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A PHARMACOLOGICAL PROMISE TOWARDS CELL CYCLE TARGET INHIBITION IN ANTICANCER DRUG DISCOVERY OF ULVAN ISOLATED FROM Ulva lactuca(L).

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ABSTRACT

Objective: To prescreen the in vivo antiproliferative activity of the marine green alga Ulva lactuca Family Ulvaceae using the eukaryotic model organism Dictyostelium discoideum. Method: In the present study to investigate the effect of ulvan which was isolated from Ulva lactuca marine green alga was selected for phytochemical and antiproliferative activity. Antiproliferative activity was determined by inhibition of cell growth using Dictyostelium discoideum as a eukaryotic model organism. Result: Preliminary phytochemical screening of ethanolic extract of Ulva lactuca(EEUL) showed the presence of alkaloids, carbohydrates, sterols, saponins, tannins, proteins and amino acids, mucilage, flavonoids and absence of volatile oil, fixed oils. Total phenolic and flavonoid content were found to be 13.678±1.6mg/ml and 1.25±0.06mg/ml respectively. Percentage of yield of ulvan obtained was 10% and its UV, IR spectral studies were performed. Total uronic acid was found to be 18.8%w/w. The antiproliferative assay using the eukaryotic model organism Dictyostelium discoideum showed that the ulvan is a good inhibitor of cell growth. The inhibition of cell growth by ulvan 4,5,6 µg/ml were found to be 23,19,15X10^4 cells/ml on 4 th day. Conclusion: Ulva lactuca have been used in medicine due to various biological activities and as a food. This study indicates that the ulvan possesses potential anti mitotic and anti proliferative activity. The presence of ulvan and the attributed reported anti-oxidant activity appears to contribute to its activity. Further investigation requires to confirm this activity.

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INTRODUCTION

Marine plants are the most important source of life saving drugs and have been widely used for the treatment of diseases in traditional way for several years [1]. An interaction between ancient medicine and biotechnological tools is to be established towards newer drug development. The interface between cell biology, structural chemistry and in vitro assays will be the best way available to obtain valuable leads. The value of marine plants lies in the potential access to extremely complex molecular structure that would be difficult to synthesize in the laboratory. In spite of an increasing awareness and expenditure of resources, the incidence of chronic diseases like cardiac, cancer, diabetes etc. has not declined and in fact is rising at an alarming rate. Cancer may be the most feared disease of our time and the number of deaths continues to increase steadily. Marine plants especially sulphated polysaccharide derived from them represent a vast potential resource for anticancer drugs and continue to be subject to extensive screening worldwide in an attempt to develop still more effective anticancer treatment .[2]

Ulva lactuca L. (Family: Ulvaceae) has been used in medicine due to various biological activities includingantiulcer, anticancer, antibacterial, antiviral, laxative, antifungal, anti-inflammatory, anti-oxidant activity, anti-coagulant activity, antinociceptive, antiperoxidative, antihyperlipidemic, hepato protective, antiprotozoal, leishmanicidal activities, hypercholesterolemia, immunostimulant activities and anti-adhesive properties. The rich composition of sulphated polysaccharide in Ulva lactuca appears to contribute to their biological potential. Previous chemical investigations have shown the presence of alkaloids, carbohydrates, sterols, saponins, tannins, proteins and amino acids, mucilage, flavonoids. Most of the therapeutic properties of this alga are attributed to sulphated polysaccharide ulvan that has received considerable attention due to their pharmacological effects namely antioxidant activity[3].Ulvan is one of the widely studied phytochemical with demonstrated health potential due to its antioxidant, anticancer, and anti-inflammatory properties [4].

MATERIALS&METHODS

Dictyostelium discoideum ,SM/5 medium, 0.7%DMSO, Std drug, Micro pipette, Peptone water (10g peptone, 10g glucose, 5g Nacl), Falcon tubes,Remi ultra-centrifuge, incubatore, Glass spreader, Petridish, Stereo Microscope(Olympus), Haemocytometer, Autoclave, Laminar air flow.

Collection and authentication of the marine green alga U.lactuca

Thallus of the seaweeds Ulva lactuca linn. selected for our study was collected from Rameshwaram coastal area near Uppoor, Ramanathapuram District, Tamil Nadu, India during the month of July 2015 and was authenticated by Dr.M.Ganesan, Scientist of Marine Algal Research station, CSMCRI (Central Salt & Marine chemicals Research Institute) Mandapam Camp, Tamil Nadu, India, Dr.Stephen, Department of Botany, American college, Madurai and Dr.Sasikala Director (Retd) of Siddha Central Research Institute, Arumbakkam, Chennai.

