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ORIGINAL ARTICLE

Antioxidant and Anti-Inflammatory Effects of a Methanol Extract from the Marine Sponge *Hyrtios erectus*

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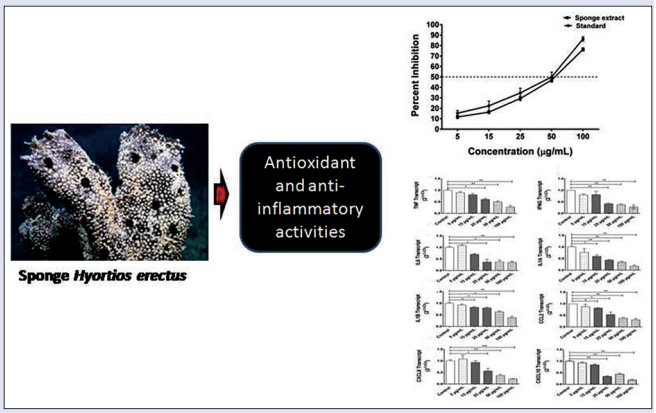
ABSTRACT

Background: The marine environment, due to its phenomenal diversity, is a rich natural source of many biologically active compounds. **Objective:** Marine sponge *Hyrtios erectus*, collected from North Bay of South Andaman Sea, was screened for potential antioxidant and anti-inflammatory activities. **Materials and Methods:** The antioxidant activities of the methanol (MeOH) extract of the sponge at different concentrations (0–100 μg/mL) were determined by measuring the free radical-scavenging activities. The anti-inflammatory activities of the extract were determined by measuring the inhibitory effect of the extract on albumin denaturation and inducible nitric oxide (NO) production. Quantitative real-time polymerase chain reaction was used to investigate the effect of the sponge extract on the expression of eight proinflammatory cytokine genes. **Results:** Our results suggested that the MeOH extract of the sponge exhibited antioxidant activity against 2,2-diphenyl-1-picrylhydrazyl-free radicals, superoxide anions, and hydroxyl radicals. More than 50% inhibition (half inhibitory concentration) was recorded with concentration of 50 μg/mL of the sponge extract. Extract of the sponge at a concentration of 25 μg/mL inhibited NO production by a macrophage cell line *in vitro* by 91.22% ± 5.78%. The sponge extract induced downregulation of eight proinflammatory cytokine genes in breast cancer Michigan Cancer Foundation-7 cell line. **Conclusion:** The secondary metabolites present in the MeOH extract of the sponge showed the potential antioxidant and anti-inflammatory activities. Further studies are required to identify the bioactive compounds.

Key words: Antioxidant, marine sponge, proinflammatory cytokines, tumor necrosis factor

SUMMARY

- Marine sponge *Hyrtios erectus*, collected from North Bay, South Andaman Sea, India, showed potential antioxidant and anti-inflammatory activities
- Methanol extract of the sponge exhibited antioxidant activity against 2,2-diphenyl-1-picrylhydrazyl-free radicals, superoxide anions, and hydroxyl radicals
- Extract of the sponge, at a concentration of 25 μg/mL, inhibited nitric oxide production by a macrophage cell line *in vitro* by 91.22% ± 5.78%
- The sponge extract induced downregulation of eight proinflammatory cytokine genes in Michigan Cancer Foundation-7 cell line.



Abbreviations used: DPPH: 2,2-diphenyl-1-picrylhydrazyl; DMEM: Dulbecco's modified eagle's medium; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; EDTA: Ethylenediaminetetraacetic acid; NBT: Nitroblue tetrazolium; PMS: Phenazine methosulfate; DMSO: Dimethyl sulfoxide.

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INTRODUCTION

Marine environment is being recognized to be a very rich natural source of bioactive metabolites with potent pharmacological importance.[1] Biological diversity of certain marine ecosystems, such as coral reefs, is higher than that of tropical rain forests.[2] Many marine organisms including sponges have a sedentary lifestyle and evolved the ability to produce diverse toxic compounds to protect themselves from predators or to fight against competitors.[3,4] Among all marine organisms, sponges are getting most attention in pharmaceutical industry because of the vast majority of bioactive secondary metabolites; they produce anticancer, antiviral, antibacterial, antifungal, antiprotozoal, anti-inflammatory, immunosuppressive, neurosuppressive, neuroprotective, and a range of other bioactivities.[5] It is reported that almost 40% of all marine natural products come from sponges.[6] The major sources of most of the sponge-derived compounds are from the microorganisms which harbor on their surfaces and intercellular spaces.[7,8] Bioactive compounds produced by sponges vary considerably between different species and locations, ranging from some that produce no compound to others that produce large amount of the compounds.[9]

