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Comparative evaluation of the bioactivities of marine sponges *Callyspongia fibrosa* and *Dysidea fragilis* from the south west coast of India

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ABSTRACT

The bioactivity role of marine sponges *Callyspongia fibrosa* and *Dysidea fragilis* were explored in the current study. Seven strains of symbiotic microbial symbionts were isolated from the fresh samples of marine sponges, and their antimicrobial efficiency was evaluated. It is noted that the antibacterial potential of methanolic extracts of marine sponges towards *Staphylococcus aureus* and *Pseudomonas aeruginosa* displayed a higher inhibition zone compared to the standard antibiotic ampicillin. In the case of symbiotic bacterial strains, the isolated Yellow (Y) strain GPC exhibited the highest inhibition. In the antioxidant assay, the IC_{50} values were determined as 38.64 µg/ml and 199.26 µg/ml for *Callysongia fibrosa* and *Dysidea fragilis*. TLC was carried out using the chloroform and methanol (7:3) as the mobile phase, and completed chromatograms were then visualized in long UV (366 nm). The extracts of *C. fibrosa* produced three bands while *D. fragilis* produced only two bands at 0.4 cm and 9.5 cm, and the solvent front was 12.7 cm. The FT-IR analysis of crude methanol extracts of the sponges, *Callyspongia fibrosa* and *Dysidea fragilis* showed nearly 11 and 12 peaks, respectively. The peaks produced by *C. fibrosa* at 3350.33 cm^{-1}, 1624.50 cm^{-1}, 1091.41 cm^{-1}, 1413.03 cm^{-1} and 1331.32 cm^{-1}. Frequencies recorded were 3350.33 cm^{-1} displayed O-H stretching (alcohol), 1624.50 cm^{-1}reported C=C stretchings, 1091.41 cm^{-1} exhibited C-O stretching (1°alcohol), 1413.03 cm^{-1}showed O-H bending, and 1331.32 cm^{-1}displayed C-H bending. In the case of *D. fragilis* frequencies, 3361.11 exhibited OH stretching, which indicates the presence of alcohol; peak at 1631.31 reported a C=C stretching and a CH bending was noted at 1331.32. The peak at 1095.67 reported a C-O stretching, indicating the presence of primary alcohol.

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1. Introduction

Ocean represents a varied resource for natural products, and the diversified environment offers immense potential as a source of marine drugs (Blunt *et al*., 2005). A wide variety of bioactive compounds with antibacterial, antiviral, antifungal activities has been synthesized from marine organisms, including sponges, molluscs, polychaetes, bryozoans, and ascidians (Roch *et al*., 1996). Sponges are reported to be extreme sources of natural compounds, consisting of secondary metabolites of therapeutic relevance (Ancheeva *et al*., 2017). Sponges synthesize a wide range of secondary metabolites to compensate for their limited physical defensive mechanisms (Mehbub *et al*., 2014). Antibiotics are medications that destroy or slow down the growth of bacteria, and the use of antibiotics is enhanced due to acute infections. The pathogenic bacteria becoming resistant to drugs has initiated the isolation of novel antibacterial compounds from marine sponge resources. Microbial symbionts are reported to possess many antimicrobial drugs. Most of the antibiotics used in recent days are of terrestrial origin. According to the study of WHO, ten million people will die of antibiotic resistance by 2050 (Baquero, 2008). Developing more effective antibiotics is the only way to tackle antibiotic-resistant bacteria. These include natural products obtained from marine organisms (Selvin and Lipton, 2004). Marine sponges are considered an essential source for producing novel drugs with pharmacological potential because they produce diverse chemical compounds (Perdicaris *et al*., 2013).

Free radicals are compounds capable of causing harm to the body when their level becomes too high. The marine environment has a rich source of antioxidant compounds because molecules that are derived from seaweed, sponges, and marine microbes possess antioxidant properties (Balakrishnan *et al*., 2015). Bioactive compounds with antioxidant effects have been identified in marine sponges (Utkina, 2009). Phytochemicals are also reported to favour protection against various disorders associated with increased oxidative stress and protect the cells from oxidative stress damage.

The porous body of the sponges accounts for their symbiosis, and all kinds of sponges exhibits symbiosis. It was challenging to observe a sponge that is not occupied is smaller invertebrates and some fishes as shelter. Their microbes mediate many of the functions played by sponges. The microbes in sponges are about 40% of the sponge tissue volume (Taylor *et al*., 2007). The marine sponges, namely *Callyspongia* (Cladochalina) *fibrosa* (Ridley and Dendy, 1886) and *Dysidea fragilis* (Montagu, 1814) from South West coast of India were screened for symbiotic bacterial association and bioactivities. Analysis of biochemical extracts of these marine sponges was also carried out to find their antimicrobial and antioxidant properties. The current study aimed to isolate the symbiotic microorganisms from the selected marine sponges and analyze their bioactivity towards selected pathogenic strains.

