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# Assessment of antibacterial and antioxidant activity of soft coral *Cladiella pachyclados* from Port Blair, Andamans

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**ABSTRACT**

The study was undertaken to assess and evaluate the potential antibacterial and antioxidant activity of soft coral *Cladiella pachyclados* isolated from the coast of Port Blair, Andamans. The collected soft coral was identified taxonomically, and its methanolic extract (SCE-1) was subjected to the quantification of MIC, antibacterial (by agar diffusion well method) and antioxidant activities (by DPPH scavenging method, reducing power assay, FTC method). The SCE-1 exhibited moderate antibacterial activity against the tested pathogens. The organic groups present in SCE-1 were analyzed by FT-IR spectral peaks, which further exemplified the presence of various biological compounds of varying complex configurations.

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## 1. Introduction

The expansive and boundless frontiers of the marine domain, subjugated by marine bio-communities, are suffused by thriving under coarse caustic environments conditions like high salinity, pH fluctuations, variable pressure and nutrient availability (Li *et al*., 2017; Cherian *et al*., 2019; Eranhottu *et al*., 2021). Marine invertebrates, especially soft corals, are principally known to produce chemically defined secondary metabolites possessing ecological functions such as anti-predatory protection, as they are devoid of mechanical defenses (Datta *et al*., 2015; Alarfi *et al*., 2019). Most of these chemical substances have unique, extraordinary structures with exceptional multifarious pharmacological applications (Sun *et al*., 2018; Turner *et al*., 2018). The order Alcyonacea consists of soft corals subsisting pervasively in tropical marine waters, predominantly inner reefs or intertidal zones (Chanmethakul *et al*., 2010). They possess stinging cells (or nematocysts) devoid of a rigid protective skeleton (as in the case of scleractinians) and enthrall allelopathic capabilities of various chemical manufacturers (Liang *et al*., 2013), sanctioning them to endeavor and trimming down their palatability via the production of chemical substances, mucus or terpenoids, majorly aimed against predators (Zubair *et al*., 2016). The members of order Alcyonacea are proverbial for the production of terpenoids and their derivatives (Ata *et al*., 2003; Berrue and Kerr, 2009), displaying the properties of immuno-modulation, anti-cancerous and anti-fouling, conclusively earmarking soft corals as conceivable therapeutics.

Surprisingly, about40% of catalogued chemical scaffolds listed in different databases are innate compounds, along with nearly half of the novel drugs are based on natural origins or fabricated on natural architectures (Newman and Cragg, 2007). Comprehensive and lucid experimental studies have proffered an upscale advantage to marine products over terrestrial metabolites in their chemical uniqueness and structural configurations (Kong *et al*., 2010). An investigative revelation, based on the Dictionary of Marine Natural Products (DMNP), has accentuated the source of production of about 70% of metabolites in marine invertebrates. Different species of soft corals have been documented as the producers of >5800 secondary metabolites (Li *et al*., 2019), such as rare and unusual fatty acids (Imbs *et al*., 2012; 2013), terpenoids (Elkhawas *et al*., 2020; Sikorsky *et al*., 2020), quinones (Sunassee *et al*., 2012), alkaloids (Hou *et al*., 2019; Xu *et al*., 2019; Souza *et al*., 2020), glycosides (Dembitsky, 2005; 2006) and steroids (Dembitsky *et al*., 2018; Vil *et al*., 2018; Hou *et al*., 2019; Dembitsky, 2020); each of these evincing anti-cancerous and other vital pharmacologic activities (Di Costanzo *et al*., 2019; Wali *et al*., 2019; Matulja *et al*., 2020). The genus *Cladiella* has been well known in synthesizing bioactive compounds like sesquiterpene, cladidiol and diterpenes such as polyanthellin-A, cladiella peroxide and (6E)-2,9,epoxyeunicella-6,11(12)-dien-3-ol (Ata *et al*., 2004). The molecules of eunicellin diterpenes isolated from Red Sea coral, *Cladiella pachyclados* exhibited effective anti-invasive and anti-migratory activity against prostate cancer and enhanced wound healing properties (Hussan *et al*., 2011).The present study aims to assess and quantify the potentiality of soft coral species *Cladiella pachyclados* in antibacterial and anti-oxidative settings for applicative insights in drug conjugation and pharmacology.

