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Original Article

Anticancer activity of methanol extract of *Jania Rubens* Linn. Against Ehrlich as-cites carcinoma induced Balb/C Mice

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ABSTRACT

Cancer is a malignant disease that is characterized by rapid and uncontrolled formation of abnormal cells which may mass together to form a growth or tumor or proliferate throughout the body. Next to heart disease cancer is a major killer of mankind. Present study aims at a preliminary phytochemical screening and anticancer evaluation of methanolic extract of *Jania Rubens* against Ehrlich Ascites Carcinoma in animal model. The alga Jania Rubens Linn was collected manually at the Devgad Island, near Karwar of the Arabian Sea during May. The powdered sample of alga was subjected to methanol extraction (maceration). The methanolic extract of *Jania Rubens* given orally to mice at the dose of 250 and 500 mg/kg body weight for 14 days caused significant ($p< 0.001$) reduction in body weight, packed cell volume and when compared to the mice of the EAC control group. All the values were expressed as mean ± SEM. Means of treated groups were compared with those of control group using one-way analysis of variance (ANOVA) followed by Turkey's test, control group is compared with normal group using Student's *t* test. The Methanol extract of *Jania Rubens* (250,500 mg/kg) has been showed significant anticancer activity as compared to standard cyclophosphamide in EAC induced cancer model.

Key words: *Jania Rubens;* Cytotoxicity; MEJ (Methanol Extract of Jania Rubens), EAC (Ehrlich Ascites Carcinoma)

INTRODUCTION

Cancer is one of the leading causes of mortality worldwide and the failure of conventional chemotherapy to affect major reduction in the mortality indicates that new approaches are critically needed. The new and recent approaches of chemotherapy serve as an attractive alternative to control the cancer. [1] Recently, the major focus of research in chemotherapy for cancer includes the identification, characterization and development of new and safe cancer chemo preventive agents. A large number of agents including natural and synthetic compounds have been identified as having some potential cancer chemotherapeutic value. [2] A number of natural products have been studied for anticancer activity on various experimental models. This has resulted in the availability of nearly 30 effective anticancer drugs. [3] Natural products are playing an important role as a source of effective anticancer agents and it is significant that 60% of currently used anticancer agents are derived from natural sources, including plants, marine organism and micro-organism. [4-5] The mechanism of interaction between many secondary metabolites and cancer cells has been studied extensively. [6] The relevance of the sea as a tool to discover novel anticancer compounds was validated by the discovery, development and marketing approval of 1-beta-D-arabinofuranosylcytosine (ARA-C).[7] ARA-C is a basic component in the curative setting of acute myeloid leukaemia. [8] Moreover, the search for novel deoxicytidine analogues led to the identification and development of 2, 2'-difluoro-deoxycytidine, gemcitabine. Clinical data has demonstrated its important role in palliative therapy for pancreatic and non-small cell lung cancers. [9-10] The available results clearly anticipated the potential of the marine ecosystem in cancer therapy. During the last decade about 2500 new metabolites with antiproliferative activity have been reported; a recent review discussed 68 new

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marine derived anticancer chemical entities, most of them with undetermined modes of action. [11]

MATERIALS AND METHODS

Collection and Identification of Plant material: The alga *Jania Rubens Linn* was collected manually at the Devgad Island, near Karwar (14°49'12"N 74°7'12"E) of the Arabian Sea during May 2004. After carefully removing the associated algae, the material was washed with fresh water, dried in shade and weighed (1.9 kg). The herbarium specimen were carefully prepared and deposited in Department of Pharmacognosy, BLDEA's College of Pharmacy under the code No BLDE-2004-006. The taxonomy was performed through the courtesy of Dr. B. B. Chaugule, Professor and Head of Department, Department of Botany, Poona University Pune and the specimen was identified as *Jania Rubens (Linn)* of the family *Corallinaceae (Rhodophyceae)* (red algae) & of the class *Florideophyceae*.

