ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 5, Issue, 02, pp. 124-128, February, 2013

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

In vitro Determination of Marine Sponge *Hyrtios erectus* Secondary Metabolite Effect against Human Breast and Larynx Cancer Cell Lines

[1]Ramachandran, M. [2]Titus Immanuel, [1]Manley Backyavathy, P. and [1*]Balwin Nambikkairaj

[1]Department of Zoology, Voorhees College, Thiruvalluvar University, Vellore, Tamil Nadu, India
[2]Fisheries Division, Central Agricultural Research Institute, Port Blair, A and N Islands, India

ARTICLE INFO

Article History:
Received 19th November, 2012
Received in revised form
24th December, 2012
Accepted 17th January, 2013
Published online 14th February, 2013

Key words:
Hyrtios erectus,
Antitumor,
Cytotoxicity,
Methanol extract,
Zoochemical screening.

ABSTRACT

Marine sponges are rich sources of novel secondary metabolite and they are potential drug molecule to antitumor and antiprolific drug development. *Hyrtios erectus* was extracted and purified through different solvent fractions methods. Pure compound was obtained as brownish amorphous powder. The positive test for dragendorff reagent was indicated as alkaloid group. Cytotoxicity was tested on Normal (Vero) cell line non tumor cells, Human Breast cancer cell line (MCF-7) and Human epithelial larynx cancer cell line (Hep-2) using microculture tetrazolium (MTT) assay for anticancer activity. Sponge pure compound was non-toxic to (Vero) cells but highly cytotoxicity to (53%) MCF-7 cells and low toxic to Hep-2(32%) was recorded at 25µg/ml concentration. Each concentrations express individual results of cells viability with cytotoxicity increase and decrease of their concentration level of pure compound. In this research further investigation require for this sponge purified compound in order to chemical structure elucidations as well as pre clinical study for anticancer activity.

Copy Right, IJCR, 2013, Academic Journals. All rights reserved.

INTRODUCTION

Marine sponge are rich source of structurally novel and biologically active secondary metabolites considered to be true "chemical factories" producing hundreds of unique chemical compounds with different biological activities have been isolated. Sponges are simple, multicellular, sessile invertebrates with no true tissue layers or organ; they are cytotoxic, inhibit cell proliferation and could be used as chemotherapeutics (Bergquist, 1978). These compounds differ structurally and act on different cytoskeleton elements, but have similar anti prolific and antitumor activities. Moreover, many antitumor drug isolated and their structure determined, but their biological roles and activities are still largely unknown (Bacus and Green, 1974; Kornprobst,2005). *Hyrtios erectus* synonym *H.erecta (*taxonomy: phylum *Porifera*, class *Demospongiae*, order *Dictyoceratida*, family *Thorectidae)* is an animal colonies, sedentary, grayish in color, irregularly rounded, asymmetrical, attached to sea bottom by means of masses of spicules.

These colonies are attached on the dead coral stones in shallow water inter-tidal areas at the depth of 10-20m. Sponge secondary metabolites show interesting biological activities, for example calyculins from *Discodermia calyx* (Sipkema *et al*., 2005), discodermolide from *D. dissolute* (Kato *et al*., 1986), latrunculins from *Latrunculia magnifica* (Gunasekera, 1990; Kashman, 1980; Kashman *et al*., 1985; Spector, 1983), and spongistatins from *Spongia sp*. and *Spirastrella sp*. (Spector, 1989; Petit, 1993). The secondary metabolites from the genus *Hyrtios sp* particularly *Hertios erectus* synonym *H.erecta* have been investigated extensively (Petit *et al*., 1994; Kobayashi *et al*., 1994; Miyaoka, 2000; Pettit *et al*., 1998; Yonghong, 2007). Previous chemical investigations of different *Hyrtios sp.* and their associated microorganisms revealed the presence of numerous structurally unique natural products including scalarane sesterterpenes, acyclic triterpenes, indole alkaloids and macrolides in addition to steroids. Many of these compounds possess interesting biological activities. spongistatins, the most important metabolites of the genus *Hyrtios* discovered to date, showed powerful anticancer activity (Youssef, 2002; Pettit, 1998). The chemical investigations, which afforded the antineoplastic agents sesterstatins (Longeon *et al*., 2011; Pettit *et al*., 1998). Cytotoxicity assays are a widely used method in vitro toxicology studies. It is not only rapid and standardized, but also a sensitive and inexpensive method to measure drug induced alterations in metabolic pathways or structural integrity which may or may not be related to directly to cell death (Pettit and Tan, 2005; Habib and Karim, 2011). Recent developments in the field of marine antitumor research with emphasis on the signal transduction pathways of oncogenesis, clinical status of the marine derived antitumor compounds and discuss the potential challenges and probable strategies to combat the gap between marine compound isolation and successful antitumor drug development.

