Anticancer activity of ethanolic extract of Zostera Marina

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ABSTRACT

Objective: To estimate the anticancer activity of ethanolic extract of Zostera Marina. **Materials and Methods**: Insilico toxicology was evaluated by PASS. The anticancer activity of Zostera marina lamouroux was evaluated by MTT assay against Human colorectal carcinoma cell line (HCT116). **Results and Discussion:** Insilico toxicological results are shown the significant anticancer activity and less adverse effect**.** The invitro results of cytotoxicity study (MTT assay) suggested that plant extract showed toxicity in nature after the treatment period of 24hrs with IC50 value of 255.34µg/ml.**Conclusion**: The anticancer activity of ethanolic extract of Zostera Marina was evaluated by insilico and invitro method and both results are correlated.

K**ey words:** Zostera Marina; ethanolic extract; colon cancer; methanol extract; MTT assay.

INTRODUCTION

Cancer remains a major hazard to our society, despite significant advances in diagnosis and treatment. The number of cancer patients in developing and undeveloped countries is expected to climb by up to 70% in the near future, posing a major threat to all of us. Due to poor to moderate living standards and insufficient medical facilities, the Indian subcontinent's cancer problem is growing in size. Lung, breast, colon, rectum, stomach, and liver cancers are the most common

cancers in Indians. India is currently rising at a rapid pace and will most likely become a developed country within a few decades, allowing it to participate in global growth. As a result, it's critical to research the state of cancer in India so that advanced measures can be made to stem the tide in the near future. In light of these facts, efforts have been made to investigate the state of cancer in India, including its causes, prevention strategies, economic impact, and comparison to the global situation.

SEAGRASS

Natural products have played a significant part in the treatment of human illnesses throughout history. The fabled discovery of penicillin, for example, changed the world. Sea grass is a marine chemical with antibacterial and antimicrobial characteristics that can be used to cure cancer.

The in-vitro anti-cancer activity of the seagrass species against numerous human cancer cell lines, including malignant melanoma, lung, cervical, carcinoma, and colorectal tumours, was evaluated in this study, which was claimed to be the first of its type. Sea grass not only has antimicrobial qualities, but there is also substantial evidence that it has potent anti-cancer properties.

Seagrass is one of the marine angiosperm groups that can remain completely submerged and complete their life cycle in a coastal environment. Seagrass has been used for a range of medicinal purposes in traditional medicine, including wound healing, fever, stomach aches, muscle problems, and skin ailments. They've also been employed in biomedical applications like anti-cancer, anti-diabetic, anti-inflammatory, anti-fungal, anti-bacterial, and anti-viral.

Seagrass, a plant that grows in tidal environments, has unique secondary metabolites that act as anticancer bioactive chemicals. Because the majority of secondary metabolites are produced by organisms in order to adapt to their surroundings, the hunt for secondary metabolites that can act as anticancer bioactive has primarily focused on creatures that live in harsh environments. The tidal zone is one of the harshest ecosystems. Alkaloid, terpenoid, polyphenol, and flavonoid are phytochemical substances found in ethyl acetic extract of fresh seagrass leaves(1). Flavonoid chemicals are thought to have anticancer properties. Flavonoid substances derived from phenolic groups, such as Morin, quercetin, myricetin, and taxifolin, have been shown to inhibit cancer cell proliferation at low concentrations while increasing apoptosis at high concentrations. As a result, more research is needed to identify anticancer chemicals in fresh seagrass leaves.

Morin, quercetin, myricetin, and taxifolin are flavonoid compounds generated from phenolic groups that have been proven to suppress cancer cell proliferation at low concentrations while inducing apoptosis at high ones. As a result, greater research into anticancer compounds in fresh seagrass leaves is required.

Phenolic has anticancer properties as well. Phenols can be dissolved in both polar and non-polar solvents. Phenolic chemicals have been shown to inhibit the proliferation of HeLa cells and the T47D breast cancer cell line. Caffeic acid in phenols can promote apoptosis by lowering ant apoptosis protein expression.

COLORECTAL CANCER:

Colorectal cancer (CRC) is a type of cancer that develops in the colon or rectum(2-3). Blood in the stool, changes in bowel movements, weight loss, and exhaustion are all possible signs and symptoms.

