy may be attributed to the belief that herbal drugs provide benefits over allopathic medicines while being less toxic. Since conventional therapies have devastating side effects, there is a continuous need to search for new herbal cures for cancer [3]. Apoptosis, or programmed cell death, is one of the most finely coordinated regulatory functions for maintaining homeostasis in the living organism. It involves continuously checking the cellular integrity and cascade-like events of self-destruction when the organism's integrity is endangered. Morphological hallmarks of apoptosis are nuclear condensation, cell shrinkage, membrane blebbing, and the formation of apoptotic bodies. Biochemical features accompany these changes, including DNA fragmentation and the proteolytic cleavage of various 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 [4, 5]. With only about 72 species and 13 genera, seagrasses play key ecological roles in fisheries production, sediment accumulation, and stabilization [6] and have direct value to humanity as food, feed, green manure, and medicine [1-7]. In addition, phytochemical analyses of seagrass species have shown that they are potential sources of antioxidants [8,9], antibacterial, antifungal, and anti-inflammatory agents [8,10], and sources anticancer compounds. The present investigation evaluated the antiproliferative potential possessed by the seagrass ethanolic extract of *Cymodocea serrulata* against different human cancer cell lines.

2. MATERIALS AND METHODS

2.1 Sample Collection

Algal samples were collected from Thanjavur district, East coastal region, Tamil Nadu. The w*et alg*al species were identified by standard according to their morphologies [11]. W*et alg*al species were first washed with seawater to remove debris like sand, sea shells, pieces of wood, and tiny stones. It was shade dried for 24 hours and then dried in a tray drier at 60°C to remove the water content. The dry algae obtained was finely chopped into pieces and then ground into a fine powder using a mortar and pestle. Microwave drying makes the drying process faster without any degradation of cell components.

2.2 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 the Soxhlet apparatus for 24 h. The extracts were filtered with Whatman 40 filter paper and then concentrated using a rotary evaporator to produce a semi-solid mass. Each solvent extraction method was repeated thrice for the purpose of accuracy. Finally, the residues obtained were stored in a refrigerator for further analysis.

2.3 Phytochemical Screening

The preliminary phytochemical evaluation of seagrass was carried out on extract prepared by successive extraction methods in Soxhlet. Initially , the previously dried powdered (50 gm) was successfully extracted in a Soxhlet apparatus with ethanol. The resultant extracts were evaporated to dryness under a vacuum. These extracts were subjected to a chemical test to identify different phytoconstituents, viz. alkaloids, carbohydrates, phenolics, flavonoids, proteins, amino acids, saponins, mucilage, resins, etc., as per standard procedure[12-14]. Alkaloids, carbohydrates, tannins and phenols, flavonoids, gums and mucilage, fixed oils and fats, and saponins were qualitatively analyzed.

2.4 Tumour cell lines

Cell lines of different tissue origins were used, such as SHP-77 (human lung adenocarcinoma epithelial cell line) and MCF7 cell line (Human breast adenocarcinoma cell line Cells were cultured in MEM (Minimum Essential Media) supplemented with Sodium Bicarbonate, EDTA, and FCS (Foetal Calf Serum) and incubated in a 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 the medium was replaced fortnightly.

2.5 MTT assay [15]

Antiproliferative effects were measured in vitro by using MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium omide]) assays. After treatment, the living cells were assayed by adding 20 µl of 5 mg/ml MTT solution. Finally, the reduced MTT was assayed at 545 nm wells with untreated cells 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 medium's highest DMSO concentration (0.3%) did not significantly affect cell proliferation. Extracts that demonstrated potent activity (growth inhibition > 50%) were selected for further in vitro testing (dose-response curve and cytotoxicity). A checkerboard method was applied to study the interactions between acridones and doxorubicin. 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) (Table1)

Table 1: Showed antiproliferative activity			
FIX < 0.5	Synergism	1 < FIX < 2	Indifferent effect
FIX = 0.51-1	Additive effect	FIX > 2	Antagonism