Free radicals damage cells and antioxidants inhibit the production of free radicals by blocking the oxidation of other molecules. Bioactive

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compounds with antioxidant potential have been reported from marine sponges.[10,11] Secondary metabolites derived from marine sponges also showed anti-inflammatory activities. Some examples of sponge-derived metabolites with anti-inflammatory activities include cavernolide from *Fasciospongia cavernosa*,[12] contignasterol from *Petrosia contignata*,[13] and cyclolinteinone from *Cacospongia linteiformis*.[14]

Research on marine sponges for the identification of bioactive compounds has been intensified over the last decade. However, there are very few reports on the screening of sponge species from North Bay of South Andaman Sea, India. The marine sponge *Hyrtios erectus* is blackish and is attached to the sea bottom by means of masses of sand-filled fibers.[15] However, this sponge species from South Andaman Sea water has not been investigated before. Therefore, the purpose of the present study was to investigate the potential antioxidant and anti-inflammatory effects of the secondary metabolites produced by the sponge *H. erectus*. We used the methanol (MeOH) extract of the sponge for the purpose. The effect of the sponge extract on the expression of eight proinflammatory cytokine genes was also investigated.

MATERIALS AND METHODS

All the present experiment complies with all relevant institutional and national animal welfare guidelines and policies.

Reagents

All chemicals were purchased from Sigma-Aldrich (USA) unless stated otherwise. Cell culture plastics were from MIDSCI, USA.

Sample collection and preparation of the methanol extract

The marine sponge *H. erectus* was sampled in North Bay of South Andaman Sea, India, in December 2013 through scuba diving. The taxonomy details of the sponge samples were studied thoroughly, and a voucher specimen was deposited to Division of Fisheries Sciences, ICAR-Central Island Agricultural Research Institute, Andaman and Nicobar Islands.

The bioactive compounds were extracted using MeOH. The extract was filtered through Whatman filter paper No. 1 (GE Healthcare, UK) and pooled into a rotary evaporator (STRIKE 202, Germany). Then, the filtrate was concentrated under reduced pressure below 50°C into a thick mass. The crude extract was redissolved in MeOH:$CHCl_3$ (1:1) mixture and was partitioned into four solvents (dichloromethane, MeOH, chloroform, and hexane), followed by evaporation of the aqueous layer. All the fractions were subjected to bioassays (scavenging assays and nitric oxide [NO] production assay as described below) to determine the antioxidant and anti-inflammatory activities. Among the four fractions, MeOH-soluble fractions showed that antioxidant and anti-inflammatory activities and other fractions were found ineffective. On the basis of the results, the MeOH-soluble fraction was taken for the current study. The MeOH extract was dried and stored at −20°C until use.

2,2-diphenyl-1-picrylhydrazyl-free radical-scavenging assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH)-free radical-scavenging activity of sponge extracts at different concentrations (5, 15, 25, 50, and 100 μg/mL) was measured as described previously.[16] Briefly, 0.1 mM solution of DPPH was added to 3 mL of the sponge extract solutions and stirred vigorously. Then, each mixture was kept in the dark for 30 min, the absorbance was measured at 517 nm against a blank, and the percentage inhibition was calculated using the following equation.

$$\text{Percent inhibition}\,(\%)=\frac{(A_0-A_1)}{A_0}\times 100 \quad (1)$$

where A_0 is the absorbance of the control (solution without sponge extract) and A_1 is the absorbance of the sponge extract samples.

Superoxide anion-scavenging assay

Measurement of superoxide anion-scavenging activity of sponge extract was based on the method described previously.[17] The sponge extract solutions were incubated with 1 mL of nitroblue tetrazolium (NBT) reaction mixture containing phosphate buffer (20 mM, pH 7.4), nicotinamide adenine dinucleotide (reduced form, 73 μM), NBT (50 μM), and phenazine methosulfate (PMS, 15 μM) for 5 min at ambient temperature. Subsequently, the absorbance at 560 nm was measured against an appropriate blank containing everything, except the sponge extract. The inhibition % was calculated using equation 1.

