

Research Article

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

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### 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* (09 mm), *Sarcina lutea* (15 mm), *Streptococcus pyogenes* (12 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, gorgonians, 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 2,305,143sq.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)

Mumbai island city is located off the west coast of India (between longitude 18051' and 19033' N and long 72043' and 73001' E). The Arabian Sea blesses Mumbai with a 100 km long coastline. Mumbai island city is located off the west coast of India (between longitude 18051' and 19033' N and long 72043' and 73001' E). The study by Zodape and others has extensively explored bioactive compounds from marine organisms on and around the marine coast in Mumbai. They studied the jellyfish, sponges, puffer fish, and crabs collected from the marine west coast off Mumbai and their antimicrobial, antifungal, pesticidal, anti-mycobacterium, and biomedical studies. They found that Box Jellyfish *Chiropsoides buitendijki* (Dolnar and Zodape 2023), marine sponge *Suberites carnosus* (Johnston), and

*Sigmadocia fibulata* (Schmidt) (Zodape and Bhadekar 2021; Bhadekar and Zodape 2021a; Bhadekar and Zodape, 2021b; Bhadekar and Zodape 2021c), fish *Tetraodon fluviatilis* (Zodape, 2018), crab *Atergatis integerrimus* (Lamarck) (Zodape, 2014), and *Leptodius exaratus* (Zodape *et al.*, 2008) had antibacterial, antifungal, pesticidal, anti-mycobacterium and biomedical properties. Marine invertebrate diversity is also high in India, and some groups may show a similar trend to that for corals described above. However, there are not enough studies on this group in India (Venkataraman and Wafar, 2005). Therefore, the present study aimed to explore the effect of crude extract of coral *J. delicata* collected from Mumbai coasts to investigate its antibacterial, antifungal and anti-mycobacterium properties.

## 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

(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.diphtheria* 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-Varde College, Goregaon 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 *Ellisellidae*. 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 nehrenbergi*, and *Dendro nephthya hemprichi* showed antibacterial activity. It was found that *D. hemprichi* extract showed the highest antimicrobial 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.*, *Astrogorgia 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 antimicrobial activity against at least one UTI pathogens (Sabdono *et al.*, 2022). Five dominant soft corals collected from Red Sea showed varied cytotoxic and anti-

bacterial activities. The crude extract of coral *Nephthea elatensis* 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 subtilis* 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; Citaet *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 (Khatab *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 eltor* 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 (Khatab *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 *Glaucom*, *Sarcophyton trocheliophorum*, *S. polydactyla*, *Sooglossus gardineri*, *Litophyton arbo-reum*, *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

(Kelman *et al.*, 1998; Harder, 2003; Norrby, 2005). Three hard corals *Favites sp.*, *Galaxea fascicularis* and *G. astreata*, were extracted with water and a combination of dichloromethane and methanol against six pathogenic microbes for their antimicrobial property. The organic extracts showed antimicrobial activity from the Disc diffusion method, while the aqueous extracts showed no activity (Qaralleh *et al.*, 2014). The study on octocorals (Kapustina *et al.*, 2014) *Junceella sp.* showed antibacterial inhibition against *Escherichia coli* but was inactive against *Bacillus megaterium*. Amore specific study was carried out by Kumar *et al.* (2012) and found evidence that the methanolic extract of *Junceella juncea* showed inhibited growth of bacterial strain *Salmonella typhi* followed by *Escherichia coli*, *Vibrio cholerae* and *Shigella flexneri*. Khattab *et al.* (2016) noted that the extract of soft coral *S.polydactyla* has more effective antimicrobial inhibitory activity than other soft corals. Crude extract of 23 corals and sponges showed considerable antibacterial activity isolated from the natural environment (Kelman *et al.*, 2009). The coral *Pocillopora damicornis* releases antibacterials due to mechanical stress (Norrby, 2005). Crude extract of stony corals showed no to weak antimicrobial activity, whereas the extract of soft corals possesses strong antibacterial activity (Kelman *et al.*, 2006). Table1 shows the effect of crude extract of *J.delicata*