## 2. Materials and Methods

### 2.1. Sampling, preservation and identification

The soft coral sample was collected by hand picking method from the intertidal region of Science Center, Port Blair, South Andaman (Fig. 1) in January 2021. Collected samples were washed with sterile sea water, coded as SC-1 and kept at -20°C in an ice box until processing. The tissue samples and skeletal elements called sclerites (dissolved in 5% sodium hypochlorite) were isolated for morphological and internal (structure and sizes of sclerites) identification using referral taxonomic keys of Rao and Kamala Devi (2003) & Janes and Lee Mei Wah (2007).

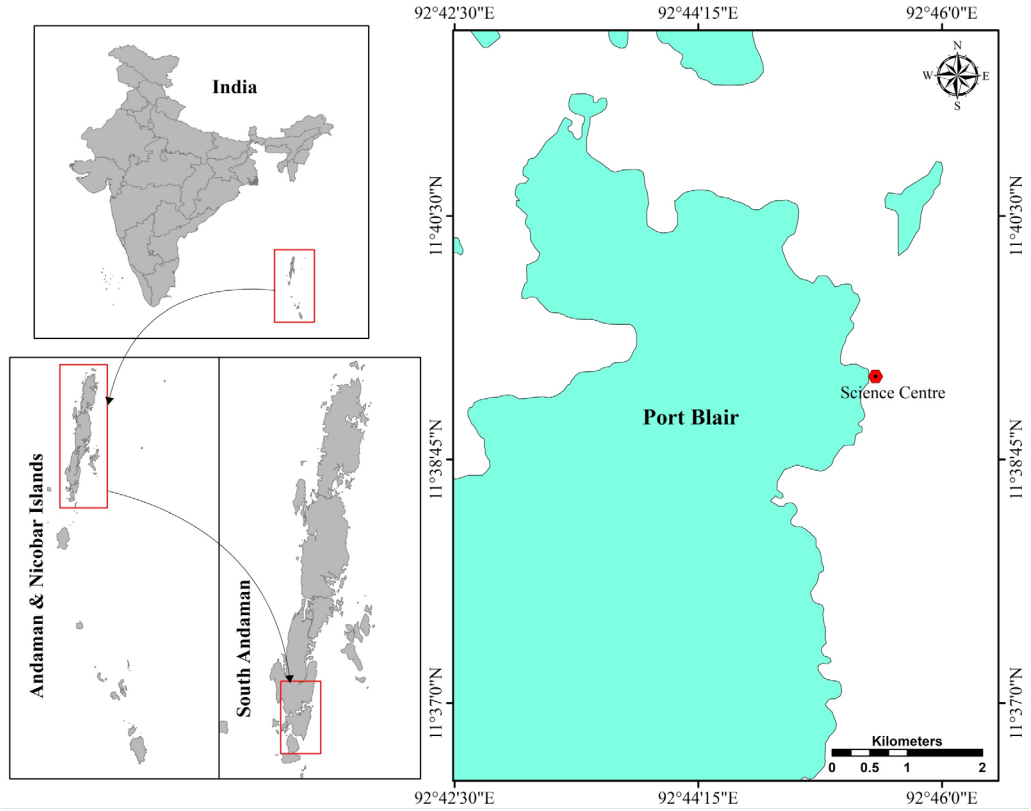


**Fig. 1.** Map of Andaman island showing the study area

### 2.2. Preparation of extracts

The frozen soft coral samples were defrosted, and sized into small pieces; about 10 g macerated tissue was added to 80 ml methanol and kept at room temperature for 24 hrs. The extract (SCE-1) was filtered using Whatman no.1 filter paper. The resulting filtrate was dried using a rotary evaporator at 40˚C, and the final concentration was made to 10 mg/ml.

### 2.3. FT-IR (Fourier transform infrared spectroscopy)

FT-IR was employed to assess functional groups of soft coral extract (SCE-1). The dried powder of soft coral was diluted with KBr (Potassium bromide; mass ratio 1:100). The spectrum was recorded, and measurements were documented on Perkin Elmer FT-IR spectrometer Spectrum Two (Perkin Elmer Life and Analytical Sciences, CT, USA) at diffuse reflectance mode; resolution 4 $cm^{-1}$ (Cherian *et al*., 2019).