Preparation of Extracts: The dried algae materials were crushed into powder using Wiley mill and the powdered sample of alga was subjected to methanol extraction (maceration) for 3 days. The supernatant was filtered using Whatman filter paper, concentrated and dried under reduced pressure. Fresh methanol was added each time and the extraction was carried thrice. The concentrated extract was used for anticancer activity. The preliminary phytochemical studies of methanol extract of *Jania Rubens* were performed to detect the presence of alkaloids, glycosides, flavonoids, lipids, Phenolic compounds, steroids and terpenoids. The extract showed the positive test for steroids, lipids. [12]

Chemicals and Instruments: EAC cell lines, Tryphan Blue and PBS were procured from HI media Laboratories, Mumbai. Cyclophosphamide were obtained from Cadila Healthcare Ltd., Kundaim Industrial Estate, Goa, India. Meltzer Biomedical Research Microscope**,** Haemocytometer, C.O.D incubator (Thermlab, Mumbai) and Refrigerator centrifuge (MPW-350R), Research centrifuge (Remi industries, Mumbai). All the other chemicals and solvents used were of AR grade.

Animals and Animal Care: Female Balb/c mice (20-25 g) were used for this activity. The mice were housed in standard polyacrylic cages and maintained under standard laboratory conditions (temperature 25±2^{0}C, relative humidity 65±10% and in 12 dark/light cycle), free access to standard dry pellet diet (Pranav Agro Industries, Sangli) and water *ad libitum.* All procedures described were reviewed and approved by the Institutional Animal Ethical Committee B.L.D.E.A's College of Pharmacy, Bijapur, Karnataka, India (B.L.D.E.A's COP/ IAEC, Clear/ BPC/1109/67/10-11).

Acute Toxicity Studies: Female Balb/c mice weighing 20-25 Gms were selected randomly for this study. Oral acute toxicity was performed as per OECD- 425 guidelines. [13] The animals were fasted overnight provided only water. Methanol extract of *Jania Rubens* was administered orally at the dose level of 5mg/kg body weight and the animals were observed for 24h for their behavioural changes or mortality. If no mortality is seen, the animals were further observed for 14days. If mortality was observed in 2 to 3 animals, then the dose was assigned as the toxic dose. If mortality was not observed in any of the animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose; 50, 300, 2000 and 5000mg/kg body weight. The animals were observed for toxic symptoms such as behavioural changes, locomotion, convulsion and mortality for 72hr. However in the present study no mortality was observed with any of the above dose.

Experimental design

Transplantation of tumor: EAC were obtained from Life Sciences, Manipal University, Manipal, India. The EAC cells were cultured in the peritoneal cavity of the healthy Balb/c mice by intraperitoneal transplantation of 0.1ml of ascetic fluid. After every 15 days, the ascetic fluid was withdrawn and each animal received 0.1ml of tumour cell suspension containing 1×10^6tumour cells intraperitoneal to get third generation of tumour bearing animals. These animals were selected for *in vitro* and *in vivo* experiments. [14]

Treatment Protocol of various Groups (G) of Animals: Female tumor inoculated Balb/c mice were divided into 5 groups containing 6 animals in each group. The first group serves as normal group contains normal Balb/c mice. All the animals were transplanted with EAC cell lines intraperitoneal except normal group, for the development of ascetic tumor. [15] This was taken as day 0. The animals were grouped as follows:

G1- Normal control, receive 0.9% NaCl solution; p.o.

G2- Tumor control, receive 0.9% NaCl solution; p.o.

G3- Positive control (standard), receives Cyclophosphamide (5 mg/kg) BW; p.o.

G4- Methanol extract of *Jania Rubens* (MEJ) at 250 mg/kg BW; p.o.

G5- Methanol extract of *Jania Rubens* (MEJ) at 500 mg/kg BW; p.o.

All these treatments were given 24 hr after the tumor inoculation, once daily for 14 days.