(Grasshoff, 1999) on different bacterial and fungal strains using Kirby Bauer's Disc diffusion method (Hudzicki,2009). It was observed that coral *J.delicata* showed a zone of inhibition against all bacterial strains, viz. *K.pneumoniae* (15 mm), *E. coli* (15 mm), *S.typhi* (14 mm), *P. aeruginosa* (09 mm), *S.lutea* (15 mm), *S.pyogenes* (12 mm), *C.diphtheriae* (27 mm), *S.aureus* (28 mm), whereas antifungal activity was observed in *Penicillium sp.* (10 mm), *C. albicans* (14 mm) and no activity was noted in *Aspergillus sp.*. Table 2 and Fig.1 show the effect of crude extract of *J.delicata* on *M. tuberculosis* strain H37 Rv: ATCC No.27294.The results were also compared with the standard drugs using Microplate AlamarBlue Assay (MABA).The sensitivity of standard drugs against *M. tuberculosis* was noted as Isoniazid (1.6 µg/ml), Ethambutol (1.6 µg/ml), Pyrazinamide (3.125µg/ml), Rifampicin (0.8µg/ml), and Streptomycin (0.8µg/ml) respectively. The sensitivity of the extract of *J. delicata* against *M. tuberculosis* was noted as 25µg/ml. The present results confirmed that the crude extract of coral *J.delicata* contained bioactive compounds that showed antibacterial, antifungal and antimycobacterial properties. However, this study showed novelty against *Mycobacterium tuberculosis*. No studies have revealed *antimycobacterial* activity against corals, particularly the studied coral *J.delicata*. The present study showed that the crude extract of

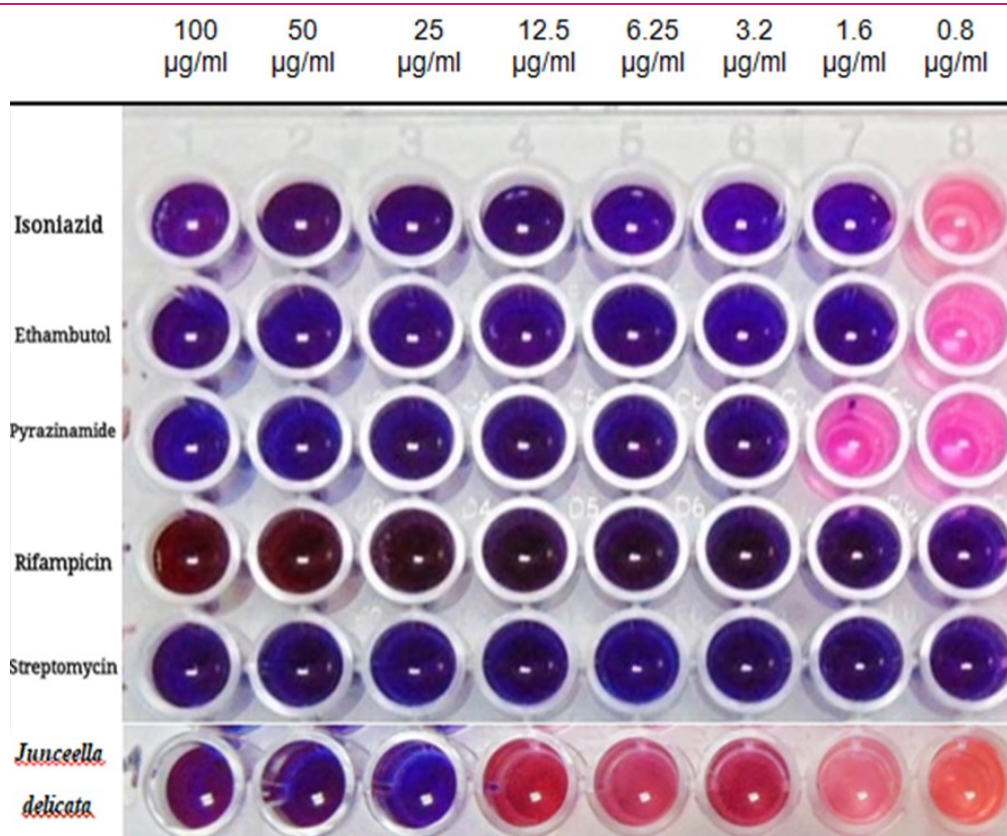
**Table 1.** Effect of crude extract of *Junceella delicata* showing zone of inhibition against bacterial and fungal strains

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

**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)

Sr. No.	Sample	Concentration ( µg/mL)							
		100	50	25	12.5	6.25	3.12	1.6	0.8
1	Isoniazid	S	S	S	S	S	S	S	R
2.	Ethambutol	S	S	S	S	S	S	S	R
3.	Pyrazinamide	S	S	S	S	S	S	R	R
4.	Rifampicin	S	S	S	S	S	S	S	S
5.	Streptomycin	S	S	S	S	S	S	S	S
6.	<i>Junceella delicata</i>	S	S	S	R	R	R	R	R

\*S-Sensitive\*R- Resistant



**Fig.1.** Showing the antimycobacterial effect of standard drugs and crude extract of *Junceella delicata* on *Mycobacterium tuberculosis*

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.

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## Conflict of Interest

The authors declare that they have no conflict of interest.

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