IJBPAS, September, Special Issue, 2021, 10(9): 614-624
ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)
'A Bridge Between Laboratory and Reader'
www.ijbpas.com

EVALUATION OF ANTICANCER ACTIVITY OF TAXILLUS TOMENTOSUS PLANT USING ALBINO MICE

RAMBABU BODA[1*], HARIKIRAN LINGABATHULA[2] AND RAKESHKUMAR JAT[3]

1: Research Scholor, SJJT University, Jhunjhunu-churela road, churela, Jhunjhunu, Rajasthan, India-333001

2: Associate Professor, Department of Pharmacology, Princeton College of Pharmacy, Chowdaryguda (V), Ghatkesar (M), Telangana State, India-500088

3: Associate Professor, Department of Pharmacology, SJJT University, Jhunjhunu-churela road, Jhunjhunu, Rajasthan, India-333001

***Corresponding Author: Rambabu Boda: E Mail: rambabuboda34@gmail.com**

Received 25th June 2021; Revised 28th July 2021; Accepted 29th Aug. 2021; Available online 25th Sept. 2021

https://doi.org/10.31032/IJBPAS/2021/10.9.1047

ABSTRACT

Plant based drugs are useful to treat different ailments with less side effects compare to synthetic drugs. Anticancer agents which are available in the market are having various side effects apart from their therapeutic effectiveness. For this, we have estimated the anticancer activity of *Taxillus tomentosus* against EAC (Ehrlich ascites carcinoma) induced cancer in Swiss albino mice. We have prepared the ethanolic leaf extract of *Taxillus tomentosus* (TTEE) and their ethyl acetate fraction (TTEAF) and n-hexane fraction (TTNHF). These extract and fractions were tested at 50 and 100 mg/kg body weight doses against EAC induced mice using paclitaxel (PCTXL) at 5 mg/kg dose as standard, assessed the various antitumor and hematological parameters and compared to EAC control. The test extract and fractions resulted in decreased bodyweight of animals, especially at higher dose. The test substances also exposed lowered tumor volume, viable tumour cell count and packed cell volume compared to EAC control mice. The treatments of test TTEE, TTEAF and TTNHF have enhanced the mean survival time and

percentage increased life span compared to EAC control, indicating anticancer activity. Also, the test substances returned the all-hematological parameters such as hemoglobin content, red and white blood cells, and differential leucocyte count. Hence, the test extract and fractions have shown anticancer activity against EAC induced mice which was comparable to standard drug PCTXL and TTNHF was the most promising fraction representing anticancer activity. Future research on isolation and purification of *Taxillus tomentosus* might be useful to obtain most promising agents to treat cancer.

INTRODUCTION

Medicinal plants are the source of outdated health care systems around the world. Pharmacological studies have discovered that the worth of medicinal plants acts as a potential source of biologically dynamic compounds. Phytochemical herbal formulations are a principal compound in the innovation and development of drugs. WHO clarification that more than Eighty percentage (80%) of the world's population depends on plants to meet their basic health desires **[1]**.

Cancer is primary to cause of death widespread and has become a main public health problem in developed countries. Cancer growth is a multistep process, during collect genetic abnormalities by which cells, especially in tumor suppressor genes and transforming gene, causative to uncontrolled proliferation. These abnormalities make available several enlargement advantages. Definitely, the conversion of a tumor cell from a normal cell to frequently involves mutations in the cell genome. There were 6 key changes that take place throughout the transformation to a cancer cell from a normal cell, these structures may be measured hallmarks of cancer **[2]**.

Medicines for the treatment of malignant tumors of plant derivation have been carefully studied to extend drugs for the treatment of a variety of human tumors. Medicinal plants used to treat cancer include *Acalypha fruticosa, Terminalia chebula, Catharanthus roseus, Embelia ribes,* and *Tylophora indica*. The extracts used to treat breast cancer are *Scopolendra subspinipes, Radix glycyrrhizae* and *Squama manitis*. The drugs used for treating pancreatic cancer are Emblica officinalis and Nigella sativa **[3]**.

Taxillus tomentosus is a plant belonging to the family loranthaceae. Recent research studies revealed that this plant has various pharmacological activities, such as antidiabetic, cardioprotective, hepatoprotective, anti-urolithic, neuroprotective, and antistress,

nootropic activities **[4-6]**. In the present study, we evaluated the anticancer activity of ethanolic leaf extract and, ethyl acetate and n-hexane fractions of *Taxillus tomentosus* using Ehrlich Ascites Carcinoma (EAC) inoculated mice by estimating various parameters.

