



Original Article

Antitumour Activity of *Ecteinascidia venui* Meenakshi, 2000 against Dalton's Lymphoma Ascites

S Sankaravadivu¹, R Jothibai Margret^{2,*}, V K Meenakshi^{3,*}¹ Department of Chemistry, V.O.Chidambaram College, Tuticorin, India^{2*} Department of Chemistry, Pope's College, Sawyerpuram, Tuticorin, India^{3*} Department of Zoology, A.P.C. Mahalaxmi College for Women, Tuticorin, India

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The aim of this study is to investigate the *in vitro* cytotoxicity and antitumor effect of the ethanol extract of colonial ascidian *Ecteinascidia venui* Meenakshi, 2000 in Swiss albino mice against Dalton's Lymphoma Ascites. The viability of DLA cells to varying concentrations of the extract was assessed. *In vivo* antitumor activity of the extract at doses of 100,150, 200 and vincristin 80 mg/kg body weight against DLA bearing mice was evaluated. Solid tumor volume, median survival time, percentage increase in life span, packed cell volume, viable, non-viable cell count and haematological parameters were determined by adopting standard procedures. 100 percent cytotoxicity to DLA cells was observed at a concentration of 0.80 mg/ml. Administration of the extract resulted in significant decrease in tumor, packed cell volume, viable cell count and increased non-viable cell count, median survival time, thereby increasing the life span of the tumor bearing mice. Restoration of hematological parameters - Hb, RBC, WBC and differential count to normal levels in extract treated mice was also noted. The results suggest that the ethanol extract of *Ecteinascidia venui* exhibit significant cytotoxic and antitumor activity in DLA bearing mice comparable to that of the reference standard, vincristin.

Key words: Colonial ascidian. *Ecteinascidia venui*. Cytotoxicity. DLA.

1. INTRODUCTION

Cancer is considered as one of the serious diseases which is featured by abnormal or uncontrolled growth. Today, cancer is one of the leading causes of death worldwide. A total of 1,660,290 new cancer cases and 580,350 deaths were predicted in the United States in 2013¹. Cancer chemoprevention was first defined as “a

Corresponding author *Sankaravadivu S
V.O.Chidambaram College, Tuticorin, India
E Mail: sankarisankaran86@gmail.com

strategy of cancer control by administration of synthetic or natural compounds to reverse or suppress the process of carcinogenesis”². Drugs obtained from medicinal plants play a crucial role in the treatment of cancer and most of the plant secondary metabolites and their derivatives have been applied to combat cancer^{3,4}. Nature is the most valuable source of therapeutic compounds as enormous chemical diversity is present in millions of species of plants, animals, marine organisms, and microorganisms⁵. Especially marine organisms such as ascidians, sponges and soft corals containing symbiotic microorganisms are a rich source of bioactive compounds⁶⁻⁸. In recent times, the demand for more effective and safer therapeutic agents for the chemoprevention of human cancer has increased. Ascidian-derived natural products have yielded promising drug leads, among which ET-743 from *Ecteinascidia turbinata* was approved as a drug with the trade name Yondelis against refractory soft-tissue sarcomas⁹. Various species of ascidians from Indian water has been proved to exhibit antimicrobial, antiproliferative, antitumour, immunomodulatory, analgesic, anaesthetic, antipyretic, antiinflammatory, antidiabetic, hepatoprotective, antifertility, wound healing, CNS depressant, cardioprotective and hyperlipidemic activities¹⁰⁻⁴¹. Review of literature reveals that only chemical investigation of the ethanolic extract of *Ecteinascidia venui* has been carried out so far⁴²⁻⁴⁴. Hence, the present study was designed to evaluate the antitumor activity of the ethanolic extract of colonial ascidian *Ecteinascidia venui*.

2. MATERIALS AND METHODS

Specimen collection and identification

Ecteinascidia venui was collected from the hull of ships during dry docking in the month of May 2013. Epibionts and particles of shell, coral fragments attached to the colony were carefully removed.

