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ORIGINAL ARTICLE

Studies on the Antibacterial, Antifungal and Antitubercular Activities of Bioactive Compounds of Hermit Crab *Clibanarius infraspinatus* (Hilgendorf, 1869) of West Coast of Mumbai

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ABSTRACT
The Hermit crabs of species Clibanarius infraspinatus were collected by hand picking method during low tide from intertidal zone of Alibaug beach, Raigad, West Coast of Mumbai. The crabs were brought to the laboratory. The crabs were washed with ordinary water and the outer shell was removed. The crabs were minced in mortal and pestle and the mixture was heated three times by adding an equal volume of methanol. The solution obtained was centrifuge and the supernatant was processed in a Rotary vacuum evaporator. The semisolid crude extract was used for antibacterial and antifungal activity. The test was carried out on gram-positive and gram-negative bacterial strains – Staphylococcus aureus, MRSA, Streptococcus oralis, Fusobacterium nucleatum, Klebsiella pneumoniae; fungal strains – Candida albicans and Candida tropicalis by minimum inhibitory concentration (MIC) method and Mycobacterium tuberculosis H37RV: ATCC No- 27294 by Microplate Alamar Blue Assay (MABA) method. From the above study it is confirmed that, the crude extract of Clibanarius infraspinatus have antibacterial, antifungal, and anti-tuberculosis property.
Keywords: *Hermit crab, Alibaug beach, Crude extract, antibacterial, antifungal, antitubercular property.*

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INTRODUCTION

Marine world offers an extremely rich resource for novel compounds, but it also represents a great challenge that requires a multidisciplinary approach to bring the marine chemical diversity up to its therapeutic potential. Moreover, numerous marine organisms live in complex habitats exposed to extreme conditions. These challenges allow them to adapt to their new environmental surroundings and produce a variety of secondary metabolites [1]. Bioactive compounds are not only limited to invertebrates but have also been found in larger marine animals. From marine animals like crabs, tunicates, sponges, soft coral, sea hare, nudibranchs, bryozoans, sea slugs & marine fishes many bioactive compounds have been extracted for their medicinal applications [2, 3]. For example, holothurians such as sea cucumbers contain anti-tumor and anti-fungal properties and are useful as cancer chemotherapeutic drugs. Many marine organisms produce different bioactive compounds including sunscreen from corals, certain sponges produce antibiotics, cytotoxic and paralytic compounds (avarols from some sponges are known to inhibit the AIDS virus), anti-coagulants from crinoids, and even the male contraceptive pill from the proboscis worm. Among these organisms, crustaceans have developed a high potential to provide valuable nutritive products [4]. Marine invertebrates including crabs have developed a high innate immune system which includes both humoral and cellular responses in their body to defend against pathogenic organisms [5]. Marine crabs contain several secondary metabolites in their different organs and tissues that have antibacterial, and antifungal activity [6, 7, 8, 9]. Chitin is an important by-product derived from the crab shell which has wide and effective health applications including antibacterial, antifungal, and antiviral agents, and is also very useful in experimental and pharmaceutical research [10].

