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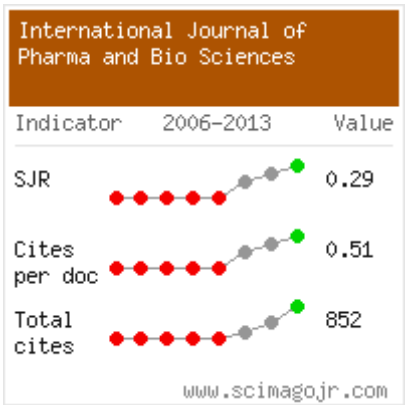

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ANTI-LEUKEMIC STUDY OF ETHANOLIC EXTRACT OF *SARGASSUM ILICIFOLIUM* IN HL-60 CELL LINE INDUCED SWISS ALBINO MICE

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ABSTRACT

This study was conceived to screen the anti-leukemic activity of Ethanolic extract of S*argassum ilicifolium i*n HL-60 cell line induced Swiss albino mice. In order to achieve this goal, the Anti-Leukemic activity of 80% ethanolic extract (200 mg/kg) body weight of *Sargassum ilicifolium* was evaluated against HL-60 tumor cell lines in mice. After the 5th day of tumor inoculation, the extract was administered daily for 14 days. Mice were sacrificed for observation of antitumor activity. The effect of *Sargassum ilicifolium* on the body weight of HL-60 bearing mice and simultaneous alterations in the hematological profile and liver biochemical parameters (lipid peroxidation, GSH, SOD and Catalase) were estimated. The *Sargassum ilicifolium* treated groups showed that the decrease in body weight of HL-60 bearing mice and the hematological profile reverted towards normal levels in extract treated mice. Treatment with ethanolic extract of *Sargassum ilicifolium* restored the serum biochemical parameters towards normal levels and decreased the levels of lipid peroxidation and increased the levels of reduced glutathione, SOD and Catalase. The ethanolic extract of 80% w/v *Sargassum ilicifolium* exhibited antitumor effect by modulating lipid peroxidation and augmenting antioxidant defense system in HL-60 cell line induced mice.

KEYWORDS: ***Sargassum ilicifolium,*** **80% w/v Ethanolic extract, HL-60 cell line, Anti-leukemic activity GSH, SOD, Glutathione and Catalase**

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INTRODUCTION

Cancer is the second leading cause of death worldwide next to cardiovascular diseases. Conventional therapies including chemotherapy and radiation therapy pose serious side effects and at best, merely extend the lifespan by a few years. There is thus an increasing demand to utilize alternative approaches in cancer chemoprevention [1]. Dietary habits and nutrition are known to play an important role in the prevention of cancer. Numerous epidemiological studies have shown that a diet high in vegetables and fruits is associated with reduced risk of most cancers [2]. Brown algae consist of various biological active substances which are characteristic to their chemical properties, of these polysaccharides and lipids from *Sargassum* species in particular have biological activities like anti-tumor activity and also used in the treatment of treating goiters, testicular pain, swelling, edema, urinary infections and sore throat [3, 4]. The present research work highlight the new innovations of research on marine drug *Sargassum ilicifolium* for novel and potent biomolecules. In developing nations like India people depend upon botanical medicines for their basic health care needs. At the same time herbal medicine is becoming popular due to its free toxicity and side effects. Hence the marine drug *Sargassum ilicifolium* which prove the claim on scientific basis can open new vistas for using it as a phytomedicine in the treatment of leukaemia..

MATERIALS AND METHODS

Plant collection and authentication

The plant material of whole part used for the investigation was collected from Chennai, kovalam in the month of November 2006. The plant was identified and authenticated by Prof. P. Jayaraman. Ph.D., Plant Anatomy Research Centre (PARC) Medicinal Plant Research Unit NO. 4-II street, Sakthi Nagar, West Tambaram, Chennai 600 045.

Preparation of extracts

The seaweeds were a shade dried and coarsely powdered. About 1 kg powdered drug was extracted successively by maceration with different solvents like hexane, chloroform, ethyl acetate and 80% ethanol. After 72 hrs of maceration it was filtered. The marc was dried each time before extraction with another solvent. After complete extraction, the extracts were concentrated by distilling off the solvent and then evaporated to dryness on a water-bath. Colour of the extracts was observed and percentage yield was calculated on the
air dried basis.

Animals

Swiss *albino* mice (18-25g) were procured from Tamil Nadu veterinary college, Chennai, India. They were housed in standard micro loan boxes with standard laboratory diet and water ad libitum. The experimental protocol was approved by the institutional animal ethics committee (IAEC) Vels University, Chennai, India.