Hydroxyl radical-scavenging assay

The scavenging activity of the sponge extract for hydroxyl radicals was measured by the method previously reported.[18] The reaction mixture was prepared by mixing 2-deoxy-2-ribose (2.8 mM), phosphate buffer (0.1 mM, pH 7.4), ferric chloride (20 μM), ethylenediaminetetraacetic acid (EDTA, 100 μM), hydrogen peroxide (500 μM), ascorbic acid (100 μM), and various concentrations (0–100 μg/mL) of the sponge extract solution in a final volume of 1 mL. After incubating the reaction mixture for 1 h, an aliquot of the reaction mixture (0.8 mL) was added to 2.8% trichloroacetic acid solution (1.5 mL), followed by thiobarbituric acid solution (1% in 50 mM sodium hydroxide, 1 mL) and sodium dodecyl sulfate (0.2 mL). The mixture was then heated (20 min at 90°C) to allow the color to develop. After cooling, the absorbance was measured at 532 nm against an appropriate blank solution including all reaction components but the sponge extract. The percentage of inhibition was calculated using equation 1.

In vitro anti-inflammatory assay

In vitro anti-inflammatory activity of the sponge extract was determined by its ability to inhibit albumin denaturation and to inhibit NO formation by the macrophages.

Inhibition of *in vitro* albumin denaturation

Inhibition of *in vitro* albumin denaturation by the sponge extract was assessed as described previously.[19] In brief, 2 mL of 1 mM bovine serum albumin (BSA) solution was mixed with 2 mL of the sponge extract solutions. The mixtures were incubated at 27°C in an incubator for 15 min followed by incubation at 60°C in water bath for 10 min for denaturation. Then, the mixture was allowed to cool and turbidity was measured spectrophotometrically at 660 nm. The percentage of inhibition of denaturation was calculated using equation 2.

$$\%\,\text{Inhibition of denaturation}=100\times\frac{\text{Mean absorbance of sample}}{\text{Mean absorbance of control}}-1 \quad (2)$$

Inhibition of nitric oxide production

Cell lines and cell culture

The mouse macrophage cell line (RAW 264.7) was obtained from National Centre for Cell Sciences (NCCS), Pune, India. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine, 100 mU/mL penicillin, and

100 μg/mL streptomycin. Cell cultures were incubated in a humidified atmosphere of 5% CO_2 in air and 37°C.

Nitrite measurement

The cells were plated at a density of 2×10^5 cells/well in a 24-well plate. After incubation for 24 h, the cells were stimulated with 1 μg/mL of lipopolysaccharide (LPS) in the presence and absence of various concentrations of sponge extract for 24 h. The concentration of nitrite, a stable metabolite of NO was measured in the culture supernatant by the Griess assay as described previously.[20]

Cell viability assessment

To ensure that the inhibition of nitrite production was not due to cytotoxicity of the sponge extract, the cytotoxic potential of the sponge extract on RAW 264.7 cells was tested using (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide) MTT assay.[21] For the MTT assay, RAW 264.7 cells were seeded in a 96-well plate in basal medium (DMEM + 10% FBS + antibiotics), and after 24 h of adherence, the cells were treated with various concentrations (0–100 μg/mL) of the sponge extract and incubated for 24 h at 37°C in an atmosphere of 5% CO_2 in air. After the incubation period, the medium was removed and 100 μL MTT solution (1 mg/mL) was added to each well and the samples were allowed to incubate for 4 h under condition mentioned above. Then, the MTT-containing media were removed and the formazan crystals were dissolved with 200 μL of dimethyl sulfoxide. Absorbance was measured at 570 nm using a microplate reader. The cell viability percentage was calculated as described previously.[21]

Effect of the sponge extract on the expression of proinflammatory cytokine genes in breast carcinoma cell line (Michigan Cancer Foundation-7)

Cell lines and cell culture

The breast adenocarcinoma cell line (Michigan Cancer Foundation [MCF]-7) was obtained from NCCS, Pune, India. The cells were cultured in DMEM supplemented with 10% FBS, 2 mM glutamine, 100 mU/mL penicillin, and 100 μg/mL streptomycin. Cell cultures were incubated in a humidified atmosphere of 5% CO_2 in air and 37°C.

