INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
ISSN: 2277-7881; IMPACT FACTOR – 6.014; IC VALUE:5.16; ISI VALUE:2.286
VOLUME 8, ISSUE 8(6), AUGUST 2019

STUDIES ON IN VIVO AND IN VITRO IMMUNOMODULATORY ACTIVITY OF EXTRACTS OF THE MARINE SPONGE AXINELLA CARTERI

Vishnu D. Bansode
Department of Zoology
S.S. & L.S. Patkar College
Goregoan (W), Mumbai

Asha J, Rao
Department of Zoology
Bhavan's College
Andheri (W), Mumbai

Sanjay P. Jumale
Department of Zoology
Bhavan's College
Andheri (W), Mumbai

Abstract

Marine sponges are well known for their medicinal value. The present study involved the investigation of immunomodulatory activities of extracts of marine sponge Axinella carteri. The whole body Methanol and Chloroform: Methanol (2:1) extracts and the different fractions of A. carteri were tested for their effects on phagocytosis by In vitro and In vivo for its immunomodulatory potential. The A. carteri extracts in in-vitro study were found to show immune-suppression at low concentrations only in the hexane fraction while at higher concentration it possesses immune-stimulant activity for all the fractions. The pattern of activities observed in the male and female mice were comparable for the In Vitro studies. The highest phagocytic index was seen for the Chloroform: Methanol (2:1) hexane fraction as compared to the control. No particular pattern was evident for the other fractions. The standard drug, Cyclophosphamide showed the highest activity

Keywords: Marine Sponge Axinella Carteri; Immunomodulatory; Phagocytosis.

Introduction

Since ancient times, marine environment has been documented to be a valuable source of bioactive metabolites including antioxidants, polyunsaturated fatty acids (PUFAs) and sterols[1]. Marine sponges are one of the few a rich source of biologically active secondary metabolites with novel chemical structures. These naturally occurring bioactive substances often have no matches on earth. Sponges are found to be a rich source of bioactive compounds with anti-inflammatory[1,2] antitumor, immunosuppressive or neurosuppressive,

antimalarial, antibiotic and antifouling agents[3-8] Immunomodulators are biological or synthetic substances which can stimulate, suppress or modulate any of the immune system[9] The immunomodulatory activities of the methanolic extract derived from the dried sponge Spongosories halichondriodes with medicinal properties have been reported.[10]
The present study was attempted to assess the effect of extracts from the sponge Axinella carteri for their immunomodulatory activity on yeast Candida albicans and polymorpho nuclear leucocytes of human blood through In vitro bioassay. An In vivo bioassay for immunomodulatory activity of the sponge extract was evaluated on swiss albino mice of both the sexes.

Materials and Methods
The sponge Axinella carteri was collected from subtidal areas of the Arabian Sea at Khar Danda, Mumbai, India (latitude: 18.79 N and longitude: 72.92 E).

Preparation of Sponge Extracts:
10g each of the dried sponge sample was soaked in 200ml of methanol and 200ml of chloroform:methanol (2:1) and kept standing for 24 hrs[11]. Solvents were removed by squeezing the sponge samples and filtered through Whatman filter paper number 1. The solvents were evaporated at low pressure using Buchi Rotavapor R-200 (USA) at 45 °C. The resultant compound was finally dried in a vacuum desiccator and labelled as crude methanol and crude chloroform:methanol extracts. These crude extracts were fractionated using hexane (non-polar), acetone (semi polar) and n butanol (polar). The crude extracts were also partially purified using DEAE Cellulose column chromatography using 0.2, 0.4, 0.6, 0.8 and 1.0 M NaCl in phosphate buffer.