MATERIALS AND METHODS

Sponge collection

Sponge tissue was collected by scuba divers at (10-20m) deep along the South Andaman Sea coast (North Bay and Pongibalu region11°30'N and 92°39'E). The sponge collection was made in the month of June, 2011. Samples were kept instead plastic packs in ice boxes before freezing at -20°C until analysis. Sample was preserved in 100% methanol solvent. Taxonomy details were identified and deposited at the Central agricultural research institute, Fisheries division, Port Blair. A voucher specimen is under the accession number (PB5985).

***Corresponding author:** insilicobrain@gmail.com

Extraction/Fractionation/Isolation

The fresh sponge *Hyrtios erectus* (3kg weight) was chopped into small pieces and filled in glass containers soaked in methanol (3 x 2.5L) which was shacked well twice daily around one weak. Methanol extract was filtered by No.1 Whatman filter paper (GE Healthcare, 125mm, UK) pooled into rotary evaporator (STRIKE 202, Germany). Methanol extract was concentrated under reduced pressure 45°C, which was further dried under high vacuum (42g). The residual mass was further extracted with methanol-chloroform (80:20%). Complete drying the methanol-chloroform extract, a residual mass of (23g) was obtained. Methanol extract showing promising activity in vitro models, it was fractionated into four fractions, i.e. the hexane(2g), chloroform(4g), n-butanol (6g)soluble and n-butanol (11g) insoluble fractions. The hexane fraction was found to be a complex mixture and was of low yield. Moreover, hexane and chloroform fractions were mixed combined fraction was included for purification of its components was achieved by chromatography over column of silica gel. One pure compound was obtained after re-chromatography over flash silica gel (230-400 mesh) of the combined chloroform and hexane fraction (Lakshmi *et al.,* 2012). Thin Layer Chromatography (TLC) analysis was carried out for identifying purified compound spot using aluminium sheet precoated silica gel 60 F254 or on glass precoated RP-18 F254 plates (Merck, Darmstadt, Germany).

Zoo chemical analysis

The Zoochemical analysis absence of an established protocol for the screening of chemical constituents in animal tissues, the qualitative analysis for the possible bioactive components present in the sponge samples were done using established phytochemical screening protocol (Edeoga , 2005).

Cytotoxicity Evaluation

The cell lines Human Breast cancer(MCF-7), Human epithelial larynx cancer(Hep-2) and Non tumourous normal cells(Vero) were obtained from American type culture collection (ATCC) Manassas, VA, and cultured in Dulbecco's modified eagle's medium supplemented with10% fetal bovine serum (FBS), 2mM glutamine and 100 mg/ml Penicillin-Streptomycin incubated at 37°C, humidified atmosphere with 5% CO_2. Sponge purified compound was subjected into invitro analysis of cytotoxicity using MTT {(3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (Sigma, St. Louis, USA)} assay. Obtained cells were grown at concentration of 5 x 103 cells/well in 96 well plates. After24 h, cells were washed with fresh medium and were treated with different concentrations of crude extract (25, 50, 100 and 500mg/ml). The cells without the addition of sponge purified compound were taken as control. After 24 h incubation, 100 ml of MTT solution was added and further incubated for4 h at 37 °C. Eventually 100 ml DMSO was added to solubilize the formazan salt formed and the amount of formazan salt was determined by measuring the OD at 540 nm using microplate reader. The relative cell viability was determined by the amount of MTT converted into formazan salt. Viability of cells was quantified as a percentage compared to that of control. The experiment was carried out in triplicate and the data were expressed as mean from these three sets of experiments (Chairman, 2012).

