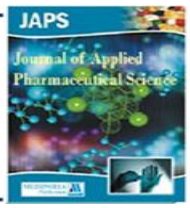
Journal of Applied Pharmaceutical Science
Available online at www.japsonline.com



ISSN: 2231-3354
Received on: 04-01-2012
Revised on: 09:01:2012
Accepted on: 13-01-2012

Antifungal and Cytotoxic Activities of Some Marine Sponges Collected from the South East Coast of India

D. Isaac Dhinakaran, Manohari .V, Atchya .B, Tamilselvi .K and A. P. Lipton

D. Isaac Dhinakaran†, Manohari .V, Atchya .B, Tamilselvi .K
Department of Biotechnology, Ayya Nadar Janaki Ammal College, Sivakasi- 626124, TN, India,

† *Manonmanium sundaranar university,Centre for marine science and Technology (CMST), Rajakkamangalam.*

A. P. Lipton
Vizhinjam Research Centre of Central Marine Fisheries Research Institute,Vizhinjam - 695 521, Kerala, India.

For Correspondence
D. Isaac Dhinakaran
Email: isaacdhina@yahoo.co.in

ABSTRACT

The present work describes the biological activities using the marine sponges collected from kanyakumari. The sponges are such as the *Callyspongia diffusa, Echinodictyum gorgonoides, Callyspongia reticutis, Gelliodes cellaria, and Thalysias vulpine.* It is revealed that the sponges showed the antifungal activity against the various fungal strains such as the *Aspergillus niger, Pencillium notatum,and Candida albicans* by using the agar well diffusion method. The sponge crude extracts seems to have effective cytotoxic property that was detected by Brine shrimp assay. Hence it is assumed that the marine sponges act as the vital source for the development of anticancer drugs.

Keywords: *Callyspongia Diffusa, Echinodictyum Gorgonoides, Callyspongia Reticutis, Gelliodes Cellaria,* and *Thalysias Vulpine*

INTRODUCTION

Sponges, exclusively are aquatic and mostly marine, are found from the deepest oceans to the edge of the sea. There are approximately 15,000 species of sponges in the world, of which, 150 occur in freshwater, but only about 17 are of commercial value (Brusca and Brusca, 1990). A total of 486 species of sponges have been identified in India. In the Gulf of Mannar and Palk Bay a maximum of 275 species of sponges have been recorded. The distribution of sponges in other area are in Gulf of Kutch – 25 species; and Orissa coast – 54 species (Thomas ,1998). Technologies have been developed to produce novel products from marine sponges; which could contribute to human healthcare (e.g. bioactive compounds that can be used for new medicines), A variety of natural products from the marine sponges have been found to exhibit remarkable antitumour and anti-inflammatory activities (Edrada *et al.*, 2002). It has been proved that marine organisms are excellent source of bioactive secondary metabolites and number of compounds of originated from marine organisms had been reported to possess *in vitro* and *in vivo* immuno stimulatory activity (Donia and Hamann 2003).

Marine sponges are rich source of pharmacologically active compounds that can potentially be used as medicines to cure human diseases, and the isolation of bioactive compounds from sponges have tremendous effect. Antitumour studies were conducted with 19 marine natural products in a number of experimental and clinical models proved that sponges act as an excellent source for bioactive compounds (Azevedo *et al*., 2008). Among the groups of marine organisms, sponges are the most diverse and abundant, due to their soft bodies and sedentary life styles.These marine invertebrates have evolved chemical defense mechanisms against other invading organisms, which involve the production of secondary metabolites (Li kam wah *et al*., 2006). Recently, studies suggested that some bioactive compounds isolated from marine organisms have been shown to exhibit anti-cancer, anti-microbial, anti-fungal or anti-inflammatory and other pharmacological activities (Venkateswara rao *et al*., 1998). So the aim of the study to screen out the antifungal and cytotoxicity effects of the sponges collected from the south east coast of India.

MATERIALS AND METHODS

Collection of Sponges

The marine sponges were collected in kanyakumari, Tamilnadu from the fish nets using by catch method. Then they were stored in sterile containers containing sea water and transferred to the laboratory under sterile conditions. Then sponges were identified based on their taxonomic position.

