Vol 2 Issue 12 June 2013

Impact Factor : 1.2018 (GISI)

ISSN No :2231-5063

Monthly Multidisciplinary Research Journal

GoldenResearch Thoughts

IMPACT FACTOR : 0.2105

Welcome to ISRJ

RNI MAHMUL/2011/38595 **ISSN No.2230-7850**

Indian Streams Research Journal is a multidisciplinary research journal, published monthly in English, Hindi & Marathi Language. All research papers submitted to the journal will be double - blind peer reviewed referred by members of the editorial Board readers will include investigator in universities, research institutes government and industry with research interest in the general subjects.

International Advisory Board

Editorial Board

Address:-Ashok Yakkaldevi 258/34, Raviwar Peth, Solapur - 413 005 Maharashtra, India
Cell : 9595 359 435, Ph No: 02172372010 Email: ayisrj@yahoo.in Website: www.isrj.net

Golden Research Thoughts
Volume 2, Issue. 12, June. 2013
ISSN:-2231-5063
Available online at www.aygrt.isrj.net

ORIGINAL ARTICLE

CYTOTOXIC AND ANTIOXIDANT ACTIVITY OF MARINE SPONGE ASSOCIATED BCATERIUM Bacillus cereus

S. BRAMMAVIDHYA AND G. USHARANI

Department of Microbiology, Faculty of Agriculture, Annamalai University, Chidambaram , Tamilnadu, India
Assistant Professor, Department of Microbiology, Faculty of Agriculture, Annamalai University, Chidambaram Tamilnadu, India

Abstract:

The present study was aimed to evaluate the antioxidant and anticancer activity of the marine sponge Hyatella cribriformis associated bacteria Bacillus cereus SBS02 isolated from Gulf of Manner, Tamilnadu, India. Hyatella cribriformis containing bacterial extracts were tested for Hep-2 cell line. The percentage viability of the cell line was carried out by using colorimetric method. The cytotoxicity of the partially purified protein sample 70 kDa and 8kDa on Hep-2 cell was evaluated by MTT assay. Both the protein sample has significant cytotoxic effect on Hep-2 cell line in the concentration range between 3.906 µl to 1000 µl using MTT assay. R2 value of protein 70 kDa was 0.997 and for 8 kDa was 0.964. The above proteins were relatively evaluated for the radial scavenging activity using DPPH. The compound concentration was increased from 2 to 10 mg/ml, the DPPH scavenging rate of 70 kDa protein was 25%, 40%, 65%, 80% and 95.4% and similarly 8 kDa was 25.3%, 48.69%, 84.7% and 96.1% respectively.

KEYWORDS:

Marine sponge, Scavenging activity, Cytotoxicity and Hyatella cribriformis.

INTRODUCTION

The role of natural products from all sources in drug discovery has been reviewed recently (Gullo, et al., 1994). More comprehensive coverage has been given to plants over the past 10 years (Kinghorn et al., 1993; Chadwick, et al., 1990). Thus, Farnsworth et al. (1985) have reported that at least 119 compounds derived from 90 plant species can be considered as important drugs. Over that past 10 years, there has been a resurgence of interest in the investigation of natural materials as a source of potential new chemotherapeutic agents, but there are now signs that this interest is once more waning in favour of new approaches to drug discovery. Drugs of natural origin have been classified as original products, products derived semi synthetically from natural products, or synthetic products based on natural product models (Cragg, 1997).

Marine sponges represent a significant component of benthic community throughout the world in terms of both biomass and their potential to influence benthic or pelagic processes (Dayton, 1974; Dayton, 1989; Gili and Coma, 1998; Maldonado et al., 2005a). Sponges included under the phylum Porifera are primitive metazoans that were thought to be the starting point for the metazoan explosion during the Precambrian about 650 million years ago (Hadzi, 1963). They are widely distributed in tropical and subtropical benthic marine habitats as well as at higher latitudes including even in freshwater lakes and streams. So far, an estimated 15,000 species have been described, but with many species yet to be described (Hooper and Van Soest, 2002).