Invitro study
Immunomodulatory activity was analysed through In vitro phagocytosis of Candida albicans by polymorphonuclear cells (PMN) slide method[12]. C. albicans cell pellets were resuspended in sterile Hanks balanced salt solution and human serum in proportion of 16:4 and cells were properly mixed in the vortex. Two or three drops of human blood obtained by finger prick method were collected on to a sterile glass slide. The clot was removed very gently and the slide was slowly drained with sterile normal saline talking care not to wash the adhered neutrophils that were invisible. The slide was flooded with predetermined concentrations crude and fractions (1-5000µg/ml) as well as DEAE fractions of samples and incubated at 37^0C for 15 min, flooded with a suspension of C.albicans and incubated at 37^0C for 1 hour and then drained, fixed with methanol and stained with Giemsa stain. The mean numbers of phagocytosed cells on the slide were determined microscopically for 100 granulocytes using

morphological criteria. This number was taken as the Phagocytic Index (PI) and was compared with the basal PI of controls. This procedure was repeated for different concentration of crude samples (1-5000µg/ml) in triplicate sets.

In vivo study

In vivo phagocytic activity was determined by carbon clearance assay[13]. Animals consisting of swiss albino mice of either sex weighing 20±02g were procured from National Toxicology Centre, Pune. The animals were acclimatized for 10 days before being used for the experiments. They were housed in a room with controlled temperature (23±2°C) and a 12 hr light/12 hr dark cycle. The animals were fed with standard pellet diet and water ad libitum.

The experimental protocol was approved by the Institutional Animals Ethics Committee of the National Toxicology Centre, Pune and conducted according to the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals, India (Research Project number 39/1616.

There mice were grouped (n=3), into control group which did not received treatment while the Standard group was treated with cyclophosphamide 30 mg/kg by intra-peritoneal route. The animals in test groups were treated with 0.2ml p.o. methanol-hexane fraction, 0.4M, 0.6M, 0.8M, 1.0M chloroform DEAE, 0.4M methanol DEAE, 0.6ml methanol DEAE, 0.8 ml methanol DEAE 1.0 methanol DEAE and standard cyclophosphamide for 5 days. On the 6th Day, mice were injected with 0.1 ml of 1% carbon suspension. Blood samples 25 µl were drawn from ROP at 0 and 15 min. The individual test was lysed with 2 ml of 0.1 % of sodium carbonate. Absorbance was measured spectrophotometrically at 675 nm for determination of optical densities. The rate of carbon clearance, termed as phagocytic index (K) was calculated by following equation.

$K = (\text{In } OD_1 - \text{In } OD_2) / t_2 - t_1$

Where, OD_1- Optical density at t_1 means blood collected at 0 min.

OD_2- Optical density at t_2 means blood collected at 15 min.

Results and discussion

In-vitro study

The crude chloroform:methanol extract and the three fractions of Axinella carteri exerted immunosuppressive activity. The suppression of phagocytic activity was observed even at low concentrations of the sponge extracts. No immune-stimulation was observed in any of the fractions (Tab 1 -9).

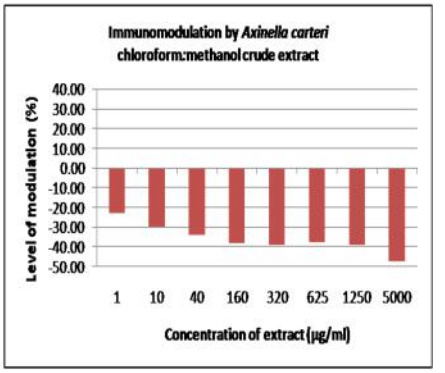
The crude methanol extract exhibited immunostimulatory effect at 33.93% of magnitude. The immune-stimulation was observed upto 160µg/ml of extract but in decreasing way. The crude extract exerted immunosuppressive effect with increase in concentration. The butanol fraction exhibited immune-stimulation at 4.53% magnitude at 1µg/ml to 1.62% magnitude at 10µg/ml and

with increase in concentration of extract exhibited immunosuppressive effect. The hexane fractions exhibited immunosuppression and immunostimulatory activity was not observed. The acetone fraction exhibited immunostimulatory effect at 22.54% of magnitude at lower concentration of 1µg/ml and exerted immunosuppressive effect at increasing concentrations.