Experimental design

To determine the effect of the sponge extract on expression of proinflammatory cytokine mRNAs, MCF-7 cells were cultured in DMEM with 10% FBS in the presence of different concentrations of the sponge extract. Cells cultured in the absence of sponge extract were used as the control. After 24 h, the cells were harvested and washed in PBS and total RNA was extracted. Expression of eight proinflammatory cytokine genes, i.e., tumor necrosis factor-α (*TNF-α*), interferon-λ (*IFNG*), interleukin-6 (*IL6*), interleukin-1α (*IL1A*), interleukin-1β (*IL1B*), chemokine (C-C Motif) ligand 2 (*CCL2*), chemokine (C-X-C Motif) ligand 8 (*CXCL8*), and chemokine (C-X-C Motif) ligand 10 (*CXCL10*) were studied by quantitative real-time polymerase chain reaction analysis (PCR).

RNA preparation and reverse transcription-quantitative polymerase chain reaction analysis

Total RNA was extracted from MCF-7 cells that were either untreated (control) or exposed to various concentrations of the sponge extract (5, 15, 25, 50, and 100 μg/mL) using the RNAeasy Mini Kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's instructions. Quality of the RNA was determined using a NanoDrop spectrophotometer, and the concentration of the RNA was determined by Qubit® RNA HS Assay Kit (Thermo Fisher Scientific, Life Technologies) as per the manufacturer's protocol.

Reverse transcription of RNA was performed from 1 μg total RNA in the presence of RNase inhibitor, random hexamer primers (50 ng/μL), deoxynucleotides (10 mM), SuperScript III reverse transcriptase (200 U/μL), and reverse transcriptase buffer in a 20 μL final reaction volume using SuperScript III First-Strand Synthesis System for RT-PCR Kit (Invitrogen, Life Technologies, IN, USA).

Relative quantification of the genes was performed using Power SYBR green PCR Master Mix (2X) (Applied Biosystems) in Taqman ABI 7900 Real-Time PCR System (Applied Biosystems). Relative expressions of eight proinflammatory cytokine genes (*TNF*, *IFNG*, *IL6*, *IL1A*, *IL1B*, *CCL2*, *CXCL8*, and *CXCL10*) were studied. The housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was used as the endogenous control. The thermal cycling conditions for real-time PCR were one cycle of 50°C for 2 min (AmpErase uracil-N-glycosylase activation) and 95°C for 10 min (AmpliTaq Gold activation), followed by 40 cycles of 95°C for 15 s (denaturation) and 60°C for 1 min (annealing and extension). Fold changes were determined using the $2^{-\Delta\Delta Ct}$ method. Primer sequences used in this experiment are provided in Table 1.

Statistical analysis

All the statistical analysis was performed by one-way analysis of variance with Dunnett's posttest.[22] Data were expressed as mean ± standard deviation. GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA, USA) was used to analyze the data.

RESULTS

In vitro antioxidant activity of the sponge extract

In the present experiment, *in vitro* antioxidant activity of the sponge extract was evaluated by DPPH-free radical-scavenging assay, superoxide anion-scavenging assay, and hydroxyl radical-scavenging assay. *In vitro* antioxidant activity of sponge extract in various concentrations is presented in Table 2. More than 50% inhibition (half inhibitory concentration [IC_{50}]) was recorded with concentration of 50 μg/mL of the sponge extract.

The DPPH-free radical-scavenging effects of the sponge extract at different concentrations are shown in Table 2. Sponge extract at all

Table 1: Information on primers used in the real-time polymerase chain reaction

Gene name	Primer sequences (5′-3′)
TNF	F: 5'-CTCTTCTCCTTCCTGATCGTGGCA-3'
	R: 5'-GCCAGAGGGCTGATTAGAGAGAGG-3'
IFNG	F: 5'-GAACTGTCGCCAGCAGCTAAAACA-3'
	R: 5'-GCAGGACAACCATTACTGGGATGC-3'
CCL2	F: 5'-CTCAAACTGAAGCTCGCACTCTCG-3'
	R: 5'-TTATAACAGCAGGTGACTGGGGCA-3'
IL1A	F: 5'-GGGCTTGCACACACCTTCTTCTAC-3'
	R: 5'-AGTAGCCCTCTACCAAGGACCAGA-3'
IL1B	F: 5'-ACAGGCTGCTCTGGGATTCTCTTC-3'
	R: 5'-TAAGCCATCATTTCACTGGCGAGC-3'
CXCL8	F: 5'-CCTTGTTCCACTGTGCCTTGGTTT-3'
	R: 5'-GTGCTTCCACATGTCCTCACAACA-3'
CXCL10	F: 5'-GTCTCAATATTAGAGTCTCAACCCCCA-3'
	R: 5'-TGGAGAAGGAGTTCATAGCTGGGC-3'
GAPDH	F: 5'-GACAACGAATTTGGCTACAGCAAC-3'
	R: 5'-GGGTCTCTCTCTTCCTCTTGTGCT-3'