Identification up to the species level was carried out based on the key to identification of Indian ascidians⁴⁵. A voucher specimen AS 2247 has been submitted in the ascidian collections of the Museum of the Department of Zoology, A. P. C. Mahalaxmi College for Women, Tuticorin - 628002, Tamilnadu, India

Plate 1.

Systematic position

Ecteinascidia venui belongs to Phylum: Chordata, Subphylum: Urochordata, Class: Ascidiacea, Order: Enterogona, Suborder: Phlebobranchia, Family: Perophoridae, Genus: *Ecteinascidia*, Species: *venui*.

Experimental animals

Swiss albino mice weighing 20-25 g were collected from Central Animal House, Dr. Raja Muthiah Medical College, Annamalai University, Chidambaram, Tamilnadu. The animals were fed with normal mice chow and water ad libitum in air-controlled room with constant 12 hours of dark light schedule, room temperature (24±2 °C) and 60 - 70 % humidity. The experimental work was done as per the rules and regulations of Animal Ethical Committee, Government of India.

Cells for cytotoxic study

Dalton's Lymphoma ascites (DLA) cells were purchased from Adayar Cancer Institute, Chennai, India. The cells were maintained as ascites tumors in Swiss albino mice.

Preparation of powder and extract

Colonies of *Ecteinascidia venui* were dried at 45° C, powdered, soaked overnight in 100 ml 70% ethanol and centrifuged at 10,000 rpm at 4°C for 10 minutes. The supernatant was collected and evaporated to get a residue, which was used for *in vitro* and *in vivo* studies. It was suspended in 1% gum acacia blended with vanillin and administered orally using intra gastric catheter for animal experiments.

In vitro cytotoxic activity to DLA cells

Various concentrations (0.05, 0.10, 0.20, 0.40, 0.60, 0.80 and 1.00 mg/ml) of *Ecteinascidia venui* extract were prepared. DLA cells (1×10^6 cells) were incubated with the extract at 37°C for 3hr in a final volume of 1ml. Trypan blue dye exclusion method was followed for testing the viability of the cells⁴⁶.

Experimental protocol

Five groups of six healthy adult Swiss Albino mice were used for study. DLA cells maintained as ascites tumors were aspirated, washed thrice in normal saline counted using a hemocytometer and cell suspension of 1million cells/ml was prepared. DLA cells (1×10^6 cells/mouse) were injected intraperitoneally for 9 days. Group I was administered with normal saline and treated as tumor control. Group II, III, IV, V received 100, 150, 200 and 80 mg/kg body weight of the extract and Vincristin respectively. Body weight of the animal was observed on the 10th day. One set of animals were sacrificed and the weight of the vital organs such as spleen, thymus, liver, kidney, lungs were recorded 24 hr after the last dose of the drug and expressed as relative organ weights. The extract was administered for 30 days and the experiment was continued with the remaining set of mice. Parameters such as Hb, RBC, WBC and differential count were estimated using the blood collected from caudal vein⁴⁶.

Induction and measurement of solid tumor Volume

DLA cells (1×10^6 cells/mouse) were injected subcutaneously to the right hind limbs of five groups of animals to induce tumor. Vernier callipers was used to measure the radii of tumors at an interval of 5 days for one month starting with 15th day. The tumor volume was calculated using the formula $V = \frac{4}{3} r_1^2 r_2$, where 'r1' and 'r' represent the major and minor diameter respectively⁴⁷.

Effect on Median survival time, lifespan, packed cell volume, viable and non-viable cell Count

Median survival time and percentage increase of life span (% ILS)

Daily mortality was recorded for six weeks by monitoring the effect of the extract on tumor growth. Percentage increase in lifespan (% ILS) was calculated by the following equation. $MST \% = \frac{\text{Median survival time of treated group} - \text{Median survival time of control group}}{\text{Median survival time of control group}} \times 100$
 Median survival time (MST) = (Day of first death + Day of last death) / 2
 Increase in lifespan = $\frac{T - C}{C} \times 100$

Packed cell volume

The ascetic fluid was collected from the peritoneal cavity of the dissected mice. Packed cell volume was determined by centrifuging a known volume at 10,000 rpm for 5 minutes in a graduated centrifuge tube.

