Antimicrobial activity with special reference to antimycobacterial activity of the coral, *Junceella delicata* (Grasshoff, 1999) collected from Madh island, West coast of Mumbai, India

Meenakshi Prakash Borate  
Department of Zoology, S.S. & L.S. Patkar College of Arts & Science, and V. P. Varde College of Commerce & Economics, Goregaon West, Mumbai-400104 (Maharashtra), India  
G.V. Zodape*  
Department of Zoology, S.S. & L.S. Patkar College of Arts & Science, and V. P. Varde College of Commerce & Economics, Goregaon West, Mumbai-400104 (Maharashtra), India

*Corresponding author. Email: drgaumvz5@gmail.com

How to Cite

Abstract
The world now needs new antimicrobial drugs because many infections are resistant to existing treatments. Many microbial infections pose a significant global health challenge and a critical need for new, effective treatment. Soft corals are being explored for their potential as sources of new antimicrobial drugs. The present study aimed to determine the antimicrobial, antifungal, and antimycobacterial properties of the crude extract of coral *Junceella delicata*, collected from Madh island, Mumbai. The crude extract was prepared by adding an equal volume of methanol: dichloromethane (1:1) for 24 hours in the water bath at 45°C. The sample was filtered through Whatman filter paper No. 1 and was subjected to concentrate in a rotary vacuum evaporator at 45°C. Further, antimycobacterial drugs viz. Isoniazid, Ethambutol, Pyrazinamide, Rifampicin, and Streptomycin were used to compare the activity with crude extract of the coral. It was observed that Coral *J. delicata* extract showed a zone of inhibition against all the bacterial strains viz., *Klebsiella pneumonia* (15 mm), *Escherichia coli* (15 mm), *Salmonella typhi* (14 mm), *Pseudomonas aeruginosa* (12 mm), *Streptococcus pyogenes* (11 mm), *Corynebacterium diphtheria* (27 mm), *Staphylococcus aureus* (28 mm), whereas antifungal activity was observed in *Penicillium sp.* (10 mm), *Candida albicans* (14 mm) and no activity was observed in *Aspergillus sp.* The *Mycobacterium tuberculosis* (MTB) strain showed sensitivity at (25µg/ml of coral extract, nearly close to the standard antituberculosis drug pyrazinamide (3.2µg/ml). The above results indicated that the crude extract of *J. delicata* had antibacterial activity nearly against the standard antituberculosis drug Pyrazinamide. The study suggests the crude extract of coral *J. delicata* can be used as an antimicrobial agent to cure different diseases, including tuberculosis.

Keywords: Antibacterial, Antimycobacterial, Antifungal, Coral, Gorgonia, *Junceella delicata*

INTRODUCTION
Antimicrobial-resistant drugs have become a serious issue, and there is a serious urge to search for new antimicrobial drugs from natural sources. Marine organisms produce secondary metabolites to protect against predators and other pathogens and can be a potential source of antimicrobial substances. Marine biology is intriguing and offers immense potential for drug development. Since the 1960s, there has been an increasing demand for medications to treat novel diseases and eradicate resistant microbial strains (Hana et al., 2016). This has sparked an interest in finding new natural product sources. In terms of biodiversity, the marine environment is one of the most diverse and complicated ecosystems to be investigated for various reasons (Kumar et al., 2012). Secondary metabolites like steroids, terpenoids, isoprenoids, nonisoprenoids, quinones, brominated chemicals, nitrogen heterocyclics, and nitrogen sulfur heterocyclics are bioactive compounds derived primarily from marine organisms (Datta et al., 2015). Bioactive compounds produced by marine organisms such as poriferans, cnidarians, annelids, arthropods, mollusks, and echinoderms are gaining attention due to their antiviral, antimicrobial, antiprotozoal, antifungal, antihelminthic, and anticancer proper-
ties (Zapata and Amemiya, 2000). Over the past few years, the healthcare and livestock system has steadily increased alarming signals against antibiotics used against different bacterial strains. (Chinemerem et al., 2022). Therefore, it is necessary to find new antimicrobial agents from natural sources. Among these marine organisms, gorgonian, commonly known as sea fans, have garnered significant attention due to their intricate structures and symbiotic relationships, making them a potential source of unique bioactive compounds. The marine soft coral genus Xenia is known for its diverse array of 199 terpenes, including 14 sesquiterpenes, 180 diterpenes, and 5 steroids (Ng et al., 2020)