### 2.4 Anti-oxidative assays

#### 2.4.1. Reducing power

The reducing power of SCE-1was analyzed by following the protocols of Keshari *et al*. (2018) with slight modifications. The extracts (50 and 100 µg/ml) (diluted in 1.0 ml distilled water) were mixed with sodium phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and $K_3Fe(CN)_6$ (potassium ferricyanide, 2.5 ml; 1%) followed by incubation at 50˚C for 20 minutes. After incubation, TCA (trichloroacetic acid, 2.5 ml; 10%) was added to the mixtures and centrifuged (3000 rpm; 10 minutes). Following centrifugation, the upper layer (2.5 ml volume) was extracted and mixed with $FeCl_3$ (ferric chloride, 0.5 ml; 0.1%) + 2.5 ml milli-Qwater. The absorbance was read at 700 nm, and the results were expressed as ascorbic acid equivalents.

#### 2.4.2. Free radical scavenging activity

The free radical scavenging ability of SCE-1was evaluated by using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical (Locatelli *et al*., 2009) with slight modifications. In brief, SCE-1(50 and 100 µg/ml) (diluted in 3 ml ethanol) was mixed with DPPH (3 ml) in ethanol (200 µM) and incubated for 30 minutes under dark conditions. Ascorbic acid was used as a comparative set. The absorbance was recorded at 517 nm and percent inhibition (I%) was calculated employing the following equation:

$$I\% = \frac{Ao - Ae}{Ao} \times 100$$

where, $A_0$= absorbance of the blank sample and $A_e$= absorbance of the tested sample.

#### 2.4.3. Ferric thiocyanate method (FTC)

For the FTC method (Samimifar, 2019) under slight variations, the SCE-1 (100 µg/ml) was mixed with linoleic acid (2.5%) in ethanol (4 ml), phosphate buffer (8 ml, 0.05 M, pH 7.0), milli-Q water (4 ml) and kept at 40˚C under dark. Aliquots (0.1 ml) were mixed with 70% ethanol (9.7 ml) and 30% ammonium thiocyanate (0.1 ml). After 3 minutes, 0.1 ml $FeCl_2$ (ferrous chloride, 20 mM) in 3.5% HCl (hydrochloric acid) was added and the reaction absorbance was noted at 500 nm every 24 h until a maximum value was reached. Control and standard (ascorbic acid) setup were run parallel.

### 2.5. Antibacterialassay

#### 2.5.1. Pathogenic strains

Human pathogens, *Shigella dysenteriae* type 5 NK2440, *Shigella boydii* type 1 NK2379, *Aeromonas hydrophila* IDH1585, *Vibrio fluvialis* IDH 02036, maintained in our microbial laboratory, were used. Nutrient agar and nutrient broth medium were used for bacterial culture and incubated at 37˚C for 24 hours. The culture turbidity was adjusted according to the standard 0.5 McFarland solution.

#### 2.5.2. Determination of MIC (Minimum Inhibitory Concentration)

The MIC values were evaluated with minor modifications by broth microdilution assay (NCCLS, 2008) (Reda *et al*., 2021). The initial dissolution of samples was done in DMSO with subsequent dilutions in sterile broth media. Five-fold dilutions were prepared, and microbial suspensions were inoculated in each well (performed in 96-well plate), followed by incubation for 24 h at 37˚C. The assay was undertaken in a nutrient broth medium following Hammer *et al*. (1999) with slight modifications. Briefly, one milligram (1mg) pure compound (present in SCE-1) was dissolved in DMSO (50 µl). The initial concentration (10 µl) was added, followed by 190 µl tested microbial suspension ($5\times 10^5$ CFU/ml). Serial dilutions were performed and adjusted to the final volume (200 µl) of each well by adding the microbial suspensions. A microbial growth control setup was run parallel by adding the same volume of DMSO (devoid of test compound) for the elimination of possible antibacterial effects of the solvent. The well plates were carefully mixed and incubated (37˚C, 24 h). Turbidity was taken as an indicator of microbial growth and no microbial growth was expounded as antibacterial activity (Shin *et al*., 1998).

#### 2.5.3 Agar well diffusion assay

The agar well diffusion method is one of the most extensively used methods for the evaluation of antimicrobial activities of microbial or plant extracts (Magaldi *et al*., 2004; Valgas *et al*., 2007). Briefly, the overnight grown bacterial inoculum with the cell density ($2\times10^8$ CFU/ml, 0.1 ml) was spread evenly on Mueller Hinton agar plates and wells were created by gel puncture. Subsequently, different amounts of SCE-1 (50 and100 µg/ml) were added to the pre-cut wells along with positive control (chlortetracycline, 30 mcg) and negative control (methanol) incubated for 24 h at 37˚C. The size of ZOI (zone of inhibition) was measured by the antibiotic zone scale (HiMedia).