ZOSTERA MARINA (Marine eelgrass)

Zostera marina is a flowering vascular plant species that is one of many types of seagrass. This species is primarily known in English as eelgrass, with sea wrack being a less common name, and refers to the plant after it has broken free from the submerged wetland soil and drifted free with the ocean current and waves to a coast seashore. It is a saline soft-sediment submerged plant native to marine habitats over the northern latitudes of North America and Europe, from subtropical to subpolar regions. This species is the Northern Hemisphere's most widespread marine flowering plant. It lives in cooler ocean waters in the north Atlantic and it dies out during the summer in the warmer southern regions of its range. It grows in the arctic region and is exposed to ice for several months each year. It is the only type of seagrass found in Iceland. Bays, lagoons, estuaries, beaches, and other coastal habitats are all good places to look for it. Each ecotype has its own set of habitat requirements. It lives in the sublittoral zone, where the water is calmer and it is rarely exposed to the air. It anchors in sandy or muddy substrates via rhizomes, and its leaves catch particle material in the water, which collects around the plant bases and builds up the top layer of the seabed. Seagrass contain polyphenols, rosmarinic acid, luteolin sulphated derivatives and it also contains flavonoids, terpenoids.

The current research focuses on a thorough examination of the seagrass Zostera.L. Many botanical reviews have revealed that this seagrass has powerful therapeutic characteristics, including anti-oxidant and antibacterial properties(4-5). Few investigations on the anti-cancer

activity of this seagrass have been conducted to yet. As a result, the anti-cancer effects of this plant (seagrass), which has a wide range of pharmacological activity, should be checked.

The plan of work on the present study includes the following:

- ❖ Successive extraction of seagrass Zostera.L by hot continuous extraction (Soxhlet extraction) method using ethanol as the solvent.
- ❖ To perform Qualitative analysis of the extracted sample.
- ❖ To evaluate the invitro cytotoxic activity of ethanol extract of Zostera.L on Human colon cancerous cell line (HCT116) by MTT assay.

DOCKING STUDIES

A computer-aided software programme called PASS makes predictions about the range of biological activity of various substances based on those assumptions. The examination of structure-activity connections for more than 250,000 physiologically active chemicals, including pharmaceuticals, drugs-substances, leads, and potentially harmful substances, ultimately determines the prediction outcome. Over 4000 different biological activities, such as therapeutic effects, toxic effects, adverse effects, enzyme interactions, mechanism of action, and more, can be predicted by PASS online. One of the best programmes for providing early signals about a compound's potential utility is this one.

A chemical compound's range of biological activities, which shows the results of its interactions with numerous biological molecules, is called the biological activity spectrum. The likelihood that the anticipated compound falls into the group of active compounds is assessed using the probability "to be active" (Pa) metric. The likelihood that the projected chemicals fall under the category of inactive compounds is assessed using the probability "to be inactive" (Pi) formula.

METHODS AND MATERIALS

Collection of plant :

Seagrass Zostera .L was collected from coastal regions of Nellore, AP. It was collected in the month of January 2022, where the chemical constituents show the almost activity. The plant material was identified and authenticated by **Mr. Illaya Perumal,** Annakili Amma research institute Medavakam, Chennai.

Methods: Soxhlet extraction

Preparation of extract:

The plant was shade dried at room temperature and prepared an herbarium for authentication purpose and the extraction purpose. About 100 grams of dried product was packed into Soxhlet apparatus and subjected to extraction sequentially with 500 ml of ethanol followed by pet ether, ethyl acetate, benzene. The extraction was continued until the color of the solvent in siphon tube become colorless. Extracts of ethanol, pet ether, benzene, ethyl acetate, were subjected to evaporation by Rota evaporator at below 60 C. we have collected the extracts respectively and the percentage yield from Zostera .L using different solvent is given as below

Table no: 1 Percentage yield of extracted plant Zostera .L from different solvents

Extracts	**Plant material used for extraction [g]**	**Yield [g]**	**Percentage yield [g]**
ethanol	30	9.5	15.80
Ethyl acetate	30	4.0	6.50
Pet ether	30	1.5	2.40
benzene	30	0.9	1.29

Qualitative analysis:

We have performed qualitative analysis of the Zostera marina lamouroux by the following functional test in the table no2. All the test shows positive except carboxylic acid test shows negative.

Table no: 2 Identification of the functional group

S.No	**Name of the chemical test**	**Results**
01	Carbohydrates- Benedict's test:	Positive
02	Ketones and aldehydes- DNPH test	Positive
03	Phenols- Ferric chloride test	Positive
04	Hydrocarbons- Reaction with sulphuric acid	Positive
05	Carboxylic acids- Sodium bicarbonate test	Negative

METHODOLOGY:

IN-VITRO ANTICANCER ACTIVITY:

The anticancer activity of Zostera marina lamouroux was evaluated by MTT assay against Human colorectal carcinoma cell line (HCT116). The test sample details are shows in table no 2.