The total length of the coastline in India is approximately 8014 km, including the coastline of two oceanic islands, namely Andaman-Nicobar and Lakshadweep. India has a huge Exclusive Economic Zone of 2,305,143 sq.km. and territorial waters of 55889 sq. km. (Jaiswar and Kulkarni, 2005). The Indian subcontinent offers the potential to pursue marine biotechnology research to discover novel biologically active compounds that can be used in a large spectrum of human diseases and, in this context, bio-resources used for sustainable development. Indian laboratories have focused on bioactive substances from marine animals such as horseshoe crabs, green mussels, sponges and corals to characterise novel molecules. The Department of Biotechnology (DBT) has been promoting marine biotechnology in India for the last one and a half decades. Many sponsored R&D programs on marine biotechnology lead to product and process development of viable technology for commercial production systems. The work done by National Institute of Oceanography, Goa (Tilvi and Naik, 2006) and others involves the bioactivity of compounds collected from the coast of South and Southeast India. The Indian coast has a variety of sensitive ecosystems such as lagoons, sand-dunes, coral reefs, mangroves, seagrass beds and wetlands (Ingole 2005).

MATERIALS AND METHODS

Sample collection
During the low tide, the coral Junceella delicata were collected from Madh Island, Malad West Mumbai, Maharashtra (19° 8’ 47652" N, 72° 47’ 17.8116" E). The debris was removed during collection. The samples were collected by hand picking, washed twice with seawater, rinsed with distilled water and stored in ice cubes until they were transferred to the deep freezer at 8°C at the Patkar-Varde college, Goregaon West, Mumbai.

Identification of coral
Preliminary identification was done by examining the shape and size of the sclerites and by reviewing the literature (Fabricius and Alderslade, 2001). Dr. Swapnaja Mohite, Professor and Head, Department of Fisheries Biology, College of Fisheries, Shirgaon, Ratnagiri, Maharashtra, confirmed the identification.

Preparation of crude extract
The coral sample was removed from the deep fridge, bloated with blotting paper, and dried in the shed for 48 hours. After that, the sample was ground with a blender and then macerated by adding an equal volume of methanol: dichloromethane (1:1) for 24 hours in the water bath at 45°C. The aliquot mixture obtained was filtered through Whatman filter paper No. 1. The homogenate was centrifuged at 10,000 rpm for 15 minutes in a cold centrifuge (Remi centrifuge serial No. VCDX- 5983) at -8°C and supernatant was collected. The aliquot was concentrated in a rotary vacuum evaporator at 45°C. The resultant extract was subjected to a Millipore filter system, dried in a vacuum desiccator, and stored in the refrigerator at – 20°C until further use.

Ethical approval
Ethical approval was sought from the Principal Chief Conservator of Forest, Nagpur (Desk-22(8)/Res/CR-25

Borate, M.P. & Zodape, G. V. / J. Appl. & Nat. Sci. 16(2), 697 - 703 (2024)
Borate, M. P. & Zodage, G. V. / J. Appl. & Nat. Sci. 16(2), 697 - 703 (2024)

(22-23)/1431/(22-23) and final approval was taken from the Maharashtra State Biodiversity Board, Nagpur (MSBB/Desk-5/825/2022-23) for collection of J. delicata samples. The voucher specimen of J. delicata was submitted to the repository at the Zoological Survey of India, Western Regional Office, Pune (ZSI-WRC Misc/18), India.

**Procurement of bacterial and fungal cultures**

The pure cultures of bacteria, K. pneumonia NCIM 5656, E. coli NCIM 2931, S. typhi NCIM 2051, P. aeruginosa NCIM 5210, S. lutea NCIM 2103, S. pyogenes NCIM 2608, C. diptheria MTCC 116, S. aureus NCIM 5345 and fungus i.e. Aspergillus sp. NCIM 596, Penicillium sp. NCIM 1065 and C. albicans NCIM 3471 were collected from the Department of Microbiology, Patkar-Varade College, Gorgaon West, Mumbai. The Mycobacterium tuberculosis (Vaccine strain, H37 RV strain): ATCC No- 27294 was purchased and procured from the Central Research Laboratory, Maratha Mandal’s NGH Institute of Dental Sciences and Research Centre, R.S. No. 47A/2, Bauxite Road, Belgaum, India.

**Antibacterial and antifungal activity**

The antibacterial and antifungal activity was assessed by using Kirby Bauer's disc diffusion method (Hudzicki, 2009) and the antimycobacterial assay was evaluated by using Microplate Alamar Blue Assay (MABA) as proposed by (Lourenço 2007).