Another chemical compound found in crab shells is Glucosamine, which is used in the treatment of Osteoarthritis [11]. Recent studies showed that the multi-drug-resistant bacteria and other marine animals living in unsanitary conditions have developed ways of protecting themselves against other pathogenic microorganisms [12]. Many workers have evaluated the biologically active products that are derived from crab shells and different organs and tissues [13, 14]. They have studied the bioactive compounds present in marine organisms and their role in controlling several diseases. The presence of secondary metabolites in marine invertebrates including specific proteins, peptides, and others possess antibacterial, antiparasitic, antiviral, and anticancer activities [15, 16, 17]. In India, many research organizations like the Marine Products Export Development Authority (MPEDA) working on antibiotics in aquaculture, particularly in shrimp farming [18]. National Institute of Oceanography (NIO) in collaboration with Central Drug Research Institute (CDRI), Lucknow, has taken a program on "Development of Potential Drugs from the seas around India". Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Indian Institute of Chemical Technology (IICT), Hyderabad, and ten other laboratories are working on bioactive compounds present in marine organisms [19]. The Ministry of Earth Sciences (MoES), New Delhi, supports this program and under this program, the therapeutic potential of several isolated and identified compounds has been explored and there are hopes of a few of the lead compounds identified reaching the drug stage. Several new compounds from marine origin are now under clinical trials for drug development. 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. The study carried out by Zodape and others has extensively explored the marine coast in and around Mumbai. They studied the Jelly Fish, Sponges, Puffer fish, and crabs collected from the marine west coast of Mumbai, India, and studied its antimicrobial, antifungal, Pesticidal, anti- mycobacterium and biomedical studies. They found that Box Jelly *Fish chiropsoides buitendijki,* marine sponge *Suberites carnosus* (Johnston), and Sigmadocia *fibulata* (Schmidt), fish *Tetraodon Fluviatilis,* crab *Atergatis integerrimus* (Lamark), and *Leptodius exaratus* showed antibacterial, antifungal, Pesticidal, anti-mycobacterium and biomedical properties [20, 21, 22, 23, 24]. Therefore, the present study has been undertaken to explore the effect of crude extract of Hermit crab *Clibanarius infraspinatus* collected from Mumbai coasts to investigate the presence of antibacterial, antifungal, and anti-mycobacterium properties.

MATERIALS AND METHODS

Method of Collection

The samples of Hermit crab *Clibanarius infraspinatus* were collected by hand picking from the intertidal zone of the Alibaug beach, Raigad District, 18.6344°N 72.8642°E; West Coast of Mumbai, and washed twice with seawater and then rinse with distilled water and stored in ice cubes until they were transferred to the deep freezer at 8^0 C at the Department of Zoology, S.S. & L.S. Patkar College of Arts & Science, and V. P. Varde College of Commerce & Economics, Goregaon West, Mumbai-400104.

Identification of Hermit crab

Preliminary identification was done by examining the shape and size and by reviewing the literature. The confirmation of identification was done at the ICAR CMFRI, Versova, Andheri (West), Mumbai 400061.

Extraction of Hermit crab sample

The crabs were removed from the ice cubes and the outer shell was removed. The crabs were minced in mortar and pestle and the mixture was heated three times for 24 hours in a hot water bath at 45^0 C by adding an equal volume of methanol. 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 -8^0 C and the supernatant was collected. The aliquot was concentrated in a rotary vacuum evaporator at 45^0 C. The resultant compound was subjected to a Millipore filter system and finally dried in a vacuum desiccator and stored in the refrigerator at – 20 ^{0} C till further use.

a) Ethical approval

The Ethical approval was sought from the Maharashtra State Biodiversity Board, Nagpur, Maharashtra, for the collection of Hermit crab samples for research purpose (No.: MSBB/Desk-5/Research/807/2022 - 23).

b) Procurement of Bacterial and Fungal Cultures

The pure culture of bacterial and fungal strains was *Staphylococcus aureus, Streptococcus oralis, MRSA, Fusobacterium nucleatum*, *klebsiella pneumonia,* Mycobacterial strain *Mycobacterium tuberculosis* and, fungal strains *Candida albicans* and *Candida tropicalis* were 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-590010, India.

c) Anti-bacterial and anti-fungal activity

The Anti-bacterial and anti-fungal activity were assessed as Minimum Inhibitory Concentration (MIC) using [25], and the anti-mycobacterium assay was performed by using Microplate Alamar Blue Assay (MABA) as proposed by [26].

RESULT AND DISCUSSIONS

TABLE-1: Effect of Crude Extract of Hermit Crab *Clibanarius infraspinatus* and Standard Drugs Against Bacterial And Fungal Strains.