***In-vitro* screening of anticancer agent**

Tryphan Blue Exclusion Method (Cell viability test): This is one of the methods to assess cytotoxicity of anticancer compounds. It comes under preliminary screening of anticancer compounds. *In vitro* short term cytotoxicity of MEJ was assayed by determining the percentage viability of EAC cells using the Tryphan blue exclusion technique. A living cell membrane has the ability to prevent the entry of certain dyes into the cell. Hence, the viable cells remain unstained and can be easily distinguished from the dead cells that take up the dye and appear blue under the light microscope. Percent cytotoxicity was calculated after comparing with the untreated control. [16]

Procedure: On the 15th day, the Ascetic fluid was withdrawn aseptically from peritoneal cavity of the mice which were induced with EAC cell lines, washed with phosphate buffer saline (PBS) and centrifuged for 15mins at 1,500 rpm in a cold centrifuge. The pellet was re-suspended with PBS and the process was repeated three times. Finally the cells were suspended in a known quantity of PBS and the cell count was adjusted to $1x10^6$ cells/ ml. 0.1ml of this suspension was taken in Eppendorf tubes and 0.1 ml of different concentrations of MEJ and Cyclophosphamide was added and incubated at 37^0C for 3 h. After 3h, the Tryphan blue exclusion assay was performed by counting the cells using haemocytometer, percentage viability was determined and the CTC50 value was calculated.

$$\% \text{ Cytotoxicity} = 100 \text{ X } (T_{dead} - C_{dead}) / T_{total}$$

Where, T_{dead} is the number of dead cells in the treated group, C_{dead} is that in the control group, and T_{total} is the total number of cells in the treated group.[15] Avoid the exposure of cells to trypan blue for a period longer more than 30 minutes. In this case it is possible to observe an increase in the dead cell population (trypan blue positive) due to the trypan toxicity.

***In-vivo* screening of anticancer agent:**

Effect of methanol extract of *Jania Rubens* on physical parameters of EAC bearing mice: The weight of each animal in all groups was recorded on tumor inoculation day and then for every 3^{rd} day up to 15^{th} day, each animal was then sacrificed and organ body weight ratio was measured. [17]

Effect of methanol extract of *Jania Rubens* on Hematological parameters of EAC bearing mice [18-19]: After the 24 h of last dose treatment, the blood was collected by retro-orbital puncture under slight anesthesia (diethyl ether) condition from all the fasted animals. The blood obtained was divided into two portions. First portion was taken in EDTA tubes so as to prevent coagulation and then was subjected to α-Swelab cell counter for the determination of the hematological parameters such as Red blood cells (RBC), White blood cells (WBC), platelets (PLT), Hemoglobin (Hb) etc.

Statistical analysis: All the values were expressed as mean ± SEM. Means of treated groups were compared with that of control group using one-way analysis of variance (ANOVA) followed by Turkey's test, control group is compared with normal group using Student's *t* test. [22]

RESULTS

***In vitro* anticancer activity:** The extract dose showed significant dose dependent inhibition of EAC cells and it was found that the drug is cytotoxic and produced 50% population of cell death at a concentration of 64.22µg/ml against EAC cell lines. The result was compared with Cyclophosphamide & the IC50 were found to be 90µg/ml respectively against EAC cell lines [Figure 1-2].

***In vivo* anticancer activity**

Weight variation parameter: After the tumour transplantation, there was increase in body weight in EAC bearing mice due to accumulation of ascetic fluid. The MEJ treated animals at the doses of 250 and 500 mg/kg inhibited the increase in body weight indicating an arrest of tumour cell proliferation and growth. The tumour inoculated animals exhibited significant weight gain as (p<0.001) compared to the normal group by day 15. Cyclophosphamide administration significantly reduced (p< 0.001) weight gain on day 15 as compared to control. It was also found that the 250mg/kg (p<0.001), and 500mg/kg (p<0.001) produces similar effect as that of Cyclophosphamide [Table 1], [Figure 3].