The DEAE fractions of methanol and chl:meth showed similar pattern but the activity observed was approximately 1.5 times higher than that of solvent fractions. Immunostimulatory activity at lower concentrations and immunosuppression at higher concentrations were observed with extracts of the sponge Halichondria panicea[14,11]. The results in the present study are contrary to those reported for the activity using salivary gland extracts of Octopus[15]. Components extracted from the marine sponge Aurora globostellata from Tuticorin, India showed immunosuppressant potential[3]. It has been reported that immunostimulants increase the resistance against diseases in fishes and therefore there was an excessive interest in the modulation of the non-specific immune response of fish to elevate the general defence barriers[16,17]. The use of immune stimulants for prevention of diseases in fish is considered as an alternative and promising area[18]. Immunomodulatory effects of bioactive natural products from marine sources are very poorly studied and reported. The results of the In vitro PMN function test showed a significant decrease in the percentage of phagocytosis and phagocytic index for successive crude and fractionated extracts.

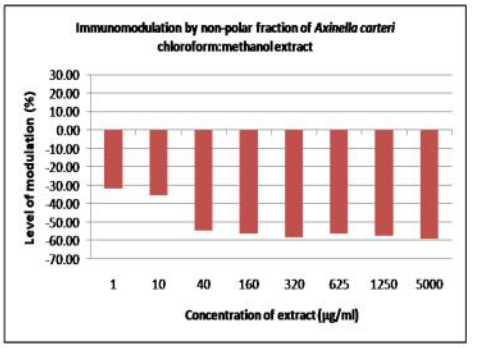
The results indicate that these extracts suppressed the phagocytic efficacy of the PMN cells by causing more engulfment of the Candida cells, thereby stimulating a non-specific immune response. These outcomes are encouraging enough to pursue structure elucidation of the active components.

Concentration of *Axinella carteri* chloroform: methanol crude extract (µg/ml)	Phagocytosis %	Phagocytic index	% Modulation
1	70	1.73	-22.83
10	83	1.57	-30.08
40	78	1.47	-34.18
160	65	1.38	-38.19
320	63	1.37	-39.06
625	58	1.40	-37.65
1250	55	1.36	-39.12
5000	75	1.17	-47.62



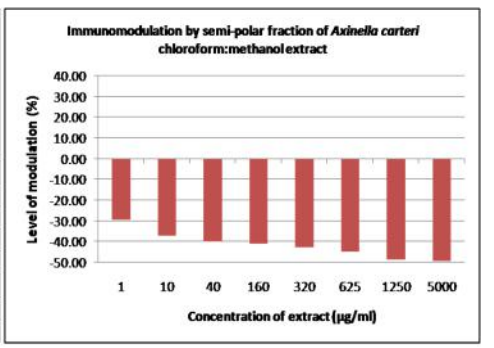
Tab: 1 Immunomodulation by Axinella carteri chloroform:methanol crude extract.

Concentration of non-polar fraction of *Axinella carteri* chloroform: methanol extract (µg/ml)	Phagocytosis %	Phagocytic index	% Modulation
1	68	1.53	-31.72
10	61	1.44	-35.60
40	55	1.02	-54.55
160	43	0.98	-56.40
320	43	0.93	-58.47
625	40	0.98	-56.47
1250	38	0.95	-57.71
5000	35	0.91	-59.18



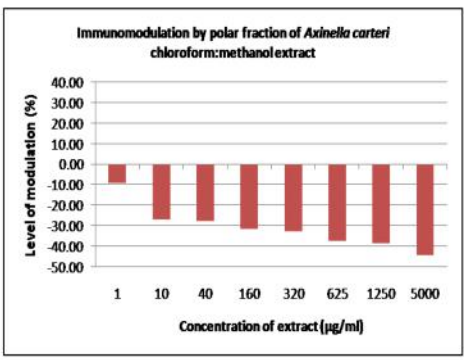
Tab: 2 Immunomodulation by non-polar hexane fraction of Axinella carteri chloroform:methanol extract.

Concentration of semi-polar fraction of *Axinella carteri* chloroform: methanol extract (µg/ml)	Phagocytosis %	Phagocytic index	% Modulation
1	87	1.57	-29.70
10	79	1.41	-37.27
40	73	1.34	-40.07
160	69	1.32	-41.12
320	68	1.28	-42.88
625	65	1.23	-45.05
1250	69	1.14	-48.89
5000	69	1.13	-49.53



Tab: 3 Immunomodulation by semi-polar acetone fraction of Axinella carteri chloroform:methanol extract.