### 2.6 Statistical analysis

All experiments were performed in triplicate and evaluated using one-way ANOVA and Duncan's multiple range tests (IBM SPSS® Statistics version 22.0, NY).The p value < 0.05 was considered statistically significant and graphs were constructed by Sigmaplot version 10.1 (USA).

## 3. Results and Discussion

Marine milieu circumscribes diverse ecosystems embodying a wide range of living forms, each within imitable chemical and biological features with rare and pronounced therapeutic flairs and potentialities. Marine natural compounds, principally obtained from marine sponges and soft corals, are remarkably proficient against human malignancies and medical studies (Alves and Diederich, 2021; Saide *et al*., 2021).

In this study, the collected soft coral sample (SC-1) was morphologically identified as *Cladiella pachyclados* (Klunzinger, 1877), belonging to the order Alcyonacea. The morphological characteristics of SC-1 are listed in Table 1.

The FT-IR spectrum (Fig. 2) revealed the presence of various functional groups, each pertaining specifically to the organic compounds present in SCE-1. 3752-3497 $cm^{-1}$ (hydroxyl (-OH) group), 2927-2364 $cm^{-1}$ (alkynyl C≡C stretch), 1644 $cm^{-1}$ (C=O stretch of amide region), 1164 $cm^{-1}$ (C-N vibrations of aromatic amines). Also, moderate stretching was observed in peaks at 1127, 1089, 1007, 894, 812, 654 and 505 $cm^{-1}$ related to alkene groups (C=C stretching), polyphenolic compounds, aliphatic amines (C–N stretch vibrations), alkene groups (C–H stretch), respectively. The reductive ability of SCE-1 in the $Fe^{3+}$-$Fe^{2+}$ transformation was investigated and compared with ascorbic acid (Fig. 3). The reducing power of SCE-1 increased with increasing concentrations exhibiting high activity. The dose-dependent increase in the transformation of $Fe^{3+}$ to $Fe^{2+}$ was exhibited with increasing concentrations of SCE-1 as the comparative standard ascorbic acid also followed a similar trend with respect to the reducing capacity. An increase in the absorbance indicated increased antioxidant activity, thus exhibiting the reducing power of SCE-1. The $Fe^{3+}$- $Fe^{2+}$ reaction reduction is mainly attributable to the presence of reductones that exerts an anti-oxidative action via breakage in the free radical chain by donating hydrogen atom (Gordon, 1991).

Furthermore, the distinct absorption capacity of DPPH decreases significantly in reaction with the radical scavengers via the donation of hydrogen atoms, which

**Table 1.** Map of Andaman island showing the study area

| Soft coral | Characteristics | Sample |
|---|---|---|
| *Cladiella pachyclados* (SC-1) | **Colony shape**- Broccoli of colony size varied in size, lobate form with short, inconspicuous stalk. Colony colour Brown. Surface with numerous pores.<br>**Polyps**- Monomorphic, rounded lobed capitulum.<br>**Sclerites**- Dumbbell-shaped and small biscuit-like sclerite forms were commonly found. |  |