Procedure Mice were divided into 5 groups

S.no	Groups	Type of Extract
1	Group I (Normal Control)	Animal was provided with food and *ad libitum*
2	Group II (Cancer control)	Animals were inoculated with HL-60 cell lines 1×10^6 cells/animal
3	Group III (Standard)	Received 5-Flurouracil 20mg/kg (*IP*)
4	Group IV (EAESI)	Ethanolic extract (200mg/kg) body weight (Orally)
5	Group V (EESI)	Ethanolic extract (200mg/kg) body weight (Orally)

Induction of cancer [5]

HL-60 cell lines were initially purchased through the courtesy of Amala cancer institutes, Kerala, India. They were maintained by weekly intraperitoneal (*IP*) inoculation of $1x10^6$ cells/mice.

Effect on mean survival time (MST) and average body weight of tumor bearing mice [6]

Animals were inoculated with 1×10^6 cells / mice on '0' day and the drug treatment was

started on the 5th day of tumor cell line inoculation and all treatments were continued for 10 days. The animals were subjected to the analysis of mean survival time (MST) of each group (n=6) and changes in body weight. The tumor efficacy was compared between groups of animals treated with different extracts of *Sargassum ilicifolium* and cancer control. MST was noted with reference to the control. Survival time of the treated group was compared with those of the control groups (C) using the following formula and results are shown in [Graph.no.1].

Increase in life span ILS (%) = T-C/C x 100

Where T = Number of days treated animals survived and C = Number of days control animals survived.

Effect of different extracts of Sargassum ilicifolium Hematological parameters of HL-60 bearing mice [7]

In order to detect the influence of drugs on the hematological status of cancer cell lines bearing mice, comparison was made amongst groups of mice on the 10th day after inoculation. Blood was drawn from each mice in the conventional way and the white blood cell count, red blood cell count, hemoglobin, and packed cell volume were determined. The ascetic fluids were collected on the 11th day and smeared. The smear was stained with Giemsa stain for cytological studies.

Hemoglobin concentration of whole blood [8]

The concentration of hemoglobin was measured by the usual procedure using Shali's haemometer. The blood sample was drawn into the pipette up to the 20cumm mark and transferred to the rectangular cell containing a little amount of N/10 HCL placed in haemometer (Hellige Charlie homemaker No. 304-B, Hellige, USA). After 5 minutes, a color comparison was made with a standard color prism of haemometer. If the color of the solution was high, distilled water was added to this solution and mixed using a stirrer until a good color was obtained The final reading of the solution in the tube was noted. From the Corbett reading, hemoglobin in g/100ml of blood or its percentage was calculated.

R.B.C count

Blood was taken up to 0.5 marks in the RBC pipette and excess blood was wiped off from the pipette was the tip. The pipette was then filled to 101 marks with RBC diluting fluid. The RBC pipette was horizontally shaken and a drop of resultant mixture was discharged under the cover glass of a Neubauer counting chamber (Neubauer, Feinoptic, Germany). Number of erythrocytes in 80 small squares were counted under the light microscope[6,7] . The number of cells in 1 ml of undiluted blood was calculated using the standard formula and the reports are shown in [Graph; no. 2].

Erythrocyte count/ml = N×1×200/0.02

Where N = Number of cells in 80 small squares (Dilution)

W.B.C count

Blood was drawn up to 0.5 marks in the WBC pipette, diluted with WBC diluting fluid up to 11 marks and mixed properly. The resultant mixture was charged under the cover slip in the Neubauer chamber and the number of cells in four-corner block (each block is subdivided into 16 squares) was counted. The total leukocyte count per ml of blood was calculated by multiplying the average number of cells in the four blocks by 200.

Packed cell volume (PCV)

Using a Pasteur, the wintrobe tube was filled with blood, starting at its bottom and withdrawing the pipette as the tube is filled from below upwards. The blood column was brought to be 'O' mark. Air bubbles, if any were removed from the top of the column of blood so that it stands exactly at 'O'. The tube was centrifuged for about 20 minutes at 2560 RPM [8]. The reading of the packed cells was taken, the tubes again centrifuged for 5 minutes and reading was noted. Final reading was recorded when three consecutive readings were identical, i.e., when the red cells have been fully packed.