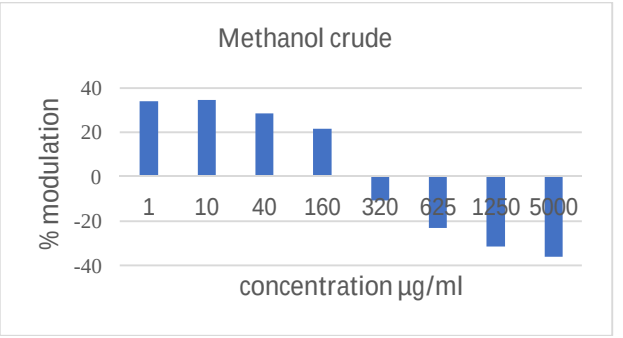
Concentration of polar fraction of *Axinella carteri* chloroform: methanol extract (µg/ml)	Phagocytosis %	Phagocytic index	% Modulation
1	84	2.04	-9.12
10	74	1.63	-27.03
40	63	1.62	-27.72
160	62	1.53	-31.60
320	61	1.51	-32.67
625	60	1.40	-37.50
1250	57	1.38	-38.47
5000	52	1.25	-44.39



Tab: 4 Immunomodulation by polar n butanol fraction of Axinella carteri chloroform:methanol extract

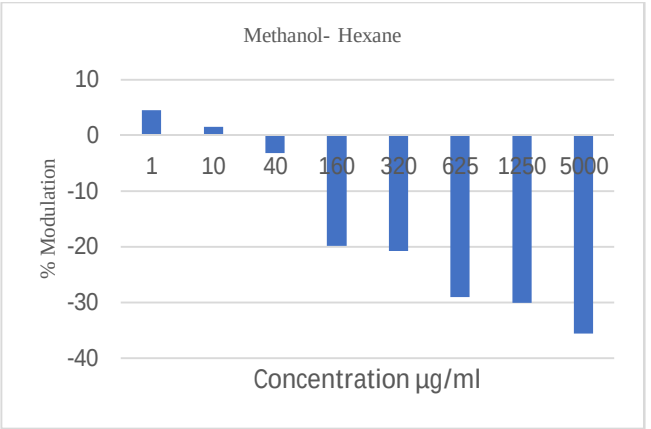
Tab: 5 Immunomodulation by Axinella carteri methanol crude extract.

Conc. Of A. carteri crude methanol(µg/ml)	Phagocytosis %	Phagocytic index	% Modulation
1	88	3	33.93
10	87	3.01	34.44
40	73	2.88	28.42
160	62	2.73	21.69
320	61	2	-10.71
625	61	1.72	-23.16
1250	58	1.53	-31.5
5000	37	1.43	-36.05



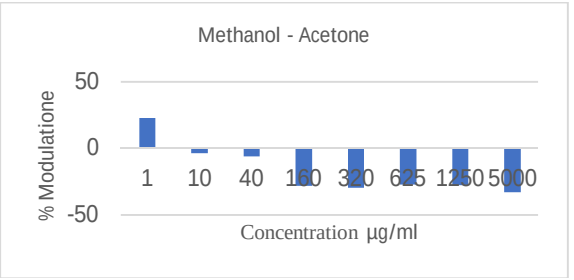
Tab: 6 Immunomodulation by hexane fraction of Axinella carteri methanol crude extract.