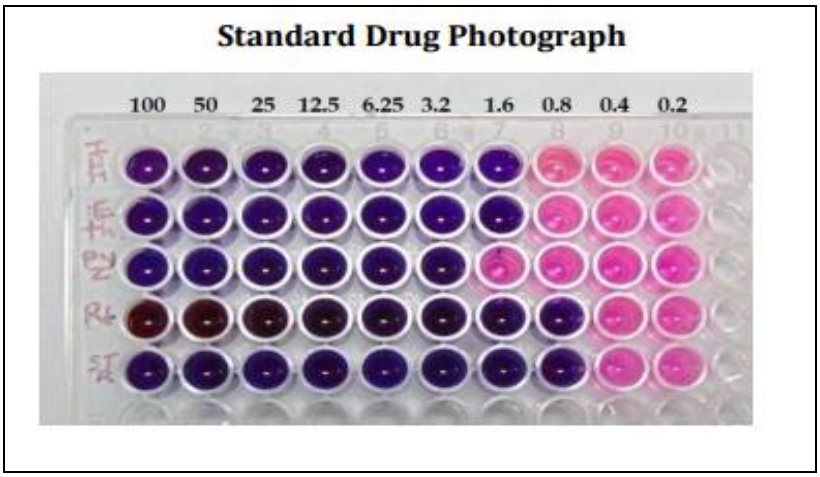
Sr. No.	Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml	0.4 µg/ml	0.2 µg/ml
1.	*S. aureus* + Ciprofloxacin	S	S	S	S	S	S	S	S	S	R
2.	*S. aureus* + crude extract	S	S	S	S	R	R	R	R	R	R
3	*MRSA* + Ciprofloxacin	S	S	S	S	S	S	S	S	R	R
4.	*MRSA* + crude sample	S	S	R	R	R	R	R	R	R	R
5.	*S. oralis*+Ciprofloxacin	S	S	S	S	S	S	S	S	S	R
6.	*S. oralis*+ crude sample	S	S	S	R	R	R	R	R	R	R
7.	*Klebsiella pneumoniae* + Ciprofloxacin	S	S	S	S	S	S	S	S	S	R
8.	*Klebsiella pneumoniae* + crude sample	S	S	R	R	R	R	R	R	R	R
9.	*F. nucleatum*+ Monofloxacin	S	S	S	S	S	S	S	S	S	S
10.	*F. nucleatum*+ crude sample	S	R	R	R	R	R	R	R	R	R
11.	*C. albicans* + Fluconazole	S	S	S	S	S	S	S	S	R	R
12.	*C. albicans* + crude sample	S	S	S	S	S	S	R	R	R	R
13.	*C. tropicalis* + Fluconazole	S	S	S	S	S	S	S	S	R	R
14.	*C. tropicalis* + crude sample	S	S	S	S	S	S	S	R	R	R

*S-Sensitive *R- Resistant

Table-2: Showing Effect of Crude Extract of Hermit Crab *Clibanarius Infraspinatus* And Standard Drugs On *Mycobacterium Tuberculosis* Strain H37 Rv: Atcc No-7294 Using Microplate Alamar Blue Assay (Maba)

Sr. No	Sample	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.12 µg/mL	1.6 µg/mL	0.8 µg/mL	0.4 µg/ml	0.2 µg/ml
1	Isoniazid	S	S	S	S	S	S	S	R	R	R
2.	Ethambutol	S	S	S	S	S	S	S	R	R	R
3.	Pyrazinamide	S	S	S	S	S	S	R	R	R	R
4.	Rifampicin	S	S	S	S	S	S	S	S	R	R
5.	Streptomycin	S	S	S	S	S	S	S	S	R	R
6.	Hermit crab *Clibanarius infraspinatus*	S	S	S	S	S	S	S	R	R	R

*S-Sensitive *R- Resistant



A

B

Photograph 1: Photograph showing, A) Standard drug concentration on *Mycobacterium tuberculosis.* B) Effect of crude extract of hermit crab *Clibanarius infraspinatus* on *Mycobacterium tuberculosis*