Effect of Ethyl acetate and 80% w/v Ethanolic extract of Sargassum ilicifolium on the HL-60 cell line induced mice [9, 10]

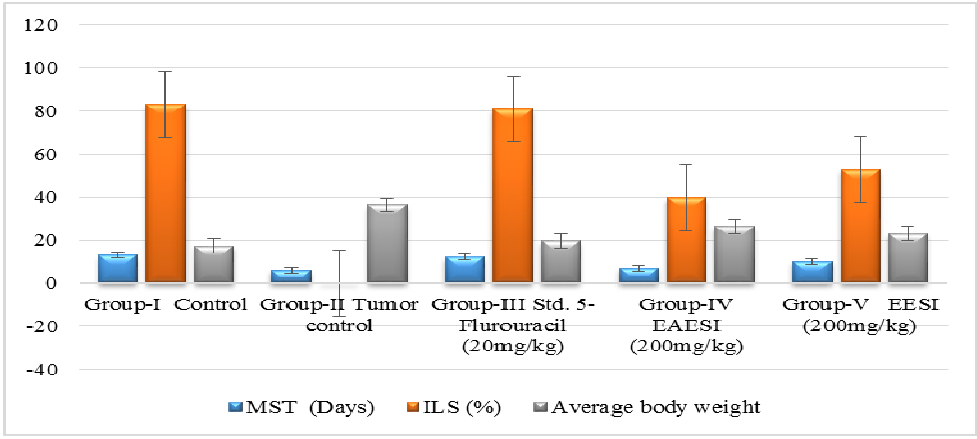
The liver was excised, rinsed in ice cold normal saline followed by. 15 m Tris-HCL (pH-7.4). Bottled and weighed, the homogenate was processed for estimation of lipid peroxidation, GSH, SOD and CAT. Assay for lipid peroxidation was carried out by the measurement of thiobarbituric acid reactive substances (TBARS) in the tissue the pink

chromogen produced by the reaction of malondialdehyde, which is a secondary product of lipid peroxidation reaction with thiobarbituric acid were estimated at 532nm. Reduced glutathione (GSH) in the tissue was assayed by the method of Ellman. GSH estimation is based on the development of yellow color when 5, 5-dithiobis (2-Nitro benzoic acid) dinitrobis benzoic acid was added to compounds containing sulphydryl group. SOD was assayed by the method of Kakkar. The assay was based on the 50% inhibition of formation of NADH-Phenazine methosulphate notable tetrazoliumat 520nm. The activity of CAT was assayed by the method of Abei. Proteins were estimated by the method of Lowry [11,] Using bovine serum albumin as a standard and the results are shown in [Graph; no. 3].

RESULTS

Graph 1

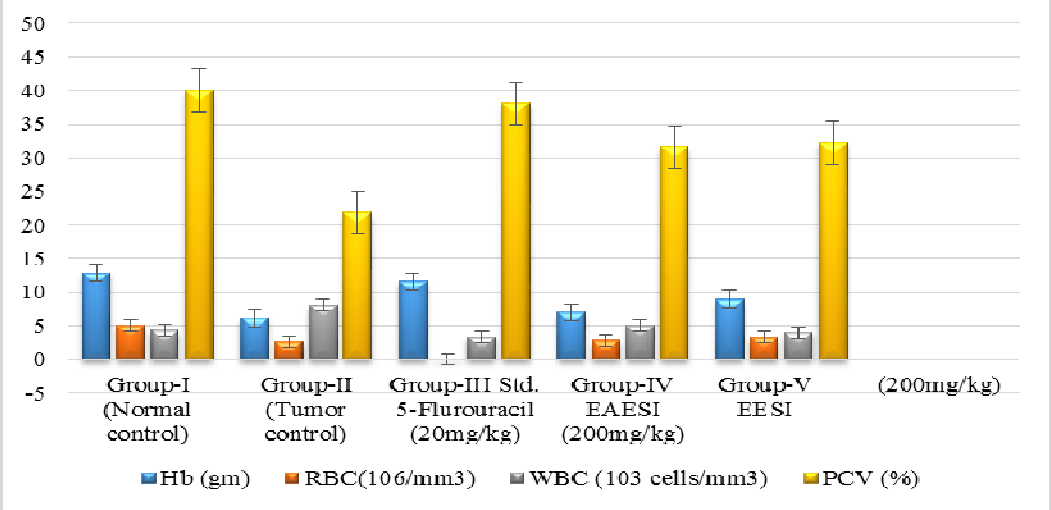
Effect of Ethyl acetate and 80% Ethanolic extract of Sargassum ilicifolium on Mean Survival Time (MST) and Average Body weight of HL-60 cell line bearing mice