Conc. Of A. carteri methanol Hexane(µg/ml)	Phagocytosis %	Phagocytic index	% Modulation
1	82	2.32	4.53
10	76	2.28	1.62
40	71	2.17	-3.17
160	69	1.8	-19.77
320	67	1.78	-20.71
625	61	1.59	-29.01
1250	60	1.57	-30.06
5000	52	1.44	-35.61



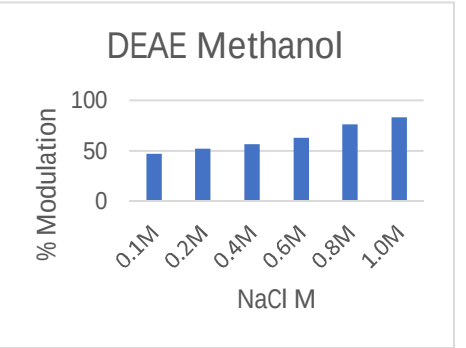
Tab: 7 Immunomodulation by acetone fraction of Axinella carteri methanol crude extract.

.Conc. Of A. carteri methanol - Aetone(µg/ml)	Phagocytosis %	Phagocytic index	% Modulation
1	98	2.74	22.54
10	86	2.15	-3.97
40	79	2.1	-6.19
160	71	1.61	-28.32
320	69	1.58	-29.48
625	64	1.63	-27.46
1250	44	1.61	-27.96
5000	40	1.5	-33.04



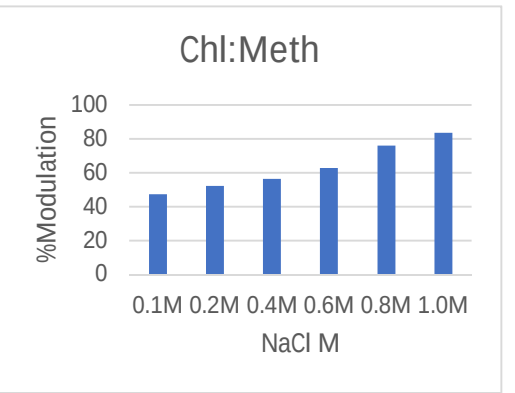
Tab: 8 Immunomodulation of Methanol DEAE Fractions

Conc. Of A. carteri DEAE methanol	Phagocytic index	% Modulation
0.1M	0.15	33.33
0.2M	0.18	44.44
0.4M	0.24	58.33
0.6M	0.35	71.43
0.8M	0.42	76.19
1.0M	0.65	84.62



Tab 9: Immunomodulation of Chl:Meth DEAE Fractions

Conc. Of A. carteri DEAE Chl:meth	Phagocytic index	% Modulation
0.1M	0.11	47.37
0.2M	0.14	52.38
0.4M	0.16	56.52
0.6M	0.19	62.96
0.8M	0.2	76.19
1.0M	0.21	83.61



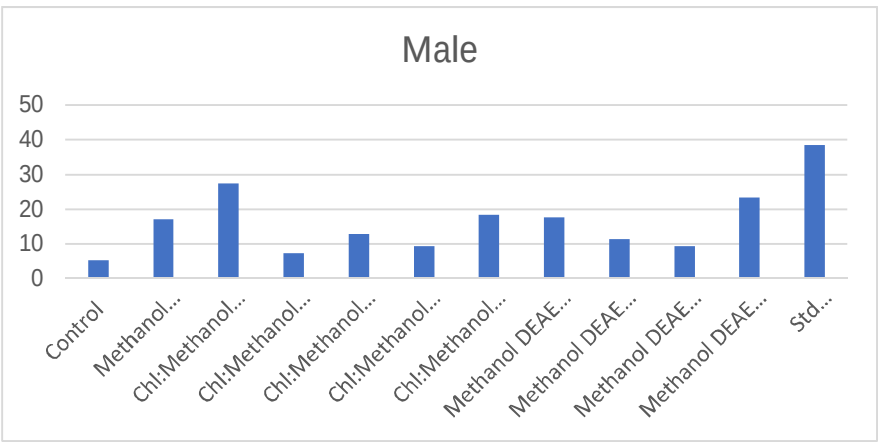


Fig 1: In vivo Phagocytosis index in male albino mice using carbon clearance assay in partially purified extracts of A.carteri.