MATERIALS AND METHODS

Plant material

The recent healthy, sickness-free leaves of *Taxillus tomentosus* were collected from the hills of Tirumala, Tirupati, India, and were authenticated by Dr. M. Madhava Chetty, Department of Biological Sciences, Sri Venkateshwara University, Thirupati, Andhrapradesh.

Extraction and Fractionation

The leaves of *Taxillus tomentosus* plant were shade dried for Two weeks and ground into a rough powder by using a grinder. The 100 g of powder of plant leaves were macerated for 24 hours with continuous stirring in 500 mL of ethyl alcohol employing a mover and shaker at 28^{o}C. Then, the supernatant was recovered by filtration through muslin cloth and along with Whatman paper. Further, the filtrates were totally dried by rotary vacuum evaporator. The ethanol extract were evaporated to dryness at room temperature to produce the ethanol extract **[7]**. The ethanol extract of *Taxillus tomentosus* (TTEE) were subjected to fractional process by partitioning the aqueous suspension of the drug with ethyl acetate and n-hexane to get respective fractions such as ethyl acetate fraction (TTEAF) and n-hexane fraction (TTNHF). Further, the extract and fractions were stored at -4^{o}C until further use.

Acute toxicity study

The acute toxicity studies of TTEE, TTEAF and TTNHF were conducted as per the guidelines of OECD 423 i.e., acute oral toxicity (Acute toxic class method). This acute toxicity class technique is a stepwise process with the practice of 3 female mice. The main principle of this test is constructed on a stepwise process with the use of the minimum number of animals (5mg/kg, 50mg/kg, 300mg/kg and 2000mg/kg). The constituent is directed orally to a group at one of defined doses 5mg/kg. Each step using 3 mice of single-sex (Female). Presence (or) absence of compound-related death rate of the animals dosed at 1 step will conclude the next step i.e., 50mg/kg, 300mg/kg and 2000mg/kg.

Animals

The 8 weeks old female Swiss albino mice were taken from Mahaveer Enterprises, Hyderabad, India. The mice were kept 1 week for adaptation before starting the experiments. The mice were housed in

polypropylene mice cages in a room, provide water and feed *ad libitum* with controlled temperature (25 ± 2 °C), humidity (55 ± 5%) and a 12 h light/dark cycle during the adaptation and experimental periods.

Experimental Design

The animals, weighed approximately20–25 g were chosen and randomly divided into 9 groups (*n* = 10). Group 1-Normalgroup, Group 2-EAC control, Group 3-Standard Paclitaxel drug (PCTXL) 3 mg/kg, Group 4-TTEE 50mg/kg and Group 5-TTEE 100mg/kg, Group 6-TTEAF 50mg/kg and Group 7-TTEAF 100mg/kg, Group 8-TTNHF 50mg/kg and Group 9-TTNHF 100mg/kg body weight of mice. All the test and standard drugs are injected intraperitoneally. The ascitic fluid from EAC bearing mice was withdrawn with sterile syringe and aseptically by intraperitoneal route. The normal group 1was not inoculated with tumor cells, while other groups were injected with (EAC) Ehrlich Ascites Carcinoma cells (0.2mL of 2×10^6 cells/mouse) intraperitoneally. This was taken as day 0 and the experiment was started after 24 hr. From the first day, and recorded the Bodyweight of each animal and 100 µL/mouse per day of sterile saline was directed intraperitoneally to the negative control group (EAC-bearing mice). The test drugs at doses of 50 mg/kg and 100 mg/kg were administered each day to the treated groups and the standard drug PCTXL at a dose of 5 mg/kg was administered to each animal from the positive control group by intraperitoneal route. The pharmacological treatment lasted for 9 days. 14 days after the treatment, 5 mice from each group were dissected for the study of the antitumor parameters. The remaining 5 mice from each group were reserved to check the Mean survival time (MST) of EAC tumor-bearing hosts **[7]**.

Ehrlich Ascites Carcinoma Model

The EAC cells procured from UCPSc, Kakatiya University, Warangal, were maintained and broadcasted by serial intraperitoneal transplantation of EAC cells in a germ-free environment. The EAC cells propagated for 12-14 days were used in the experiment. The tumor cell cultures for EAC were start from mouse Ehrlich Ascites with at least 1 passage *in vitro* prior to use. The ascitic fluid is withdrawn using an sterile syringe with18 gauge needle. Tumor viability was finding by trypan blue exclusion test and cells were calculated using hemocytometer. The ascitic fluid was diluted with suitable normal saline to get a concentration of $1x10^7$ cells/mL of tumor cell suspension. From this stock suspension 0.25 mL (2.5 million cells/mice)

was injected i.p to find ascitic tumor **[8]**.