ISOLATION OF ULVAN

The algae were washed under tap water thoroughly to remove the foreign particals and air dried under shade. Then dried algal flakes were pulverized. The polysaccharides were firstly isolated by steeping dried Ulva lactuca powder (50gm) in 1 L of distilled water for 3 hours at 80^{o}C. The aqueous extract was then clarified by centrifugation at 10,000 rpm at 4^{o}C for 20 minutes and subsequent filtration through filter paper No.3 to remove insoluble materials. The polysaccharide in the aqueous extract was precipitated with two volumes of ethanol and recovered by filtration. Then the polysaccharide was precipitated [5].

Preliminary phytochemical screening

Preliminary phytochemical screening was carried out using appropriate solvent extract of the alga to identify the presence and absence of various phytoconstituents like alkaloids, carbohydrates, sterols, etc., [6, 7]

Identification of ulvan

Ulvan was identified by using UV_VIS Spectroscopy and IR spectroscopy[8,9].

Determination of total phenolic content

The total phenolic content in EEUL was determined spectrophotometrically by Folin-Ciocalteu method [10].calibrating against gallic acid standards and expressing the results in gallic acid equivalent and defined as mg gallic acid /L.

Determination of total flavonoid content[11]

The flavonoid content of EEUL was estimated by aluminium chloride method. In this method, aluminium chloride complexes with flavonoids of C3-C5 hydroxyl group and to produce intense colour in acidic medium. The intensity of the colour is proportional to the amount of flavonoids and can be estimated as quercetin equivalent at wavelength of 415nm.

Determination of uronic acid

Samples containing up to 200 nmol of glycosyluronic acid are dissolved or suspended in 0.4 ml of water or dilute acetate buffer in small glass tubes, and 10 ml of 4 M sulfamic acid-potassium sulfamate (pH 1.6) is added and mixed thoroughly. Analytical grade (96.4% assay) H2,SO4, containing 75 mM sodium tetraborate (2.4 ml) is then added, and the solution is stirred vigorously by vortex mixing. The solutions are heated to near 100°C for 20 min in a boiling water bath with the tubes capped with marbles. The tubes are placed in an ice bath to quickly cool the reaction mixtures to ambient temperature. After cooling, 80 µl of 0.15% (w/v) m-hydroxydiphenyl in 0.5% (w/v) NaOH is overlaid and then stirred in vigorously by vortex mixing. The pink colour develops to completion in about 5 to 10 min and is stable for about 1 h before fading results in some loss in colour. Absorbance is read at 525 nm or, if browning is still observed, the reaction mixture can be scanned from 400 to 700 nm and the peak absorbance can be integrated after subtraction of background from 430 to 650 nm. [12]

ANTIPROLIFERATIVE ACTIVITY [13]

In a agar plate 0.5- 1 ml klebsiella culture media was spread over the plate using glass spreader followed by Distyostelium discoideum AX2 strain spores and allowed to dry and kept at 22^0C in incubator. Allowed to grow for 2-5 days, Dictyostelium discoideum to form a lane with clear the bacterium, Harvested by centrifugation at 185 rpm .These cells were then used to prepare 5 $x10^4$ cells/mL cultures which were transferred into Falcon tubes. These cell cultures were then treated with different concentration of Ulvan samples. Cisplatin (3mM/ml) was used as a standard drug.All these cultures were grown with shaking at 185 rpm at 21°C. A positive control (lacking plant extract sample) for normal cell growth was also grown. All the cultures prepared contained 0.7% DMSO. An aliquot of each culture was removed at 24 h interval (up to120 h) and the cells were counted using a hemacytometer. Growth rates for different cultures were then plotted as cell number versus time. Maintain aseptic condition throughout procedure.

RESULTS

- Preliminary phytochemical screening of appropriate solvent extract of the alga showed the presence of alkaloids, carbohydrates, sterols, saponins, tannins, proteins and amino acids, mucilage, flavonoids and absence of volatile oil, fixed oils.
- Percentage of yield of ulvan obtained was 10%.
- UV spectrum of ulvan showed the maximum absorption at360,200 nm.
- IR Spectrum of Ulvan showed carboxylate structure group at 1610(sharp) and 1429 (weak), Sulphate ester at 1257, C- O Strecting of Rhamnose & Glucuronic acid maximum absorption band at 1055, Sugar cycle at 848 and 792.
- Total phenolic content was found to be 13.678 ± 1.6 mg/ml.
- Total flavonoid content was found to be1.25 ± 0.06 mg/ml.
- The content of uronic acid was determined and found to be 18.8 % w/w.
- Anti Proliferative Activity using D. discoideum (Table-1).
  Anti Proliferative Activity of Ulvan on D.discoideum (Figure-1).