F: Forward primer; R: Reverse primer; *TNF*: Tumor necrosis factor; *IFNG*: Interferon, λ; *CCL2*: Ligand 2; *IL1A*: Interleukin-1α; *IL1B*: Interleukin-1β; *CXCL8*: ligand 8; *CXCL10*: Ligand 10; *GAPDH*: Glyceraldehyde 3-phosphate dehydrogenase

Table 2: *In vitro* antioxidant activity of the sponge extract

Concentration µg/mL	DPPH radical-scavenging activity inhibition (%)	Superoxide anion-scavenging activity inhibition (%)	Hydroxyl radical-scavenging activity inhibition (%)
5	19.02±02.65	26.33±01.53	17.67±04.04
15	21.67±01.53	29.67±04.51	31.00±03.06
25	33.67±04.04	37.33±02.52	37.34±01.53
50	56.33±04.52	59.67±06.66	53.02±01.25
100	66.01±02.05	68.32±04.35	69.67±02.08

The scavenging activities are represented as percentage of inhibition and values are presented as mean±SD (n=3). SD: Standard deviation; DPPH: 2,2-diphenyl-1-picrylhydrazyl

chosen concentrations showed free radical-scavenging activity in a concentration-dependent manner. Sponge extract was able to reduce the DPPH to give a percentage of inhibition 19.02 ± 2.65, 21.67 ± 1.53, 33.67 ± 4.04, 56.33 ± 4.52, and 66.01 ± 2.05 at concentrations of 5, 15, 25, 50, and 100 µg/mL, respectively. The IC_{50} was below 50 µg/mL.

The superoxide anion radical-scavenging activity of the sponge extract at different concentrations is presented in Table 2. A concentration-dependent scavenging pattern was also observed in this case. The percent inhibition of superoxide anion was 26.33 ± 1.53, 29.67 ± 4.51, 37.33 ± 2.52, 59.67 ± 6.66, and 68.32 ± 4.35 at concentrations of 5, 15, 25, 50, and 100 µg/mL, respectively. The IC_{50} was below 50 µg/mL.

The hydroxyl radical-scavenging assay of the sponge extract at different concentrations is presented in Table 2. The inhibition of hydroxyl radical was 17.67% ±4.04%, 31.00% ±3.6%, 37.34% ±1.53%, 53.02% ±1.25%, and 69.67% ±2.08% at concentrations of 5, 15, 25, 50, and 100 µg/mL, respectively. The IC_{50} was below 50 µg/mL.

In vitro anti-inflammatory activity

As denaturation of proteins is a well-known cause of inflammation, the ability of the sponge extract to inhibit BSA denaturation was investigated and the percent inhibition of protein denaturation is presented in Figure 1. The sponge extract inhibited the denaturation of BSA in a concentration-dependent manner. The inhibition of BSA denaturation was 11.67% ± 1.53%, 16.33% ± 1.57%, 29.34% ± 2.08%, 47.00% ± 2.21%, and 76.56% ± 2.53% at concentrations of 5, 15, 25, 50, and 100 µg/mL of sponge extract, respectively, with IC_{50} of 55 µg/mL.

The inhibitory activity of the extract on NO production by induced macrophage cell line (RAW 264.7) is presented in Table 3. The inhibition of NO production was 67.94% ± 8.64%, 86.45% ± 5.39%, 91.22% ± 5.78%, 91.09% ± 4.67%, and 87.57% ± 6.34% at concentrations of 5, 15, 25, 50, and 100 µg/mL of sponge extract, respectively. The viability of the RAW 264.7 macrophages was 88.34% ± 5.33%, 86.08% ± 3.84%, 91.65% ± 5.11%, 89.30% ± 3.18%, and 86.52% ± 4.31% at concentrations of 5, 15, 25, 50, and 100 µg/mL of sponge extract, respectively. The viability of the RAW 264.7 macrophages was not significantly lower than in nontreated cells, indicating that the inhibition of the NO production was not due to cytotoxicity of the sponge extract.