% Viability = (Test OD Value / Control OD value) X 100

Statistical Analysis

The experimental data were expressed as mean ± sd. The significance of difference among the various treated groups and control group were analyzed by means of one-way ANOVA. The level of significance was set at $p < 0.05$. IC50 (inhibitory concentration which caused 50% inhibition) were estimated using linear regression method of plots of the percent of cell viability against the concentration of the tested compounds.

RESULTS

The pure compound was isolated from marine sponge 'hexane' with 'chloroform' combined fraction and brownish amorphous powder respectively. The positive tests of the pure compound are determined by the method of dragendorff reagent which was identified alkaloid group. In this research three cancer cell lines are (Vero) normal cell line non tumor cells, (MCF-7) Human Breast cancer cell line and (Hep-2) Human epithelial larynx cancer cell line selected for anticancer assays. In this study, the normal Vero cell line expressed 91% cells viability and 8% of inhibition of cancer cells was found at low concentration 25mg/ml of pure compound are shown in Table.1. The (MCF-7) breast cancer cell line low viability 46% and cytotoxic 53% was observed, even though 14% of viability and 85% of cytotoxic in 500mg/ml concentration level was recorded (Fig.1-2). Similarly result of (Hep-2) cancer cell line cells viability 67% and cytotoxicity 32% was obtained at 25mg/ml concentration. Low percentage of viability 20% and high cytotoxicity 70% was found at 500mg/ml concentration on (Hep-2) cell line. The pure compound was expressed potent cytotoxic activity 53% at 25mg/ml against (MCF-7) cancer cell line than Hep-2 cancer cell line. Minimum effective concentration of pure compound of sponge was non-toxic to (Vero) cells but highly toxic to 53% (MCF-7) cells and low toxic to 32% (Hep-2) cells were recorded(IC_{50}) at 25µg/ml concentration of the pure compound (Fig. 3-4). Pure compound was highly potent effect on cervical cancer cells could be inhibit on the cells proliferative activity. Similarly increase the dose could increase the activity on cancer cells is dose dependent manner. In the presence of 400 mg concentration of compound titrated on cancer cells contribute low percentage cells viability and high