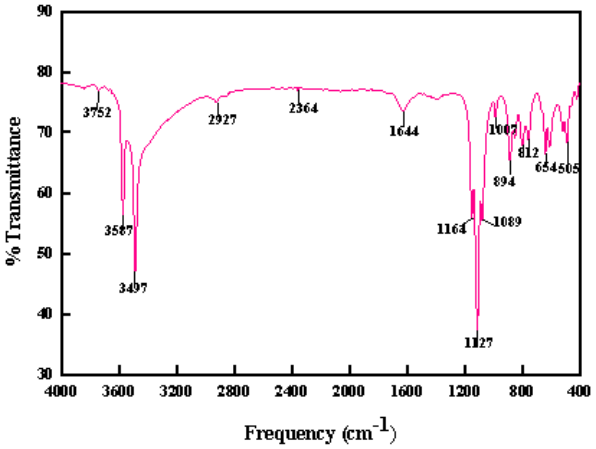


**Fig. 2.** FT-IR spectra of SCE-1

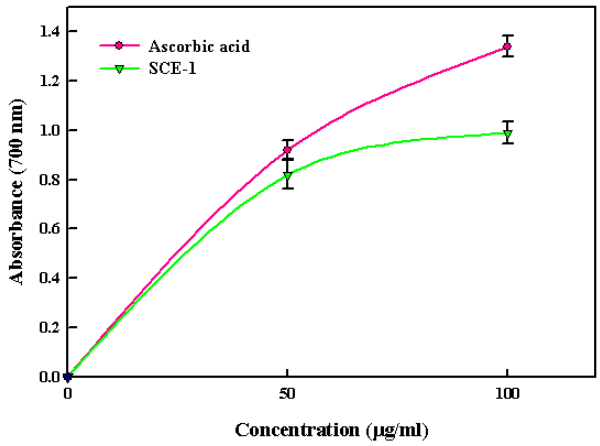


**Fig. 3.** Reducing power of SCE-1
(Results are means ± SD of triplicate values)

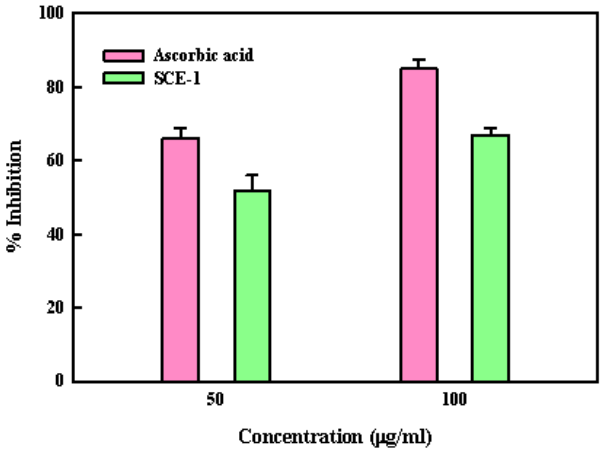


**Fig. 4.** Free radical scavenging activity of SCE-1 (DPPH method)
(Results are means ± SD, $p < 0.05$)

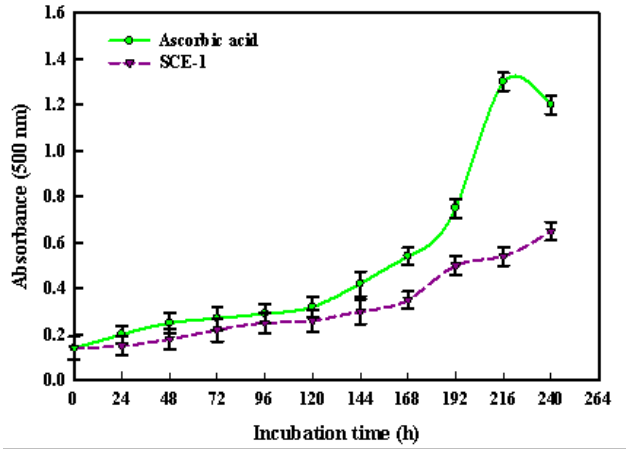


**Fig. 5.** Antioxidant activity of SCE-1 quantified by FTC method
(Results are means ± SD of triplicate values)

converts the former into a stable diamagnetic molecule (Pal *et al*., 2010). The principle underlines the reaction of an antioxidant molecule with the stable free radical DPPH, converting it into 1, 1- diphenyl-2-picryl hydrazine (Sreejayan and Rao, 1996). The neutralization of DPPH occurred by the electron transfer initiated by the molecule of antioxidants present in the extract (here, SCE-1). The reduction capacity of DPPH was determined by detecting a visible effect on scavenging free radical potential, indicative via colour change from purple to yellow at 517 nm, inferring increased scavenging effect of SCE-1 at increased concentrations (Fig. 4). Also, the levels of peroxide during the initial stage of lipid oxidation were measured by FTC method (Fig. 5). The most reactive molecule of ROS system, the hydroxyl radical evokes severe damage to the bio-molecule system complex (Gutteridge, 1984) causing great deal of oxidative damage to cellular molecules of lipids, DNA and proteins (Spencer *et al.,* 1994). Iron ions usually arouse lipid peroxidation via the Fenton reaction; hastening it by the decomposition of lipid peroxide into the hydrogen abstracter radicals of peroxyl and alcoxyl type and perpetuating chain reaction mechanistic steps of lipid peroxidation (Halliwell and Gutteridge, 1989). The hydroxyl radicals ($OH^-$) generated in the living cell play a significant role in cellular injury at the inflammatory sites in oxidative stress-derived diseases.