Table no: 3 Details of Samples received

Sl. No.	Sample Name/Code	Concentrations	Cell line
1	PLANT EXTRACT	5(31.25,62.5,125,250and500)	HCT116

BACKGROUND OF THE STUDY:

MTT assay is a colorimetric assay used for the determination of cell proliferation and cytotoxicity, based on reduction of the yellow-coloured water-soluble tetrazolium dye MTT to formazan crystals. Mitochondrial lactate dehydrogenase produced by live cells reduces MTT to insoluble formazan crystals, which upon dissolution into an appropriate solvent exhibits purple colour, the intensity of which is proportional to the number of viable cells and can be measured spectrophotometrically at 570nm. (6).

MATERIALS:

Cell lines: **HCT116- Human Colon cancer cell line (NCCS, Pune),** Cell culture medium: MC'COYS-5A media- (#AL057S, Himedia), Adjustable multichannel pipettes and a pipettor (Benchtop, USA), Fetal Bovine Serum (#RM10432, Himedia), MTT Reagent (# 4060, Himedia) DMSO (#PHR1309, Sigma) Cisplatin D-PBS (#TL1006, Himedia) 96-well plate for culturing the cells (From Corning,USA) T25 flask (# 12556009, Biolite - Thermo) 50 ml centrifuge tubes (# 546043 TARSON), 1.5 ml centrifuge tubes (TARSON), 10 ml serological pipettes (TARSON) 10 to 1000 ul tips (TARSON)

EQUIPMENTS:

Centrifuge from (Remi: R-8^{o}C), Pipettes: 2-10µl, 10-100µl, and 100-1000µl, Inverted microscope (Biolinkz, India), 37°C incubator with humidified atmosphere of 5% CO_2 (Healforce, China) 96 well microplate reader (ELX-800, BioTek, USA)

STEPS FOLLOWED:

Seed 200µl cell suspension in a 96-well plate at required cell density (20,000 cells per well), without the test agent. Allow the cells to grow for about overnight. Add appropriate concentrations of the test agent (Mentioned in the results - Excel sheet). Incubate the plate for 24hrs at 37°C in a 5% CO_2 atmosphere. After the incubation period, takeout the plates from incubator, and remove spent media and add MTT reagent to a final concentration of 0.5mg/mL of total volume. Wrap the plate with aluminium foil to avoid exposure to light. Return the plates to the incubator and incubate for 3 hours. (Note: Incubation time varies for different cell lines. Within one experiment, incubation time should be kept constant while making comparisons) Remove the MTT reagent and then add 100µl of solubilisation solution (DMSO). Gentle stirring in a gyratory shaker will enhance dissolution. Occasionally, pipetting up and down may be required to completely dissolve the MTT formazan crystals especially in dense cultures. Read the absorbance on a spectrophotometer or an ELISA reader at 570nm and 630nm uses a reference wavelength. **The IC_{50} value** was determined by using linear regression equation (7)

i.e., Y =Mx+C. Here, Y = 50, M and C values were derived from the viability graph.

STEPS INVOLVED IN PASS:

Step 1: Navigation of the PASS online web page:

Any web browser can be used to access the pass directly by typing "PASS Prediction" into the search bar. Use the online program's prediction page for the free registration and log-in to use the component of interest prediction feature (8).

Step 2: Drawing the structure of molecule:

The 2D structure of the components, which serves as the foundation for the prediction, is used by the PASS online prediction tool as an input; as a result, the structure can be created using Chemsketch version 12 and uploaded to the PASS website as a (*.mol) file, or it can be created directly on the website using JAVA, which makes use of the drawing programme Marvin Sketch.

Step III: Prediction output:

The input structure's activities, which were drawn in the second stage, are now compared with the structures in the program's database that have known activities. As the principal of estimation, the Bayesian approach, the prediction tool predicts the Pa: Pi ratio of the input substance. The result is shown in the table 4-9 as several biological activities ranked in descending order of their likelihood ratios.

RESULTS:

Table no: 4 Details of possible pharmacological activity of Rosmarinic acid

SMILES : **C1=CC(=C(C=C1CC(C(=O)O)OC(=O)C=CC2=CC(=C(C=C2)O)O)O)O**

0,956	0,003	Membrane integrity agonist
0,938	0,003	Feruloyl esterase inhibitor
0,921	0,002	Antihypoxic
0,836	0,003	Monophenol monooxygenase inhibitor
0,799	0,005	Antidiabetic
0,804	0,020	CYP2J substrate
0,787	0,011	GST A substrate
0,779	0,004	Reductant
0,785	0,012	Membrane permeability inhibitor
0,766	0,004	Pyruvate decarboxylase inhibitor
0,763	0,005	Preneoplastic conditions treatment
0,780	0,024	Chlordecone reductase inhibitor
0,779	0,024	Mucomembranous protector
0,757	0,008	Linoleate diol synthase inhibitor
0,751	0,004	APOA1 expression enhancer
0,745	0,001	3,4-Dihydroxyphenylacetate 2,3-dioxygenase inhibitor
0,745	0,003	Free radical scavenger
0,744	0,005	TNF expression inhibitor
0,735	0,002	4-Hydroxybenzoate 3-monooxygenase inhibitor
0,745	0,013	JAK2 expression inhibitor
0,730	0,005	MMP9 expression inhibitor
0,722	0,005	Antimutagenic
0,719	0,005	Lipid peroxidase inhibitor
0,725	0,021	Mucositis treatment
0,752	0,049	Ubiquinol-cytochrome-c reductase inhibitor

0,705	0,003	4-Coumarate-CoA ligase inhibitor
0,710	0,051	Gluconate 2-dehydrogenase (acceptor) inhibitor

Table no: 5 Details of possible adverse and toxic effects of Rosmarinic acid

0,797	0,014	Hematemesis
0,739	0,015	Urine discoloration
0,748	0,059	Shivering
0,707	0,041	Ulcer, aphthous

Table no: 6 Details of possible pharmacological activity of Luteolin

SMILES : C1=CC(=C(C=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O)O

0,978	0,001	Chlordecone reductase inhibitor
0,965	0,003	Membrane integrity agonist
0,964	0,003	HIF1A expression inhibitor
0,953	0,002	Membrane permeability inhibitor
0,952	0,002	2-Dehydropantoate 2-reductase inhibitor
0,947	0,001	Aryl-alcohol dehydrogenase (NADP+) inhibitor
0,947	0,003	Aldehyde oxidase inhibitor
0,942	0,001	P-benzoquinone reductase (NADPH) inhibitor
0,940	0,001	Antimutagenic
0,940	0,002	Kinase inhibitor
0,942	0,005	CYP2C12 substrate
0,936	0,002	Peroxidase inhibitor
0,935	0,002	HMOX1 expression enhancer
0,932	0,002	CYP1A inducer
0,927	0,001	NADPH-ferrihemoprotein reductase inhibitor
0,923	0,002	UGT1A6 substrate
0,918	0,003	Anaphylatoxin receptor antagonist
0,914	0,001	SULT1A3 substrate

0,916	0,004	TP53 expression enhancer
0,913	0,001	CYP1A1 inducer
0,912	0,002	UGT1A9 substrate
0,909	0,002	Histidine kinase inhibitor
0,906	0,001	Glycerol dehydrogenase (NADP+) inhibitor
0,902	0,001	2-Dehydropantolactone reductase (A-specific) inhibitor
0,904	0,004	CYP1A substrate
0,901	0,003	Vasoprotector
0,897	0,001	Cystathionine beta-synthase inhibitor
0,889	0,002	MAP kinase stimulant
0,889	0,002	Alcohol dehydrogenase (NADP+) inhibitor
0,884	0,004	CYP1A1 substrate
0,881	0,002	Monophenol monooxygenase inhibitor
0,878	0,001	Quercetin 2,3-dioxygenase inhibitor
0,877	0,002	Beta-carotene 15,15'-monooxygenase inhibitor
0,873	0,007	Antiseborrheic
0,873	0,011	Ubiquinol-cytochrome-c reductase inhibitor
0,866	0,005	Apoptosis agonist
0,859	0,002	AR expression inhibitor
0,858	0,002	NADPH oxidase inhibitor
0,857	0,001	Testosterone 17beta-dehydrogenase inhibitor
0,855	0,002	UGT1A10 substrate
0,854	0,003	Sulfotransferase substrate
0,853	0,003	4-Nitrophenol 2-monooxygenase inhibitor
0,850	0,003	CYP1A inhibitor
0,849	0,002	Antihemorrhagic
0,846	0,002	UGT1A3 substrate
0,857	0,018	Aspulvinone dimethylallyltransferase inhibitor
0,842	0,004	27-Hydroxycholesterol 7alpha-monooxygenase inhibitor
0,841	0,004	UGT1A substrate
0,836	0,001	Chalcone isomerase inhibitor
0,830	0,003	Histamine release inhibitor
0,833	0,006	JAK2 expression inhibitor
0,826	0,003	UGT1A1 substrate
0,820	0,002	CYP1A1 inhibitor