Preparation of sponge extracts

Crude extracts were prepared using the method of (Bakus and Green,1981). The sponges were dried in air for 2 days, and then 10 grams of sponge tissue was soaked in 200 ml of methanol for 5 hours. The solvent was removed after squeezing the sponge and filtered through Whatman filter paper No. 1. The solvent was evaporated at low pressure using a Buchi Rotavapor at 60ºC and the extract was stored in refrigerator for further use.

Brine shrimp cytotoxicity

Dried cysts of *Artemia salina* were incubated in natural sea water at 28°C-30°C under constant aeration for 48hrs. After hatching, active naupli free from egg shells were collected from brighter portion of the hatching chamber and used for the assay. 20 artemia naupli were added into each concentration of extract in 24 well microtitre plate.

Control was maintained with DMSO instead of extract. After 24 hrs, dead shrimp was counted using microscope. Larvae that did not exhibit any internal or external movement during several seconds of observation was calculated as dead. Percentage of mortality was calculated to determine the LC_{50} values of the extract using the probit scale analysis.

Antifungal activity

The warm melted and autoclaved Potato dextrose agar was poured in separate sterilized petri plates under aseptic conditions. The plates were covered and allowed to solidify. The agar plates were swabbed with the fungal organisms *Penicillium notatum, Candida albicans, Aspergillus niger*. Three wells were made by using 6mm cork borer or puncher that was sterilized with alcohol and flame. Sponge extracts at different concentrations 50µl, and 100 µl were added in the wells and the methanol solvent was used as the control. The plates were incubated at 37ºC for 2-3 days. After the completion of incubation period, the zone of growth inhibition was measured in millimeters using a caliper or ruler. The measurements were recorded.

RESULTS

The present results represents the antifungal activity and the cytotoxic effect against the brine shrimp *Artemia salina* using the various sponge crude methanolic extracts such as the *Callyspongia diffusa, Echinodictyum gorgonoides, Callyspongia reticutis, Gelliodes cellaria,* and *Thalysias vulpina* collected from the region of kanyakumari. Table 1 shows the details regarding the taxonomic position of the sponges. The table 2 describes the brine shrimp mortality rate in 24h due to the concentration of extracts from 0.1 to 1% doses. The LC_{50} values are represented in table 2. The median lethal dose of the sponge the *Callyspongia diffusa* showed that it produces the mortality rate at 5.20% concentration. The LC_{50} values of the sponge *Echinodictyum gorgonoides* and *Callyspongia reticutis* were 7.54 and 0.72 respectively. It also suggests that the crude extracts of the sponge *Gelliodes cellaria* and *Thalysias vulpina* showed significant mortality at 4.52 % and 0.52%. Then the table 3 illustrates the antifungal activity of the sponges *Callyspongia diffusa, Echinodictyum gorgonoides, Callyspongia reticutis, Gelliodes cellaria,* and *Thalysias vulpina.* The zone of inhibition was observed for two concentrations (50µl, 100 µl,) of the extracts against various fungal isolates such as *Aspergillus niger, Penicillium notatum* and *Candida albicans.* For *Thalysias vulpina* which showed the maximum inhibitory concentration against the *aspergillus niger* and *candida albicans* with the zone of inhibition at 12mm for 50µl and it was observed as 14mm for 100 µl concentrations. It does not show any effect against the *penicillium notatum*. The sponge *Gelliodes cellaria* showed the least effect against the *Aspergillus niger, Penicillium notatum* and *Candida albicans.* The other sponges *Callyspongia diffusa, Echinodictyum gorgonoides, Callyspongia reticutis,* showed moderate significant activity against the three fungal organisms.

Table 1: List of sponges collected from the south east coast of India.