Effect of the sponge extract on proinflammatory cytokine expression in breast carcinoma cell line (Michigan cancer foundation-7)

Proinflammatory cytokines are produced predominantly by activated macrophages, and they promote systemic inflammation. The effect of the sponge extract on the expression of the proinflammatory cytokine genes (*TNF*, *IFNG*, *IL6*, *IL1A*, *IL1B*, *CCL2*, *CXCL8*, and *CXCL10*) in MCF-7 cells is presented in Figure 2. Following treatment, expressions of all the proinflammatory cytokines were downregulated. Significant decrease in the expressions of *IL1A*, *IL1B*, and *CCL2* was observed at a sponge concentration as low as 15 µg/mL, while the reduction in the expression level of the rest of the proinflammatory cytokines required a little higher concentration of the sponge extract (25 µg/mL) [Figure 2]. The results of this study demonstrated that the sponge extract contains some bioactive compounds which have the potential to inhibit the expression levels of proinflammatory cytokines.

Table 3: Inhibitory activity of sponge extract on lipopolysaccharide-activated nitric oxide production in RAW 264.7 macrophages

Concentration (µg/mL)	Percentage NO inhibition	Percentage cell viability
5	67.94±08.64	88.34±05.33
15	86.45±05.39	86.08±03.84
25	91.22±05.78	91.65±05.11
50	91.09±04.67	89.30±03.18
100	87.57±06.34	86.52±04.31

Values are presented as mean±SD (n=3). NO: Nitric oxide; SD: Standard deviation

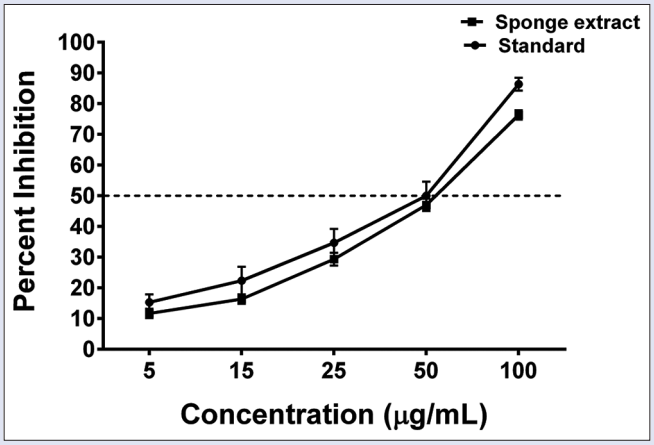

Figure 1: Inhibition of denaturation of bovine serum albumin by the sponge extract. Inhibition of *in vitro* albumin denaturation was used to evaluate the anti-inflammatory activity of the sponge extract. The values and error bar represent average and standard deviation of three independent sets of experiments. Diclofenac acid was used as the standard

DISCUSSION

There is increasing evidence that suggest that the marine environment contains different classes of biologically active compounds with strong antioxidant and anti-inflammatory activities.[23] Marine sponges are very rich source of structurally diverse natural products including alkaloids, steroids, terpenes, peptides, macrolides, and polyketides.[24] Several screening investigations have demonstrated that crude extracts from marine sponges display potent antioxidant and anti-inflammatory