2. Materials and Methods

2.1. Sponge collection and Identification

Sample specimens of marine sponges were collected from the rocky shore regions of Kovalam beach (Lat. 8°22'0.01"N; Long. 76°59'48.01"E), Southern West coast, India at a depth of 6 to 7 m. Surface ornamentation, form, colour, resiliency and symbiotic biological associates were also recorded at the time of collection. Taxonomic identification was carried out by studying the prepared spicule's nature (Sivaleela, 2014) using pertinent literatures and keys: Demospongiae of the Gulf of Mannar and Palk Bay (Thomas, 1986); Systema Porifera: A guide to the classification of sponges (Hooper and Van Soest, 2002); Sponguide: Guide to sponge collection and identification and compared it with the original description of the species in World Porifera Database (http://www.marinespecies.org/porifera).

The experimental samples were cut and removed from the collected sponges with a dive knife. The removed individual pieces were transferred to separate sterile plastic collection bags, brought to the surface, maintained at ambient seawater temperature, and transported to the laboratory on the same day of collection. Fresh samples were used to isolate symbiotic bacteria and further bioactivity studies. Spicule analysis was also conducted to confirm the identity. The colour, shape, and consistency of the specimen are noted. The type and arrangement of mineral spicules in the endoskeleton were analysed (Selvin *et al*., 2009). For taxonomical identification, the sponge tissue's head, bottom, interior and surface regions were taken and boiled with concentrated HNO_3 until the liquid became clear with dissolved cellular components to extrude spicules. The sample tube was filled with distilled water and kept for at least three days to settle the spicule to the bottom. Then it was washed many times and examined under Labomed LX500 electron microscope. Identification was confirmed using taxonomic standards.

2.2. Isolation of sponge-associated bacteria

Isolation of symbiotic bacteria from collected sponges were done as per (Gandhimathi *et al*., 2008). For this, nearly 1 cm3 of sponge tissue was removed directly from the internal mesohyl area by sterile scissors in aseptic conditions on a sterile ceramic tile. Autoclaved 0.85% saline (1 ml) solution was poured and well squeezed the specimens. The homogenization procedures continued until the sponge exudates were obtained (Bauer *et al*., 1996). The squeezed exudates were pour-plated on a modified culture medium of Zobell Marine Agar 2216 and incubated for 24 hrs at 37°C (Hatano *et al*., 1989). Morphologically distinctly observed bacterial colonies were sub-cultured and maintained on ZMA (HiMedia) at 4°C.

2.3. Extract preparation

Sponge specimen was cut into small pieces for crude extract preparations and placed in 500 ml of methanol. Sponge tissues and solvent were transferred to capped containers, kept at room temperature for three days, and constantly agitated. The sponge tissue was removed from the container, and solvents were squeezed from the tissue using a sterile cheese cloth. The extract was evaporated to dryness under a vacuum at room temperature.

2.4. Screening of antibacterial susceptibility test

The *in vitro* antibacterial susceptibility of the extracts against bacterial isolates was carried out by disc diffusion method on Muller Hinton agar plates (Hi-media) (Maldonado *et al*., 2005). For this, about 1/3rd portion of petri dishes (100 mm) were prepared with Muller-Hinton agar medium up to 2 mm thickness as a single layer and allowed to solidify for 15 minutes. After solidification, 0.1 ml of 18 h bacterial shake culture was surface inoculated using sterile cotton swabs and set for 5 minutes. The microorganisms used were clinical isolates maintained in the Department of Zoology, University of Kerala, including Gram-negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and Gram-positive bacterium, *Staphylococcus aureus*. Before starting the experiment, these were activated in nutrient broth and subsequently purified using the agar streak plate method.

The sterile discs (6 mm diameter, Hi-media) which were impregnated with the desired concentration of test compound at 30 µl, were placed on the inoculated agar surface using autoclaved forceps. Assays were carried out in triplicate sets with appropriate controls. After incubation at 37°C for 24 h, the area of the inhibition zone (mm^2) was calculated using a Hi Antibiotic Zone Scale-C (PW-297, Hi-media), and the antibacterial potential of extracts were compared with those of standard antibiotics (Hi-Media) like ampicillin (30 mcg/disc).

2.5. Screening of antioxidant assay

The *in vitro* antioxidant assay of the samples was done using the DPPH radical scavenging activity (Bartikova *et al*., 2014). The extract was screened for its antioxidant capacity by considering its DPPH radical scavenging activity. It is based on the scavenging of DPPH by antioxidants, which, upon a reduction reaction, decolorizes the DPPH methanol solution and measures the reducing ability of antioxidants towards the DPPH radical. The reference compound used is ascorbic acid. The samples MeOH solutions (200 µL) at different concentrations were added to 1 mM DPPH/MeOH solution (50 µL). The reaction mixture was shaken, and the absorbance of the remaining DPPH was measured at 517 nm after 30 minutes. The antioxidant compounds react with DPPH radicals through a hydrogen atom donation mechanism and cause colour decay from purple to yellow. The radical scavenging activity was determined by comparing the absorbance with blank (100%) containing only water DPPH and solvent. The assay experiments were done in three replicates.

2.6. Screening test of extracts for thin layer chromatography

Thin layer chromatography in aluminum sheets of 10 x 20 cm silica gel plates (Merck, Germany) coated with silica gel were used for screening the extracts from the samples. The prepared methanol extract was spotted on the TLC plate using a sterile capillary tube. The plates were then developed in closed glass chambers saturated with the developing solvents (chloroform: methanol (7:3).