Haematological parameters: The control group animals has shown significant increase in WBC (p<0.01) , PCV and decrease in RBC, MCH, PLT, Hb and lymphocytes levels as compared to normal group. The PCV and WBC were found to decreases significantly in animal treated with the MEJ at almost all the doses tested when compared to EAC tumour control, Similarly, RBC, Hb, PLT, MCH and lymphocytes, which were decreased after EAC inoculation, were found to be significantly return to the normal levels in the animals treated with the MEJ, which indicates the anticancer nature of the extract [Table 2-3]..

DISCUSSION

In the present pharmacological evaluation the methanol extract of *Jania Rubens* was extensively investigated for its anticancer activity against EAC induced cancer in mice. The Methanol extract of *Jania Rubens* (250,500 mg/kg) has been showed significant anticancer activity as compared to standard cyclophosphamide in EAC induced cancer model. The anticancer properties of the extracts may be due to sterols. The present study points to the potential anticancer activity of *Jania Ruben* that might be a promising chemotherapeutic agent against EAC tumours. All the results obtained imply that further research is needed for isolation of active principles possessing the therapeutic activity and clinical study for evaluation of safety and efficacy of the drug needs to be assessed.

CONCLUSION

The most of the marine based medicines have shown promising agents for the treatment of the cancer with safety, efficacy and economical. In the present investigation, an attempt has been made for the extraction of *Jania Rubens* and their evaluation for anticancer activity. Qualitative chemical tests were conducted for all the methanolic extracts of algae of *Jania Rubens* Linn to identify the various phytoconstituents. The methanolic extract shows presence of steroids and lipids. The anticancer properties of the extracts may be due to sterols. The extract posses' potent anticancer activity against EAC cell lines but the mechanism by which the drug produces the effect remains unknown. Further studies are therefore required to elucidate the mechanism of action.

