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# STUDIES ON THE PESTICIDAL ACTIVITIES OF BIOACTIVE COMPOUNDS OF INTERTIDAL CRAB *ATERGATIS INTEGERRIMUS* (LAMARCK) OF WEST COAST OF MUMBA

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**ABSTRACT:** During low tide the specimens of crab were collected from Colaba (TIFR and NCPA) costal area. After collection, the crabs were transported for acclimatization in glass aquarium (12 x 12 x 18 cm.) containing 25 L Sea water collected from the site to the laboratory of Patkar College Goregaon (west), Mumbai during the monsoon season and acclimatized for 2 days at room temperature before use. After acclimatization, live crabs were selected and whole body, carapace, hepatopancreas and remaining body tissues were dissected out. The dissected parts where weighed and were crushed separately in an equal volume of a mixture of methanol and acetic acid (w/v). Finely crushed crabs were homogenized with a mixture of 80% methanol and 1% acetic acid by heating in water bath at 50°C for half an hrs. The mixture was defatted with dichloromethane and crude extract was processed for evaluation of bioactivity. In case of cockroaches *(Periplaneta Americana)* and rice weevils *(Sitopbilus oryzae)* 100 % mortality was noted after 24 hours of exposure to the crude extracts. The 100 % mortality was observed in fish *Gumbusia affinis* at a concentration of 3.0 ml and 4.0 ml of crude extract of carapace and hepatopancreas whereas, no mortality of the crude extract of crab was observed in whole body, carapace, hepatopancreas and remaining body tissues on mosquito larvae.

**Keywords:** Bioactivity, crude extract, Rice weevil, Cockroach, Fish, Mosquito larvae

## INTRODUCTION :

Marine natural products have attracted the attention of biologists and chemists the world over for the past five decades. As a result of the potential for new drug discovery, marine natural products have attracted scientists from different disciplines, such as organic chemistry, bioorganic chemistry, pharmacology, biology and ecology (Boslow, et al, 1969). The search for new metabolites from marine organisms has resulted in the isolation of more or less 10,000 metabolites (Fuesetani 2000), many of which are endowed with pharmaco dynamic properties. To date approximately 16000 marine natural products have been isolated from marine organisms and reported in approximately 6800 publications. In addition to these, there are approximately another 9000 publications which cover synthesis, reviews, biological activity studies, ecological studies etc (Grein, et al., 1958). From marine toxins that impact public health concerns to the search for new drugs from the sea, the study of biologically active marine natural products has profoundly influenced the course of discovery in fields ranging from pharmacology to cancer medicine (Yasumoto, 1993).

In recent years, a significant number of novel metabolites with potent pharmacological properties have been discovered from the marine organisms. Due to resistance of the pathogens to the existing drugs and also due to the more and more powerful mutants, there exists all the more need to look for compounds with stronger biological activities (Rinehart, et al, 1990). Many bioactive compounds have been extracted from various marine animals like crabs, tunicates, sponge, soft coral, sea hare, nudibranchs, bryozoans, sea slugs & marine fishes (Donia 2003; Halvorson, 1998). For example sea cucumbers contain holothorians which are anti-tumor and anti-fungal and are useful as cancer chemotherapeutic drugs. Other known uses of several marine organisms include production of sunscreen from corals, antibiotics, cytotoxic and paralytic compounds from certain sponges (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. Bioactive compounds are not only limited to invertebrates, but have also been found in larger marine animals.

Medical professionals and the public are eager for new sources of medicines that can address some of our top health issues (Krasil, 1962). Currently, a number of drugs from marine organisms are undergoing clinical trials as anticancer treatments (Gerard, et al., 1999). In addition, scientists have isolated and chemically characterized many unique compounds that have exhibited possible efficacy against fungal infections, Alzheimer's, strokes, tuberculosis, Cystic fibrosis, viral infections, and other diseases. Many bioactive molecules produced by marine invertebrates have exhibited potent anti-viral and anti-tumor activity (Gustafson, et al, 2004). Additionally, while bacterial infections can be treated with antibiotics, relatively few compounds are available to treat viruses, parasites, and fungi, which are responsible for thousands of deaths each year (Hashomoto, 1979). It is envisioned that continued exploration and research will lead to many organisms with unusual structures and unique compounds with medicinal promise.