Most chemotherapeutic drugs are directed against actively dividing cells and so the malign cells

will be influenced to the larger extent than normal cells. These drugs will affect normal cells in the dividing stage and therefore patients experience in the adverse side effect. Targeted cancer therapy uses drug that specifically should attack cancer cells, and hence may have fewer side effects (Rad and Sabodno, 2009). In these project, one cancer cell line used for anticancer screening.

Relatively few works were carried out to investigate antioxidant properties of marine natural products isolated from sponges (Tziveleka et al., 2003; Amigo et al., 2008). The aim of this work was to study the distribution of sponges containing antioxidants in the phylum porifera and to investigate marine natural products responsible for an antioxidant activity of sponge associated bacteria.

2. MATERIALS AND METHODS

2.1. Sample collection

Sample of Hyatella cribriformis marine sponge was collected from Mandapam, Gulf of Mannar, Tamilnadu. The sponge samples soon after collection was transferred to a sterile polyethylene bag and transported at 4ºC to the laboratory for the isolation of associated microbes. On reaching the laboratory, the invertebrate was brought to room temperature and cut aseptically into small pieces (2 x 2cm) using a sterile scissors. Finally, sample in sterile seawater was homogenized using sterilized mortar and pestle in a laminar flow chamber.

2.2. Isolation of bioactive potential bacteria from marine sponge

The sponge sample was homogenated and serially diluted upto 10-6 dilutions and plated on the surface of Zobell marine agar. The plates were incubated at room temperature for 24 - 48 hrs. The isolated bacterial strains were tested for the antibacterial activity against Staphylococcus aureus, Vibrio cholerae, Salmonella paratyphi, Klebsiella pneumoniae, Salmonella typhi, Proteus mirabilis, Klebsiella oxytoca, Escherichia coli, Pseudomonas aeruginosa and Lactobacillus bulgaricus. The most potential strain was identified using biochemical test and 16S rRNA sequencing.

2.3. Cytotoxic activity [(3, 4, 5 – dimethylthiazol-2yl) - 2, 5, diphenyltetrazoliumbromide (MTT) assay]

The human liver carcinoma cancer cell line (Hep-2) and cells were grown as monolayer culture in MEM medium and incubated at 37°C in a 5% of CO2 atmosphere. Hep-2 was seeded in 24 well plates at a concentration of 5×103 cells/mL for 48 hrs. After the incubation, the culture medium was replaced with 100 mL serum free medium containing various concentrations (3.906, 7.8125, 15.625, 31.25, 62.5, 125, 250, 500 and 1000 µg/ml) of bacterial extracts at 24 - 48 hrs. After that, the medium was refreshed with 100 µL of serum free medium (MEM) and 200 µL of MTT [5 mg/mL of (3, 4, 5 – dimethylthiazol - 2yl) - 2,5-diphenyltetrazoliumbromide] was added and incubated for 6 - 7 hrs in 5% CO2 incubator for cytotoxicity. After incubation added 1ml of DMSO in each well and mixed by pipette and leave for 45 seconds, it showed purple colour formation. The O.D was read at 595 nm by taking DMSO as a blank.

Cell viablity = Mean OD/control OD x100

2.4. Antioxidant activity

1,1-Diphenyl-2-picryl hydrazyl (DPPH) Free Radicals Scavenging Assay

The scavenging activity for DPPH free radicals was measured according to the procedure of Zhao et al. (2006). To 2 mL of distilled water, 1mL of 0.1 mM DPPH solution in ethanol and 0.5 mL of purified protein sample from Bacillus cereus was added. The mixture was shaken vigorously and allowed to reach a steady state for 30 min at room temperature. Decolourization of DPPH was determined by measuring the decrease in absorbance at 517 nm and the DPPH radical scavenging effect was calculated according to the following equation:

$$\% \text{ scavenging rate} = (1- (A1 - A2)/A0) \times 100$$

3. RESULTS

3.1. Cytotoxic activity

The purified protein 70 kDa and 8 kDa from Bacillus cereus SBS02 isolated from the marine sponges samples were seeded with the Cancerous Hep2 (Hepato cellular liver carcinoma) cell lines. The observations revealed that the sample treated with Hep2 cell lines give out different ranges of cytotoxicity as showed in Fig - 1and 2.