The study carried out by [27] on gram-positive and gram-negative bacteria, showed antimicrobial activity. This antibacterial property was possibly observed due to its lipid composition, charge density, and structure of lipopolysaccharides of the cytoplasmic membranes of bacteria. Another study on crab shells showed a wide range of applications in medicine with a primary focus on antimicrobial agents [28]. Crab hemolymph have antimicrobial properties due to the presence of peptides and proteins present on their cell surface [29, 30, 31, 32, 33]. Reports on the effect of antibacterial agents present in crabs against several pathogens were studied by [34, 35, 36, 9]. The study carried out by [7] have successfully investigated the potency of crab shells and hemolymph and their compounds have antimicrobial agent property. The chitosan showed inhibitory effect against bacteria and it acts as a good source of antimicrobial properties. This inhibitory property may be because of its ability to disrupt the barrier properties through the electrostatic interaction between chitosan derivatives and negatively charged bacterial cell surfaces in gram-negative bacteria [37, 38]. The presence of proteins in the granular and semi-granular hemocytes of crabs are found more effective in inhibiting the growth of both gram-positive and gram-negative bacteria exhibiting antibacterial mechanisms [39]. The potent antibacterial and antifungal activities of the invertebrate worm Polychaete, *Perinerei scultrifera*, have been reported by [40, 34]. The antimicrobial activities of crab extract increase with increasing concentration of chitosan [41]. The minimum inhibition concentration value was calculated to be 12.5 µg/ml. The MIC of haemolymph from *Maydelliathelphusa* mud crabs ranged between 6.25 µg/ml and 12.5 µg/ml against *S. pneumoniae* and *K. pneumoniae*, respectively [42]. The present study was undertaken to examine the effect of crude extract of Hermit crab *Clibanarius infraspinatus* to find antibacterial and anti-fungal properties. Table No. 1 shows the effect of crude extract of Hermit crab *Clibanarius infraspinatus* on different bacterial and fungal strains by using Minimum Inhibitory Concentration (MIC) by [25], Table No. 2 and Photograph No.1 and No. 2 showing the anti-mycobacterium assay by using Microplate Alamar Blue Assay (MABA) as proposed by [26]. From Table No.1, The results were compared with the standard drugs showing sensitivity against the bacterial and fungal strains as Ciprofloxacin (0.4 µg/ml), Monofloxacin (0.2 µg/ml) and Fluconazole (0.8 µg/ml) respectively. The (MIC) values of bacterial strains (Sensitivity) were reported as *Staphylococcus aureus*(12.5µg/ml), *MRSA* (50µg/ml), *Streptococcus oralis* (25µg/ml), *Fusobacterium nucleatum* (100µg/ml), *Klebsiella pneumonia* (50µg/ml), and fungal strain *Candida albicans* (3.12µg/ml), *Candida tropicalis*(1.6µg/ml) respectively. Table No. 2 and Photograph No.1 and No. 2 shows the effect of crude extract of Hermit crab *Clibanarius infraspinatus on Mycobacterium tuberculosis* strain H37 Rv: ATCC No- 27294.The results were compared with the standard drugs using microplate alamar blue assay (MABA). Photograph: Photograph No.1 and No. 2 showing the effect of standard drugs

and crude extract of Hermit crab *Clibanarius infraspinatus* on *Mycobacterium tuberculosis* (MTB) strain, H37 Rv: ATCC No- 27294. The sensitivity of standard drugs against *Mycobacterium tuberculo*sis (MTB) strain, H37 Rv: ATCC No- 27294 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 crude extract of Hermit crab *Clibanarius infraspinatus* against *Mycobacterium tuberculosis* (MTB) strain, H37 Rv: ATCC No- 27294 was noted (1.6µg/ml). From the above results, it is confirmed that the crude extract of Hermit crab *Clibanarius infraspinatus* contains bioactive compounds which showed antibacterial, antifungal and anti-mycobacterial property.

CONCLUSION

Many authors have studied the effect of crude extract of whole crab, including different organs like shells, hepatopancreas, and haemolymph of crabs and hermit crabs to study its bioactivity on gram-positive and gram–negative bacterial and different fungal strains. However, no reports are revealed on antitubercular property. From the above results it was found that Hermit crab *Clibanarius infraspinatus* have strong antibacterial, antifungal and anti-mycobacterial property. It also confirms that the crude extract of Hermit crab *Clibanarius infraspinatus* has drug sensitivity against anti-mycobacterial drugs. Thus, the study on Hermit crab *Clibanarius infraspinatus* highlights the significant role that it may be useful for the development of new drugs as it has extraordinary antibacterial, antifungal, and anti-tuberculosis effects, underscoring the potential of these organisms as a rich source of bioactive compounds.

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CONFLICT OF INTEREST

Authors have no conflict of interest

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