After it had run, it was removed from the developing chamber and dried in the air and the separated compounds were visualized using long-wavelength ultraviolet light. The colour changes indicate the presence of bioactive components separated in TLC plates. The Rf values are then calculated based on the distance travelled by the extract. Fourier Transform Infrared Spectroscopy (FTIR) is employed as an effective analytical instrument to detect functional groups and characterize covalent bonding information. FTIR analysis was conducted at the Central Laboratory for Instrumentation and Facilitation (CLIF), University of Kerala, to get a preliminary idea of its chemical components.

3. Results

Two species of marine sponges were collected from the Southwest coast of India and identified as *Callyspongia fibrosa* (Cladochalina) and *Dysidea fragilis* (Montagu, 1814) (Fig 1 & 2). The bacteria symbiotically associated with *Callyspongia* (Cladochalina) *fibrosa* and *Dysidea fragilis* were isolated by the pour plate method (Fig. 3). The isolates were found to be White Puffy (WP), Dark White (DW), Dark Yellow (DY), Transparent White (TW), White Linear (WL), Yellow (Y) and White Perky (WP). The isolated bacteria were characterized by biochemical screening, including indole test, mannitol motility, citrate, TSI test, lysine, arginine, urease, catalase, oxidase, and gram staining (Table 1). Seven strains of bacteria were identified from both specimens. These isolated bacterial strains were identified as Gram-positive long bacilli, Gram-positive short bacilli, GPC (micrococci), GPC (clusters, pairs, micrococci), and very large broad *bacilli*.

The antibiotic property of methanol extract was detected by the disc diffusion method. The methanolic extracts of both sponges were tested against the symbiotic bacteria associated with it and against *Staphylococcus aureus, Enteorococcus faecalis*, *Pseudomonas aeruginosa* and *Escherichia coli* respectively. The antibiotic activity of methanol extract was determined by calculating the inhibition zone diameter. The Zone inhibitions were measured by Hi Antibiotic zone scale-C (PW-297, Hi-media). The antimicrobial activity of methanol extract of marine sponges was represented in Fig. 4 & 5. The tested results showed that majority of bacterial strains were inhibited to various degrees, and the bacteriostatic potential of MSMs varied greatly based on the strains of bacteria used. The results also showed the inhibition of methanol extract of *Dysidea fragilis* against Gram-negative strain *Pseudomonas aeruginosa* compared to the standard antibiotic ampicillin. Crude methanolic extract of *Callyspongi*a produced more inhibition against *E. coli* at 37°C. *E. coli* isolate was found to be resistant to *Dysidea fragilis*. Results also indicated that the methanolic extract of both sponges successfully inhibited *Staphylococcus aureus* and *Enterococcus faecalis.* In the case of symbiotic bacterial strains, the isolated Yellow (Y) strain GPC (micrococci) exhibited the highest inhibition compared to standard antibiotics.

The antioxidant activity of the sponge extracts at varied concentrations (200, 400, 600, 800 and 1000 mg/ml) were measured by DPPH radical scavenging method and absorbance rate was measured at 517 nm using IMPLEN NP 80 touch UV-Vis- spectrophotometer. Ascorbic acid was used as standard. The reduction in absorbance and percentage of scavenging at different concentrations of the

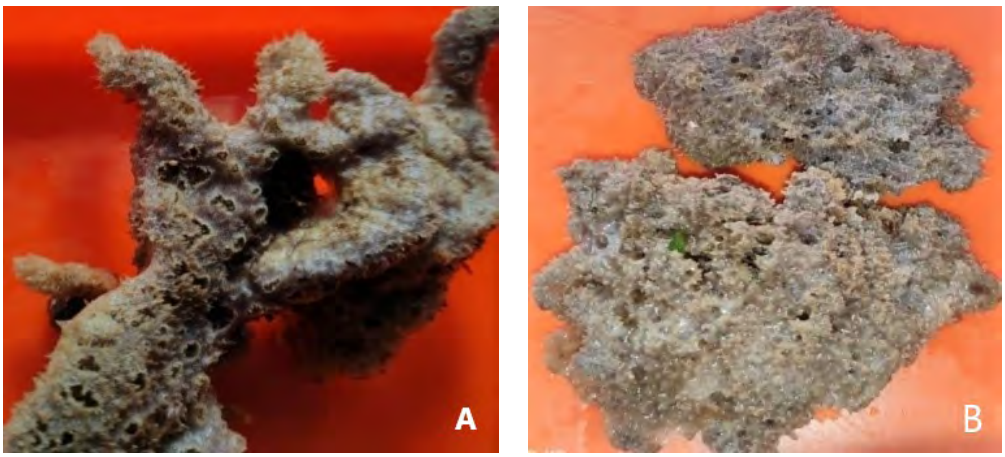

Fig. 1. Morphological characters of *Callyspongia diffusa* (A) and *Dysidea fragilis* (B)

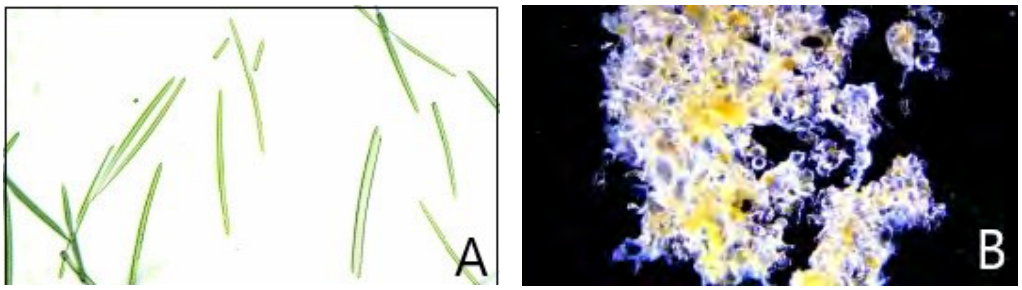

Fig. 2. Spicule morphology of *Callyspongia diffusa* (A) and *Dysidea fragilis* (B)