MST= Mean survival time
ILS= Increase in life span EAESI= Ethyl acetate extract of Sargassum ilicifolium
EESI= Ethanolic extract of Sargassum ilicifolium
n=6 Animals in each group
****P<0.05, **P<0.01 when compared with control***
Values are expressed as mean± SEM

Graph 2

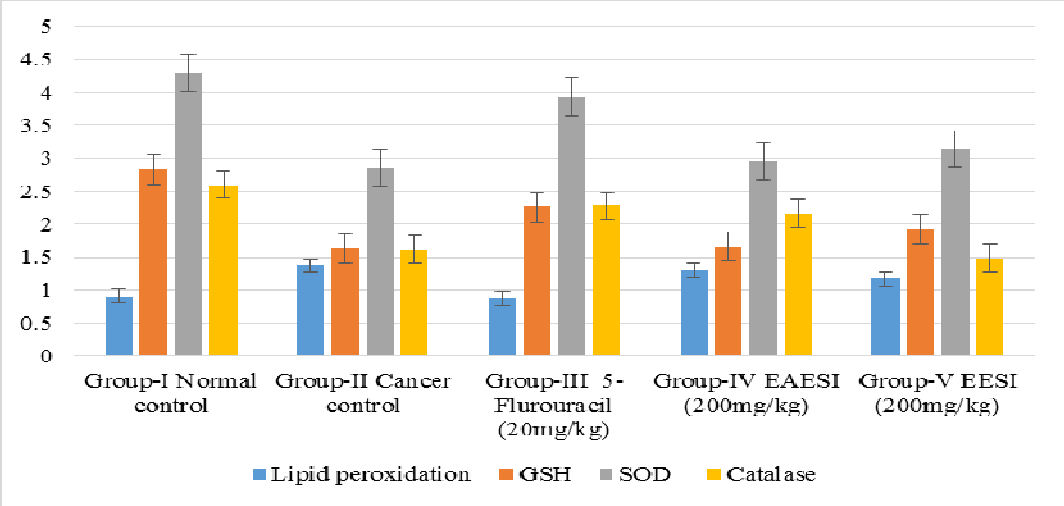
Effect of Ethyl acetate and 80% w/v Ethanolic extracts of Sargassum ilicifolium on Hematological parameters of HL- Cell line bearing mice



n=6 Animals in each group
****P<0.05, **P<0.01 when compared with control***
Values are expressed as mean± SEM

Graph 3

Effect of Ethyl acetate and 80% w/v Ethanolic extract of Sargassum ilicifolium on Biochemical parameters in HL-60 Cell line bearing mice



n=6 Animals in each group
**P<0.05, **P<0.01 when compared with control*
Values are expressed as mean± SEM

CYTOLOGICAL STUDIES OF HL-60 BEARING MICE

Figure 1

Tumor Control the cells are larger in size and showed binucleation

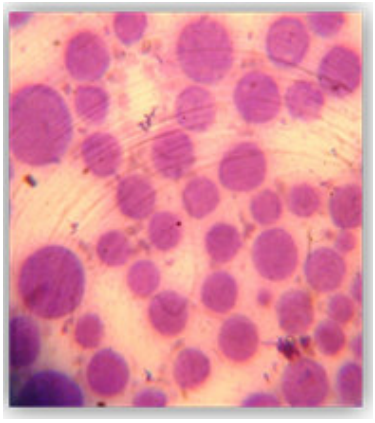


Figure 2

5-Flurouracil (20mg/kg) the cells showed the Plasmocytoid feature with varying degree of degeneration and cytoplasmic vacuolation Which is characteristic of immunoblast

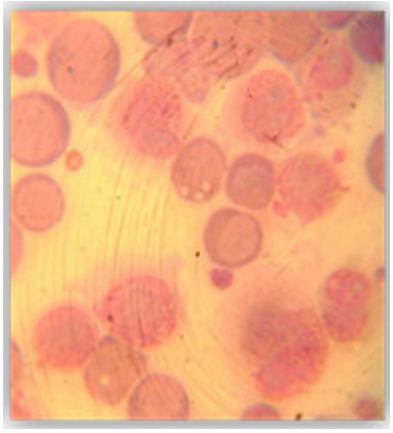


Figure 3
EAESI (200mg/kg) the cells showed, Plasmocytoid feature with varying degree of degeneration and cytoplasmic vacuolation Which is characteristic of immunoblast.