Cytotoxicity in partially purified protein sample 70 kDa was seeded with the cell line concentration (µg/ml) and cell viability (%) was 1000 (2.35), 500 (10.23), 250 (21.03), 125 (35.69), 62.5 (45.23) 31.25 (55.25), 15.625 (65.23), 7.8125 (78.89) and 3.906 (89.61) for HeP2 as given in (Table - 1). The cytotoxicity for Hep2 cell lines 8kDa was found to be 22.35, 29.63, 48.56, 54.32, 61.05, 65.55, 75.23, 78.90 and 32% ranges were observed for the concentrations 1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.8125 and 3.906 (µg/ml) respectively (Table - 2).

3.2. Antioxidant activity

The active fractions were lyophilized and used for antioxidant and anticancer activities. In the present study, 70 kDa protein from Bacillus cereus SBS02 was evaluated for its Radical scavenging activity using DPPH.

The effects of bioactive compound (70 kDa) from Bacillus cereus SBS02 on the antioxidant activity in vitro, the DPPH scavenging rate of the purified antibiotic compound was examined. When the compound concentration was increased from 2.0 to 10 mg/mL, the DPPH scavenging rate was 25%, 40%, 65%, 80% and 95.4% respectively (Fig - 3).

Fig -1: MTT assay of bioactive compound 70kDa from Bacillus cereus

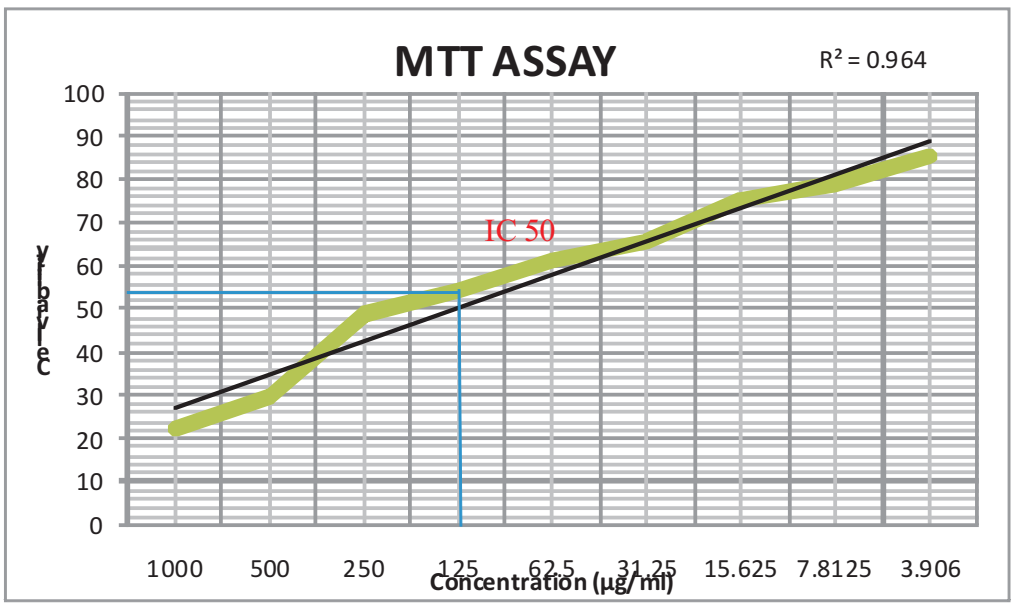


Fig -2: MTT assay of bioactive compound 8 kDa from Bacillus cereus

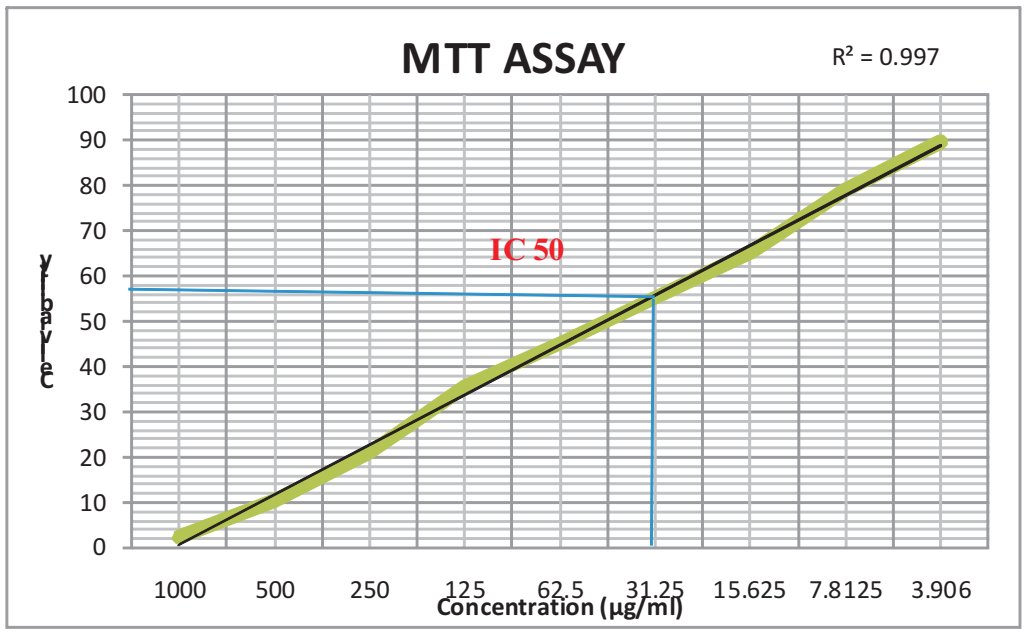
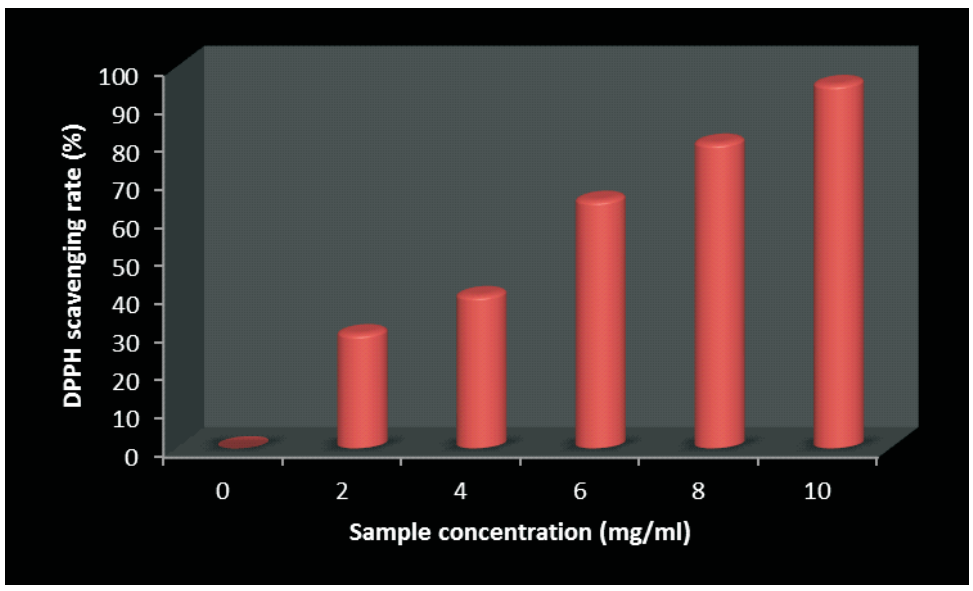
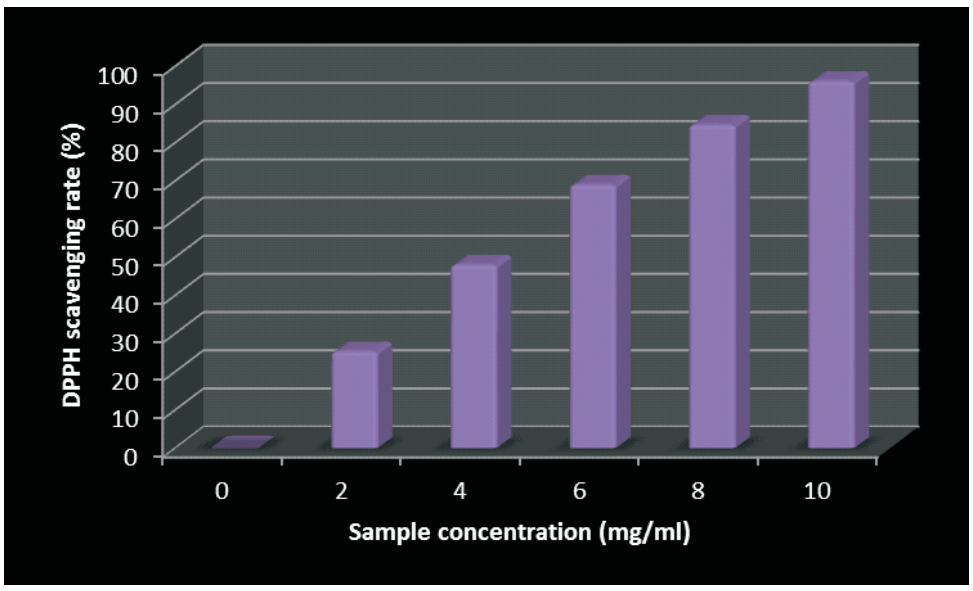