Taxonomic position of the sponges				
Name of the sponges	Phylum	Class	Order	Family
Callyspongia diffusa	Porifera	Demospongiae	Haplosclerida	Callyspongidae
Echinodictyum gorgonoides	Porifera	Demospongiae	Poecilosclerida	Phorbasidae
Callyspongia reticutis	Porifera	Demospongiae	Haplosclerida	Callyspongidae
Gelliodes cellaria	Porifera	Demospongiae	Haplosclerida	Niphatidae
Thalysias vulpine	Porifera	Demospongiae	Poecilosclerida	Lophomnidae

Table 2: Mortality of Brine shrimp exposed for 24 hours to different concentrations of methanol extract from sponge extracts (± represents standard deviation).

Conc of extracts (%)	Mortality %				
	Cally spongia diffusa	***Echinodictyum gorgonoides***	***Callyspongia reticutis***	***Gelliodes cellaria***	***Thalysias vulpine***
Control	Nil	Nil	Nil	Nil	Nil
0.1	*15.0* ±0.54	*10.0* ±0.32	*10.2*±0.28	*15.6* ±0.50	*20.0* ±0.10
0.2	*30.0* ±0.70	*20.0* ±0.70	*30.4*±0.64	*20.*4±0.58	*30.2* ±0.60
0.4	*40.1* ±1.20	*35.0* ±1.20	*40.0* ±1.52	*40.*4±.80	*50.0* ±1.52
0.6	*60.0* ±1.70	*50.0* ±1.70	*80.0* ±2.20	*60.2* ±1.50	*70.0* ±2.20
0.8	*70.2*±1.80	*70.6* ±1.70	*90.0* ±3.16	*70.0* ±2.10	*90.0* ±3.16
1.	*90.4* ±3.80	*90.2* ±4.0	*100*.0 ±0.0	*90.0* ±4.20	*100* .0±0.0

Table 3: LC_{50} (% extract) value of the sponge methanolic crude extracts to *Artemia salina*.

Samples	LC_{50} (in % extract)
Callyspongia diffusa	*5.20*
Echinodictyum gorgonoides	*7.54*
Callyspongia reticutis	*0.72*
Gelliodes cellaria	*4.52*
Thalysias vulpine	*0.52*

Table 4. Antifungal activity of marine sponge crude extracts.

Name of the Sponges	Zone of Inhibition (diameter in mm)					
	Aspergillus niger		***Pencillium notatum***		***Candida albicans***	
	50µl	100µl	50µl	100µl	50µl	100µl
Callyspongia diffusa	1.2	1.2	1.3	1.5	0.8	1.8
Echinodictyum gorgonoides	1.1	1.3	1	1.2	0.9	1.4
Callyspongia reticutis	0.7	1.0	0.5	0.9	1.0	1.5
Gelliodes cellaria	0.6	0.9	0.6	0.4	0.4	2.0
Thalysias vulpina	0.8	0.9	0.5	2.0	1.3	2.0

DISCUSSION

From the previous studies it was suggested that the Brine shrimp lethality assay considered to be one of the most useful tools for the preliminary assessment of biotoxicity and bioassay with cytotoxic activity against some human solid tumors. The antitumour activity of cell free extracts from sponge associate actinomycetes might be due to the presence of the active secondary metabolites alkaloids and guninine (Selvin and Lipton, 2004). The sponges *Gelliodes cellaria* and Thalysias vulpina seems to have tremendous effect of cytotoxicity. (Pawlik et al., 2002 reported that sponges are primitive marine invertebrates which contains more natural products than any other marine phylum. Many of their products have strong bioactivities including anticancer, antimicrobial , immunomodulatory, haemolytic and anti-inflammatory activities, and are often applicable for medical use. In the present study we found that the marine sponge have shown some antifungal, and cytotoxic effects. The methanolic extract of the mairne sponge Haliclona exigua showed promising antifungal activity against Candida albicans,Cryptococcus neofromans ,Aspergillus fumigatus, and Candida parapsilolis. . The marine sponges include Amphimedon viridis,Neopetrosia sp. possess antileishmanial activity. The Haliclona exigua ,is active against the rat brain nitric oxide synthase (Lakshmi et al., 2010). Hence it is assumed that the methanolic crude extracts of sponges Thalysias vulpina, Gelliodes cellaria, Callyspongia diffusa, Echinodictyum gorgonoides, and Callyspongia reticutis showed vigorous antifungal activities against Callyspongia diffusa, Echinodictyum gorgonoides, and Callyspongia reticutis showed vigorous antifungal activities against the Aspergillus niger, Penicillium notatum and Candida albicans. Marine sponge have been shown to produce many natural bioactive agents, including alkenes, and many of the sponge-derived compounds that have entered clinical and pre-clinical development are believed to be ultimately microbial in origin (Proksch *et al*., 2002). Sponges of the class Demospongiae are known to produce the largest number and diversity of secondary metabolites isolated from marine invertebrates, most of them with medically relevant biological activities and important ecological roles (Faulkner, 2002). The toxicity of sponges has been well-documented, which could be ascribed to the diverse and potent cytotoxic compounds (Lee and Quian, 2003).