DISCUSSION

Antiulcer, anticancer, antibacterial, antiviral, laxative, antifungal, anti-inflammatory, anti-oxidant activity, anticoagulant activity, antinociceptive, antiperoxidative, antihyperlipidemic, hepato protective, antiprotozoal, leishmanicidal activities, hypercholesterolemia, immunostimulant activities and anti-adhesive effect of seaweed Ulva lactuca have been reported[14-23]. It is reported recently that, the polysaccharides isolated from seaweeds have become a matter of great interest for cancer therapy. The mechanisms of their anticancer activity are related to their ability to suppress the growth of cancer cells. Cytotoxic or cytostatic effects, to enhance the immune responses, and to inhibit tumor angiogenesis[13].Polysaccharide isolated from Ulva lactuca have anti tumor activity.[14].Presence of alkaloids, flavonoids, carbohydrates, proteins etc were confirmed. Total phenolic and flavonoid content was found to be 13.678 ± 1.6 mg/ml and 1.25 ± 0.06 mg/ml. Ulvan was isolated (10%),it showed the presence of a majority of 4-O-(β-D-glucuronsyluronic acid)-L-rhamnose and small quantities of two other aldobiouronic acids tentatively identified as 3-O- and 4-O-(D-glucuronosylronic acids-D-xylose. Previous report of acidic tetrasaccharide identified as D-glucuronic acid-(1→4)-L-rhamnosyl (1→3/4)-D-glucuronosyluronicacid-(1→3) D-glucose, obtained after partial acid hydrolysis of desulfated and carboxy-reduced Ulvan, demonstrated the association of the three different sugars in the polysaccharide [24]. Total uronic acid was determined. It is reported that the ulvan has a anti tumoral activity. [25, 27]

The results of cell growth inhibition clearly showed the dose depended antiproliferattive effect by ulvan. It is assumed that this antiproliferative effect may be due to the polysachaide ulvan and antioxidant activity. So it is concluded that U.lactuca possesses antiproliferative activity without toxicity. Further studies needed to fully delineate the part they play in cancer and molecular mechanism to understand clearly. It is ongoing work in our laboratory and soon we will find systematic explanation of mechanism of action. Further investigation on advanced system, animal model and clinical trials are required to obtain drug leads.

TABLE-1: ANTI PROLIFERATIVE ACTIVITY USING D. discoideum.

DAYS	CELL GROWTH X10^4 (Cells/ml)				
	CONTROL	CISPLATIN 3mM/ml	ULVAN 4µg/ml	ULVAN 5µg/ml	ULVAN 6µg/ml
0	5	5	5	5	5
1	18	7	12	10	8
2	23	8	13	11	10
3	50	10	17	14	11
4	126	13	23	19	15

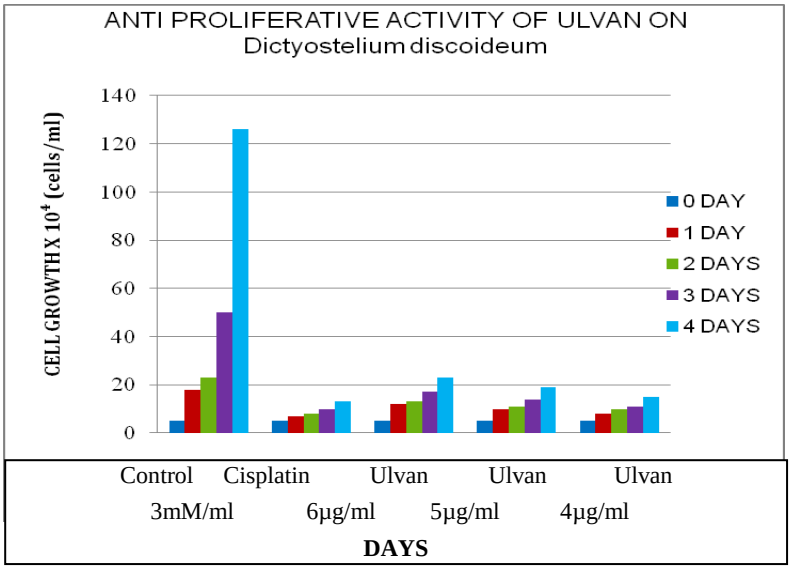

FIGURE-1.

Conflict of interest statement

We do not have any conflict of interest.

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