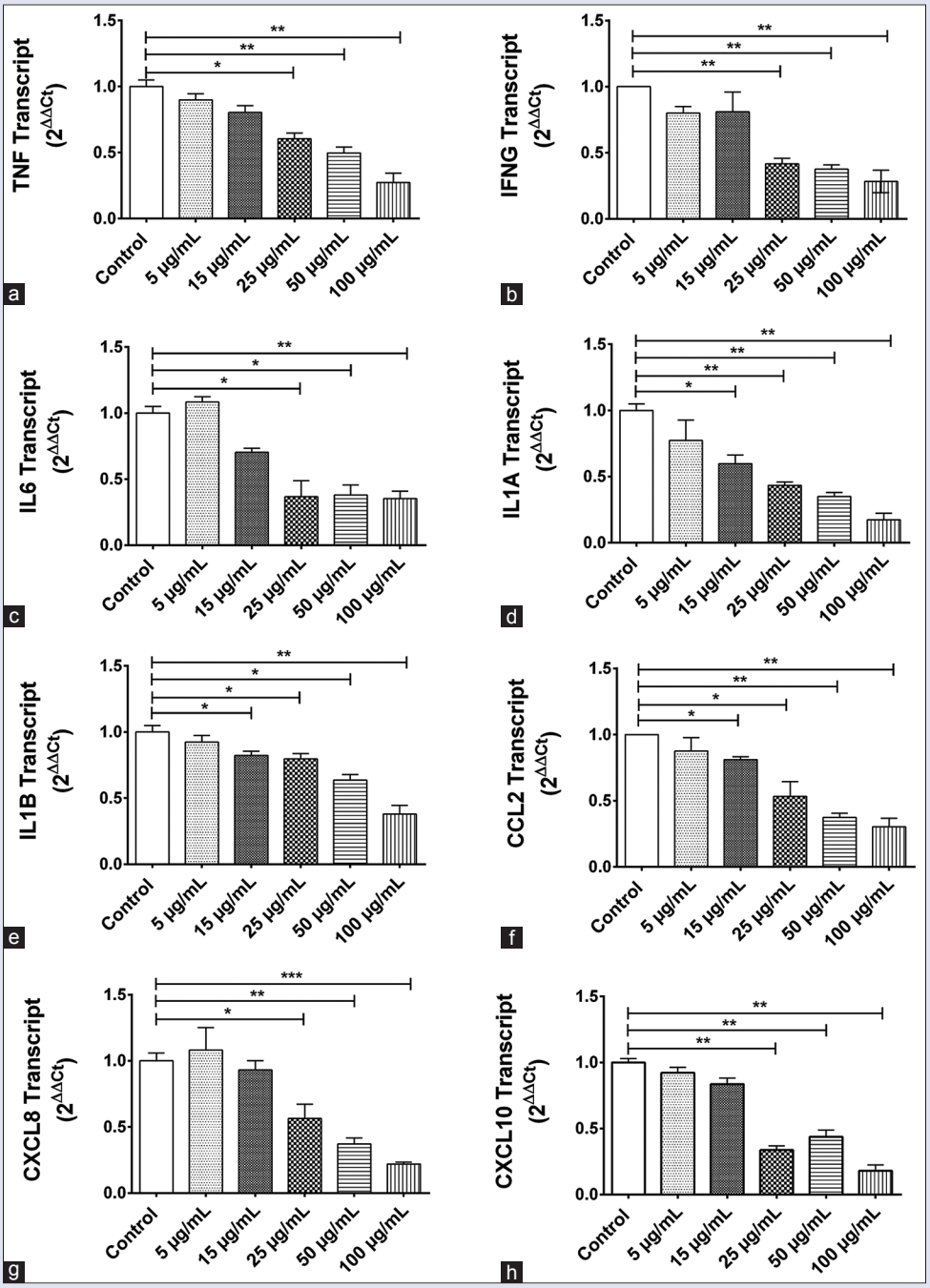

Figure 2: Effect of the sponge extract on the expression of proinflammatory cytokine transcripts in Michigan cancer foundation-7 Cells Real-time reverse transcription polymerase chain reaction analysis was performed to analyze the expression of eight proinflammatory cytokine genes. (a) Tumor Necrosis factor, alpha (*TNF*), (b) interferon, gamma (*IFNG*), (c) interleukin-6 (*IL-6*), (d) interleukin-1, alpha (*IL1A*), (e) interleukin-1, beta (*IL1B*), (f) chemokine (C-C Motif) ligand 2 (*CCL2*), (g) chemokine (C-X-C Motif) ligand 8 (*CXCL8*), and (h) chemokine (C-X-C Motif) ligand 10 (*CXCL10*). The values and error bars represent average and standard deviations of three independent sets of experiments. One-way analysis of variance followed by Dunnett posttest was performed to find out significant difference between control and treatments. *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$

properties.[25,26] In the present work, screening of the antioxidant and anti-inflammatory effects of the MeOH extract of the sponge *H. erectus* isolated from North Bay of South Andaman Sea, India, was carried out. The sponge extract exhibited excellent antioxidant and anti-inflammatory activities and induced downregulation of eight proinflammatory cytokine transcripts in a breast carcinoma cell line (MCF-7).