0,822	0,003	APOA1 expression enhancer
0,823	0,005	UDP-glucuronosyltransferase substrate
0,816	0,004	Cholestanetriol 26-monooxygenase inhibitor
0,816	0,003	CYP1A2 inhibitor
0,813	0,003	Pectate lyase inhibitor
0,807	0,002	UGT1A7 substrate
0,805	0,002	CYP19 inhibitor
0,808	0,004	CYP2B5 substrate
0,799	0,002	2-Enoate reductase inhibitor
0,798	0,002	NOS2 expression inhibitor
0,808	0,016	Mucomembranous protector
0,791	0,007	CYP3A4 inducer
0,783	0,002	CYP19A1 expression inhibitor
0,777	0,004	MMP9 expression inhibitor
0,775	0,004	Antioxidant
0,783	0,014	Antineoplastic
0,772	0,003	UGT1A8 substrate
0,769	0,001	Iodide peroxidase inhibitor
0,771	0,009	Alkane 1-monooxygenase inhibitor
0,764	0,002	CYP1B1 inhibitor
0,762	0,002	1-Alkylglycerophosphocholine O-acetyltransferase inhibitor
0,763	0,003	CYP1B substrate
0,758	0,003	Xenobiotic-transporting ATPase inhibitor
0,758	0,004	CYP2A4 substrate
0,755	0,002	Alcohol dehydrogenase [NAD(P)+] inhibitor
0,756	0,004	Cardioprotectant
0,755	0,003	Free radical scavenger
0,752	0,002	UGT2B15 substrate
0,754	0,003	Leukotriene-B4 20-monooxygenase inhibitor
0,757	0,008	CYP3A inducer
0,749	0,004	UGT2B12 substrate
0,750	0,007	CYP1A2 substrate
0,746	0,003	Hemostatic
0,746	0,005	Pin1 inhibitor

0,745	0,005	Insulysin inhibitor
0,771	0,033	Testosterone 17beta-dehydrogenase (NADP+) inhibitor
0,740	0,004	Lipid peroxidase inhibitor
0,736	0,004	Tetrahydroxynaphthalene reductase inhibitor
0,735	0,003	Beta glucuronidase inhibitor
0,755	0,030	Gluconate 2-dehydrogenase (acceptor) inhibitor
0,721	0,002	SULT1A1 substrate
0,720	0,004	Nitrite reductase [NAD(P)H] inhibitor
0,719	0,004	CYP2C9 inducer
0,715	0,005	UGT2B substrate
0,713	0,003	CF transmembrane conductance regulator agonist
0,727	0,019	Dehydro-L-gulonate decarboxylase inhibitor
0,710	0,005	Antiseptic
0,741	0,037	CYP2J substrate
0,706	0,005	Cytoprotectant
0,707	0,020	Fibrinolytic
0,715	0,030	CYP2J2 substrate

Table no: 7 Details of possible adverse and toxic effects of Luteolin

0,824	0,004	Genotoxic
0,819	0,016	Reproductive dysfunction
0,823	0,028	Shivering
0,804	0,017	Toxic, vascular
0,752	0,014	Urine discoloration
0,734	0,012	Endocrine disruptor
0,713	0,039	Ulcer, aphthous

Table no: 8 Details of possible pharmacological activity of 7,3'-disulfate luteolin

SMILES :

C1=CC(=C(C=C1C2=CC(=O)C3=C(C=C(C=C3O2)OS(=O)(=O)O)O)OS(=O)(=O)O)O

0,982	0,001	Hemostatic
0,970	0,001	SULT1A3 substrate
0,969	0,002	Sulfotransferase substrate
0,969	0,003	CYP2C12 substrate
0,957	0,002	Benzoate-CoA ligase inhibitor

0,938	0,001	SULT1A1 substrate
0,923	0,001	Beta-carotene 15,15'-monooxygenase inhibitor
0,906	0,004	Chlordecone reductase inhibitor
0,885	0,001	Coenzyme-B sulfoethylthiotransferase inhibitor
0,888	0,006	HIF1A expression inhibitor
0,873	0,004	Kinase inhibitor
0,867	0,004	Membrane permeability inhibitor
0,860	0,003	Glutathione S-transferase substrate
0,854	0,003	Monophenol monooxygenase inhibitor
0,850	0,000	SULT1E1 substrate
0,847	0,004	UDP-glucuronosyltransferase substrate
0,844	0,008	Anaphylatoxin receptor antagonist
0,839	0,005	Arylsulfate sulfotransferase inhibitor
0,816	0,001	1-Alkylglycerophosphocholine O-acetyltransferase inhibitor
0,815	0,004	Peroxidase inhibitor
0,813	0,003	4-Methoxybenzoate monooxygenase (O-demethylating) inhibitor
0,807	0,004	Histidine kinase inhibitor
0,792	0,001	Iduronate-2-sulfatase inhibitor
0,786	0,005	Vasoprotector
0,813	0,033	Membrane integrity agonist
0,772	0,001	Endoglycosylceramidase inhibitor
0,777	0,009	2-Dehydropantoate 2-reductase inhibitor
0,753	0,003	UGT2B substrate
0,734	0,002	Laxative
0,754	0,024	Sugar-phosphatase inhibitor
0,730	0,002	Cholecystokinin agonist
0,713	0,003	Mannosyl-glycoprotein endo-beta-N-acetylglucosaminidase inhibitor
0,714	0,005	P-benzoquinone reductase (NADPH) inhibitor
0,710	0,004	Histamine release inhibitor
0,705	0,003	Antihemorrhagic
0,707	0,007	NADPH-ferrihemoprotein reductase inhibitor
0,702	0,004	Aryl-alcohol dehydrogenase (NADP+) inhibitor
0,705	0,025	TP53 expression enhancer