Viable and non viable cell count

Trypan blue (0.4% in normal saline) dye was used to stain the cells. The cells which did not take up the dye were counted as viable and those which took the stain as non viable.

Effect on hematological parameters

Parameters such as haemoglobin, RBC, WBC and differential count were recorded with the blood collected from caudal vein of the experimental mice at the end of thirty days.

Statistical Analysis

Values were expressed as mean \pm SEM. The statistical analysis was done by one-way analysis of variance (ANOVA) followed by Dunnett's test. P-values less than 0.05 were considered to be significant.

3. RESULTS AND DISCUSSION

Cytotoxic activity to DLA cells

In the present investigation cytotoxicity of *Ecteinascidia venui*, a common biofouling colonial ascidian was assayed against DLA cells. Cytotoxicity of the ethanolic extract of *Ecteinascidia venui* is given in Table 1. Administration of the extract at a

concentration of 0.05, 0.10, 0.20, 0.40, 0.60, 0.80 and 1.0 mg/ml produced 8, 15, 40, 76, 93 and 100 percent cytotoxicity to DLA cells respectively. Percentage cytotoxicity was observed to be dose dependent. Ethanolic extract of *Ecteinascidia venui* was found to be 100% toxic at a concentration of 0.80 mg/ml to DLA cells.

Effect on Relative Organ Weight

The activity of the ethanolic extract of *Ecteinascidia venui* on the weight of body and relative organs in DLA tumor bearing mice is given in Table 2. A comparison of the result indicates a dose dependent decrease in bw of the animals treated with the extract. The reduction observed in Group III, IV and V was significant when compared to that of control. Relative organ weight of spleen, thymus, liver, kidney and lungs showed a negligible change in the groups treated with the extract compared to control. The decrease in body weight registered for group IV was same as that of group V treated with 80 mg/kg bw of Vincristin. Spleen, thymus, liver and kidney are vital organs necessary for the stimulation and production of immune related cells to destroy abnormal cells, by cell mediated immune response. Treatment with the extract did not show any change in these organs, indicating normal functioning of the immune system to overcome the stress induced by tumor growth which may be because of the bioactive components like phenols and flavonoids present acting as antioxidants. GC-MS report of colonial ascidian *Ecteinascidia venui* have shown the presence of compounds like Eicosane, Tetradecyloxirane Nonadecane, Methyl ester of (E,E)-9,12-Octadecadienoic acid, methyl ester of (E)-9-Dodecanoic acid with anticancer and antioxidant activity⁴².

Effect on Solid Tumor Volume

Table 3 shows the results of the antitumor activity of the extract of *Ecteinascidia venui* on solid tumor

volume of DLA bearing mice. On 25th and 30th days, a significant dose dependent decrease in tumor volume was noticed in the experimental groups III, IV and V compared to tumor control. On all the days of the experiment, the tumour volume observed in group IV was nearer to that of the group treated with the standard drug. Ascitic fluid acts as a direct nutritional source to tumour cells⁴⁸. It increases the tumour size as was noted in DLA bearing mice. Treatment with the extract significantly decreased solid tumor volume indicating inhibition in the growth and multiplication of tumor cells. This may be due to the presence of compounds present in extract of *Ecteinascidia venui* inhibiting mitosis, DNA synthesis or replication via pathways¹².

Effect on Median Survival Time, Percentage increase of Life Span, Packed cell volume, Viable and Non viable cell count

The effect of *Ecteinascidia venui* extract on median survival time, percentage increase of life span, packed cell volume, viable and non viable cell count in DLA tumor bearing mice is shown in Table 4. There was a highly significant increase in the median survival time, life span in a dose dependent manner in experimental groups. The median survival time observed for group V was also highly significant when compared to control. The percentage of increase in life span recorded for group IV treated with 200 mg/kg body weight of extract was very highly significant and higher than that of standard drug. A significant dose dependent reduction in packed cell volume, viable cell counts were noticed in group II, III and IV compared to group I. Group V treated with standard drug also showed a highly significant decrease. Non viable cell count increased in a significant and proportionate manner in the experimental groups compared to that of the control. This may be due to cytotoxic effect on tumor cells or by leading to macrophage activation and

inhibition of vascular permeability by the extract as has been evidenced in plants⁴⁹⁻⁵¹. Similar observations have been reported with the ethanolic extract of *Phallusia nigra* and *Microcosmus exasperatus* by earlier workers^{12, 18}.