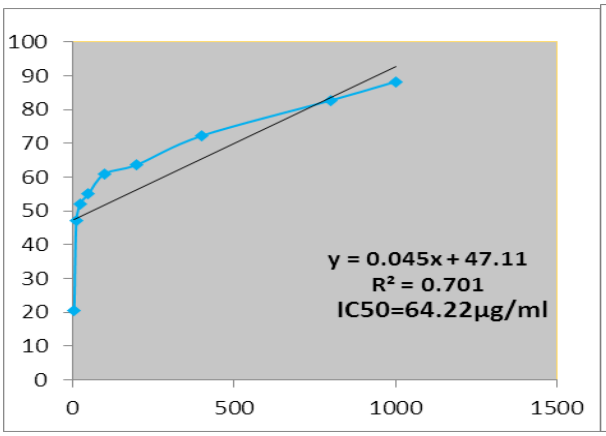


Figure 1: IC50 of MEJ on EAC cell lines

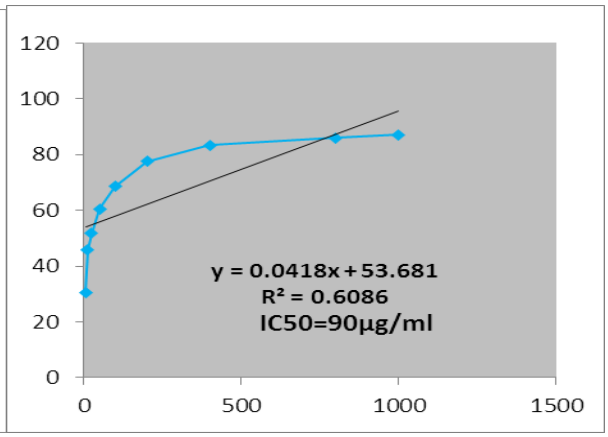
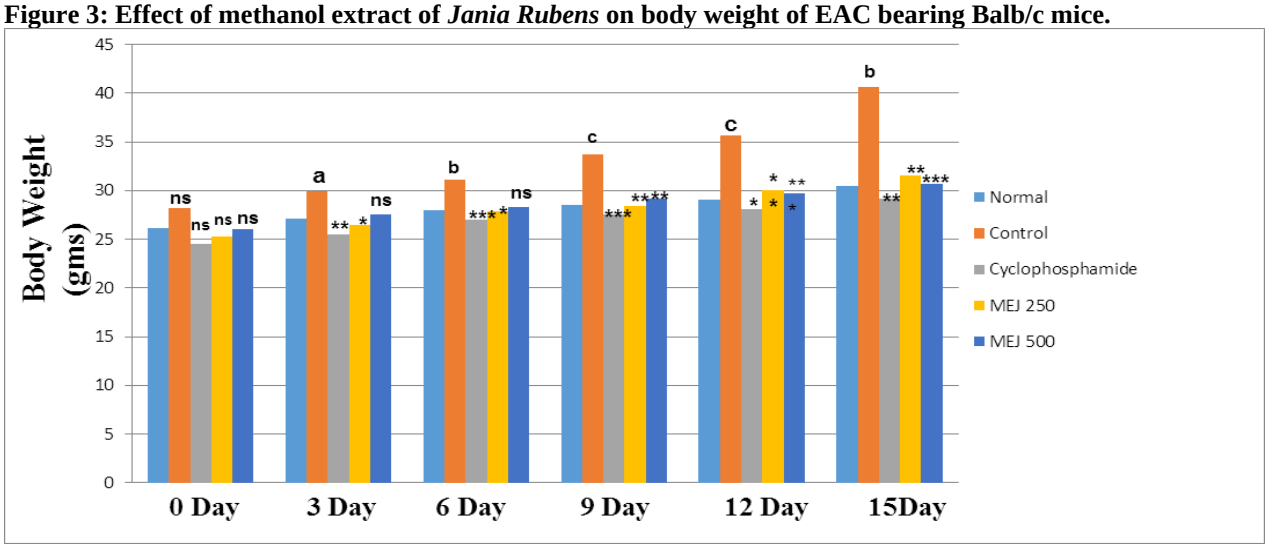


Figure 2: IC50 of Cyclophosphamide on EAC cell line

Figure 3: Effect of methanol extract of *Jania Rubens* on body weight of EAC bearing Balb/c mice.



All values are expressed as mean ± SEM, n=6. **P*<0.05, ***P*<0.01, ****P*<0.001 as comparison to control group One Way analysis of variance (ANOVA) followed by multiple comparison Turkey's test. [a]*P*<0.05, [b]*P*<0.01, [c]*P*<0.001, compared to normal group (student's *t* –test). MEJ-Methanol extract of *Jania Rubens*.Cyclophosphamide.

Table 1. Effect of methanol extract of *Jania Rubens (MEJ)* on body weight of EAC bearing Balb/c mice

Treatment groups	Body weight (Gms)					
	0 day	**3rd day**	**6th day**	**9th day**	**12th day**	**15th day**
Normal (0.9% saline)	26.11 ±0.478	27.16 ±0.454	27.97 ± 0.450	28.57 ± 0.5043	29.05 ± 0.5168	30.43 ± 0.333
Control (0.9% saline)	28.26± 0.541^{ns}	29.92 ± 0.832^{a}	31.12 ± 1.085 ^{b}	33.74 ±0.850 ^{c}	35.64 ± 1.169 ^{c}	40.59 ± 1.554 ^{b}
Cyclophosphamide (5 mg/kg)	24.55 ± 0.827^{ns}	25.53 ±0.664**	27.01 ±0.762***	27.56 ±1.098***	28.14 ±1.275*	29.13 ± 1.375**
MEJ (250mg/kg)	25.32 ± 0.827 ^{ns}	26.5 ± 0.618*	27.87 ± 0.963*	28.37 ± .798**	30.07 ±0.7590**	31.61 ± .5875**
MEJ 500mg/kg	26.03 ± 1.141 ^{ns}	27.57± 1.066^{ns}	28.27 ± 1.066 ^{ns}	29.14 ± 1.191**	29.73 ±0.9834***	30.69 ± 1.571***