Table 1. Cultural characters of sponge-associated bacteria

Biochemical tests	WP	DW	DY	TW	WL	Y	WPY
Indole	-	-	-	-	-	-	-
MNM	F/m	F/Nm	F/Nm	F/m	F/Nm	F/Nm	F/m
Citrate	-	+	+	-	-	-	-
TSI	A/A gas	-	K/K gas	A/A gas	K/K gas	K/K gas	A/A gas
Lysine	+	+	+	+	-	-	+
Arginine	-	+	-	-	-	+	+
Urease	-	-	-	+	+	-	-
Gram Stain	G + long *bacilli*	G + short *bacilli*	GPC, clusters, pairs *(micrococci)*	Very large broad *bacilli*	G+ long *bacilli*	GPC (*micrococci*)	G + short *bacilli*
Catalase	-	-	-	-	-	-	-
Oxidase	+	-	-	- (40%)	+	-	+

WP: White puffy; DW: Dark white; DY: Dark yellow; TW: Transparent white; WL: White linear;Y : Yellow; WPY: White perky; F/m: fermentable motile, F/Nm: Fermentable non-motile; A/A: Acid; K/K: Alkaline; MNM: mannitol motility; G: Gram

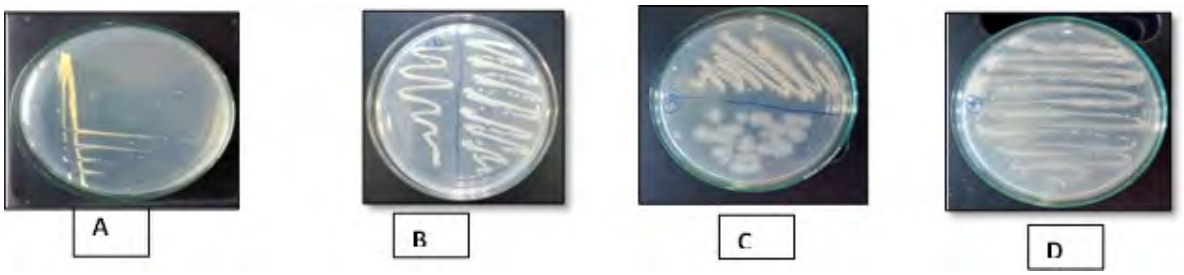

Fig. 3. Bacterial Colonies: A. Yellow colony B. white linear and white perky colonies C. white puffy and dark white colonies D. Transparent White

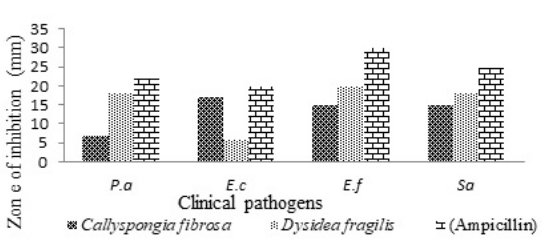

Fig. 4. Zone of inhibition (mm) of sponge extracts against clinical pathogens

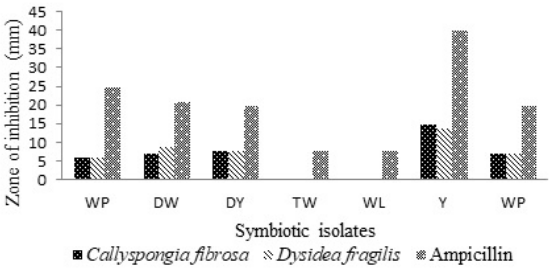

Fig. 5. Zone of inhibition (mm) of sponge extracts against symbiotic isolates

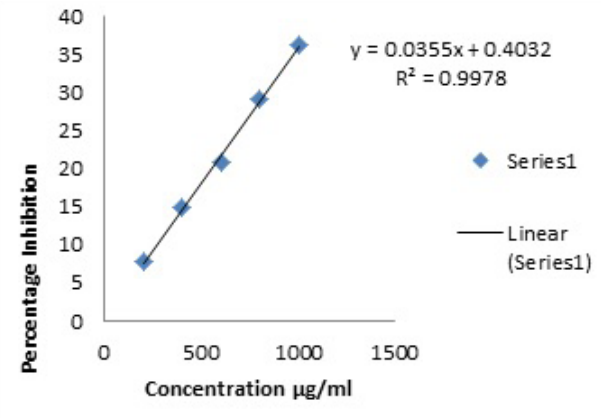

Fig. 6. Antioxidant activity of *Callyspongia fibrosa*

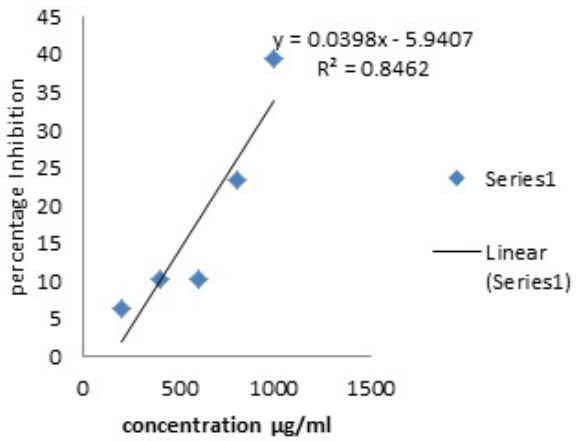

Fig. 7. Antioxidant activity of *Dysidea fragilis*

Table 2. Rf values of TLC solvent system for methanol extract of *Callyspongia fibrosa* and *Dysidea fragilis*