Table no: 9 Details of possible adverse and toxic effects of 7,3'-disulfate luteolin

0,835	0,005	Carcinogenic
0,829	0,007	Non mutagenic, Salmonella
0,725	0,020	Teratogen
0,717	0,020	Embryotoxic
0,706	0,043	Toxic

In this study, given test compound is evaluated to analyze the cytotoxicity effect on HCT116 cell lines. The concentrations of the test compound used to treat the cells are shows in the table no 10.

Table no: 10 Details of test compound concentrations

S.No	Test Compounds	Cell Line	Concentration treated to cells
1	Untreated	HCT116	No treatment
2	Standard (C)	HCT116	25µM of CISPLATIN
3	Blank	-	Only Media without cells
4	Test-PLANT EXTRACT	HCT116	5(31.25,62.5,125,250 and 500µG/ml)

GRAPH:

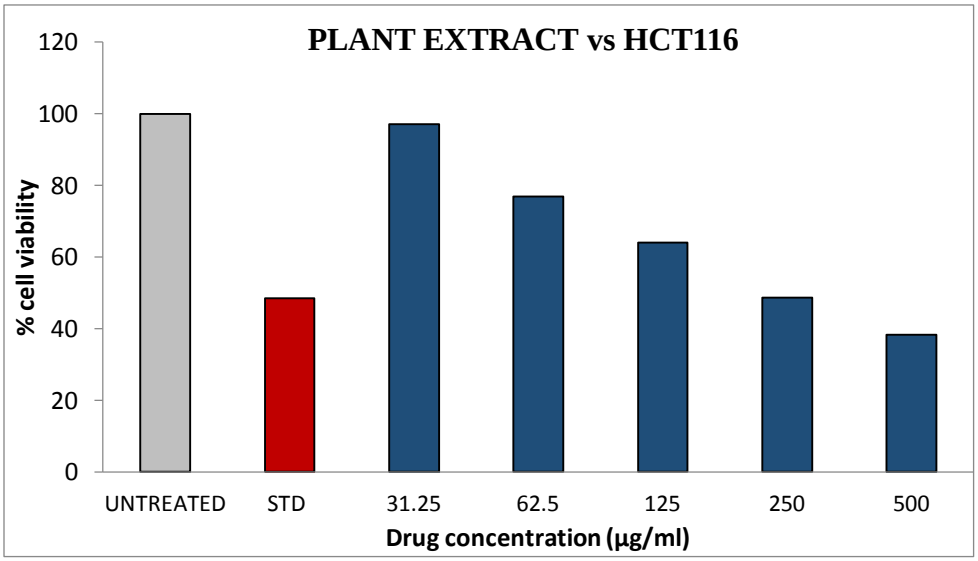

Fig: 1 Plant extract vs HCT116

Table no: 11 IC_{50} Concentration and % cell viability Ethanol extract of Zostera L

CONCENTRATION UNIT: µG/ML INCUBATION: 24HRS EXTRACTS								
CONCENTRATION	**BLANK**	**UNTREATED**	**STD**	**31.25**	**62.5**	**125**	**250**	**500**
ABS READING 1	0.044	0.925	0.487	0.905	0.741	0.614	0.474	0.398
ABS READING 2	0.04	0.957	0.468	0.924	0.725	0.621	0.485	0.375
MEAN ABS	0.042	0.941	0.4775	0.9145	0.733	0.6175	0.4795	0.3865
MEAN ABS (SAMPLE-BLANK)		0.899	0.4355	0.8725	0.691	0.5755	0.4375	0.3445
STANDARD DEVIATION		0.022627417	0.013435029	0.013435029	0.011313708	0.004949747	0.007778175	0.016263456
STANDARD ERROR		0.016	0.0095	0.0095	0.008	0.0035	0.0055	0.0115
CELL VIABILITY %		**100**	**48.44**	**97.05**	**76.86**	**64.01**	**48.66**	**38.32**