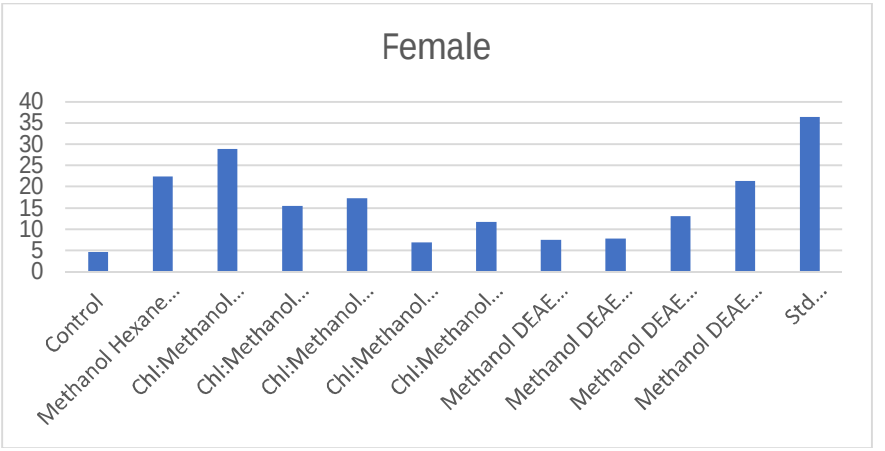


Fig 2: In vivo Phagocytosis index in female albino mice using carbon clearance assay in partially purified extracts of A.carteri.

In-vivo study (Fig 1 & 2)

The in-vivo study of phagocytosis index by carbon clearance was done on swiss albino male and female mice. High phagocytic activity was observed in Hexane extract with a value of 28.96% in female and 27.43 % in male. It was followed by 1ml DEAE Methanol extract having a value of 23.46% in male and 21.35% in female. The highest phagocytic activity of 38.55% in male and 36.41% in female albino mice was observed in Standard cyclophosphamide. No in-vivo phagocytic activity was detected in acetone extract, crude methanol and crude chloroform methanol extracts of A.carteri.

The new strategy for the treatment of various diseases like infections, tumours autoimmune diseases etc. can be developed through studies on immune system. The enhancement of immune system is called immune-stimulation and implies stimulation of the function and efficiency of the granulocytes, macrophages and complement etc., while immunosuppression implies mainly to reduce resistance against infections, stress or chemotherapeutic factors. In the present In-vitro study the extracts of sponge Axinella carteri in Hexane, acetone, DEAE methanol, DEAE chloroform and crude chloroform:methanol extract was found to supress the immune system. The crude methanol extract showed immunostimulatory activity at lower concentration while at higher concentrations it was found to reduce the phagocytic effect thus causing immunosuppression. The A.carteri extract can thus enhance phagocytic activity of polymorphonucleocytes (PMN) cells by causing more engulfment of Candida albicans. This extracts can thus be used in therapeutic medicines to improve the functioning of immune system.

In-vivo study by carbon clearance extract revealed that Hexane extract and Crude methanol extract in lower concentration can stimulate immune response. DEAE chloroform extract can increase the immunostimulation with slight increase in concentration while DEAE methanol was found to be immunosupressent even with slight increase in concentration. It has been suggested that in In-vivo assay macrophages probably secrete a number of cytokines which in turn stimulate other immunocytes and give host the defence ability to encounter the infectious stress[19].

4 Conclusions

The study shows that the extracts of A.carteri are dose dependent and their effect on the immuno response can be determined by studying the effect of different doses. The A. carteri extracts in In-vitro study was found to show immuno suppresion. In-vivo study also indicates immuno stimulant effect but only in DEAE chloroform and DEAE methanol extract at low concentration while crude chloroform was not found to have any effect on immune system. The standard cyclosphosphamide was found to show high activity compared to other extracts. The use of A.carteri extract needs further detailed investigations for understanding its role in immunomodulation and its use in therapeutic medicine.

