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IN-VIVO ANTICANCER ACTIVITY OF RED ALGAE (*GELIDIELA ACEROSA* AND *ACANTHOPHORA SPICIFERA*)

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ABSTRACT: The Gulf of Mannar, is a unique marine habitat with diverse of macroalgae. Macroalgae are primitive non flowering marine plants, which have rich sources of bioactive compounds (i.e., proteins, peptides, amino acid, polysaccharide, alkaloids etc). The selected red algae *Gelidiela acerosa* and *Acanthophora spicifera* collected from the Gulf of Mannar, southeast coast of India. In the present study, the anticancer potential of the methanol (crude) extract from *G. acerosa* and *A. spicifera* was tested for probable anticancer activity in Dalton's Ascitic Lymphoma (DAL) cells. The cells were tested in Swiss albino mice. The results show that *G. acerosa* and *A. spicifera* algae extract were the most effective against DAL cells in mice respectively had significant anticancer activity and it might be a good candidate for further investigation in order to develop a natural compound as an anticancer agents, which can be utilized for the production of potential anticancer drug and novel pharmaceutical leads.

INTRODUCTION: Cancer is one of the most dangerous threats to human being in the world. Chemotherapy is the only standard remedy for cancer treatment. Most of the anticancer drugs currently used in chemotherapy are cytotoxic to normal cells and cause immunotoxicity; this affects not only the tumor development, but also aggravates patient's recovery. The discovery and identification of new anticancer drug with low side effects on immune system have become an essential purpose in much research in immunopharmacology [1].

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Hence, so many researches were interested to finding new drugs from terrestrial plants, marine organisms/microorganisms including marine macroalgae etc. Now a day, drug discovery has been developed greatly in finding a pure organic compounds or crude extracts to provide new lead. Marine algae have been historically an extremely rich source of pharmacologically active metabolites with antineoplastic, antimicrobial and antiviral effects [2, 3]. And various biological effects are found in marine algae [4].

The Marine macroalgae are ecologically and commercially important to many regions in the world, especially in India. They are a valuable food resource which contains low calories and rich vitamin, minerals, proteins, polysaccharides, steroids and dietary fibers [5, 6]. They were also considered as important as traditional remedies [7]. Macroalgae have been one of the richest and most

prospective sources of bioactive metabolites [28] and their discovery has significantly prolonged [7, 8]. The algae are synthesizing a variety of compounds such as Carotenoids, Terpenoids, xanthophylls, chlorophyll, vitamins and amino acids etc [9].

Macroalgae act as allelopathic, antimicrobial, antifouling and herbivore deterrents, or as ultraviolet screening agents [10]. They are also used by the pharmaceutical industry in drug development to treat diseases like cancer, Acquired Immune-Deficiency Syndrome (AIDS), inflammation, pain, arthritis, infection for virus, bacteria and fungus [11]. Currently, algae represent about 9% of biomedical compounds obtained from the sea [12].

The low side effects of bioactive compounds were isolated from natural resources; scientists are interested in working on them to finding new medications. Finding anticancer agents from plant sources started in the earliest 1950s with the discovery and development of vinca alkaloids, vinblastine and vincristine and the isolation of the cytotoxic podophyllotoxins [13]. Marine algae are one of the natural resources in the marine ecosystem. They contain various thousands of compounds have been isolated from macroalgae populations [14]. Recently the marine algae contained antiviral [15], antibacterial, antifungal [16] and antitumor[4] potentials, among numerous others. And then the polysaccharides and peptides compounds were isolated from macroalgae have become a substance of great interest for cancer therapy. The mechanisms of their anticancer activity are related to their ability to suppress the growth of cancer cells [17].

Previous studies have proved that macroalgae possess broad range of biological activities such as antibiotics [18]. Since, the demand for screening of natural bioactive compounds has widened in interest of research. To date, research on anticancer activity substance from red algae species is rather and limited of Gulf of Mannar, southeast coast of India. Therefore the present study was aimed to evaluate in anticancer activity of marine red algae against Dalton's Ascitic Lymphoma (DAL) cells in mice. This preliminary research studies reported here in could serve as a basic to isolated and identify the anticancer compounds from red algae extracts as a source of natural anticancer agents for pharmaceutical leads.