Fig 3: Scavenging effect on DPPH radicals by bioactive compound from Bacillus cereus



The effects of bioactive compound (8kDa) from Bacillus cereus SBS02 on the antioxidant activity in vitro, the DPPH scavenging rate of the bioactive compound cerein was examined. When the compound concentration was increased from 2.0 to 10mg/mL, the DPPH scavenging rate was 25.3%, 48, 69%, 84.7% and 96.1% respectively (Fig. 4).

Fig 4: Scavenging effect on DPPH radicals by bioactive compound from Bacillus cereus



5. DISCUSSION

Antioxidant refer to any substance that hinder s the reaction of a substance with dioxygen and any substance that inhibits free radical reaction (Soniya, 2003; Jarrar et al., 2012; Ezeigho et al., 2011). Nowadays, antioxidants have gained more importance on account of their positive effects, as health promoters in the treatment of cancer. Many antioxidant compounds which are naturally occurring in plant sources have been identified as free radical scavengers (Yaha et al., 2000; Tava and Avato, 2006). In the present study, in vitro antioxidant activity of the marine sponge associated Bacillus cereus secreting protein compounds showed potential free radical scavenging activity.

Developing countries which is induced by oxidative stress (Valetin et al., 2011). Hence, antioxidants need thorough search especially safer compounds from the plant sources. Increased oxidative stress encountered in body due to their environmental hazard or important in the body metabolism due to various disease conditions including drugs or having insufficient antioxidants. That are present in herbs, spices and are responsible for inhabiting or preventing the deleterious consequence of oxidative stress.

Cytotoxicity assays are widely used method invitro toxicology studies. It is not only rapid and standardized, but also sensitive and inexpensive method to measure drug induced alterations in metabolic pathways integrity which may or may not be related directly to the cell death (Tava and Avato, 2006).

Crude extract of four Bacillus species isolated from the sponge Amphimedon ochrocea were subjected to cytotoxicity screening against cancer cell lines HepG-2, HCT,MCF-7.The IC 50 values of the bacterial extract against cancer cell line were in the range of 4.3-4.6 9µg/ml. In the present study the protein extract from Bacillus cereus different cytotoxicity effect on HeP-2 cell line.

REFERENCES

1)Dayton, P. K., 1974. Biological accommodation in the benthic community at McMurdo Sound. Antarctica. Ecol. Monogr., 44: 105–128

2)Dayton, P. K., 1989. Interdecadal variation in an Antarctic sponge and its predators from oceanographic climate shifts. Science., 245: 1484–1486

3)Gili, J. M. and Coma, R., 1998. Benthic suspension feeders: their paramount role in littoral marine food webs. Trends Ecol. Evol., 13:316–321

4)Maldonado, M., Carmona, C., Velasquez, Z., Puig, A., Cruzado, A., Lopez, A. and Young, C. M., 2005a. Siliceous sponges as a silicon sink: an overlooked aspect of benthopelagic coupling in the marine silicon cycle. Limnol. Oceanogr., 50: 799 – 809.