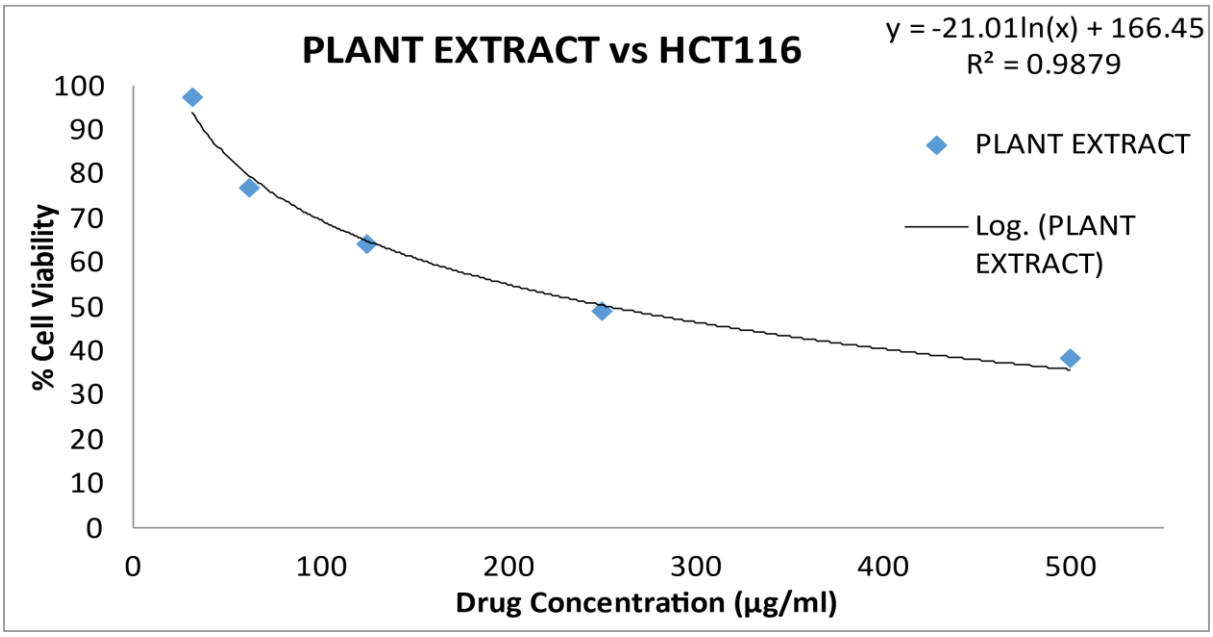

Fig 2: % cell viability vs concentration in µG/ml of Ethanol extract of Zostera L

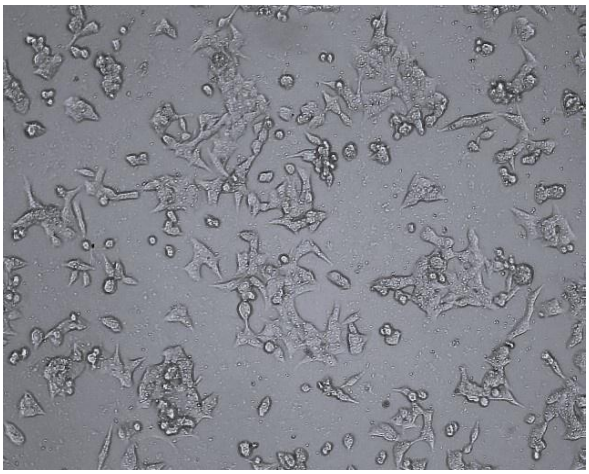

Fig 3a: Untreated

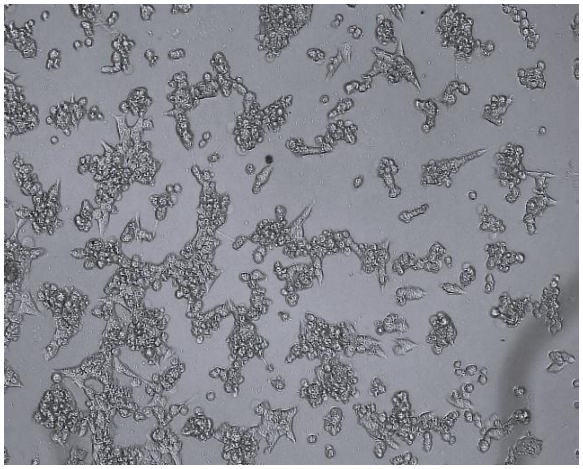

Fig 3b: Standard(25µM/ml)