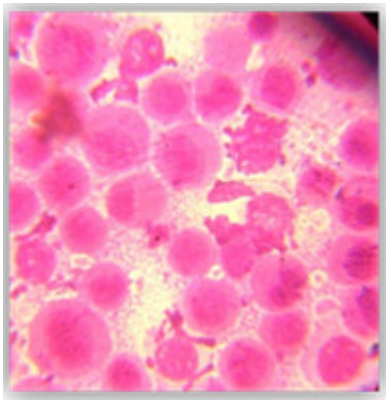
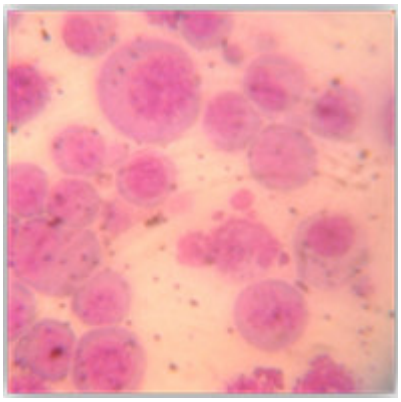


Figure 4
EESI (200mg/kg) the cells showed, Plasmocytoid feature with varying degree of degeneration and cytoplasmic vacuolation which is characteristic of immunoblast



DISCUSSION

The present study was carried out to evaluate the Anti-leukemic effect of 80% Ethanolic extract of *Sargassum ilicifolium* on HL-60 bearing mice. A significant enhancement of mean survival time, inhibition of the average body weight increase, minimum adverse effects on the evaluation of the hematological parameters showed a minimum adverse effect in mice treated with ethyl acetate and 80% w/v ethanolic extract of *Sargassum ilicifolium* after 14 days. The reliable criteria for judging the value of any anticancer drug is the prolongation of life span and decrease the WBCs from blood. The present study revealed the Anti-leukemic effect of ethyl acetate and 80% w/v ethanolic extract of *Sargassum ilicifolium* against the Cell line HL- 60 induced in Swiss *Albino* mice. The most common problems encountered in cancer chemotherapy are myelosuppression and anemia. Anemia occurring in tumor bearing mice is mainly due to iron deficiency or due to hemolytic or other myelopathic conditions. Tumor bearing mice treated with 80% Ethanolic extract of *Sargassum ilicifolium* showed restoration in hemoglobin content, RBC and WBC count near to normal, which indicated the protective action of the extract on the haemopoietic system. The antitumor activity of the extract was comparable to that of 5- Fluorouracil which is commonly used as an active antitumor agent in a vast series of preclinical and clinical studies. In Graph no. 1 the hematological study revealed that 80% EESI treated and subsequent anti-tumor inhibition resulted in appreciable improvements in hemoglobin content, RBC and WBC counts [Graph no. 2] compared with control group. The improvement in hematological profile of the HL-60 Cell line

bearing mice following the treatment with extract could be due the action of different phytoconstituents present in the extract. The levels of LPO, GSH, SOD and Catalase were summarized in [Graph no. 3]. The levels of lipid peroxidation in liver tissue significantly increased in HL-60 control mice (1.38 n moles MDA/g of tissue) as compared to the normal mice (0.91 n moles MDA/g of tissue). Treatment with 80% w/v ethanolic extract of *Sargassum ilicifolium* (200mg/kg) body weight significantly decreases the LPO levels at 1.30 and 1.17 n miles MDA/g of tissues. The GSH count in liver tissues of normal mice was found to be 2.83 mg/kg wet tissue. Inoculation of HL-60 drastically decreased the GSH content to 1.63 mg/g wet tissue. Whereas treatment with 80%w/v ethanolic extracts of *Sargassum ilicifolium* (200 mg/kg) GSH levels were reversed to normal (1.92 mg/g wet tissue) respectively. The SOD levels are lower in the HL-60 control mice was significantly decreased 2.85 units/mg proteins when compared with normal mice (4.30 units/mg proteins). Administration of the 80% Ethanolic extract of *Sargassum ilicifolium* significantly increased the SOD levels (3.14 units/mg proteins in tissues) at the dose level of 200mg/kg body weight. The biochemical assessment like LPO, GSH, SOD and Catalase good tool to measure the anti-leukemic activity of the targeted drug and also hemoglobin content, RBC and WBC count showed the significant role.