5)Gullo, V. P. (1994): The discovery of Natural Products with Therapeutic Potential, Butterworth-Heinemann, Boston.

6)Kinghorn, A. D. and Balandrin, M. F. (1993): Human medicinal agents from plants, ACS, 205-207.

7)Chadwick, D. J. and Marsh J. (1990): Bioactive compounds from plants. In: Ciba Foundation Symposium, 154, John Wiley and Sons, Chichester, Sussex, U.K.
8)Farnsworth, N. R.; Akerele, O.; Bingel, A. S.; Soejarto, D. D. and Guo, Z. (1985): Bull. WHO, 63, 965 – 981.
9)Cragg, G. M.; Newman, D. J. and Snader, K. M. (1997): J. Nat. Prod., 60, 52-60
10)Hadzi, J., 1963. The Evolution of the Metazoa I-XII, Edn 1st., Pergamon Press, Oxford.
11)Hooper, J. N. A. and Van Soest, R. W. M., 2002. Systema Porifera. A guide to the classification of sponges, vol. 1. Plenum Publishers, New York, N.Y.
12)Chanas, B., Pawlik, J. R., Lindel, T and Fenical, W. 1997. Chemical defence of the Caribbean sponge Agelas clathrodes (Schmidt). J. Exp. Mar. Biol. Ecol., 208: 185 –196.
13)Rotruck .J. K, Pope A. L, Ganther . H. E, Swanson. A. B, Hafeman, D. G, Hoekstra. W. G. 1973. Selenium and Diabetes: More bad news for supplements science, 179: 588 - 590.
14)Habib, M. R and Karim, M. R. 2011. Evaluation of antitumor activity of Calotropis gigantean L. root bark against Ehrlich ascites carcinoma in swiss albino mice. Asian Pac. J. Trop Med., 4(10): 786 - 790.
15)Soniya, J. 2003. Exploration of sponge associated actinomycetes for the development of potent novel antibacterial agent (M.sc dissertation), Thirunelveli: MS University: P.201
16)Jarrar, N., Abu – Hijleh, A and Adwan, A. 2012. Antibacterial activity of Rosmarinus officinalis alone and in combination with cefuroxime against methicillin – resistant Staphylococcus aureus. Asian Pac. J. Trop Med., 3(2): 121 – 123.
17)Ezeigho,Ezeja,MI,Madubui.K.G,Ifekwe.D.C,Ukweni.I.A,Udeh.N.E,Akomas.S.C. 2011. Antidiarrhoeal activity of leaf methanolic extract of Rauwolfia serpentine. Asian Pac J Trop Med., 2(6): 430 - 432.
18)AL – Yaha, M.A and Adel – Sattor, E. 2000. Pregnane Glycoside from Caralluma russeliana. J. Nat. Prod., 63:1450 – 1453.
19)Tava, A and Avato, P. 2006. Chemistry and biological activity of triterpene saponins from Medicago species. Natural Prod Commun., 1: 1159 - 1180.
20)Valetin, B. B., Vinod, V and Beulah, M. C. 2011. Biopotential of secondary metabolites isolated from marine sponge Dendrilla nigra. Asian Pac. J Trop Dis., 1(4): 299 - 303.
21)Rad, O. K and Sabdono, A. 2009. Bacterial symbionts of reef Invertibrates as a sustainable source of marine natural products. Curr Res Bac., 2(1): 7 – 13.
22)Tziveleka, L. A., Vagiar, C and Roussis,V. 2003. Natural products with anti HIV activity from marine organisms. Curr Top Med Chem., 3: 1512 - 1535.
23)Amigo, J. M., Bautista, M., Cruz, T., Coello, J., Maspoch, S and Balanco, M. 2008. Study of pharmaceutical samples by NIR, Chemical image and multivariate analysis. Trends in Analytical Chem., 27(8): 696 - 713.