MATERIALS AND METHODS:

Preparation of algae extract: The *G. acerosa* and *A. spicifera* belongs used in this study to red algae. The macroalgae were collected freshly from Gulf of Mannar, Southeast coast of India (Long N 9°16.313 Lat E 79°00.073). The collected samples were immediately rinsed with water to remove all kind of epiphytes. The macroalgae was shade dried at room temperature for 3-4 days. The shade dried macroalgae was powdered individually and used for experiment. The powdered samples (15.0 g) were soaked in 100 mL methanol for 2 days at room temperature twice, then filtered and evaporated under reduced pressure below 40°C. The crude samples were subjected to anticancer assay with DAL cells bearing in mice.

Acclimation of animal: The healthy adult male Swiss Albino mice (20-25g) sizes were used throughout the experiments. the experiment room was maintained at micro nylon boxes at suitable environmental condition i.e., temperature at 25 ±2 °C and 12 hours light / dark cycles with standard laboratory diet and water *ad libitum* [19]. This study was conducted after obtaining institutional ethical clearance (Proposal Number: K. Durai/ MKU/IAEC/KMCP/60/2012) as per standard practice. After sufficient period of acclimatization, they were used to anticancer activity experiment.

Grouping of animals: Swiss albino mice were divided into five groups of each six mice. Four groups (G_2-G_5) of animals were injected with DAL cells (1×10^6 cells/mouse) intraperitonealy and remaining one group (G_1) was treated as a normal control group [20].

- Group 1 served as Normal Control (G_1)
- Group 2 served as Cancer Control (G_2)
- Group 3 served as Positive Control, was treated with injection flurouracil at 20mg/kg body weight, oral route (G_3) [21]
- Group 4 served as Treatment Control, which was treated with methanol extract of

G. acerosa at 200mg/kg body weight, in i.p, (as per LD_{50} value) (G_4).

- Group 5 served as Treatment Control (G_5), which was treated with (methanol extract of *A. spicifera*) at 200mg/kg body weight, in i.p, (as per LD_{50} value).

In the present investigation, treatment was given after 24 hrs inoculation, once daily for 14 days. At the end of 15th days sacrificed in all five groups was observed. The blood was withdrawn in all mice separately by Retro-Orbital plexus methods and the collected blood was stored in refrigerator at 4°C [27].

Cancer cell count: The fluid (100 µl) from the peritoneal cavity of each animal was withdrawn by sterile syringe and diluted with 800µl of ice cold normal saline or sterile phosphate buffer solution (PBS) and 100µl of tryphan blue and total numbers of the living cells were counted using haemocytometer [22].

Total number of cells per µl = average no of cell × dilution factor 2×10^4

Animal weight: All mice were weighted from the beginning to 15th day of the experiment; average increase in body weight on the 15th day was calculated.

Life span (%): The effect of methanolic extract of *G. acerosa* and *A. spicifera* on cancer cells growth and the life span (%) were calculated as follow.

ILS (%) = [(Mean survival of treated group/Mean survival of control group)-1] × 100

Mean survival time (MST) = [1st Death + Last Death] / 2

Haematological parameters: Such as WBC, RBC, and Platelet count and haemoglobin count of all five groups were analysed (pentra-120 Automated Hematology Analyzer). Similarly; Total cholesterol (TC), Triglycerides (TG), Aspartate amino transferase (AST), Alanine amino transferase (ALT) and Alkaline phosphatise (ALP) were analyzed the blood serum.

- All biochemical investigation was done by using COBAS MIRA PLUS-S Auto analyzer from Roche Switzerland.
- Haematological test was carried out in COBAS MICROS OT 18 from Roche
- Biochemical investigation of blood sample were analysed in added Hi-Tech instruments MAX-MAT used for auto analyzer.

Statistical analysis: The total experimental results were expressed as the mean ± S.E.M. The haematological and biochemical parameters were subjected to statistical analysis by one way analysis of variance to determine the significant difference between the groups. ANOVA was done with graph pad prism software. The data were accepted as statistically significant different was obtained at $p< 0.05$.