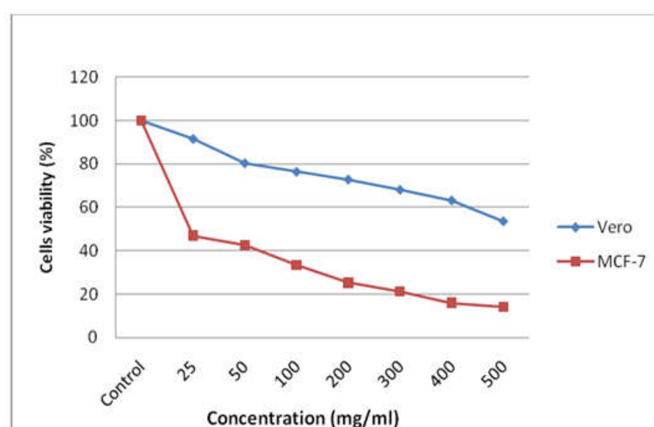


Fig. 1. Cells viability interpretation of Vero and MCF-7 cell line

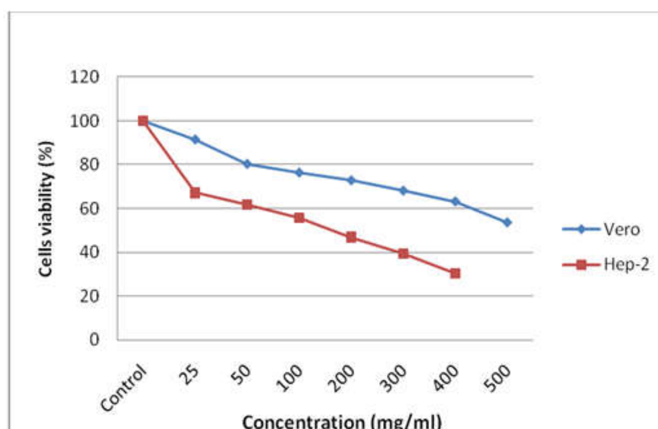


Fig. 2. Cells viability interpretation of Vero and Hep-2 cell line

Table 1. Cells viability assay with sponge pure compound against normal (Vero) cell line, Breast Cancer cell line (MCF-7) and Human Epithelial Larynx cancer cell line (Hep-2)

Sponge Pure compound Concentration mg/ml	Non tumorous -Normal cell line (Vero)			Human Breast Cancer cell line (MCF-7)			Human Larynx Cancer cell line (Hep-2)		
	Optical Density value (OD)	Cells viability (%)	Cytotoxicity (%)	Optical Density value (OD)	Cells viability (%)	Cytotoxicity (%)	Optical Density value (OD)	Cells viability (%)	Cytotoxicity (%)
Control	0.956±0.03	100	0	1.532±0.12	100	0	0.899±0.06	100	0
25	0.876±0.03	91.63	8.36	0.720±0.04	46.99	53.00	0.605±0.06	67.29	32.70
50	0.769±0.04	80.43	19.56	0.655±0.05	42.75	57.24	0.557±0.02	61.95	38.04
100	0.731±0.06	76.46	23.53	0.512±0.06	33.42	66.57	0.501±0.03	55.72	44.27
200	0.697±0.05	72.90	27.09	0.388±0.05	25.32	74.67	0.423±0.04	47.05	52.94
300	0.652±0.03	68.20	31.79	0.326±0.04	21.27	78.72	0.355±0.03	39.48	60.51
400	0.605± 0.04	63.28	36.71	0.245±0.02	15.99	84.00	0.275±0.03	30.58	69.41
500	0.513±0.05	53.66	46.33	0.218±0.01	14.22	85.77	0.187±0.04	20.80	79.19