All values are expressed as mean ± SEM, n=6. $^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.001$, compared to normal group (student's *t* -test) **P*<0.05, ***P*<0.01,****P*<0.001 as comparison to control group One way analysis of variance(ANOVA) followed by multiple comparison Turkey's test. MEJ - Methanol extract of *Jania Rubens*, ns- not significant.

Table 2: Effect of methanolic extract of *Jania Rubens* on hematological parameters of EAC bearing Balb/c mice

Treatment groups	WBCs ($X10^6$ /ml)	RBCs ($X10^9$ /ml)	Hb (gm/dl)	PLT ($X10^3$ /µl)	PCT (%)
Normal (0.9%Saline)	6.550 ± 0.295	10.285 ± 0.2028	16.72 ± 0.602	1063.47 ± 160.4	0.44.75 ± 0.008
Control (0.9% saline)	17.120 ± 4.615^{c}	6.075 ± 0.39^{b}	8.60 ± 0.694^{b}	641.30 ±112.2^{a}	0.2877 ± 0.0209^{c}
Cyclophosphamide (5 mg/kg)	7.860 ±3.316***	9.613 ± 1.158^{ns}	13.83 ± 0.368 ^{ns}	973.7 ±150.8 ^{ns}	0.3050 ± 0.0338 ^{ns}
MEJ (250mg/kg)	7.200 ±2.538**	8.774 ± 0.9321^{ns}	13.22 ± 1.680 ^{ns}	875.0 ± 49.60 ^{ns}	0.1560 ± 0.02561 **
MEJ (500mg/kg)	6.935 ± 0.558^{ns}	10.198 ± 0.1647 ^{ns}	14.84 ±0.3172 ***	950.4 ± 7.194 *	0.1820 ± 0.005831 ^{ns}

All values are expressed as mean ± SEM, n=6. $^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.001$, compared to normal group (student's *t* -test). **P*<0.05, ***P*<0.01, ****P*<0.001 as comparison to control group One way analysis of variance (ANOVA) followed by multiple comparison Turkey's test MEJ- Methanol extract of *Jania Rubens*, Cyclophosphamide, ns-not significant.

Table 3: The effect of methanolic extract of *Jania Rubens* on hematological parameters of EAC bearing Balb/c mice

Treatment groups	GRANULOCYTES (%)	LYMPHOCYTES (%)	MCV	PCV	MCH
Normal (0.9%Saline)	7.363 ± 1.223	62.05 ± 2.038	52.65 ± 1.076	18.43 ± 1.078	18.93 ± 0.318
Control (0.9% saline)	12.46 ± 1.666[a]	31.38 ± 2.707[b]	62.48 ± 3.224[a]	36.57 ±2.77 [ns]	10.05 ± 0.195[ns]
Cyclophosphamide (5 mg/kg)	8.608 ± 1.183 [ns]	66.28 ± 1.020 [ns]	45.05 ± 3.109***	20.40 ± 1.09 [ns]	18.60 ± 0.250 [ns]
MEJ (250mg/kg)	9.760 ± 3.474[ns]	64.14 ± 7.985*	47.00 ± 1.374 [ns]	24.88 ± 0.80 *	17.34 ± 0.1435 [ns]
MEJ (500mg/kg)	8.940 ± 2.815***	65.04 ± 6.087 [ns]	48.44 ± 1.263 [ns]	21.97 ± 1.02 [ns]	17.98 ± 0.1655 [ns]

All values are expressed as mean ± SEM, n=6. [a]$P<0.05$, [b]$P<0.01$, compared to normal group (student's *t* -test). *$P<0.05$, **$P<0.01$, ***$P<0.001$ as comparison to control group One way analysis of variance (ANOVA) followed by multiple comparison Turkey's test MEJ- Methanol extract of *Jania Rubens*, Cyclophosphamide, ns-not significant.