Body weight of animals

The mice were weighed on the day of tumor injection and noted as 0^{th} day. Then weigh the body weight of EAC inoculated mice once in 2 days of the post inoculation period, the % increase in body weight was calculated. The formula for calculation of percentage increase in body weight was, % increase in body weight = (Animal Weight on respective day/ animal weight on day 0) -1 x 100 **[9]**.

Mean survival time (MST) and Percentage Increase in life span [%ILS]

The major parameters to evaluate the antitumor activity were mean survival time and percentage increased life span. For this purpose, total number of days a mouse survived from the day of tumor injection was counted. Subsequently the MST was calculated as Mean survival time = (Day of first death + Day of last death)/2. The %ILS was calculated as %ILS = [(Mean Survival Time of treated group/ Mean Survival Time of control group)-1]x100follows. An improvement of life span by 25% or more ended that of control was measured as current antitumor response **[10]**.

Tumor volume, Packed cell volume and Viable tumor cell count

The parameters like tumor volume, viable cell count and packed cell volume were estimated to assess the anticancer activity in EAC injected mice. On 14 day of the tumor inoculation, the animals were dissected and the peritoneal ascitic fluid was taken into a measuring cylinder and the volume of the ascitic fluid was measured and also measure the packed cell volume and compared in all the treated and EAC control groups **[11]**. The number of viable and non-viable cells present in ascetic fluid in all the groups were measured by dye exclusion method using 0.4% Trypan Blue solution **[12]**.

Hematological parameters

In order to detect the influence of extract and fractions on the hematological status of EAC bearing mice, the parameters such as white blood cell count, red blood cell count hemoglobin content and differential leukocyte count such as lymphocytes, neutrophils and monocytes. For this, on the 14^{th} day of tumor inoculation, the blood was withdrawn from animals in each group by retro-orbital puncture and different haematological parameters were estimated.

Statistical analysis

The statistical analysis was executed by one-way ANOVA subsequently bonferroni posttests. The data was represented as mean ± standard deviation (S.D.) of 3 independent experiments. Test significance was designated by *$p<0.01$ and **$p<0.001$ compared to EAC control.

RESULTS AND DISCUSSION

The present investigation assesses the anticancer activity of *Taxillus tomentosus* ethanolic extract (TTEE) and their ethyl acetate fraction (TTEAF) and n-hexane fraction (TTNHF) at the doses of 50 and 100 mg/kg body weight using EAC bearing mice by various antitumour and hematological parameters.

Acute toxicity study

Acute toxicity study was performed according to the OECD guide line 423 using 3 female mice per step. Mice were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours and up to 72 hours. All the changes were systematically recorded with individual records being maintained for each mouse. Observations included changes in skin, mortality and general behavioral pattern. There was no death was observed till the end of study till the 2000 mg/kg dose for all the 3 test substances. The same was reported with the ethanolic extract of *Taxillus tomentosus* **[5]**. So, the 2 doses were selected for the anticancer activity studies viz., 50 and 100 mg/kg body weight.

Body weight

The percentage increased body weight of animals were calculated on 1, 3, 5 and 7^{th} days compared to 0^{th} day of post treatment in EAC control, PCTXL and test drugs. The observations were shown in the Table 1. All the three test substances have shown considerable reduced body weight of animals. Among the three test substances, TTAEF at 100 mg/kg dose have shown significant reduced body weight ($p<0.001$), where as TTNHF at both 50 and 100 mg/kg doses have shown significant decrease in body weight after 7 days ($p<0.001$) which are comparable to standard drug PCTXL. The ethanol extract of *Sargassum tenerrimum* have also shown the decreased body weight of EAC bearing mice should contribute the anticancer activity **[11]**.