TLC solvent system	*Callyspongia fibrosa*			*Dysidea fragilis*	
	A1	A2	A2	B1	B2
Distance travelled by band(cm)	0.4	9	10.8	0.4	9.5
Total distance travelled by solvent front (cm)	12.2	12.2	12.2 cm	12.7	12.7
R_f value	0.0328	0.7377	0.8852	0.03149	0.748

samples were calculated. The obtained data were expressed as mean±SD (Table 3A and B). The IC_{50} values were 38.64 µg/ml and 199.26µg/ml for *C. fibrosa* and *D. fragilis*. The IC_{50} showed that *Callyspongia fibrosa* has low $IC_{50,}$ indicating higher antioxidant activity (Fig. 6 & 7).

Thin layer chromatography was carried out using chloroform and methanol (7:3) as the mobile phase. Completed chromatograms were then visualised in long UV (366nm). The extracts of *C. fibrosa* produced three bands (A1, A2, and A3) in the TLC plate coated with silica at distances 0.4 cm, 9 cm, and 10.8 cm, respectively (Fig 5A). The solvent front travelled 12.2 cm. Rf value of each of the bands was then calculated. Rf value was obtained as 0.0328, 0.7377 and 0.8852 for the bands A1, A2, and A3 (Fig 8). While considering *D. fragilis,* it produced only two bands at 0.4cm and 9.5cm and the solvent front was found to be 12.7cm.

The FT-IR analysis of crude methanol extracts of the sponges, *Callyspongia fibrosa* and *Dysidea fragilis* showed nearly 11 and 12 peaks, respectively (Fig.8). The peaks produced by *C. fibrosa* at 3350.33 cm^{-1}, 1624.50 cm^{-1}, 1091.41 cm^{-1}, 1413.03 cm^{-1} and 1331.32 cm^{-1} were considered. Frequencies having 3350.33 cm^{-1} displayed O-H stretching (alcohol), 1624.50 cm^{-1} reported C=C stretchings, 1091.41 cm^{-1} exhibited C-O stretching (1°alcohol), 1413.03 cm^{-1}showed O-H bending, and 1331.32 cm^{-1}displayed C-H bending. In the case of *D. fragilis* frequencies 3361.11 exhibited OH stretching, which indicates the presence of alcohol; peak at 1631.31 reported a C=C stretching and a CH bending was noted at 1331.32. The peak at 1095.67 reported a C-O stretching, indicating the presence of primary alcohol.

4. Discussion

Marine habitats have excellent potential for the untapped reservoir of novel drug leads enriched with the unique structure of complex lead innovative compounds that could be used against various infectious diseases (Santavy *et al*., 1990). The present study discusses marine sponges'