RESULTS: The intraperitoneal inoculation of DAL cells in the mice produces increased proliferation of cells. The methanol extract of *G. acerosa* and *A. spicifera* samples was reduced the cancer cell count to $1.88\pm0.30\times10^6$, $1.80\pm0.25\times10^6$ cells in the treated mice. The methanol extract of *G. acerosa* and *A. spicifera* treated mice survived upto 35 days where as cancer control mice survived upto 20 days only. And packed cell volume in cancer control mice was found 31.40±3.25% to be high.

The oral dose administration of Methanol extract of *G. acerosa* and *A. spicifera* extract had reduced the Packed Cell Volume 22.40±1.70% and 23.26±1.85% respectively. Whereas the methanol extracts of *G. acerosa* and *A. spicifera* at the dose of 200mg/kg body weight increased by 82% and 84% respectively.

Therefore, the extract treatment was reduces the tumor weight and hence increased the life span of cancer induced mice (**Table 1**).

TABLE 1: THE EFFECT OF *G. ACEROSA* AND *A. SPICIFERA* EXTRACT ON THE LIFE SPAN, BODY WEIGHT AND CANCER CELL COUNT OF CANCER INDUCED MICE.

Treatment	Number of animals	% Life Span	Increase in Body Weight grams	Cancer cell Count (mL×10^6)
G_1	6	>>30 Days	2.30±0.62	-
G_2	6	48%	7.80±0.96*	2.70±0.40*
G_3	6	92%	3.85±0.70**	1.45±0.22**
G_4	6	82%	4.25±0.88**	1.88±0.30**
G_5	6	84%	4.30±0.086**	1.80±0.25**

G_1-Normal Control, G_2-Cancer Control, G_3-Standard, G_4-Methanolic extract of *G. acerosa*, G_5-Methanolic extract of *A. spicifera*. All data vale are mean ± S.E.M (n=6); *Values are significantly different from normal control (G1) at P < 0.01; **Values are significantly different from cancer control (G2) at P < 0.01

On the subject of haematological parameters, cancer control mice showed reduced RBC count but also increased WBC count than normal group. The treatment of methanol extract *G. acerosa* and *A. spicifera* also raised the RBC count significantly to 3.35±0.68 mill/cumm and 3.15±0.60 mill/cumm respectively. Similarly both are restored, the WBC value to 12.22±1.90 cells/mL × 10^3, 12.10±1.65 cells/mL × 10^3 respectively. Haemoglobin content in cancer control mice reduced significantly when compared with normal group. But, the methanol extracts of *G. acerosa* and *A. spicifera* doses were increased Hb contents in 10.40±1.32 gm/dL, 10.25±1.05 gm/dL. The methanol extract *G. acerosa* and *A. spicifera* restored the normal platelet count in treated mice **(Table 2)**.

TABLE 2: THE EFFECTS OF *G. ACEROSA* AND *A. SPICIFERA* EXTRACT ON HAEMATOLOGICAL PARAMETERS

Treatment	*WBC cells/mL× 10^3*	*RBC Count mill/cumm*	*Hemoglobin gm/dL*	*PCV%*	*Platelets lakh/cumm*
G_1	10.65±1.60	4.60±0.96	12.65±1.30	14.40±2.45	3.46±0.96
G_2	14.45±2.50*	2.32±0.30*	7.36±0.92*	31.40±3.25*	1.75±0.62*
G_3	11.40±1.85**	4.10±0.88**	11.30±1.45**	18.40±1.50**	2.80±0.96**
G_4	12.22±1.90**	3.35±0.68**	10.40±1.32**	22.40±1.70**	2.15±0.80**
G_5	12.10±1.65**	3.15±0.60**	10.25±1.05**	23.26±1.85**	2.30±0.92**

G_1-Normal Control, G_2-Cancer Control, G_3-Standard, G_4-Methanolic extract of *G. acerosa*, G_5-Methanolic extract of *A. spicifera*. All values are expressed as mean ± S.E.M (n=6); *Values are significantly different from normal control (G_1) at P < 0.01; **Values are significantly different from cancer control (G_2) at P < 0.01.