3. RESULTS AND DISCUSSION

The phytochemicals were analyzed qualitatively using standard protocols in the different solvent extracts of seagrass. The protein, reducing sugar, phenol, tannins, amino acid, and steroids were found in all the extracts. Flavonoids, anthraquinones, and terpenoids were present in methanol and chloroform extracts. Tannins, alkaloids, amino acids, steroids, and phenol were present in the ethanol extract of *C. serrulate.* The saponins, resins, and glycosides were present only in the methanol extracts of seagrass *C.serrulata* (table-2). It is consistent with the findings of Ragupathi *et al.* (2013a[8]), who had reported the qualitative analysis of the above phytoconstituents in the ethanolic extracts of five seagrasses like *Enhalus acoroides*, *Thalassia hemprichii, Halodule pinifolia, Cymodocea serrulata* and *Cymodocea rotundata* from Chinnapallam coast of Tamil Nadu. Athiperumalsami [16] screened four seagrasses such as *Halophila ovalis, S. isoetifolium, C. serrulata, and H. pinifolia,* and reported 16 phytochemicals from benzene and petroleum ether extract of *S. isoetifolium* collected from Gulf of Mannar. The results of the present study are also in line with the results of [17], who reported the presence of ten phytoconstituents in the methanol extracts of *C. serrulata* collected from the study site. *The anticancer* activity of *Cymodocea serrulata* was studied in the different mammalian cell lines. Anticancer activity of ethanolic extract of *C. serrulata.* As well, as standard was determined through MTT cytotoxicity assay. In the preliminary study, the ethanolic extract showed a good yielding capacity of phytocompounds activity. In this regard, in the present investigation, the ethanolic extract of *C. serrulata* was studied in MCF7 and SHP-77 cell lines. Its result was labeled in Table 3 and made with the standard drug tamoxifen. The minimum cell viability (15.3%) and maximum cell inhibition (84.7%) were noted in 200 µg/ml concentration of *C. serrulata.* The IC_{50} value (65.8µg/ml and 67.6µg/ml) was calculated for the anticancer activity of the ethanolic extract of *C. serrulata* against MCF7 and SHP-77 cell lines. Tamoxifen was 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 concentrations. The percentage of cell inhibition was noted in the different concentrations of ethanolic extract of *C. serrulata*, ranging from 25 to 200 µg/ml. The lowest cell inhibition (21.5%) was recorded in the lowest concentration, and the highest cell inhibition (84.7%) was noted in the higher concentration of ethanolic extract of *C. serrulata.* The above results are consistent with the findings of [18, 19]. Only a few studies have been done on the bioactivity of seagrass and showed that seagrasses such as *Thalassia testudinum, Posidoniaoceanic,* and *Z.marina* had antibacterial [20-22], antialgal [23], antifungal [24], antiviral [25], anti-inflammatory [26] and antifouling [24] activities.

Table.2 Qualitative phytochemical analysis for the extracts of *C.serrulata*

Sl.No	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	+	+	+

+ present; - absent.

Table 3. 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

4. CONCLUSION

Herbs are extensively used for research and possess more than one chemical entity, so it has been widely used for research investigations. Anticancer properties of many natural compounds isolated from different sea extracts have been reported. Research is being conducted worldwide to find a lead compound that can block human cancer development. Nature has always been a great contributor towards this goal. Furthermore, this study has proved that the cytotoxic effects of the ethanolic extract of *C.serrulata* may be conducted in clinical trials on cancer patients. The present study concluded that *C.serrulata* have anticancer activity against SHP-77 and MCF7 cell lines. From this study, it is clear that *C.serrulata* extract has significant anti-cancer activity in a cell line. The anti-cancer activity is probably due to the presence of phenolic compounds.

5. AUTHORS CONTRIBUTION STATEMENT

B. Meena, P. Neelarathi. Conceived of the Presented Idea. P. Neelarathi. Developed the Theory and Performed the Computations. K. Durgadevi and V. Ramamurthy. Verified The Methods. Encouraged B. Meena, P. Neelarathi. To Investigate [A Specific Aspect] And Supervised the Findings of This Word. All Authors Discussed the Results and Contributed to the Final Manuscript.

6. CONFLICT OF INTEREST

Conflict of interest declared none.

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