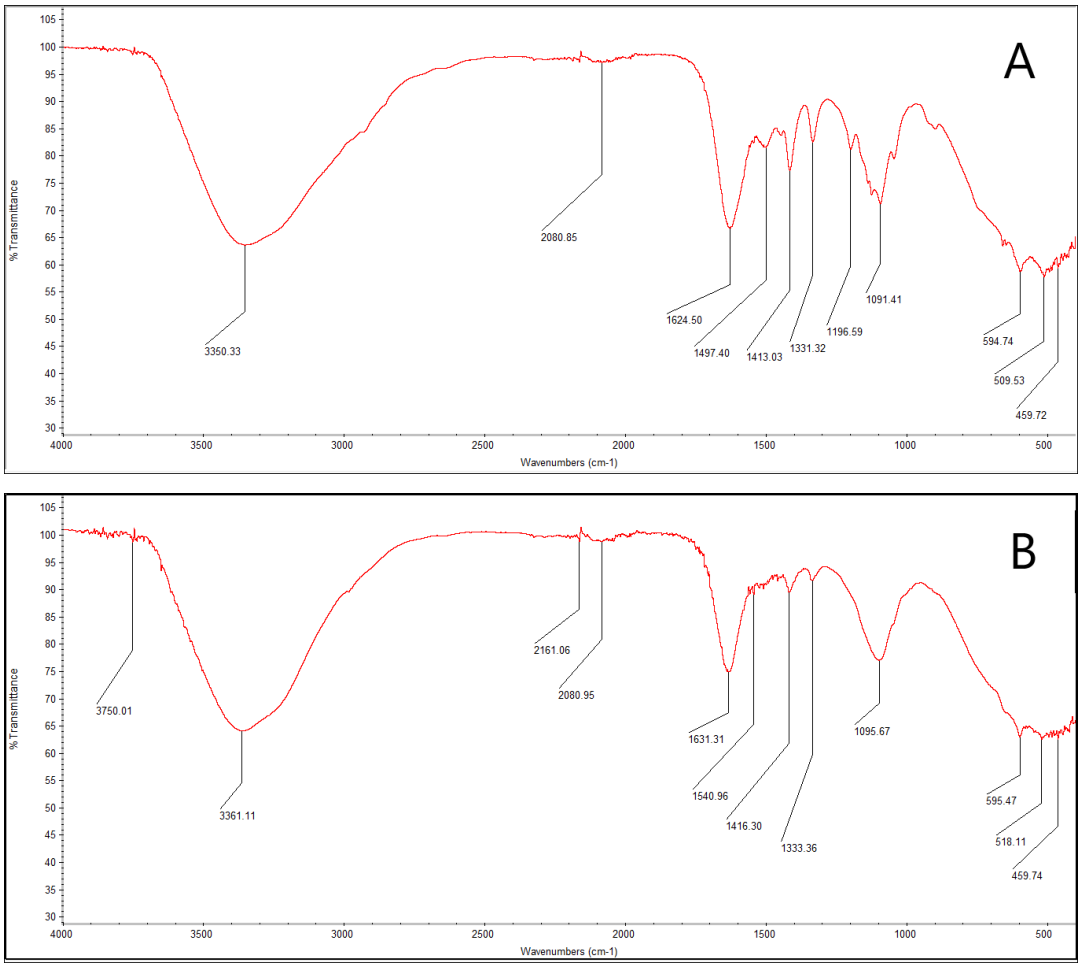

Fig. 2. FT-IR of *Callyspongia fibrosa* (A) and *Dysidea fragilis* (B)

bioactive potential and the associated symbiotic bacteria.

Combining spicules in the skeleton is an important feature for classifying sponges. Based on the morphological features and the spicules' nature, one sponge specimen was identified as *Callyspongia fibrosa* (Wilkinson, 1978). In the case of calcarans, spicules were absent and observed that the skeleton is composed of spongin fibres making the consistency very elastic and identified as *Dysidea fragilis* (Thomas *et al*., 2010).

The biopotential activity of sponge extracts was compared against multidrug-resistant human and symbiotic strains. The results indicated that marine sponges' antimicrobial potential towards *Staphylococcus aureus* and *Pseudomonas aeruginosa* exhibited higher inhibitory potentials. Marine sponges offer habitat for many other organisms. The symbiotic microorganisms associated with the sponges account for 60% of the sponge biomass (Velho-Pereira and Furtado *et al.*, 2012). Regular water pumping through the water canal system is believed to keep the sponges' interior continuously oxygenated (Boopathy *et al*., 2009). However, facultative anaerobic bacteria also were isolated from sponges (Vijayanand *et al*., 2017)

Studies also reported that sponge-associated microbes are a source of bioactive metabolites with novel, valuable, functional, and potential therapeutic applications (Gebhardt, 2002). The sponges' health, nutrition, and chemical defense depend on these microbes . In the present study, seven strains of symbiotic bacteria were identified from these sponges: six Gram +ve *bacillus* species and one *micrococcus* bacteria. Previous studies reported Gram positive and Gram-negative bacteria in sponges belonging to the genus *Callyspongia*. Marine *Bacillus* species associated with sponges have significant antimicrobial properties, similar to terrestrial *Bacillus* strains (Anand *et al*., 2006). *Bacillus* sp. (SEB32) from host sponge *Dysidea fragilis* displayed a broad range of antibacterial activity (Romanenko *et al*., 2008) *Micrococcus* strains isolated from sponges also have evident antimicrobial activity (Mohan *et al*., 2016). Many bioactive agents extracted from sponges that have undergone clinical and pre-clinical development are believed to be synthesized by some marine microbes (Proksch *et al*., 2002). Thus, the isolation and analysis of symbiotic bacteria from marine sponges are of great significance.

The antioxidant is the first line of defence against free radical damage and it is regarded as critical for maintaining optimum health and well-being for many disorders like cancer, atherosclerosis, diabetes, neurodegenerative disorders, and ageing. These compounds inhibit oxidation at low concentrations and act as free radical scavengers by converting reactive oxygen species to less reactive species. The antioxidant properties can be classified into weak, intermediate, and strong based on IC_{50} values. The IC_{50} value ranging from 50-100µg/ml would be intermediate and those having greater than 100µg/ml are considered weak antioxidants (Abdelmohsen, 2010). The IC_{50} value of the methanolic extract of *Callyspongia fibrosa* was calculated as 38.64 µg/ml compared to ascorbic acid (7.93 µg/ml) and found to possess potent antioxidant activity. The methanolic extract from *Dysidea fragilis,* having IC_{50} value of 199.26 µg/ml showed a weak radical scavenging property.

Studies showed that sponges belonging to Demosponges possess antibacterial activity (Mandal *et al*., 2009). In the current analysis, the antibacterial activity of methanol extracts from sponges was tested against symbiotic bacteria associated with them and against pathogenic bacterial strains. Thus, the antibiotic assay revealed that these sponges possess significant antibiotic properties.