Review of literature reveals that even the seawater has bactericidal properties. Recently, the Marine Products Export Development Authority (MPEDA) of India has restricted the usage of many antibiotics in aquaculture, particularly in shrimp farming (Dhar, et al, 1992). A program

on "Development of Potential Drugs from the seas around India" is an ongoing project at the National Institute of Oceanography (NIO) in collaboration with Central Drug Research Institute (CDRI), Lucknow, Indian Institute of Chemical Technology (IICT), Hyderabad, Advanced Centre for treatment, research and education in Cancer (ACTREC) and ten other laboratories. The Ministry of Earth Sciences (MoES), New Delhi, supports this program. Under this program, therapeutic potential of several isolated and identified compounds have been explored and there are hopes of few of the lead compounds identified reaching the drug stage. Although there are only a few marine derived products currently in the market, several new compounds from marine origin are now under clinical trials for drug development. While the marine world offers an extremely rich resource for novel compounds, it also represents a great challenge that requires multidisciplinary approach to bring the marine chemical diversity up to its therapeutic potential.

## MATERIALS AND METHODS :

**Method of collection:** During low tide the specimens of crab *Atergatis integerrimus (Lamarck)* were collected from Colaba (TIFR and NCPA) costal area. After collection, the crabs were transported for acclimatization in glass aquarium (12 x 12 x 18 cm.) containing sea water collected from the site to the laboratory of Patkar College Goregaon (west), Mumbai during the monsoon season and acclimatized for 2 days at room temperature before use.

**Identification of fish**: The identification of the crab was done as per Chapgar (1956)

**Preparation of crude extract:** After acclimatization, live crab *Atergatis integerrimus (Lamarck)* were selected and whole body, carapace, hepatopancreas and other body tissues were dissected out. The procedure was followed as per the manual by (Vankateshwaran, 1997). This solid was weighed and dissolved in 1% aqueous Tween- 80 solution such that the concentration of the solution corresponds to 1mg/mL and stored in screw capped vials in a refrigerator at -20$^0$C till further use.

**Collection of the animals for evaluating effects of the extracts:** To assay bioactivity of the isolated extracts, their effect was evaluated on cockroach *Periplaneta americana*, rice weevil *Sitopbilus oryzae,*; fish *Gambusia affinis (girard) and Mosquito larvae* by applying standard bioassay method (APHA. 1999).

## RESULTS AND DISCUSSIONS :

**a) Effect of the crude extracts of crab *Atergatis integerrimus (Lamarck)* on cockroach *Periplaneta americana*.** The experiments were conducted to find the effect of crude extract of whole body, carapace, hepatopancreas and remaining body tissues on cockroach *Periplaneta americana.* Data represented in Table. No 1 show the mortality of cockroach *Periplaneta americana* after exposure to different tissue extracts of crab.

It was found that, mortality of cockroach *Periplaneta americana* was noted 100% mortality was observed after 24 hrs in the crude extract of whole body, carapace, hepatopancreas and remaining body tissues at 0.1 ml concentration. From the above data it was evident that the accumulation and concentration of bioactive compounds was found in all the tissues of crab *Atergatis integerrimus (Lamarck).*

**b) Effect of crude extracts of crab *Atergatis integerrimus (Lamarck)* on Rice weevil *Sitopbilus oryzae:*** Two kinds of experiments were conducted to find the effect of crude extract of whole body, carapace, hepatopancreas and remaining body tissues on rice weevils *Sitopbilus oryzae.* Data represented by coating and by sprinkling method in Table. No. 2 & 3 show the mortality of rice weevils *Sitopbilus oryzae* after exposure to 24 hrs in different tissue extracts of crab.

By coating method it was found that, mortality of rice weevils was noted 100% in mortality was observed after 24 hrs in the crude extract of whole body, carapace, hepatopancreas and remaining body tissues at 0.1 ml concentration.