Effect on Hematological Parameters

The changes in the hematological parameters in DLA bearing mice are given in Table 5. A decrease in Hb content was observed in the tumor control whereas in the groups which received the extract, a significant dose dependent increase was noted. In the group treated with the standard drug also there was a highly significant increase. A comparative study reveals that the increase in group IV treated with 200 mg/kg bw of extract was highly significant like that of standard. RBC and Lymphocytes were decreased while WBC, Neutrophils and Eosinophils indicated an elevation in group I. On administration of the extract, the hematological parameters were brought back to that of normal control and the values were almost same as that of the standard indicating normal hemopoiesis and iron absorption. The acceptance criteria for determining the antitumour activity of a compound is the determination of circulating WBC and the life span prolongation. Usually, in cancer chemotherapy, the major problems that are being encountered are of myelosuppression and anaemia. The anemia noticed in tumor bearing mice is mainly due to reduction in RBC or hemoglobin and this may occur either as a result of iron deficiency, hemolytic or myelopathic condition⁵²⁻⁵⁴.

Table 1: Cytotoxicity of ethanolic extract of *Ecteinascidia venui* to DLA

S. No	Concentration (g/ml)	Percentage of Cytotoxicity
1	0.05	8
2	0.1	15
3	0.2	40
4	0.4	76
5	0.6	93
6	0.8	100
7	1	

Table 2: Effect of ethanolic extract of *Ecteinascidia venui* on Relative Organ Weight

Group	Dose (mg/kg)	Relative Organ Weight (g/100g body wt)					
		Body weight	Spleen	Thymus	Liver	Kidney	Lungs
I	Control	34.16±1.22	0.38±0.018	0.24±0.031	3.73±0.24	2.11±0.012	0.59±0.016
II	100	31.63±1.84	0.31±0.024	0.26±0.054	3.24±0.23	2.03±0.018	0.52±0.027
III	150	28.36±1.33	0.26±0.013	0.22±0.069	3.11±0.14	1.93±0.027	0.46±0.032
IV	200	26.93±2.04*	0.30±0.011	0.21±0.036	3.46±0.26	2.04±0.013	0.50±0.016
V	Vincristin 80	26.59±1.86	0.24±0.018	0.20±0.011	3.04±0.16	1.91±0.067	0.45±0.012

Data represented as mean±SEM, (N=6). Significance between tumour control Vs extract treated group *P<0.05.

Table 3: Effect of ethanolic extract of *Ecteinascidia venui* on solid tumor volume

Group	Dose (mg/kg)	Relative Organ Weight (g/100g body wt)					
		Body weight	Spleen	Thymus	Liver	Kidney	Lungs
I	Control	34.16±1.22	0.38±0.018	0.24±0.031	3.73±0.24	2.11±0.012	0.59±0.016
II	100	31.63±1.84	0.31±0.024	0.26±0.054	3.24±0.23	2.03±0.018	0.52±0.027
III	150	28.36±1.33	0.26±0.013	0.22±0.069	3.11±0.14	1.93±0.027	0.46±0.032
IV	200	26.93±2.04*	0.30±0.011	0.21±0.036	3.46±0.26	2.04±0.013	0.50±0.016
V	Vincristin 80	26.59±1.86	0.24±0.018	0.20±0.011	3.04±0.16	1.91±0.067	0.45±0.012

Data represented as mean±SEM, (N=6). Significance between DLA control Vs extract treated group *P<0.05; **P<0.01; ***P<0.0001

Table 4: Effect of ethanolic extract of *Ecteinascidia venui* on median survival time, life span, packed cell volume and viable and non-viable cell count