The antibacterial activity was assessed by observing growth inhibition of *Vibrio fluvialis*, *Aeromonas hydrophila*, *Shigella boydii*, *S. dysenteriae*. The MIC values of SCE-1 against tested pathogenic strains are shown in Table 2. The results shown in Table 3 and Figures 6 and 7 exhibited antibacterial activity of SCE-1 against tested pathogenic strains. SCE-1 exhibited an inhibition zone of 12 and 14 mm at 50 and 100 µg/ml, respectively, against *Vibrio fluvialis*. A moderate activity was observed against *Aeromonas hydrophila* and *Shigella boydii* compared to the positive control, chlortetracycline which inhibited the tested pathogens with larger inhibitory zones. The antimicrobial activity is influenced by several factors like pH, media composition, inoculum size, incubation time, stability of antimicrobial compounds & state and metabolic activity of organism (Jawetz, 1998).

The recent clamor of antibiotic resistance in pathogenic bacterial strains against existing medical therapies has resulted in a hysterical rise in bacterial infections (Cherian

**Table 2.** MIC value of SCE-1 against tested pathogens

| S.No. | Pathogenic strains | MIC (µg/ml) |
|---|---|---|
| 1 | *Shigella boydii* | 35±0.34 |
| 2 | *Aeromonas hydrophila* | 40±0.47 |
| 3 | *Shigella dysenteriae* | 45±0.3 |
| 4 | *Vibrio fluvialis* | 30±0.6 |

**Table 3.** Zone of inhibition size (in mm) values of SCE-1 against pathogenic strains

| S.No. | Pathogenic strains | Methanol | Chlortetracycline | SCE-1 (µg/ml) | |
|---|---|---|---|---|---|
| | | | | 50 | 100 |
| 1 | *Shigella boydii* | - | 20±1.0 | 10±1.0 | 11±1.3 |
| 2 | *Aeromonas hydrophila* | - | 23±1.5 | 11±1.0 | 12±1.0 |
| 3 | *Shigella dysenteriae* | - | 24±1.0 | 12±1.0 | 14±1.4 |
| 4 | *Vibrio fluvialis* | - | 22±1.56 | 13±1.1 | 14±1.32 |

*et al*., 2020). Besides, the superfluous side-effects of some antibiotics have resulted in the rummage for new anti-bacterials, particularly from marine life forms (Marican *et al*., 2016). Soft corals, occupying a pivotal consign in chemical ecology, possess assorted biological activities; one being antibacterial (Afifi *et al*., 2016). The vast sums of inconsistency in differential responses of antibacterial compounds are well known, owing to the presence of resistance factors in bacteria. A Gram-negative bacterium tends to be more susceptible to polar based antimicrobials, while a gram-positive bacterium is more receptive to the non-polar ones (Branen and Davidson, 1993). The variability's in antimicrobial response to both Gram-positive and -negative forms of bacteria are basically linked to their cell wall architectural components such as peptidoglycan content, cross-linking properties, the presence of translocation sites (pores, receptors, lipids) and autolytic enzymes; determining the binding, penetration and intracellular activities of antimicrobial compounds (Jawetz, 1998). Furthermore, Setyaningsih *et al*. (2012) propounded that some soft coral extracts, nil in inhibitory effects, may possess synergistic activities. The biologically active compounds isolated from natural origins are proposed in a synergism between compounds via working models of multi-compound and multi-target synergistic modules (Merzenich *et al*., 2010; Long *et al*., 2015). Tanod *et al*. (2019) proposed a plausible inference of stronger antimicrobial effects at appropriate minimum concentrations of soft coral extracts.