FTIR analysis and thin-layer chromatography of the crude extract were conducted, and the results indicated the presence of specific biologically essential components. FTIR analysis designated that alcohols and alkenes were present in the extract. Thin-layer chromatography also indicated the presence of two components with Rf factors 0.3149 and 0.7480 in *Dysidea fragilis* and *Callyspongia fibrosa* produced three bands with Rf 0.0328, 0.7377 and 0.8852, respectively. Previous studies showed that around 300 compounds, including terpenoids (Gautam and Gautam, 2021), steroids (Bindu *et al*., 2018) and peptides (Gunasekera *et al*., 1996) were extracted from *Dysidea* sp. This study shows that sponges are essential for producing diverse therapeutic bioactive compounds (Patil, 1997). This is an initial step towards developing novel drugs of marine origin, mainly from marine sponges.

5. Conclusion

The current study demonstrated the bioactive capabilities of the marine sponges *Callyspongia fibrosa* and *Dysidea fragilis*. Additional research is required to determine the precise constituents accountable for their biological activity. Marine organisms are highly susceptible to even minor alterations in their surroundings. Consequently, sponge species collected from different regions may exhibit varying levels of bioactivity. Additionally, climatic changes and human activities can impact the partnership between sponges and microbes. Therefore, doing additional research using extracts from sponges found in various places and analyzing their biological properties will result in the discovery of promising medicinal chemicals. The process of fractionation and purification will yield chemicals that have the potential to be developed into new and innovative medication candidates in the future. Overall, the sponges *Callyspongia fibrosa* and *Dysidea fragilis* possess a microbial fauna that displays significant traits relevant to industrial uses. Therefore, it is important to conduct similar investigations in scientific study and to continue them, especially in the case of these species.

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6. References

Abdelmohsen, U.R., Pimentel-Elardo, S.M., Hanora, A., Radwan, M., Abou-El-Ela, S.H., Ahmed, S., and Hentschel, U. 2010. Isolation, phylogenetic analysis and anti-infective activity screening of marine sponge-associated Actinomycetes. *Mar. Drugs*, 8: 399-412.

Anand, T.P., Bhat, A.W., Shouche, Y.S., Roy, U., Siddharth, J., and Sarma, S.P. 2006. Antimicrobial activity of marine bacteria associated with sponges from the Waters off the coast of South East India. *Microbiol. Res.,* 161: 252-262.

Ancheeva, E., El-Neketi, M., Song, W., Lin, W., Daletos, G., Ebrahim, W. and Proksch, P. 2017. Structurally unprecedented metabolites from marine sponges. *Curr.Org. Chem*., 21: 426-449.

Balakrishnan, D., Bibiana, A.S., Vijayakumar, A., Santhosh, R.S., Dhevendran, K. and Nithyanand, P. 2015. Antioxidant Activity of Bacteria Associated with the Marine Sponge *Tedania anhelans*, Indian J Microbiol., 55: 13-18.

Baquero, F. M. J. 2008. Antibiotics and antibiotic resistance in water environments.

Current Opinion in Biotechnology, 19(3): 260-5.Bartikova, H., Hanusova, V., Skalova, L., Ambroz, M. and Bousova, I. 2014. Antioxidant, pro-oxidant and other biological activities of sesquiterpenes. Curr Top Med Chem., 14: 2478-2494.

Bauer, A.W., Kirby, M. Scheris J.C. and Turuk, M. 1996. Antibiotic susceptibility testing by a standardized single disc method, *American J of Cli. P.*, 45: 493-496.

Bindu, D., Vinoth Kumar, T. and Geetharamani, D. 2018. Bioprospecting of marine sponge (*Callyspongia diffusa*) for antibacterial compound. *Asian J Pharm Clin Res*., 11(1): 150-153.

Blunt, J.W., Copp, B.R., Munro, M.H., Northcote, P.T. and Prinsep, M.R. 2005. Marine natural products. *Nat. Prod. Rep.,* 22(1): 15-61.

Boopathy, S., Ajith Kumar, T. T. and Kathiresan, K. 2009. Isolation of symbiotic bacteria and bioactive potential from the marine sponge, *Callyspongia diffusa. Indian Journal of biot.,* 8: 272-275.

Gandhimathi, R., Arunkumar, M., Selvin, J., Thangavelu, T., Sivaramakrishnan, S., Kiran, G. S., Shanmughapriya, S. and Natarajaseenivasan, K. 2008. Antimicrobial potential of sponge associated marine actinomycetes. *J of Medical Mycology.*, 18(1):16-22.

Gautam, V.Z. and Gautam, V. 2021. Antibacterial and pesticidal activity of marine sponges *Sigmadocia fibulata* (Schimdt) and *Suberites carnosus* (Johnston) collected from West Coast of Mumbai. *Saudi J Med Pharm Sci.*, 7(1): 32-38.

Gebhardt, K., Schimana, J., Müller, J., Fiedler, H.P., Kallenborn, H.G., Holzenkämpfer, M., Krastel, P., Zeeck, A., Vater, J., Höltzel, A., and Schmid, D.G., 2002. Screening for Biologically active metabolites with endosymbiotic bacilli isolated from Arthropods. *FEMS Microbiol. Lett*., 217 (2): 199-205.

Gunasekera, S.P., McCarthy, P.J., Kelly-Borges, M., Lobkovsky, E. and Clardy, J. 1996. Dysidiolide: a novel protein phosphatase inhibitor from the Caribbean sponge Dysidea etheria de Laubenfels. *J Amer Chem Soc.*, 118(36): 8759-8760.