REFERENCES

1. Kapadia GJ at el. Inhibition of Epstein-Barr virus early antigen activation promoted by 12-O-tetradecanoylphobol-13-acetate by nonsteroidal anti-inflammatory drugs. Cancer Lett 2000; 161: 221.
2. Kelloff GJ. Perspective on cancer chemoprevention research and drug development. Adv. Cancer Res 2000; 78:199.
3. Ramakrishna Y et al. Plants and novel antitumor agents: A review. Indian Drugs 1984; 21:173.
4. Cragg GM et al. Anticancer Agents from Natural Products. (Brunner-Routledge Psychology Press, London) 2005; pp.186.
5. Newman DJ et al. Natural products as a source of new drugs over the period 1981-2002. J. Nat. Prod 2003; 66:1022.
6. Leland J et al.Natural Products from Plants. (CRC press, London) 1999; pp.1581.
7. Bergmann W, Feeney R.J. Contributions to the study of marine products. The nucleosides of sponges III. Spongothymidine and Spongouridine J. Org. Chem 1985; 20:1501.
8. Wolf SN et al. High dose cytosine arabinoside and daunorubicin as consolidation therapy for acute non lymphoblastic leukemia. Blood 1985; 65:1407.
9. Moore M. Activity of gemcitabine in patients with advanced pancreatic carcinoma: a review. Cancer 1996; 78(3):633.
10. Guchelaar HJ et al. Clinical, toxicological and pharmacological aspects of gemcitabine. Cancer. Treat. Rep 1996; 22:15.
11. Mayer AMS, Gustaveson KR. Marine pharmacology in 2000: antitumor and cytotoxic compounds. Int J Cancer 2003; 105:291.
12. Wagner H et al. Plant Drug Analysis. Springer -Verlag, Berlin 1984; pp.298.
13. The organization of Economic Co-Operation and Development (OECD). The OECD Guideline for Testing of Chemicals 2008; 1.
14. Gothoskar SV, Ranadive KJ. Anticancer screening of SAN-AB: An extract of Marking out Semicarpus anacardium. Indian J Exp Biol 1971; 9(3):372.
15. Dongre SH et al. Antitumor activity of methanol extract of Hypericum hookerianum stem against Ehrlich ascites carcinoma in swiss albino mice. J Pharmacol Sci 2007; 103:354.
16. Devi PU et al. In vivo tumour inhibitory and radio sensitizing effects of an Indian medicinal plant, Plumbago rosea on experimental mouse tumours.Ind J Exp Biol 1994; 32:523.
17. Eckhardt AE et al. Inhibition of Ehrlich Ascites Tumour Cell Growth by Griffonia simplicifolia I Lectin in Vivo. Cancer Research 1982; 42: 2977.
18. Kuttan G et al. Isolation and identification of tumor reducing component from mistletoe extract (Iscador). Cancer letters 1988; 41:307.
19. Mazumdar UK et al. Antitumor activity of Hygrophila spinosa on Ehrlich ascites carcinoma and sarcoma-180 induced mice. Ind J Biol 1997; 35:473.
20. Kumar RS et al.Antitumor Activity of Prosopis glandulosa Torr on Ehrlich Ascites Carcinoma (EAC) Tumor Bearing Mice.Iranian Journal of Pharmaceutical Research 2011; 10(3):505.
21. Jeevanantham P et al. Anti-Cancer Activity of Methanol Extract of Aerial parts of Momordica Cymbalaria Hook F. against Ehrlich Ascites Carcinoma in Mice. J. Pharm. Sci. & Res 2011; 3(8):1408.
22. Naigonkar AV, Burande MD. A manual of medical laboratory technology, 2nded, Nirali Prakashan 1996; pp.284.