By sprinkling method the effect of crude extract of whole body, carapace, hepatopancreas and other body tissues of crab *Atergatis integerrimus (Lamarck)* on rice weevils *Sitopbilus oryzae* was found to be similar to that of the coating method.

**c) Protocol for evaluating effect of crab extracts on the fish *Gumbusia affinis:*** Fish *Gumbusia affinis* were collected form the drain at Andheri – Jogeshwari (near railway tract) area of Mumbai and brought to the laboratory for acclimatization. These fishes were transferred to a glass aquarium of size (18 x 12 x12 inches) containing 5 liter of stored tap water. After acclimatization of 24 hrs, the fishes were divided into 5 groups with 10 fishes in each group. These fishes were added in glass aquarium of size (18 x 12 x12 inches) containing 3 liter of stored tap water. To test the effect, the concentration of 0.1 ml to 5 ml the crude extract of whole body, carapace, hepatopancreas and remaining body tissues was added into the experimental aquarium. After adding the extract into the aquarium, behavior and mortality of the fish was recorded till 96 hours. The 100 % mortality was observed in fish *Gumbusia affinis* at a concentration of 3.0 ml and 4.0 ml of crude extract of carapace and hepatopancreas

**d) Protocol for evaluating effect of crab extracts on the Mosquito larvae*:*** Mosquito larvae were collected form the drain at Andheri – Jogeshwari (near railway tract) area of Mumbai and brought to the laboratory for acclimatization. These larvae's were transferred to a glass aquarium of size (14 x 12 x 12 inches) containing 3 liter of stored tap water. After acclimatization of 24 hours, the larvae's were divided into 5 groups with 10 larvae's in each group. To test the effect, the concentration of 0.1 ml to 5 ml of crude extract of whole body, carapace, hepatopancreas and other body tissues of crab *Atergatis integerrimus (Lamarck)* was added into the experimental aquarium. After adding the extract into the aquarium, behavior and mortality of the larvae's were recorded till 96 hours. After 96 hours, no mortality of the crude extract of crab was observed in whole body, carapace, hepatopancreas and remaining body tissues on mosquito larvae.

Zodape Kulkarni (2009) have perform the experiments on Biopotential activity of the extract isolated from intertidal crab Leptodius exaratus from Mumbai Nariman point coast and

was observed that 0.1 mg / ml of the extracts were found sufficient to obtain 100 % mortality within 2 hrs and 24 hrs for rice weevils and cockroaches respectively. 100 % mortality in case of fish with extract of hepatopancreas was observed at 96 hrs at a concentration of 3 to 5 mg/ ml where as there was no effect of the extracts on the mosquito larvae even with 5 mg /ml concentration at 96 hrs. Schooling behavior of the weevils was observed after 15 minutes of exposure. Weevils dispersed there after towards the corners of the petridishes. These weevils could not be moved afterwards and 100% mortality was noticed after 2 hours. Where as the cockroaches treated with extracts showed cleaning behavior at the initial stages of poisoning by rubbing their bodies with their legs or sides of the cages. Mrantic movements of the mouthparts were also observed. At the later stages of poisoning tremors, incoordination and convulsion leading to paralysis were noted. Cockroaches in moribund condition found dead after 24 hours. When the extracts were in direct contact with invertebrates like rice weevil and cockroach, the concederable effect was observed whereas no effect was observed when the extract was not in direct contact with the body of the animals for example mosquito larvae in aquatic medium. In the case of fish, however, remarkable mortality was observed in 96 hrs. This may be because of the respiratory mechanism of the fish which is responsible for the intake of the extract along with the water inside the body through the gills. After adding the extract in aquarium of the fishes, hypersensitivity of the fishes was observed. Increased respiratory rate indicated by rapid operculum movement of the fish was observed. Erratic movement of the fishes was also noted. At the final stages of intoxication the head region of the fishes became red in color. Similarly a red line was also observed along with the lateral line of the fish. The dead fishes was found floating in the aquarium. These results indicate that the extract of the *Leptodius exaratus* contains some toxins in them. These toxins are uniformly distributed throughout the body of the crab instead of concentrating at the particular part of the body. In the further experiments Zodape (2010) presence of bioactive compounds in snail *Achantina fulica* on Cockroaches showed cleaning behavior at the initial stages of poisoning by rubbing their bodies with their legs or sides of the cages. Marentric movements of the mouth parts were also observed and at the later stages of poisoning tremors, incoordination and convulsion leading to paralysis were noted. Cockroaches in moribid condition were found to be dead after 24hrs. The percent mortality of the cockroaches was found to be 100% when the cockroaches were fed with the extract of the *Achantina fulica* and the methanolic solution of the compounds indicating the presence of bioactive compounds in the *Achantina fulica. The studies carried out by* Zodape and Berde (2012) on the Pharmacological Activities of the Extracts of Tetraodon oblongus (Fugu Fish) from Mumbai Coast: The Effect of Tetraodon oblongus on rice weevil Sitopbilus oryzae and cockroach Periplaneta americana was evaluated and It was evident that crude extract and compounds isolated from whole body extract, liver, skin and remaining tissues shows bioactivity by inducing 100% mortality of the rice weevils after 4 hours exposure and cockroaches was inducing 100% mortality afterr 24 hours.