Group	Dose (mg/kg)	Median Survival time (Days)	Increase of life span (%)	Packed cell volume	Viable cell count 1x10 ⁶ cells/ml	Non-viable tumor cells count 1x10 ⁶ cells/ml
I	Control	20.16±0.18	-	2.84±0.012	13.42±2.37	0.58±0.011
II	100	26.81±0.13	32.98	1.86±0.054*	7.93±1.67*	0.89±0.048*
III	150	28.16±0.24*	39.68	1.32±0.027**	4.13±1.04**	1.96±0.027***
IV	200	33.46±0.18**	65.97	0.83±0.018**	2.04±0.93***	2.86±0.011***
V	Vincristin 80	31.16±0.24	54.56	0.93±0.019	2.16±0.27	2.71±0.019

Data represented as mean±SEM, (N=6). Significance between DLA control Vs extract treated group *P<0.05; **P<0.01; ***P<0.0001

Table 5: Effect of ethanolic extract of *Ecteinascidia venui* on hematological parameters

Group	Dose (mg/kg)	Hb (gm%)	RBC (million/m ³)	WBC (10 ³ cells/mm ³)	Differential count		
					Lymphocytes	Neutrophils	Eosinophils
Norm Saline		12.54±0.9	4.31±0.18	9.35±0.16	53.16±1.65	40.36±1.3	6.30±1.3
I	Control	8.06±0.14	2.91±0.26	16.84±1.84	41.36±0.71	50.27±0.1	8.16±0.1
II	100	9.68±0.38	3.46±0.73*	13.16±0.98	46.91±0.84	48.13±0.2	7.16±0.2
III	150	12.84±0.6	4.11±0.16**	11.92±0.76	50.16±0.62	42.16±0.3	7.04±0.1
IV	200	12.91±0.2	4.26±0.28**	10.84±0.67	52.16±0.74	40.11±0.2	6.84±0.1
V	Vincristin 80	12.84±0.7	4.38±0.74	11.04±0.36	51.31±0.22	43.16±0.1	5.93±0.2

Data represented as mean±SEM, (N=6). Significance between DLA control Vs extract treated group *P<0.05; **P<0.01; ***P<0.0001

4. CONCLUSION

Ethanol extract of *Ecteinascidia venui* at a dose of 200 mg/kg body weight was observed to have a highly significant antitumour activity to DLA cells. The present study showed significant percentage increase in the life span, reduction in tumor volume and improvement of hematological parameters of the host. Hence further detailed study is required to target the isolation of the active principle responsible for the activities.