Mean survival time (MST) and Percentage Increase in life span (%ILS)

The important parameters to anticancer activity *in vivo* were mean survival time and percentage increased life span (Sridhar *et al*, 2012). The calculated values of MST and %ILS were shown in the **Table 2**. The TTEE and TTEAF were significantly ($p<0.01$) increases the MST at 100 mg/kg dose whereas TTNHF was increased MST significantly at both doses 50 mg/kg ($p<0.01$) and 100 mg/kg ($p<0.001$) which was almost same as with standard PCTXL. Similarly, TTEE and TTEAF increased the %ILS significantly at 50 mg/kg ($p<0.01$) and 100 mg/kg ($p<0.001$). The TTNHF have shown

enormous increase in %ILS at both doses significaly ($p<0.001$). The PCTXL have also shown in the similar way as in case of TTNHF. The antitumour activity of ethanol extract of *Bauhinia variegata* has been evaluated up on oral administration against EAC inoculated albino mice. A significant enhancement of MST in EAC bearing mice was found with respect to the EAC control group. The test treatment was found to enhance % ILS after 14 days of injection compared to EAC control group. The ethanolic extract of *Bauhinia variegata* was originate to be a potent cytotoxic towards EAC tumor cells **[13]**.

Tumour volume, Packed cell volume and Viable tumour cell count

The results of tumour volume, viable tumour cell count and packed cell volume were shown in Table 3. The tumour volume and packed cell volume were reduced with the treatment of test extract and fractions. The treatment of TTEE and TTEAF at both doses have shown lowered tumour volume and packed cell volume significantly ($p<0.01$) compared to EAC control. The treatment of TTNHF at 50 mg/kg ($p<0.01$) and 100 mg/kg ($p<0.001$) doses lowered the tumour volume and packed cell volume when compare to EAC control group which was similar to PCTXL. The treatment of TTEAF at 100 mg/kg and TTNHF at both doses decreases the viable tumour cell count when compare to EAC control group. Among three test extract and fractions, the TTNHF have shown promising decreased activity towards all three tested parameters. The methanolic extract of *Argyreia nervosa* has been tested against EAC induced liquid tumor in mice. Significant and dose-dependant results were detected when the mice are dissected on 15th day for estimation of tumor proliferation, biochemical and hematological parameters. The extract also showed a decrease in tumor volume, packed cell volume and viable tumour cell count accounts for anticancer activity supports our study **[14]**.

Hematological parameters

Different hematological parameters such as hemoglobin, RBC, WBC, lymphocytes, neutrophils and monocytes were estimated and presented in Table 4. Compared to normal group animals, the EAC control group animals have shown abnormal changes in all the hematological parameters tested. The TTEE, TTEAF and TTNHF treated animals have brought back the values of hematological parameters to near normal values, especially at higher dose i.e., 100 mg/kg. Among these three tested substances, the TTEAF and TTNHF have shown promising results in brought back to the normal hematological parameters levels which were comparable to the standard drug

PCTXL treatment. The antitumor activity of methanol extract of *Lactuca serriola* was evaluated against EAC induced Swiss albino mice at 100 mg/kg and 200 mg/kg. Up on administration of the extract of plant, improvement in the hematological parameters such as hemoglobin content, RBC and WBC count and differential cell count and restored altered hematological parameters. It can be decided that the methanol extract of *Lactuca serriola* keeps significant antitumor activity **[15]**.

Table 1: Effect of different doses of *Taxillus tomentosus* extract and fractions on percentage increase in Body Weight in EAC inoculated mice

Treatment	Dose (mg/kg)	% Increase in Body weight as compared to '0th' day			
		Day 1	Day 3	Day 5	Day 7
EAC Control	Vehicle	1.27±0.84	3.50 ± 0.81	7.01 ± 0.63	24.22 ± 1.70
PCTXL	5	1.59±1.01	2.91 ± 2.29	5.47 ± 2.01	.7.21 ± 3.00**
TTEE	50	1.35±0.3	3.4 ± 0.8	5.32 ± 0.8	19.43 ± 0.78*
	100	1.41±0.76	3.12 ± 1.27	5.14 ± 2.67	16.23 ± 3.71*
TTEAF	50	1.45± 1.56	2.63 ± 1.62	5.26 ± 2.02	17.73 ± 4.10*
	100	1.25±0.17	2.69 ± 1.54	4.47± 2.68*	11.02 ± 3.81**
TTNHF	50	1.26±0.86	3.11 ± 1.13	5.15 ± 0.60	12.36 ± 1.24**
	100	1.77±0.75	3.03 ± 1.50	4.97 ± 1.32*	9.73 ± 2.50**

All the values are mean ± S.D. of six samples, *p<0.01 and **p<0.001 compared to EAC control

Table 2: Effect of different doses of *Taxillus tomentosus* extract and fractions on (MST) Mean survival time and (%ILS) percentage increased life span in EAC inoculated mice