CONCLUSION

The main focus of the study reveals that the marine sponges act as the potential source for the development of new active c ompounds in development of drugs. It also shows significant antifungal and cytotoxic effects.

ACKNOWLEDGEMENTS

The authors are thankful to the correspondent and the Principal, Dr. S. Baskaran of the Ayya nadar janaki ammal college for providing the facilities and the encouragement.

REFERENCES

Azevedo L.G., Peraza G., Lerner C., Soares A., Murcia N., and Muccillo B.A. Investigation of the anti-inflammatory and analgesic effects from an extract of *Aplysina caissara*, a marine sponge *Fundament. Clinical Pharmacol. 2008;* 22(5): 549-556.

Bakus G.J., Green G. Toxicity in sponges and holothurians: A geographic pattern, *Science*. 1981; 185: 951-953.

Brusca R.C., Brusca G.J. Phylum Porifera: The sponges, in *Invertebrates*, edited by A D Sinauer (Sinauer Press, Sunderland, Mass), (1990) 181-120.

Donia M., Hamann M.T. Marine natural products and their potential applications as anti-infective agents. *Lancet- Infec. Dis.* 2003; 3: 338-348.

Edrada R.A., Ebel R., Supriyono A., Wray V., Schupp P. A new highly active calyculin derivative from the marine sponge, *Theonella swinhoei*, J *Nat Prod*. 2002; 65: 1168-1172.

Faulkner D. J., Marine natural products. *Nat. Prod. Rep. 2002;* 19: 1 - 48.

Lakshmi V., Mishra S.K., Srivastava S., Chaturvedi A., Srivastava M.N., Shukla P.K. Antifungal activity of marine sponge *Haliclona exigua*. *Journal of mycology medicale*. 2010; 20: 31-35.

Lee O., Qian P.Y. Chemical control of bacterial epibiosis and larval settlement of *Hydroides elegans* in the red sponge *Mycale adherens*. *Biofouling*. 2003; 19: 171 - 180.

Li Kam Wah H., Jhaumeer-Laulloo S., Choong Kwet Yive R., Bonnard I., Banaigs B. Biological and chemical study of some soft corals and sponges collected in Mauritian waters, Western Indian Ocean. *J. Mar. Sci*. 2006; 5: 115-121.

Pawlik J.R., Mcfall G., Zea S. Does the odor from Sponges of the genus *Ircinia* protect them from fish predators. *J. Chem. Ecol*. 2002; 28: 1103–1115.

Proksch P., Edrada R.A., Ebel R. Drugs from the seas-current status and microbiological implications. *Appl. Microbiol. Biot. 2002;* 59: 125-134.

Selvin J., Lipton A.P. Biopotential of secondary metabolites isolated from marine sponges. *Hydrobiologia.* 2004; 513: 231-238.

Thomas P.A., Porifera, in *Faunal diversity in India,* edited by J R B Alfred, A K Das and A K Sanyal (ENVIS Centre, Zoological Survey of India, Kolkata), 1998; 28-36.

Venkateswara Rao J., Desaiah D., Vig P.J.S., Venkateswarlu Y. Marine biomolecules inhibit rat brain nitric oxide synthase. *Toxicology*. 1998; 129: 103-110.