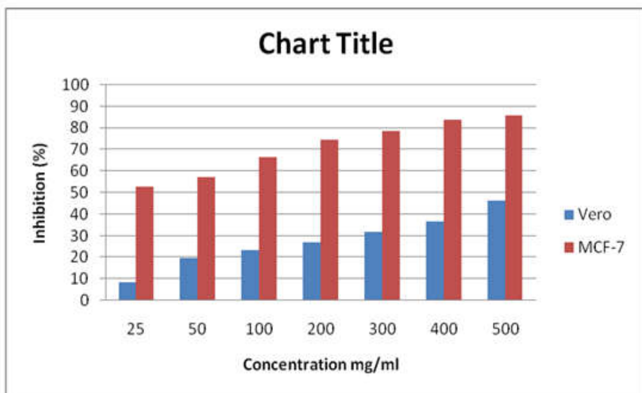


Fig. 3. Cells Inhibition analysis of Vero and MCF-7 cell line

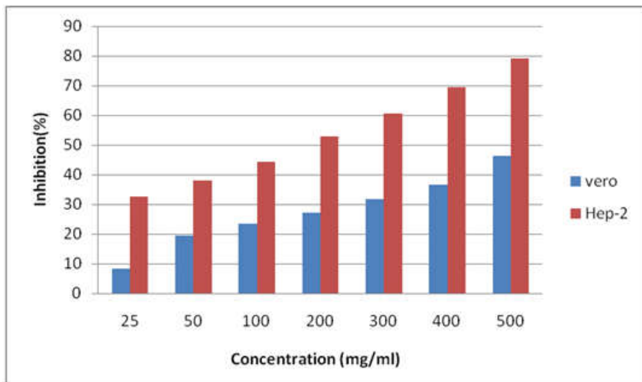
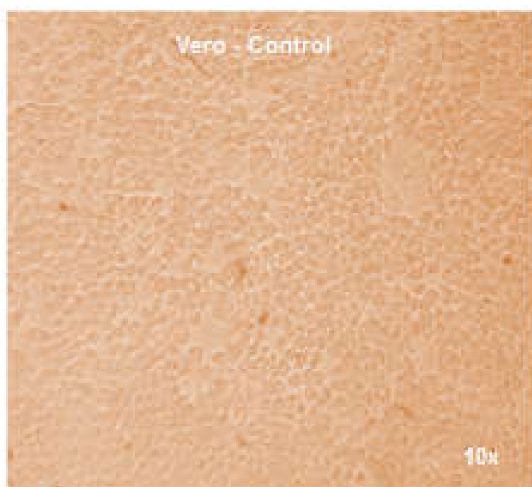
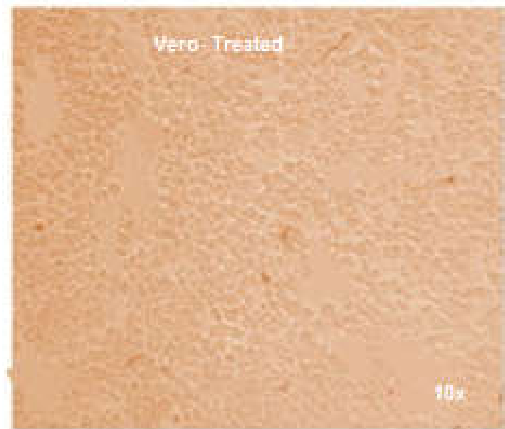


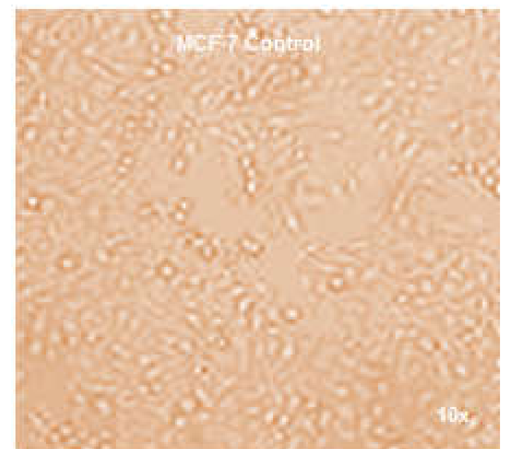
Fig.4. Cells Inhibition analysis of Vero and Hep-2 cell line