Treatment	Dose (mg/kg)	MST	% ILS
EAC Control	Vehicle	15.67±0.76	-
PCTXL	5	45.00±3.21**	187.17 ±14.75**
TTEE	50	26.00 ±2.29	65.92 ±14.44*
	100	30.17 ±2.08*	92.53 ±10.79**
TTEAF	50	28.17 ±2.35	79.77 ±13.53*
	100	31.33 ±2.48*	99.93 ±13.45**
TTNHF	50	32.83 ±3.08*	109.50 ±14.38**
	100	36.67 ±3.63**	134.01 ±11.13**

All the values are mean±S.D. of six samples, *p<0.01 and **p<0.001 compared to EAC control

Table 3: Effect of different doses of *Taxillus tomentosus* extract and fractions on tumour volume, packed cell volume and viable cell count in EAC inoculated mice

Treatment	Dose (mg/kg)	Tumour Volume	Packed Cell Volume (mm)	Viable cell count (x 10^7 cells/ml)
EAC Control	Vehicle	16.65 ± 1.21	3.23 ± 0.03	7.33 ± 0.61
PCTXL	5	4.35 ± 0.45**	0.26 ± 0.01**	0.51 ± 0.08**
TTEE	50	10.92 ± 1.01*	2.12 ± 0.09	5.52 ± 0.21
	100	8.53 ± 0.79*	1.23 ± 0.06*	4.13 ± 0.18*
TTEAF	50	9.87 ± 0.83*	1.97 ± 0.08	4.17 ± 0.28*
	100	6.93 ± 0.49*	0.73 ± 0.05*	2.54 ± 0.16**
TTNHF	50	7.53 ± 0.68*	1.13 ± 0.06*	3.43 ± 0.11**
	100	4.85 ± 0.36**	0.45 ± 0.04**	1.58 ± 0.12**

All the values are mean ± S.D. of six samples, *p<0.01 and **p<0.001 compared to EAC control

Table 4: Effect of different doses of *Taxillus tomentosus* extract and fractions on Hematological parameters in EAC inoculated mice

Treatment	Dose (mg/kg)	Hematological parameters					
		Haemoglobin (gm%)	RBC(million /mm^3)	WBC (10^3cells/mm^3)	Lymphocytes (%)	Neutrophils (%)	Monocytes (%)
Normal Control	--	14.10 ± 0.86	4.62 ± 0.38	7.86 ± 0.12	65 ± 3.31	28 ± 1.96	1 ± 0.31
EAC Control	Vehicle	5.35 ± 0.55	1.94 ± 0.08	23.60 ± 1.84	21 ± 0.67	69 ± 4.53	2.61 ± 0.91
PCTXL	5	12.31 ± 1.12**	4.23 ± 0.37**	9.92 ± 0.83**	61 ± 0.52**	32 ± 2.24**	1.32 ± 0.13**
TTEE	50	6.96 ± 0.71	2.43 ± 0.24	20.02 ± 1.73	38 ± 3.14	54 ± 5.13	2.08 ± 0.21
	100	8.36 ± 0.62*	3.27 ± 0.29*	16.38 ± 1.31*	45 ± 4.12*	45 ± 4.08*	1.75 ± 0.16*
TTEAF	50	8.44 ± 0.82*	3.17 ± 0.27*	17.74 ± 1.52*	42 ± 3.87*	48 ± 4.24*	1.95 ± 0.16*
	100	10.75 ± 1.22**	3.82 ± 0.36**	13.42 ± 1.26**	51 ± 4.76**	41 ± 3.82**	1.52 ± 0.15*
TTNHF	50	9.75 ± 1.11*	3.54 ± 0.33**	15.38 ± 1.34**	48 ± 4.24*	45 ± 4.05**	1.76 ± 0.14*
	100	11.21 ± 1.34**	4.05 ± 0.41**	10.13 ± 1.02**	59 ± 4.12**	36 ± 3.15**	1.39 ± 0.12**

All the values are mean ± S.D. of six samples, *p<0.01 and **p<0.001 compared to EAC control

CONCLUSION

The TTEE, TTEAF and TTNHF were administered intraperitoneally at 50 mg/kg and 100 mg/kg to EAC inoculated mice and assessed the different antitumour and hematological parameters. The results have clearly described that the treatment of test extract and fractions were significantly decreases the body weight, tumour volume, packed cell volume and viable tumour cell count compared to EAC control group. And also the MST and %ILS were enhanced significanltly up on the treatment of test extract and fractions against EAC control. All the hematological parameters were also brought back to near normal values and almost restored. In conclusion, the test extract and fractions showing anticancer activity against EAC induced mice. Among the three test substances, the TTNHF showed highest anticancer activity followed by TTEAF and TTEE, which were comparable to the standard PCTXL.