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6. REFERENCES

1. Siegel R, Naishadham D and Jemal A. Cancer statistics, CA: A Cancer Journal for Clinicians. 2013; 63(1):11-30.
2. Sporn MB, Dunlop NM, Newton DL, Smith JM. Prevention of chemical carcinogenesis by vitamin A and its synthetic analogs (retinoids). Federation Proceedings. 1976; 35(6):1332-1338.
3. Newman DJ, Cragg GM, Snader KM. The influence of natural products upon drug discovery. Natural Product Reports, 2000; 17(3):215-234.
4. Butler MS. The role of natural product chemistry in drug discovery. Journal of Natural Products. 2004; 67(12):2141-2153.
5. da Rocha, Lopes RM, Schwartsmann G. Natural products in anticancer therapy. Current Opinion in Pharmacology. 2001; 1(4):364-369.
6. Davidson BS. Ascidiaceae: Producers of amino acid derived metabolites. Chemical Reviews. 1993; 93: 1771- 1791.
7. Faulkner DJ. Marine natural products. Natural Product Reports. 2002; 19: 1- 48.
8. Blunt W, Copp BR, Munro MHG, Northcote PT and Prinsep MR. Marine natural products. Natural Product Reports. 2006; 23: 26- 78.
9. Ebada SS, WH Lin, Proksch P. Bioactive Sesterterpenes and Triterpenes from Marine sponges: occurrence and pharmacological significance. Marine Drugs. 2010; 8: 313-346.
10. Gopalakrishnan S, Meenakshi VK, Shanmugapriya D. Antipyretic and Analgesic activity of *Phallusia nigra* Savigny, 1816. Annals of Biological Research. 2011; 2(4): 192-196.
11. Gopalakrishnan S, Meenakshi VK, Shanmugapriya D. Chemical Investigation of the Simple ascidian *Phallusia nigra* Savigny, 1816 of Tuticorin coast by GC-MS. International Journal of Pharma and Bio Sciences. 2011; 2(4): 382-387.
12. Meenakshi VK, Paripooranaselvi M, Gomathy S, Chamundeswari KP. Antiproliferative activity of *Phallusia nigra* Savigny, 1816 against Dalton's Lymphoma Ascites. International Journal of Chemical and Pharmaceutical Sciences. 2012; 3(2): 70-75.
13. Meenakshi VK, Paripooranaselvi M, Senthamarai S, Gomathy S, Chamundeswari, KP. Antitumor and immunomodulatory activity of *Phallusia nigra* Savigny, 1816 against Ehrlich ascites carcinoma. Research Journal of Pharmaceutical Sciences. 2012; 1(2): 7-12.
14. Gopalakrishnan S, Meenakshi VK, Shanmugapriya D. Antimicrobial activity of the methanolic extract of *Phallusia nigra* Savigny. Journal of Natural Product and Plant Resources. 2012; 2(5): 579-583.
15. Gopalakrishnan S, Meenakshi VK, Shanmugapriya D. Anaesthetic activity of *Phallusia nigra* Savigny. Annals of Biological Research. 2012; 3(4):1863-1865.

16. Gopalakrishnan S, Meenakshi VK, Shanmugapriya D. Wound healing activity of the methanolic extract of *Phallusia nigra* Savigny. *International Journal of Chemical and Pharmaceutical Sciences*. 2012; 3(3): 45-51.
17. Meenakshi VK, Senthamarai S, Paripooranaselvi M, Gomathy S, Shanmuga Priya D. Chamundeswari KP. Antibacterial activity of simple ascidian *Ascidia sydneiensis* (Family: Ascidiidae) against human pathogens. *Journal of Microbiology and Biotechnology Research*. 2012; 2(6): 894-899.
18. Meenakshi VK, Senthamarai S, Paripooranaselvi M, Gomathy S, Sankaravadivu S, Chamundeswari KP. *In vitro* and *in vivo* antitumor and immunomodulatory studies of *Microcosmus exasperatus* against DLA bearing mice. *European Journal of Applied Engineering and Scientific Research*. 2013; 2(3): 18-25.
19. Gopalakrishnan S, Meenakshi VK, Shanmugapriya D. Anti-Inflammatory activity of Simple Ascidian, *Phallusia nigra* Savigny. *International Journal of Pharmaceutical sciences Review and Research*. 2013; 22(2):162-167.
20. Meenakshi VK, Paripooranaselvi M, Senthamarai S, Gomathy S, Chamundeswari, KP. Immunomodulatory activity of ethanol extracts of *Phallusia nigra* Savigny 1816, against Dalton's lymphoma ascites. *European Journal of Applied Engineering and Scientific Research*. 2013; 2(1): 20-24.
21. Meenakshi VK, Paripooranaselvi M, Sankaravadivu S, Gomathy S, Chamundeswari KP. Immunomodulatory activity of *Phallusia nigra* Savigny, 1816 against S-180. *International Journal of Current Microbiology and Applied Sciences*. 2013; 2(8): 286-295.
22. Meenakshi VK, Paripooranaselvi M, Senthamarai S, Gomathy S, Sankaravadivu S, Chamundeswari KP. Antitumor effect of ethanolic extract of *Phallusia nigra* Savigny, 1816 on S-180 tumor bearing mice. *International Journal of Pharmacological Screening Method*. 2014; 4(1): 20-25.
23. Meenakshi VK, Paripooranaselvi M, Gomathy S, Senthamarai S, Chamundeswari, KP, Sankaravadivu S. Immunostimulating activities of *Phallusia nigra* Savigny, 1816 on sarcoma-180 tumor-bearing mice. *International Journal of Medicinal Chemistry and Analysis*. 2014; 4(2): 62-69.
24. Meenakshi VK, Delighta Mano Joyce MI, Paripooranaselvi M, Gomathy S. CNS depressant activity of the simple ascidian *Microcosmus exasperatus* Heller, 1878. *International Journal of Current Microbiology and Applied Sciences*. 2013; 2(10): 16-25.
25. Meenakshi VK, Gomathy S, Senthamarai S, Paripooranaselvi M, Chamundeswari, KP. Hepatoprotective activity of the ethanol extract of simple ascidian, *Microcosmus exasperatus* Heller, 1878. *European Journal of Zoological Research*. 2013; 2(4): 32-38.
26. Meenakshi VK, Delighta Mano Joyce MI, Paripooranaselvi M, Gomathy S, Chamundeswari KP. Protective Effect of *Microcosmus exasperatus* against isoproterenol induced myocardial ischemia- A biochemical and histopathological approach. *International Journal of Pure and Applied Bioscience*. 2014; 2(1): 62-70.
27. Meenakshi VK, Delighta Mano Joyce MI, Paripooranaselvi M, Gomathy, S. Antihyperlipidemic Activity of *Microcosmus exasperatus* Heller, 1878. *Journal of Chemical,*