The previously reported scientific studies have shown that biologically active compounds and their associated potential bioactivities differ among soft coral species. Soft coral species of *Sinularia* sp. have reported promising antibacterial activity against Gram-negative and -positive bacteria. Sun *et al*. (2012) reported antibacterial activity of *Sinularia humilis* Ofwegen, isolated from the South China Sea, against the bacteria *Bacillus megaterium*. *S. depressa* Tixier-Durivault (isolated from Lingshui Bay, China) synthesized steroid compounds exhibiting strong inhibition against *S. aureus* Newman (Liang *et al*., 2013). The species of *Sinularia kavarattiensis* (isolated from the coast of Mandapam, Tamil Nadu) reported moderate to high antibacterial activity against the bacterial species of *Staphylococcus aureus* and *S. epidermidis* (Rajaram *et al*., 2014). Dobretsov *et al*. (2015) documented the antibacterial activity of two species of *Sinularia* sp. (Bandar Al-Khayran Oman) against many bacterial species like *E. coli*, *S. aureus*, *Micrococcus luteus*, *Salmonella* sp. and *Bacillus subtilis*. The crude extract of *Sinularia* sp. (isolated from Lampung, Indonesia) exhibited antibacterial activity against the bacterial cells of *S. aureus*, *B. subtilis*, *V. eltor* and *E. coli* (Putra *et al*., 2016). Afifi *et al*. (2016) reported the antibacterial efficacy of crude extract of *S. polydactyla* (Yanbu, Red Sea) against *S. aureus*.

## 4. Conclusion

The study throws an informative insight on the biological active potency of soft coral *Cladiella pachyclados* isolated from Port Blair, Andamans. A series of antibacterial and antioxidant assays exhibited promising antibacterial and anti-oxidative activity, furnishing it as novel or alternative anti-bacterials. Further comprehensive experimental

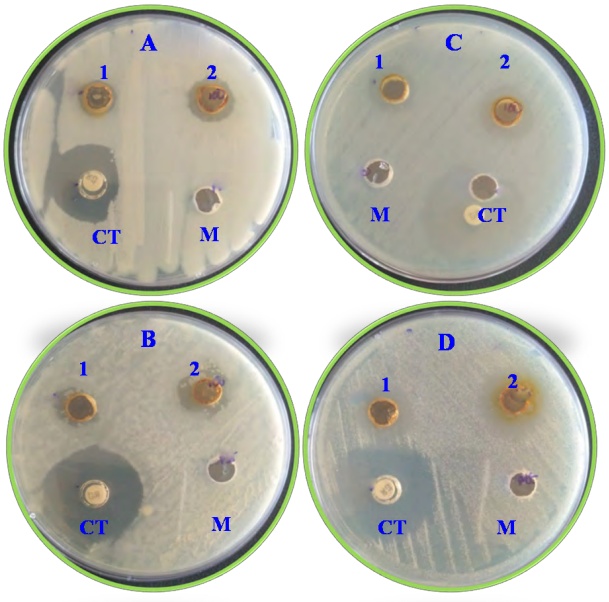


**Fig. 6.** Assessment of antibacterial activity of SCE-1 by well diffusion assay [(1) 50µg/ml, (2) 100µg/ml, (3) CT (Chlortetracycline), (4) M (Methanol)] against (A) Shigella boydii; (B) Aeromonas hydrophila; (C) Shigella dysenteriae; (D) Vibrio fluvialis

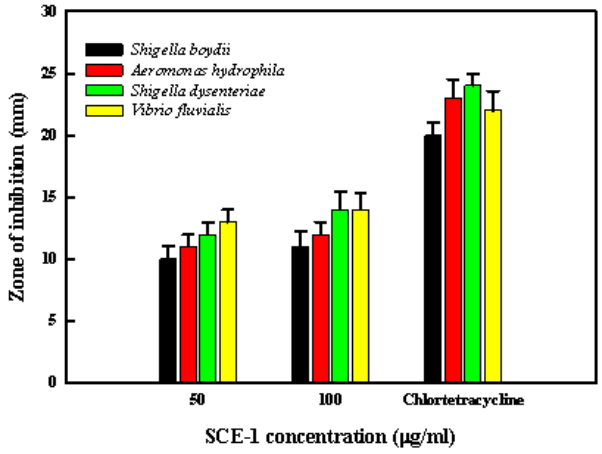


**Fig. 7.** Comparative antibacterial activity of SCE-1 (50 and 100 µg/ml) against pathogenic strains
(Results are means ± SD of triplicate measurements, $p < 0.05$)

validations are required, particularly in the purification and characterization of extracts to identify compounds responsible for the validated activities. The increased global consideration on uncovering alternative founts for bioactive activities has highlighted soft corals as one of the propitious prospects.

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