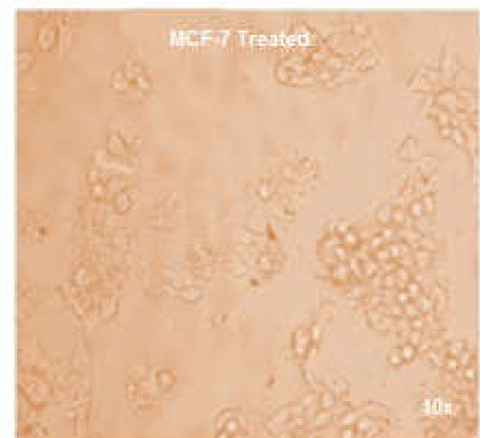
A



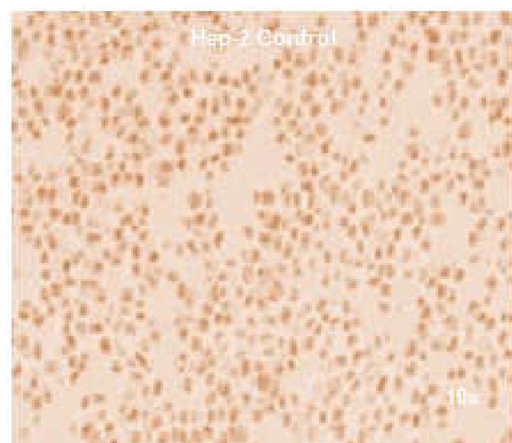
B



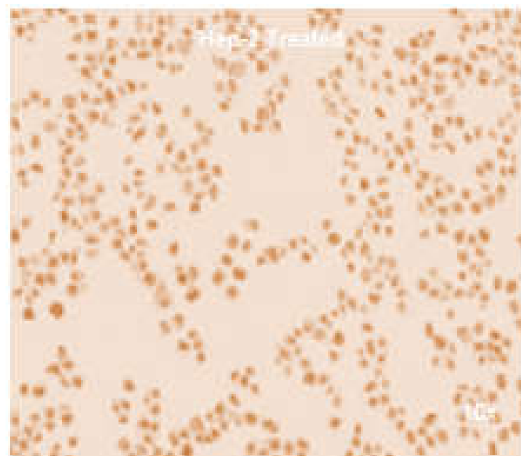
C



D



E



F

Fig. 5. Morphological changes of Normal cell line (Vero) non tumor cells control (A) viable cells in treated with pure compound (B). Cancer cells in Human Breast cancer cell line MCF-7 before treatment are (C) control cells with pure compound treated (D). Human Epithelial Larynx cancer cell line Hep-2 pre treatment as control (E) with treated (F) cells is shown in (D).

inhibitions of the cancer cells. Both of cervical and kidney cell line are treated in the same way of dose dependent manner has been expressed more potent and low effect anticancer activity. Morphological changes of control and treated cells population have been observed by inverted microscope and compared with the cells serving as control. These observations may be due to the presence of active biological compounds (Figure 5). In this case, Breast cancer cells have been proved much potent anticancer activity effect respectively.

DISCUSSIONS

Marine sponges are an important source for the potential development of effective anticancer agent(David, 1999). The results of this study shows the *Hyrtios erecta* sponge purified alkaloid compound is highly toxic to (MCF-7) breast cancer cell then (Hep-2) larynx cancer cell at low concentration (25 µg/ml) of pure compound as well as non-toxic to normal (vero) cells. Breast cancer cells viability 46% and 53% cell inhibited by the pure compound. The cells viability was low and high inhibition has been obtained from the breast cancer cell line. 67% cells viable and 32% inhibitions were found in the larynx cell line assay. In this case breast cancer cells are expressed highly inhibited and larynx cancer cell line cells revealed that lower inhibition by pure compound. But, normal cell line cells are non toxic effect against pure compound. Breast cancer cells were proved much potent anticancer activity effect on pure compound. Low dose of pure compound contributed less toxic against cancer cells as well as increase the dose of drug could be observe the high toxic of the cancer cells. Breast and larynx cancer cells could be prevent anti proliferative effects by the potent anti cancer drug of marine sponge pure compound. Previously literatures proved Sponge *Hyrtios erecta* secondary metabolite alkaloid compound prevent the human (HeLa) cervival cancer cell line, HCT-116 colon carcinoma cell line, lymphoma cancer cell line (Mohamed *et al.,* 2007). A biochemical study revealed that this compound inhibited myeloid leukemia cell differentiation protein Mcl-1, a critical player involved in survival and death decisions of individual cells (Michels and Packham, 2005). The alkaloid compound which has been isolated from various marine sponges revealed cytotoxic effect against AsPC-1 pancreatic cancer cells (Guzman *et al*., 2010). In the present study, we found that extracts from *Hyrtios erecta* exhibited significant anti-proliferative effects against a variety of human cancer cell lines. Further investigation require as well as essential to this sponge purified compound in order to chemical structure elucidations, characterization as well as pre clinical study for anticancer activity. In conclusion, it is believed that a rich source of anticancer drug candidates could be obtained from marine sponge secondary metabolites. To our knowledge, the results describe antitumor activity of marine sponge secondary metabolites promising to convey antitumor drug development. There was no statistical significant difference between control viability and treated cells.