- Biological and Physical Sciences. 2014; 4(3): 1379-1387.
28. Kohila Subathra Christy H, Jothibai Margret R, Meenakshi, VK. Antipyretic and analgesic activity of *Phallusia arabica* Savigny, 1816. International journal of medicinal chemistry and analysis. 2014; 4(3): 162-165.
 29. Kohila Subathra Christy H, Jothibai Margret R, Meenakshi VK. Antiinflammatory activity of the simple ascidian of *Phallusia arabica* Savigny, 1816. International journal of biological and pharmaceutical research. 2014; 5(7): 553-558.
 30. Kohila Subathra Christy H, Jothibai Margret R, Meenakshi, VK. Chemical screening and anaesthetic activity of *Phallusia arabica* Savigny, 1816. International research journal of pharmaceutical and applied sciences. 2014; 4(1): 24-28.
 31. Meenakshi VK, Gomathy S, Senthamarai S, Paripooranaselvi M, Chamundeswari, KP. Antifertility activity of simple ascidian, *Microcosmus exasperatus* Heller, 1878. International Journal of Pharmaceutical Sciences Review and Research. 2014; 24(1): 230-236
 32. Delighta Mano Joyce MI, Meenakshi VK, Paripooranaselvi M, Gomathy, S. Anaesthetic, analgesic and antipyretic activities of *Microcosmus exasperatus* Heller, 1878. World Journal of Pharmaceutical Research. 2015; 4(7): 1770-1779.
 33. Delighta Mano Joyce MI, Meenakshi VK, Paripooranaselvi M, Gomathy, S. Evaluation of anti-inflammatory activity of *Microcosmus exasperatus*. European Journal of Pharmaceutical and Medical Research. 2015; 2(4): 682-692.
 34. Delighta Mano Joyce MI, Meenakshi VK, Paripooranaselvi M, Gomathy, S. Wound Healing Activity of the ethanolic extract of *Microcosmus exasperatus* Heller, 1878. Journal of Environmental and Applied Bioresearch. 2015; 3(4): 226 - 229.
 35. Kohila Subathra Christy H, Jothibai Margret R, Meenakshi VK. Evaluation of wound healing activity of *Phallusia arabica*. World journal of pharmaceutical research. 2015; 4(3): 162-165.
 36. Paripooranaselvi M, Meenakshi VK, Gomathy, S. Inhibition of HLCA-549 cell proliferation and survival by ethanolic extract of *Phallusia nigra* Savigny, 1816. European Journal of Biomedical and Pharmaceutical Sciences. 2015; 2(2): 216-230.
 37. Paripooranaselvi M, Meenakshi VK. Ethanolic extract of *Phallusia nigra* Savigny, 1816 induced immunomodulations in HLCA-549 bearing mice. World Journal of Pharmaceutical Research. 2015; 4(11): 1168-1181.
 38. Shanmugapriya D, Kohila Subathra Christy H, Sankaravadivu, S. Antimicrobial activity of simple ascidism *Phallusia nigra* World Journal of Pharmaceutical research, 2015; 4(9): 822-827.
 39. Shanmugapriya D, Kohila Subathra Christy H, Sankaravadivu, S Stella Packiam. Antidiabetic activity of the ethanolic extract of a simple ascidism *Phallusia nigra* World Journal of Pharmaceutical research. 2015; 4(11):1557-1563.
 40. Shanmugapriya D, Kohila Subathra Christy H, Sankaravadivu, S Stella Packiam C. Antioxidant activity of the simple ascidism *Phallusia nigra* of Thoothukudi coast. International Journal of Pharmaceutical Chemistry. 2015; 410-412.
 41. Shanmugapriya D, Kohila Subathra Christy H, Sankaravadivu, S. Hepatoprotective activity of ethanol extracts of *Phallusia nigra* against CCl4 induced hepatotoxicity in rats. World Journal of Pharmaceutical research. 2015; 5(1):648-655
 42. Sankaravadivu S, Jothibai Margret R, Meenakshi VK. Infrared and gas chromatogram-mass spectral studies of the ethanolic extract of *Ecteinascidia*