References

1. Senthilkumar K and Kim SK (2013) Marine invertebrates natural products for anti-inflammatory and chronic diseases. Evid Based Complement Alternat Med: 1–10.
2. Azevedo L.G., Peraza G.G., Lerner C., Soares A., Murcia N. and Muccillo-Baisch AL(2008). Investigation of the anti-inflammatory and analgesic effects from an extract of Aplysina caissara, a marine sponge. Fundam Clin Pharmacol. 22: 549–556.
3. Joseph B. and Sujatha S. (2011). Pharmacologically Important Natural products from Marine Sponges. Journal of Natural Products 4: 5-12.
4. Sipkema D., Franssen Maurice C.R., Osinga R. and Tramper J. (2005). Marine Sponges as Pharmacy. Marine Biotechnology, 7: 142–162.
5. El-Shitany N., Shaala L., Abbas A., Abdel-Dayem U., Azhar E., Ali S., van Soest R. and Youssef D (2015). Evaluation of the Anti-Inflammatory, Antioxidant and Immunomodulatory Effects of the Organic Extract of the Red Sea Marine Sponge Xestospongia testudinaria against Carrageenan Induced Rat Paw Inflammation. PLoS One. 10(9).
6. Kalirajan A., Karpakavalli M. , Narayanan K. , Ambiganandham K., Ranjitsingh A. and Sudhakar S (2013). Isolation, Characterization and phylogeny of Sponge - associated bacteria with Antimicrobial and Immunomodulatory potential. Int.J.Curr.Microbiol.App.Sci, 2(4): 136-151.
7. Roy M., Ohtani I., Ichiba T., Tanaka J., Satari R. andHiga T. (2000). New Cyclic Peptides from the Indonesian Sponge Theonella swinhoei.

Tetrahedron, 56: 9079- 9092.
8. Chairman K., Jeyamala M., Sankar S., Murugan A. and Singh A. (2013). Immunomodulating Properties of Bioactive Compounds Present in Aurora globostellata. International Journal of Marine Science, 3(19):151-157.
9. Arya V. and Gupta V. (2011). A review on marine immunomodulators. International journal of pharmacy and life science: 751-758.
10. Kumar M. and Pal A. (2012). Investigation of bioactivity of extracts of Marine Sponge, Spongosorites halichondrioides (Dendy, 1905) from western coastal areas of India. Asian Pacific Journal of Tropical Biomedicine. 2(3): S1784-S1789.
11. Purushottama G.B., Venkateshvaran K., Pani Prasad K. and Nalini P. (2009). Bioactivities of extracts from the marine sponge Halichondria panicea. J Venom Anim Toxins Incl Trop Dis, 15(3): 445.
12. Kulkarni S. and Karande V. (1998). Study of the immunostimulant activity of naphthoquinone extract of leaves of Lawsonia alba Linn. Indian Drugs. 35 (7): 427-33.
13. Yan Y., Wanshun L., Baoqin H., Changhong W., Chenwei F. and Bing L. (2007). The antioxidative and immunostimulating properties of d-glucosamine. Int Immunopharmacol. 7: 29-35.
14. Asha J. Rao, Sanjay P. Jumale and Siddhi A. Malgundkar (2015). Evaluation of In vitro Immunomodulatory Potential of Extracts of the Marine Sponge Halichondria panicea. Bionanofrontier Vol.8(3): 141-144.
15. Gayathri N., Asha J Rao and Manekar A.P. (2014). Immunomodulatory Potential of Salivary Gland Extracts of Octopus. World Journal of Pharmacy and Pharmaceutical Sciences. Vol.3(5): 624-631.
16. Raa J. (2000). The use of immunestimulants in fish and shellfish feeds. In: Cruz-Suarez LE, Ricque-Marie D, Tapia-Salazar M, Olvera-Novoa MAY, Civera-Cerecedo R, (Eds.). Avances en Nutricion Acuicola V. Memorias del V Simposium International de Nutricion Acuicola. 19-22.
17. Sahoo P.K. and Mukherjee S.C. (2002). The effect of dietary immunomodulation upon Edwardsiella tarda vaccination in healthy and immune compromised Indian major carp Labeo rohita. Fish and Shellfish Immunology. 12(1): 1-16.
18. Sakai M. (1999). Current research status of fish Immunostimulants. Aquaculture. 172: 63-92.
19. Ponkshe C. and Indap M. (2002). In vivo and In vitro evaluation for immunomodulatory activity of three marine animal extracts with reference to phagocytosis. 40(12):1399-402