ACKNOWLEDGEMENTS

The first author is grateful to SJJT University, churela, Jhunjhunu, Rajasthan.

REFERENCES

[1] Karthikeyan M, Bagavathy SK, Vivek ARP, Bharath CRP, Aparna SR, Pattulingam Mangalapandi, Areejit S. A curated database of Indian medicinal plants, phytochemistry and therapeutics. *Scientific Reports* 2018; 8; 1-8.

[2] Noa R, Ran B, Moshe O, Varda R. Mutations in the p53 tumor suppressor gene. *Genes Cancer* 2011; 2(4): 466–474.

[3] Bahare S, Nanjangud VA, Bilge S *et al.* Medicinal plants used in the treatment of human immune deficiency virus. *International Journal of Molecular Science* 2018; 19(5): 1459.

[4] Mohammed NU, Mohammed WU, Firasath A. Evaluation of diabetic complications, neuro, hepato, cardio and nephron protective effects of ethanolic extract of the whole plant of *Taxillus tomentosus* in alloxan induced diabetic rats. *European Journal of Pharmaceutical and Medical Research* 2015; 2: 410-436.

[5] Silpa S, Jamal U, Jayasekhar VL. Screening of *Taxillus tomentosu*s ethanolic extract for nootropic and antistress activity in rats. *International Journal of Innovation and Applied Studies* 2014; 8: 1533-1544.

[6] Kambham V, Preethi J, Chandrasekhar KB. Antiurolithiatic activity of ethanolic extract of *Taxillus tomentosus* plant on ethylene glycol and ammonium chloride induced urolithiasis in wistar rats. *Indonesian Journal of Pharmacy* 2016; 27(2); 66 –73.

[7] Sridhar PG, Harikiran L, Apparao AVN, Narsimha RY. Evaluation of anti-cancer activity of dikamaliartane-a, a cycloartane isolated from dikamali, a gum resin. *International Journal of Pharmacy and Pharmaceutical Sciences* 2012; 4(4): 501-504.

[8] Dolai N, Islam A, Haldar PK. Methanolic extract of *Anthocephalus cadamba* induces apoptosis in Ehrlich ascites carcinoma cells in experimental mice. *Indian Journal of Pharmacology* 2016; 48: 445-449.

[9] Rajkapoor B, Jayakar B and Murugesh N. Antitumor activity of *Indigofera aspalathoides* on Ehrlich ascites carcinoma in mice. *Indian Journal of Pharmacology* 2004; 36[1]: 38-40.

[10] Rajkapoor B, Sankari M, Sumithra M, Anbu J, Harikrishnan N, Gobinath M, Suba V, Balaji R. Antitumor and cytotoxic effects of *Phyllanthus podophyllus* on Ehrlich ascites carcinoma and human cancer cell line. *Bioscience, Biotechnology, and Biochemistry* 2007; 71: 2177-2183.

[11] Satyajit P, Meenakshi SM, Ramprabhu ATJ, Ramya PR , Sujitha P. Evaluation of antitumor and antioxidant activity of *Sargassum tenerrimum* against Ehrlich ascites carcinoma in mice. *Asian Pacific Journal of Cancer Prevention* 2015; 16: 915-921.

[12] Puck and Marcus. A rapid method for viable cell titration and clone production with HeLa cells in tissue culture: The use of x-irradiated cells to supply conditioning factors. *Proceedings of the National Academy of Sciences of the*

United States of America 1995; 41: 432–437.

[13] Rajkapoor B, Jayakar B, Murugesh N. Antitumour activity of *Bauhinia variegata* against Ehrlich ascites carcinoma induced mice. *Pharmaceutical Biology* 2003; 41(8): 604–607.

[14] Bhawna S, Isha D, Sandeep K, Hema C. *In vitro* and *in vivo* evaluation of anti-tumor activity of methanolic extract of *Argyreia nervosa* leaves on Ehrlich ascites carcinoma. *Bangladesh Journal Pharmacology* 2015; 10: 399-408.

[15] Mona A, Eman E. Inhibition of Ehrlich ascites carcinoma by *Lactuca serriola* in Swiss albino mice. *Journal of Chemistry and Chemical Engineering* 2014; 8: 66-71.