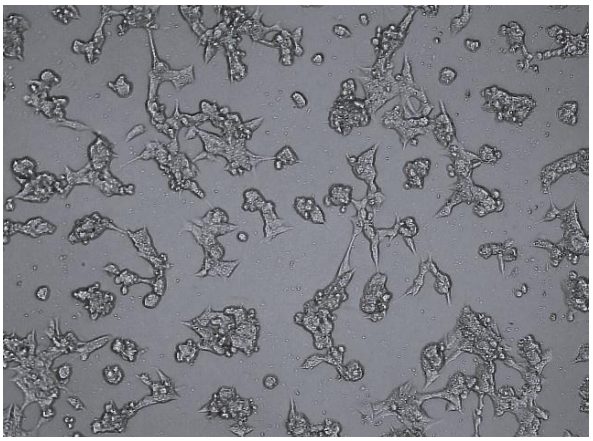

Fig 3c: Test sample (31.25µG/ml)

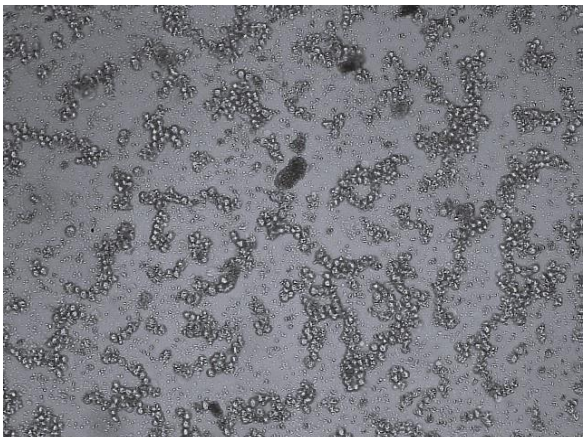

Fig 3d:Test sample (500µG/ml)

Seagrass collected from the marine region was isolated in the month of January where it shows effective activity against the colon cancer. The collected seagrass was identified and authenticated as Zostera marina. L and then it was extracted through the Soxhlet extraction apparatus, extraction procedure was continued for period of 8 hours, ethanol is the polar solvent used for the extraction. The collected extract was subjected to the evaporation by using rota evaporator.

The collected extract was dispatched for characterization and to explore the anticancer activity of colon [HCT116] present in the Zostera marina. L. In the characterization process MTT assay was chosen and performed, IC50 value is proclaimed as 255.34 microgram per ml there by the absorbance readings are calculated.

In this preclinical study we claimed that Zostera marina. L shows anticancer activity but it doesn't show potential activity noted by the standard, despite the fact that we have explored that seagrass might show anticancer activity in a better way using as a combination drug to the standard. Further study is essential to be conducted on different cell lines over the body.

CONCLUSIONS:

The results of cytotoxicity study (MTT assay) suggested that PLANT EXTRACT showed toxicity in nature after the treatment period of 24hrs with IC50 value of 255.34µg/ml. On the basis of the above result it was suggested that, the In-Vitro anticancer activity of Ethanolic extract of marine seagrass Zostera L processed significant anticancer effect. This may probably due to the presence of phytochemicals such as phenols, esters and flavonoids. Further isolation

and purification of bioactive compound from Zostera marina L may reveal the presence of potent novel anticancer agent.

REFERENCES

1. Perumal Parthasarthy, Arthanari Umamaheshwari, Ravichandran, Banupriya, Sanniyasi Elumalai., Phytochemical screening and Invitro anticancer activity of Ethyl acetate fraction of Seagrass Halodule Uninervis from Mandapam coastal Region, Rameswaram, Gulf of mannar,India., 2021, DOI:10.25004
2. Colon cancer treatment NCI., 2014
3. Priti S. Hedge, Daniel S. Chen.,Top 10 Challenges in Cancer Immunotherapy, 2020, DOI:10.1016
4. Han Gil Choi, Ji Hee Lee, Hyang Ha Park and Fotoon A. Q. Sayegh., Antioxidant and antimicrobial activity of Zostera. L Extract., 2009., 10.4490.
5. Robert j.orth, Jonathan lefcheck, karen Mcglathery et al Restoring seagrass can bring coastal bays back to life, 20 October,2020.
6. Alley, M. C et al., Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines, 1988 sep 1:48(17): 4827-33.
7. Ningning Han, Jianjiang Li 1, and Xia Li., Natural Marine Products: Anti-Colorectal Cancer *in-vitro* and *in-vivo*., 2022., 20-00349-v2.
8. Parasuraman S, Prediction of Activity Spectra for Substances. J. Pharmacol. Pharmacother. 2011; 2(1): 52-53.