The inoculation of DAL cells caused significantly increase in the level of total cholesterol, TGL, AST, ALT and ALP in the cancer control animals (G2), when compared to the normal group (G1). The treatment with *G. acerosa* and *A. spicifera* at the dose of 200mg/kg body weight reversed these changes towards the normal level. All the values were found to be significant. However the treatment with standard 5- FU at the dose 20 mg/kg body weight also produced better result in all parameters (**Table 3**).

TABLE 3: THE EFFECTS OF *G. ACEROSA* AND *A. SPICIFERA* EXTRACT ON BIOCHEMICAL PARAMETERS

Treatment	Cholesterol (mg/dL)	TGL (mg /dL)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
G_1	115.30±3.70	135.60±2.55	41.65 ±1.35	34.50 ±1.55	127.40 ±2.45
G_2	144.90±4.65*	220.40±4.85*	88.6±2.70*	62.45±2.70*	240.45±4.36*
G_3	120.50±3.90**	165.65±2.45**	56.48 ±1.85**	43.50±1.85**	160.45±2.55**
G_4	123.55±4.15**	175.30±2.85**	64.50±1.95**	47.36±1.98**	192.65±2.70**
G_5	120.50±3.25**	172.60±2.60**	62.65 ±2.35**	46.30±1.75**	190.42±2.35**

G_1-Normal Control, G_2- Cancer Control, G_3- Standard, G_4-Methanolic extract of *G. acerosa*, G_5-Methanolic extract of A. *spicifera*. All values are expressed as mean ± S.E.M (n=6); *Values are significantly different from normal control (G_1) at P < 0.01; **Values are significantly different from cancer control (G_2) at P < 0.01

DISCUSSION: The present study shows, the methanol extract of *G. acerosa* and *A. spicifera* significantly inhibited the cancer volume, packed cell volume, cancer cell (visible) count and haematological parameters in normal levels. Intraperitoneal inoculation of DAL cells in the mice produces huge cells of cancer count, which indicate development of cancer in the mice. The consistent criterion for judging the anticancer effect of methanol extract samples was reduction in viable cell count towards normal. It may be due to stimulate the immune cells activity [23].

The reliable form for judging the value of any anticancer drug is the continuation of lifespan of the animal and decreased WBC count level. The reduce RBC or haemoglobin percentage in tumor bearing mice may be due to iron deficiency (anaemia) or due to haemolytic or myelopathic conditions [24]. Usually, myelo suppression and anaemia are the major problems encountered in cancer chemotherapy [25]. The treatment of *G. acerosa* and *A. spicifera* brought back the haemoglobin content, RBC and WBC count level is significantly. The haematological parameter results are clearly indicated the *G. acerosa* and *A. spicifera* possess protective action on the hemopoietic system. The previous report shows that the presence of cancer in the human body or in the experimental animals is known to affect may function of the liver.

The significantly elevated level of total cholesterol, TG, AST, ALT, ALP in serum of cancer inoculated animal indicated liver damage and defeat of functional reliability of cell membrane [26]. Haematological and biochemical observed in cancer bearing mice by *Chondrococcus hornemanni* and *Spyridia fusiformis* extract was effective in inhibiting the tumor growth in ascetic tumor model [22].

The biochemical assessment of DAL inoculated animals showed obvious changes indicating the toxic effect of the cancer. The normalization of these effects observed in the serum treated with methanol extracts red algae *G. acerosa* and *A. spicifera* at a dose of 200mg/kg body weight supported the potent anticancer effects.

CONCLUSION: The present studies were showed a decrease in cancer cell count as a confirmatory evidence for protection against DAL bearing mice. Consequently increased life span was observed with methanol extracts treated mice. The haematological and biochemical variation observed in cancer bearing animals in this study may be due to the reduction level of cancer proliferation. Similarly, administration of red algae *G. acerosa* and *A. spicifera* extracts significantly alters this level in cancer-bearing animals. Thus, from the above observations on other parameters it was concluded that the red algae methanol extract of *G. acerosa* and *A. spicifera* possesses anticancer activity against DAL bearing mice.

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