**Table No 1.Effect of crude extract of whole crab, tissue, carapace and hepatopancreas on mortality of *Periplanata americana***

| Sr. No. | Concentration of extract taken (mL) | Whole crab | Remaining body Tissue | Carapace | Hepatopancreas |
|---|---|---|---|---|---|
| | | 24 Hrs. % Mortality | | | |
| 1 | 0.1 | 100 | 100 | 100 | 100 |
| 2 | 0.2 | 100 | 100 | 100 | 100 |
| 3 | 0.3 | 100 | 100 | 100 | 100 |
| 4 | 0.4 | 100 | 100 | 100 | 100 |
| 5 | 0.5 | 100 | 100 | 100 | 100 |
| 6 | 1.0 | 100 | 100 | 100 | 100 |
| 7 | 2.0 | 100 | 100 | 100 | 100 |
| 8 | 3.0 | 100 | 100 | 100 | 100 |
| 9 | 4.0 | 100 | 100 | 100 | 100 |
| 10 | 5.0 | 100 | 100 | 100 | 100 |
| **control** | **0.0** | **00** | **00** | **00** | **00** |

**Table No 2.Effect of crude extract of whole crab, tissue, carapace and hepatopancreas on mortality of Sitophilus oryzae *(*By coating method)**

| Sr. No. | Concentration of extract taken (mL) | 'Whole crab | Tissue | Carapace | Hepatopancreas |
|---|---|---|---|---|---|
| | | 24 Hrs. % Mortality | | | |
| 1 | 0.1 | 100 | 100 | 100 | 100 |
| 2 | 0.2 | 100 | 100 | 100 | 100 |
| 3 | 0.3 | 100 | 100 | 100 | 100 |
| 4 | 0.4 | 100 | 100 | 100 | 100 |
| 5 | 0.5 | 100 | 100 | 100 | 100 |
| 6 | 1.0 | 100 | 100 | 100 | 100 |
| 7 | 2.0 | 100 | 100 | 100 | 100 |
| 8 | 3.0 | 100 | 100 | 100 | 100 |
| 9 | 4.0 | 100 | 100 | 100 | 100 |
| 10 | 5.0 | 100 | 100 | 100 | 100 |
| control | 0.0 | 00 | 00 | 00 | 00 |