A free radical contains an unpaired electron in its atomic orbital.[27] Free radicals are involved in some signal pathways; thereby are beneficial at moderate levels, but at higher concentrations cause damage to our body by oxidative stress.[28] Antioxidants inhibit the production of free radicals by blocking the oxidation of other molecules. In the present study, it was found that the MeOH extract of the sponge could act as a potential antioxidant. The sponge extract resulted in >50% inhibition of free radicals, superoxide radicals, and hydroxyl radicals at a concentration of 50 µg/mL [Table 2]. A number of metabolites derived from marine sponges, such as indole derivatives, aromatic alkaloids,

aromatic polyketides, and phenolic compounds, have exhibited strong antioxidant potential.[10,11] Aromatic polyketides isolated from the marine sponge-derived fungus, *Aspergillus versicolor*, have shown significantly higher antioxidant capacity than that of butylated hydroxytoluene.[29] In another study,[30] with the Caribbean sponge, *Pandaros acanthifolium,* the isolated steroidal glycosides exhibited high antioxidant and cytoprotective activities.

In the present study, the MeOH extract of the sponge showed potential anti-inflammatory effect. It showed inhibitory effect on the denaturation of BSA *in vitro* [Figure 1] and on NO production by LPS-induced macrophages [Table 3]. NO promotes inflammation; therefore, any compound which shows inhibitory effect on NO production can be used as a potential anti-inflammatory compound.[31] There are several reports on anti-inflammatory effect of marine sponge extracts.[1,25,32] Several sponge metabolites with anti-inflammatory activities have been reported; such as cavernolide from *F. cavernosa*,[12] contignasterol from *P. contignata*,[13] and cyclolinteinone from *C. linteiformis*.[14] Although the mechanism of anti-inflammatory effect varies with sponge species, phospholipase A2 inhibition has been reported to be the major mechanism of anti-inflammatory properties of several sponges of order *Dictyoceratida*.[13,14]

Costantini *et al.*, 2015[25] reported that the extract of the sponge *Geodia cydonium* induced the decrease of proinflammatory cytokines in breast cancer MCF-7 cells, indicating that MCF-7 cell line can be used as an *in vitro* model to study the anti-inflammatory effect of any compound. In the present study, the effect of the sponge extract on the expression of eight proinflammatory cytokine genes in MCF-7 cells was evaluated. It was found that the sponge extract downregulated the transcripts of eight proinflammatory cytokines [Figure 2], further supporting our results on its potential anti-inflammatory properties. IL1β, IL6, and TNF-α are among the most widely investigated proinflammatory cytokines.[33] IFNγ stimulates cytotoxic T-cells, augments TNF activity, and induces NO production.[34] CCL2 and CCL8 are mediators of acute inflammation and stimulate a variety of cells including macrophages, monocytes, lymphocytes, eosinophils, and basophils.[35-37] Reduction of the proinflammatory cytokine transcripts on treatment with the sponge extract [Figure 2] indicates the presence of bioactive compounds with potent anti-inflammatory activity.

Marine sponges of the genus *Hyrtios* have been well known as a rich source of bioactive compounds. Antiproliferative and proapoptotic activities of marine sponge *H. erectus* extract on breast carcinoma cell line have been reported.[38] MeOH extract of the sponge showed cytotoxicity against three cancer cell lines HepG2, A549, and PC-3.[39] A bioactive metabolite (24methoxypetrosaspongia C) from *Hyortios* sp. showed growth inhibitory activity against hepatocellular carcinoma, colorectal carcinoma, and breast adenocarcinoma cells,[40] whereas two alkaloids (hyrtinadines C and D) from *Hyortios* sp. showed potential antimicrobial and antifungal activities.[41]

CONCLUSION

The screening of the marine sponge *H. erectus* from North Bay of South Andaman Sea, India, suggested that it is a rich source of compounds with antioxidant and anti-inflammatory properties. The secondary metabolites produced by the sponge induced downregulation of the proinflammatory cytokine transcripts. The bioactive compounds responsible for the activities need to be identified, and functional characterization of the compounds is required. This study will further add to the repertoire of bioactive compounds isolated from marine sponges and will make them potential candidates for therapeutic use in the future.

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Conflicts of interest

There are no conflicts of interest.

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