Hatano, T., Edamatsu, R. and Hiramatsu, M. 1989. Effects of the interaction of tannins with co-existing substances. VI. Effects of tannins and related polyphenols on superoxide anion radical and on 1,1- diphenyl-2-picrylhydrazyl radical. *Chem Pharm Bull*., 37: 2016-2021.

Hooper, J.N.A. and Van Soest, R.W.M. 2002. Systema Porifera: A Guide to the Classification of Sponges. Kluwer Academic/Plenum Publishers, New York, USA. 1706 p.

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

Mandal, S., Yadav, S., Yadav, S. and Nema, R.K. 2009. Antioxidants: A review. *J Chem Pharm Res*., 1(1): 102-104.

Mehbub, M.F., Lei, J., Franco, C. and Zhang, W. 2014. Marine sponge derived natural products between 2001 and 2010: Trends and opportunities for discovery of bioactives. *Mar. Drugs.*, 12(8): 4539-4577.

Mohan, G., Thangappanpillai, A.K.T. and Ramasamy, B. 2016. Antimicrobial activities of secondary metabolites and phylogenetic study of sponge endosymbiotic bacteria, *Bacillus* sp. At Agatti Island, Lakshadweep Archipelago. *Biotech. Reports.*, 11: 44-52.

Montagu G. 1814. An Essay on Sponges, with Descriptions of all the Species that have been discovered on the Coast of Great Britain. Memoirs of the Wernerian Natural History Society, 2(1):67-122.

Patil, A.D., Freyer, A.J., Killmer, L., Offen, P., Carte, B., Jurewicz, A.J. and Johnson, R.K. 1997. Frondosins, five new sesquiterpene hydroquinone derivatives with novel skeletons from the sponge *Dysidea frondosa*: Inhibitors of interleukin-8 receptors. *Tetrahedron*., 53(14): 5047-5060.

Perdicaris, S., Vlachogianni, T. and Valavanidis, A. 2013. Bioactive natural substances from marine sponges: new developments and prospects for future pharmaceuticals. *Nat. Prod. Chem. Res*., 1(3): 3-8.

Proksch, P., Edrada, R.A. and Ebel, R. 2002. Drugs from the sea current Status and microbiological implications. *Appl. Microbiol. Biotechnol*., 59: 125-134.

Ridley, S. O. and Dendy, A. 1986. Preliminary report on the Monaxonida collected by H.M.S. Challenger. Part I. *Annals and Magazine of Natural History.* 18: 325-351.

Roch, P., Hubert, F., Van Der Knaap, W. and Noël, T. 1996. Present knowledge on the molecular basis of cytotoxicity, antibacterial activity and stress response in marine bivalves. *Italian Journal of Zoology*, 63: 311-316.

Romanenko, L.A., Uchino, M., Kalinovskaya, N.I. and Mikhailov, V.V. 2008. Isolation, Phylogenetic analysis and screening of marine mollusc-associated bacteria for Antimicrobial, hemolytic and surface activities. *Microbiol. Res*., 163: 633-644.

Santavy, D.L., Willenz, P. and Colwell, R.R. 1990. Phenotypic study of bacteria Associated with the Caribbean sclerosponge Ceratoporella Nicholson. *Appl. Environ. Microbiol.,* 56, 1750-1762.

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

Selvin, J., Thangavelu, T., Kiran, G.S., Gandhimathi, R. and Priya, S.S. 2009. Culturable heterotrophic bacteria from the marine sponge *Dendrilla nigra*: isolation & phylogenetic diversity of action bacteria. *Helgoland Marine Res.*, 63: 239-247.

Sivaleela, G. 2014. Marine Sponges of Gulf of Mannar and Palk Bay. Records of the Zoological Survey of India, 114(4): 607-622.

Taylor, M.W., Radax, R., Steger, D. and Wagner, M. 2007. Sponge-associated microorganisms: evolution, ecology, and biotechnological potential. *Microbiol. Mol. Biology Rev*., 71(2): 295-347.

Thomas, P.A. 1986. *Demospongiae of the Gulf of Mannar and Palk Bay.* In: Recent Advances in Marine Biology. Today and Tomorrow Printers and Publishers, New Delhi, pp. 205-366.

Thomas, T.R., Kavlekar, D.P. and Loka Bharathi, P.A. 2010. Marine drugs from sponge-microbe association-a review. *Mar. Drugs*., 8: 1417-1468.

Utkina, N.K. 2009. Antioxidant activity of aromatic alkaloids from marine sponges *Aaptosaaptos* and *Hyrtios* sp. *Chem Nat Comp*., 6: 849-853.

Velho-Pereira, S. and Furtado, I. 2012. Antibacterial activity of halophilic bacterial bionts from marine invertebrates of Mandapam, India. *Indian J Pharm Sci*., 74: 331-338.

Vijayanand, B.W., Prasanna, H. and Rajesh, S. 2017. Antimicrobial activity of *Callyspongia diffusa* (marine sponge) associated endophytic bacterial strains. Int. J Pharm Pharm Sci., 9(7): 90-96.

Wilkinson, C.R. 1978. Microbial associations in sponges. II. Numerical analysis of sponge and water bacterial populations. *Mar. Biol*., 49(2): 169-176.