**Table No 3.Effect of crude extract of whole crab, tissue, carapace and hepatopancreas on mortality of Sitophilus oryzae *(*By sprinkling method)**

| Sr. No. | Concentration of extract taken (mL) | 'Whole crab | Tissue | Carapace | Hepatopancreas |
|---|---|---|---|---|---|
| | | **24 Hrs. % Mortality** | | | |
| **1** | 0.1 | 100 | 100 | 100 | 100 |
| **2** | 0.2 | 100 | 100 | 100 | 100 |
| **3** | 0.3 | 100 | 100 | 100 | 100 |
| **4** | 0.4 | 100 | 100 | 100 | 100 |
| **5** | 0.5 | 100 | 100 | 100 | 100 |
| **6** | 1.0 | 100 | 100 | 100 | 100 |
| **7** | 2.0 | 100 | 100 | 100 | 100 |
| **8** | 3.0 | 100 | 100 | 100 | 100 |
| **9** | 4.0 | 100 | 100 | 100 | 100 |
| **10** | 5.0 | 100 | 100 | 100 | 100 |
| **control** | 0.0 | 00 | 00 | 00 | 00 |

**Table No 4.Effect of crude extract of whole crab, tissue, carapace and hepatopancreas on mortality of Gumbusia affinis (Girad)**

| Sr. No. | Concentration of extract taken (mL) | 'Whole crab | Tissue | Carapace | Hepatopancreas |
|---|---|---|---|---|---|
| | | 96Hrs. % Mortality | | | |
| 1 | 0.1 | 00 | 30 | 40 | 10 |
| 2 | 0.2 | 20 | 30 | 30 | 30 |
| 3 | 0.3 | 20 | 40 | 40 | 30 |
| 4 | 0.4 | 40 | 30 | 50 | 30 |
| 5 | 0.5 | 50 | 60 | 40 | 40 |
| 6 | 1.0 | 40 | 60 | 50 | 40 |
| 7 | 2.0 | 60 | 80 | 60 | 50 |
| 8 | 3.0 | 60 | 100 | 50 | 90 |
| 9 | 4.0 | 90 | 100 | 60 | 100 |
| 10 | 5.0 | 90 | 100 | 80 | 100 |
| control | 0.0 | 00 | 00 | 00 | 00 |

**Table No 5.Effect of crude extract of whole crab, tissue, carapace and hepatopancreas on mortality of Mosquito larvae**

| Sr. No. | Concentration of extract taken (mL) | 'Whole crab | Tissue | Carapace | Hepatopancreas |
|---|---|---|---|---|---|
| | | 96Hrs. % Mortality | | | |
| 1 | 0.1 | 00 | 00 | 00 | 00 |
| 2 | 0.2 | 00 | 00 | 00 | 00 |
| 3 | 0.3 | 00 | 00 | 00 | 00 |
| 4 | 0.4 | 00 | 00 | 00 | 00 |
| 5 | 0.5 | 00 | 00 | 00 | 00 |
| 6 | 1.0 | 00 | 00 | 00 | 00 |
| 7 | 2.0 | 00 | 00 | 00 | 00 |
| 8 | 3.0 | 00 | 00 | 00 | 00 |
| 9 | 4.0 | 00 | 00 | 00 | 00 |
| 10 | 5.0 | 00 | 00 | 00 | 00 |
| control | 0.0 | 00 | 00 | 00 | 00 |

## CONCLUSION:

In the present investigation along with cockroach, rice weevils, fish and mosquito larvae have been used to test biopotential of the extracts isolated from the crab *Atergatis integerrimus* among the test animals used. Maximum activity of extract was observed cockroaches and rice weevils. Furthermore, bioactivity was also confirmed in extracts isolated from the crab *Atergatis integerrimus* from whole body, carapace, hepatopancreas and remaining body tissues .Therefore, toxin seems to be present in different parts of the crab *Atergatis integerrimus*. In case of cockroaches *(Periplaneta Americana)* and rice weevils *(Sitopbilus oryzae)* 100 % mortality was noted after 24 hours of exposure to the crude extracts. The 100 % mortality was observed in fish *Gumbusia affinis* at a concentration of 3.0 ml and 4.0 ml of crude extract of carapace and hepatopancreas whereas, no mortality of the crude extract of crab was observed in whole body, carapace, hepatopancreas and remaining body tissues on mosquito larvae. The results of present investigation clearly confirm the pesticidal activity of the crude extract isolated from different tissues of the crab *Atergatis integerrimus.*

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