**RESULTS AND DISCUSSION**

Gorgonian J. delicata is a soft coral species belonging to the family Elliseliidae. Many researchers have studied the antimicrobial properties against the crude extracts of different species of corals. The comparative assessment of the crude extract of soft corals Sinularia leptoclados, Sarcophyto nrehenbergi, and Dendro nephthya hemprichi showed antibacterial activity. It was found that D. hemprichi extract showed the highest antibacterial activity (El-Sawi et al., 2024). The study carried out by Malle et al. (2023) on gorgonians showed antibacterial activity against four bacteria: Escherichia coli IFO 3301, Salmonella typhimurium IFO 12529, Bacillus subtilis IFO 13719, and Staphylococcus aureus IFO 13276. The effect of six species of Gorgonian, Viminella sp., Ellisella sp., Antipathes sp., Melithaea sp., Astroergoria sp., and Junceella sp. collected from both the zones of Marine Protected Area (MPA) and non-Marine Protected Area (non-MPA) were screened for their antipathogenic potential against urinary tract infections (UTIs). The bacterial isolates exhibited antibacterial activity against at least one UTI pathogens (Sabdono et al., 2022). Five dominant soft corals collected from Red Sea showed varied cytotoxic and antibacterial activities. The crude extract of coral Nephthea eiatensis showed potent cytotoxicity against A549 cell line and antimicrobial activity against S. aureus, while S. hirta exhibited noticeable antimicrobial activities against Salmonella typhimurium and S. aureus (Alian et al., 2021). The organic extract of coral Pocillopora verrucosa found to be most effective against all selected microorganisms except Bacillus subtilius ATCC6633 and Aspergillus flavus, whereas the highest effect was observed on Fusarium solani (Hamed and Hussein, 2020). The calcined corals contain bioactive compounds acting as strong antimicrobial agents against gram-positive and gram-negative bacteria S. aureus and E. coli and the fungus Penicillium sp. (Sadeghi et al., 2020). Several recent studies have shown that bacteria isolated from tunicates, nudibranchs, sponges and soft corals produce promising antibacterial active compounds (Putra et al. 2016; Citeta et al. 2017; Ayuningrum et al. 2019; Kristiana et al. 2019). The bioactive compounds extracted from corals showed great significance in clinical ecology and also have various biological activities such as antitumor, antibacterial, antiviral and antifungal (Khattab et al., 2016).

The potent antibacterial activity was found in the methanolic extract of an Indonesian soft coral Sinularia sp. against Bacillus subtilis, Escherichia coli, Vibrio ettor and Staphylococcus aureus (Putra et al., 2016). The study carried out on bioactive materials extracted using dichloromethane: methanol mixture of soft coral Sarcophyton spp. and Sinularia polydactyla by (Khattab et al., 2016) showed the highest activity (MIC= 30–50g/ml) against two Gram-positive bacteria (Staphylococcus aureus and Bacillus spp.), while the crude extract of soft coral Xenia spp. showed the lowest activity (MIC= 200–250 g/ml) against the isolated marine Gram-positive (Escherichia coli) bacterial strains. Similar results were obtained by Khalesiet al. (2001), who demonstrated that the genus Sinularia produced antibacterial compounds that were stronger than similar compounds obtained from other genera of soft corals. Radjasa et al. (2007) reported the antagonistic properties of bacterial symbionts of the soft coral Sinularia polydactyla against Streptococcus equi and tuberculosis (TB) bacteria. Ibrahim et al. (2012) studied the antibacterial activity of different potentially pathogenic bacteria against the crude extracts of Red Sea soft corals Glaucum, Sarcophyton trocheliophorum, S. polydactyla, Soglosus gardineri, Litophyton arborreum, Lobophyton spp., Xenia spp. and Cladiella pachyclados from Egypt showed appreciable and variable antibacterial activity. In Aspergillus species, no antifungal activity was noted against 10 gorgonian extracts (Gohand Chou, 1998). Various studies have shown that soft corals and scleractinian corals possess antibacterial activity.
Table 1. Effect of crude extract of Junceella delicata showing zone of inhibition against bacterial and fungal strains