Aknowledgement

The Authors are thankful to Director, Central Agricultural Research Institute, Port Blair, for providing necessary research facilities. We are greateful to Principal, Voorhees College, Vellore, Tamilnadu, for support to this research work.

REFERENCES

Bacus G J and Green G. 1974.Toxicity in sponges and holothurians: a geographic pattern. Science., 13, 951–953.

Bergquist R P. 1978. Sponges. Universiti of Califronia Press. Berkeley.

Chairman K, Ranjitsingh A J A and Alagumuthu G. 2012. Cytotoxic and antioxidant activity of selected marine sponges. Asian Journal of Tropical Diseases ., 234-238.

David E W , Akbar T and Raymond J. 1999. Andersen. A New Acyclic Diketotriterpenoid Isolated from the Indonesian Marine Sponge Hyrtios erectus. American Chemical Society and American Society of Pharmacognosy., 12, 50-54.

Edeoga H O, Okwu D E and Mbaebie B O . 2005. Phytochemical analysis of plants. Afr J Biotechnol ., 4(7), 685-688.

Gunasekera SP, Gunasekera M, Longley RE and Schulte GK. 1990. Discodermolide: a new bioactive polyhydroxylated lactone from the marine sponge Discodermiadissoluta. J. Org. Chem ., 55, 4912–4915.

Guzman E A, Johnson J D, Linley P A, Gunasekera S E *et al*., 2010. A novel activity from an old compound: Manzamine A reduces the metastatic potential of AsPC-1 pancreatic cancer cells and sensitizes them to TRAIL-induced apoptosis. Invest. New Drugs ., 12, 123-126.

Habib M R and Karim M R. 2011. Evaluation of antitumour activity of Calotropis gigantean L. root bark against Ehrlich ascites carcicnoma in Swiss albino micr. Asian Pac J Trop Med., 4(10),786-790.

Kashman Y, Groweiss A and Shmueli U. 1980. Latrunculin a new 2-thiazolidinone macrolide from the marine sponge Latrunculiamagnifica. Tetrahedron Lett., 21, 3629–3632.

Kashman Y, Groweiss A, Lidor R , Blasberger D *et al*., 1985 . Latrunculins: NMR study, two new toxins and a synthetic approach. Tetrahedron., 41, 1905–1914.

Kato Y, Fusetani N, Matsunaga S, Hashimoto K *et al*., 1986. Bioactive marine metabolites. Part 16. Calyculin A. A novel

antitumor metabolite from the marine sponge Discodermia calyx. J. Am. Chem. Soc ., 108, 2780–2781.

Kobayashi M, Okamoto T, Hayashi K, Yokoyama N *et al*., 1994. Marine natural products. XXXII. Absolute configurations of C-4 of the manoalide family, biologically active sesterterpenes from the marine sponge Hyrtioserecta. Chem.Pharm. Bull ., 42, 265.

Kornprobst J M. 2005.Substances Naturellesd'origine Marine; Ed.; Editions Tec and Doc-Lavoisier: Paris, France.

Lakshmi V, Mishra S K, Srivastava S, Chaturvedi A *et al*., 2012. Antifungal activity of marine sponge Haliclonaexigua, Journal of mycology medicale ., 20, 31-35.

Longeon A, Copp BR, Quévrain E, Roué M, Kientz B *et al*., 2011. Bioactive Indole Derivatives from the South Pacific Marine Sponges *Rhopaloeide sodorabile* and *Hyrtios sp*. Mar. Drugs ., 9, 879–888.