CONCLUSION

The present study proved that the oral administration of 80% Ethanolic extract of *Sargassum ilicifolium* at a dose level of 200 mg/kg body weight increased the life span with minimum adverse effect compare with ethyl acetate extract. The reliable criteria for judging the value of any anticancer drug is the prolongation of life span and decrease of WBC's from blood. The Anti-leukemic activity of 80% Ethanolic extract of *Sargassum ilicifolium* against HL 60 Cell line in Swiss albino mice was understood by a minimum adverse effect on the evaluation of its hematological parameters. Further the tumor bearing mice treated with 80 % w/v extract of *Sargassum ilicifolium* improvised hemoglobin content, RBC and WBCs count near to normal which indicated the protective action of the extract. Excessive productions of free radicals have also resulted in oxidative stress, which might cause lipid peroxidation in vivo, which cause degeneration of tissues. Glutathione a potent neoplastic inhibitors have a key role in the protection process. The 80% w/v administered Ethanolic extract of *Sargassum ilicifolium* reduces the elevated levels of lipid peroxidation and increased the glutathione amount in HL-60 cell line bearing mice. The inhibition of SOD and Catalase also revealed by the administration of 80 % Ethanolic extract of *Sargassum ilicifolium* indicates the antioxidant property of *Sargassum ilicifolium.* Phytoconstituents with antioxidant principle exhibit cytotoxicity towards tumor cells. The decreased level of lipid peroxidation and significant increasing in levels of GHS, SOD and Catalase in 80 % Ethanolic extract of *Sargassum ilicifolium* treated group indicates that *Sargassum ilicifolium* is a potential inhibitor of induced oxidative stress. The presence of steroids, tannins, phenolic compounds and alkaloids in the marine algae may be responsible for the potent antitumor activity which increased the life span of tumor bearing mice. Hence all the data through an insight into the possibility of developing an Ethanolic extract of *Sargassum ilicifolium* as a novel and potent chemotherapeutic agent.

REFERENCES

1. Amin A, Alkaabi A, Al-Falasi S, Dauod SA, Chemo preventive activities of *Trigonellafoenum graecum* (Fenugreek) against breast cancer. Int.J.Cell Biology, 29: 687 – 694, (2005).
2. Steinmetz KA, Potter JD, Vegetables, fruits and cancer prevention. A review. J of Dietetic Assoc, 96 (3): 1027 – 1087, (1996).
3. Hiroyuki Noda, Hideomi Amano, Koichi Arashima, Shinziro Hashimoto, Kazutosi Nisizawa, Antitumor activity of polysaccharides and lipids from Marine

algae. Nippon suisan Gakkaishi, 55 (7): 1265-1271, (1989).
4. Kim. K.N, Ham, Y.M, Moon, J.Y, Kim, M.J., Lee, W.J. and Hyun C.G, In vitro cytotoxic activity of *Sargassum thunbergii* and *Dictyopterisdivaricata*on the HL-60 Tumor cell line. International Journal of Pharmacology, 1 (4): 1-9, (2009).
5. Ecobichon DJ, The Basis of Toxicology Testing. CRC Press, New York, 43-86, 1997.
6. OECD (Organization of Economic Co-operation Development), The OECD guidelines for testing of chemical: 420 acute oral toxicity OECD. Paris, 1 (1): 14, (2001).
7. Suffness M, Doris, J Methods in cancer research Devita V.T (Ed). Academic Press, New York, 1978: 73 - 75.
8. Balasubramanian Rajkapoor, Marmuthusankari, Mohan sumitra, Jayaraman Anbu, Narayanaswami, Harikrishnan, Manavalan Gobinath, Venkatesan Suba, Ramachandran Balaji, Antitumor activity and cytotoxic effects of Phyllanthus polyphyllus on Ehrlichs Ascites Carcinoma and Human Cancer Cell Lines. Biotechnology and Biochemistry, 71 (9): 2177-2183, (2007).
9. Verma PS, Tyagi BS, Agarwal VK, Animal physiology Company Ltd. New Delhi, 395 – 403, (2002).
10. Lowry N.J, Protein measurement with the Folin phenol reagent. J. Boil. Chem, 193 (5): 265-275, (1951).
11. Malaya Gupta, Upal K. Mazumder, Pallab K. Haldar, Chandi C. Kandar, Laxmanan Manikandanand, Senthil G.P, Anticancer activity of Indigoferaaspalathoides and Wedelia calendulaceaein Swiss Albino Mice. Iranian Journal of Pharmaceutical Research, 6 (2): 141-145, (2005).
12. Turner RA, Screening methods in pharmacology. Academic Press, London, 1965: 61-65.
13. Kulkarni SK, Handbook of Experimental Pharmacology, 2nd Edn: 78-81, (1993).