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Name of bacteria/fungus</th>
<th>Present/Absent (Antimicrobial property)</th>
<th>Zone of inhibition (Diameter in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Klebsiella pneumonia NCIM 5656</td>
<td>Present</td>
<td>15</td>
</tr>
<tr>
<td>2.</td>
<td>Escherichia coli NCIM 2931</td>
<td>Present</td>
<td>15</td>
</tr>
<tr>
<td>3.</td>
<td>Salmonella typhi NCIM 2051</td>
<td>Present</td>
<td>14</td>
</tr>
<tr>
<td>4.</td>
<td>Pseudomonas aeruginosa NCIM 5210</td>
<td>Present</td>
<td>09</td>
</tr>
<tr>
<td>5.</td>
<td>Sarcina lutea NCIM 2103</td>
<td>Present</td>
<td>15</td>
</tr>
<tr>
<td>6.</td>
<td>Streptococcus pyogenes NCIM 2608</td>
<td>Present</td>
<td>12</td>
</tr>
<tr>
<td>7.</td>
<td>Corynebacterium diphtheriae MTCC 116</td>
<td>Present</td>
<td>27</td>
</tr>
<tr>
<td>8.</td>
<td>Staphylococcus aureus NCIM 5345</td>
<td>Present</td>
<td>28</td>
</tr>
<tr>
<td>9.</td>
<td>Aspergillus sp. NCIM 596</td>
<td>Absent</td>
<td>--</td>
</tr>
<tr>
<td>10.</td>
<td>Penicillium sp. NCIM 1065</td>
<td>Present</td>
<td>10</td>
</tr>
<tr>
<td>11.</td>
<td>Candida albicans NCIM 3471</td>
<td>Present</td>
<td>14</td>
</tr>
</tbody>
</table>

Table 2. Effect of crude extract of Junceelladelicata and standard drugs on Mycobacterium tuberculosis strain H37 RV: ATCC no-7294 using Microplate Alamar Blue Assay (MABA)

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Sample</th>
<th>Concentration (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>1.</td>
<td>Isoniazid</td>
<td>S</td>
</tr>
<tr>
<td>2.</td>
<td>Ethambutol</td>
<td>S</td>
</tr>
<tr>
<td>3.</td>
<td>Pyrazinamide</td>
<td>S</td>
</tr>
<tr>
<td>4.</td>
<td>Rifampicin</td>
<td>S</td>
</tr>
<tr>
<td>5.</td>
<td>Streptomycin</td>
<td>S</td>
</tr>
<tr>
<td>6.</td>
<td>Junceella delicata</td>
<td>S</td>
</tr>
</tbody>
</table>

*S-Sensitive* R- Resistant
coral *J. delicata* had efficacy against the *M. tuberculosis* strain. In the present study, the crude extract of *J. delicata* showed antibacterial activity and antimycobacterial activity near the standard drug pyrazinamide. So, in the future, *J. delicata* may be used as an antimicrobial and an effective antimycobacterial agent.

**Conclusion**

The present study concluded that coral *J. delicata* has strong antibacterial, antifungal, and antimycobacterial properties. It also confirms that the crude extract of *J. delicata* has drug sensitivity against antimycobacterial drugs. Thus, the crude extract of *J. delicata* can be processed further for its structural determination to find the active compound that may have antibacterial, antifungal and antimycobacterial properties. The crude extract of *J. delicata* can be processed further for clinical studies in the pharmaceutical industry to develop new antibacterial, antifungal, and antimycobacterial drugs in the future.

**ACKNOWLEDGEMENTS**

The authors thank Dr. Swapnaja Mohite, Professor and Head, Department of Fisheries Biology, College of Fisheries, Shirgaon, Ratnagiri, for final species identification and confirmation. Authors are also thankful to the Co-ordinator, Department of Microbiology, S.S. and L.S. Patkar College of Arts and Science, and V. P. Varde College of Commerce & Economics, Goregaon West, Mumbai for providing bacterial and fungal strains and Maratha Mandal's Central Research Laboratory, Belgaum-590010, India, for providing the *Mycobacterium tuberculosis* (MTB) strain H37 Rv: ATCC No 27294. Thanks are also due to The Director, Principal Chief Conservator of Forest, Nagpur and The Director, Maharashtra State Biodiversity Board, Nagpur, for permitting the collection of species.

**Conflict of Interest**

The authors declare that they have no conflict of interest.

**REFERENCES**


13. Grasshoff, M. (1999). The shallow water gorganians of New Caledonia and adjacent Islands: (Coelenterata: Octocorallia), Senckenbergische Naturforschende Gesellschaft, Frankfurt am Main, 78(1/2), 1-121