Michels J J and Packham P W. 2005. Mcl-1. Int. J. Biochem. Cell Biol., 37,267-271.

Miyaoka H, Nishijima S, Mitome H and Yamada Y J. 2000. Three new scalarane sesterterpenoids from the Okinawan sponge *Hyrtios erectus*. Nat. Prod., 63, 1369.

Mohamed A. Ashour, Ehab S. Elkhayat, Rainer E , Ru Angelie Edrada *et al*., 2007. Indole alkaloid from the Red Sea sponge Hyrtios erectus. ARIKVOC ., 15, 225-231.

Petit G R, Cichacz Z A, Herald C L, Gao F *et al*., 1994. Antineoplastic agent 300. Isolation and structure of the rare human cancer inhibitory macrocyclic lactones spongistatins 8 and 9. J. Chem. Soc. Chem. Commun ., 2, 1605–1606.

Petit G R, Herald C L, Cichacz Z A, Gao F *et al*., 1993. Antineoplastic agent 293. The exceptional human cancer cell growth inhibitors spongistatins 6 and 7. Nat. Prod. Lett., 3, 239–244.

Petit G R, Herald C L, Cichacz Z A, Gao F *et al*., 1993. Isolation and structure of powerful human cancer cell growth inhibitors spongistatins4 and 5 from an African Spirastrellaspinispirulifera(Porifera). J. Chem. Soc. Chem. Commun., 24,1805–1807.

Pettit G R, Cichacz Z A , Tan R, Hoard M S *et al*., 1998. Antineoplastic agents. 386. Isolation of sesterstatins 1-3 from the marine sponge Hyrtios erecta. J. Nat. Prod., 61, 13.

Pettit G R, Cichacz Z A, Tan R, Herald D L *et al*., 1998. Czech.Antineoplastic Agents 542. The Isolation and Structure of Sesterstatin 6 from the Indian Ocean Sponge Hyrtioserecta Chem. Commun., 63, 1671.

Pettit G R, Tan R and Cichacz Z A. 2005. Antineoplastic agents. 542. Isolation and structure of sesterstatin 6 from the Indian Ocean sponge *Hyrtios erecta*. J. Nat. Prod., 68, 1253.

Pettit G R ,Tan R , Melody N, Cichacz Z A *et al*., 1998. Antineoplastic agents. 397: Isolation and structure of sesterstatins 4 and 5 from Hyrtioserecta (the Republic of Maldives). Bioorg. Chem. Lett., 8, 2093.

Pettit G R , Cichacz A, Tan R, Hoard M S *et al*., 1998. Antineoplastic agents. 386. Isolation of sesterstatins 1-3 from the marine sponge Hyrtios erecta. Journal of Natural Products., 61(1),13-6.

Sipkema D, Franssen MCR, Osinga R, Tramper J, Wijffels RH *et al*., 2005. Marine sponges as pharmacy. Mar. Biotechnol ., 7, 142–162.

Spector I, Shochet N R , Blasberger D and Kashman Y. 1989. Latrunculins-novel marine toxins that disrupt microfilament and affect cell growth: I. Comparison with cytochalasin D. Cell. Mot. Cytoskel., 13, 127–144.

Spector I, Shochet N R, Kashman Y and Groweiss A. 1983 . Latrunculins-novel marine toxins that disrupt microfilament organization in cultured cells. Science., 214, 493–495.

Yonghong Liu, Si Zhang, Jee H. Jung and TunhaiXu . 2007. Bioactive Furano sesterterpenoids from Marine Sponges. Bioactive Heterocycles ., 231-258.

Youssef D T, Yamaki R K , Kelly M and Scheuer P. 2002. Salmahyrtisol A, a novel cytotoxic sesterterpene from the Red Sea sponge Hyrtioserecta. J. J. Nat. Prod ., 65, 2-5.

****