- venui* Meenakshi, 2000. International Journal of Chemical Pharmaceutical Sciences. 2013; 4(2):84-89.
43. Sankaravadivu S, Jothibai Margret R, Meenakshi VK. Spectrophotometric studies of a colonial ascidian *Ecteinascidia venui* Meenakshi, 2000. International journal of pharmacy and biological sciences. 2013; 3(4): 159-163.
44. Sankaravadivu S, Jothibai Margret R, Meenakshi VK. Preliminary Screening and IR Spectral studies of a colonial ascidian *Ecteinascidia venui* Meenakshi, 2000. Journal of Chemical, biological and physical sciences. 2015; 5(4): 4205-4210.
45. Meenakshi VK. Biology of a few chosen ascidians. Ph.D thesis, M.S.University, Tirunelveli, 1997; 157-173.
46. Talwar GP. Hand book of Practical Immunology. In Talwar GP (Eds) National Book Trust, New Delhi, 1974; 336-339.
47. Rajesh Kumar NV, Kuttan R. Induction of apoptosis in mouse and human carcinoma cell lines by *Emblica officinalis* polyphenols and its effect on chemical carcinogenesis. Amala Research Bulletin. 2001; 21: 114-126.
48. Banu Rekha J, Jayakar B. Anti-cancer activity of ethanolic extract of leaves of *Plumeria rubra* (Linn). Current Pharma Research. 2011; 1: 175-179.
49. Vijayan P, Vinothkumar S, Badami S, Mukherjee PK, Dhanaraj SA, Suresh A. Selective invitro cytotoxicity of *Hypericum hookerianum* towards cancer cell lines. Oriental Pharmacy and Experimental Medicine. 2003; 3: 141-146.
50. Dongre SH, Badami S, Godavarthi A. Antitumour activity of *Hypericum hookerianum* against DLA induced tumor in mice and its possible mechanism of action. Phytotherapy Research. 2008; 22, 23-29.
51. Saha P, Mazumder UK, Haldar PK, Naskar S, Kundu S, Bala A, Kar B. Anti-cancer activity of ethanolic extract of *Cucurbita maxima* against Ehrlich ascites carcinoma. International Journal of Pharmaceutical Sciences. 2011; 2: 52-59.
52. Hogland HC. Hematological complications of cancer chemotherapy Semin Oncology. 1982, 9:95-102.
53. Price VE and Greenfield RE. Anemia in cancer. In Greenstein JP and Haddow A. Advances in cancer research. Academic press, Newyork. 1958; 199-200.
54. Fenninger LD and Mider GB. Energy and nitrogen metabolism in cancer. . In Greenstein JP and Haddow A. Advances in Cancer Research. Academic press, Newyork. 1954; 2: 229-253.

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