Studies on sea anemones of Goan coast- larvicidal potential of secondary metabolites

A Dissertation for

Course code and Course Title: ZOO-651

Credits: 16

Submitted in partial fulfilment of Master's Degree

MSc Zoology

by

AKSHADA ANAND PRABHUDESAI

22PO440022

Under the Supervision of

DR. PREETI PEREIRA

School of Biological Sciences and Biotechnology

Zoology

GOA UNIVERSITY

April 2024

Examined by:

Seal of the School

DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation report entitled, STUDIES ON SEA ANEMONES OF GOAN COAST -LARVICIDAL POTENTIAL OF SECONDARY METABOLITES is based on the results of investigations carried out by me in the (Zoology Discipline) at the School of Biological Sciences and Biotechnology Goa University under the Supervision of Dr Preeti Pereira and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will be not be responsible for the correctness of observations / experimental or other findings given the dissertation.

Date: 08/04/2024

Place: Goa University

Adesai

Akshada Prabhu Desai

Signature and Name of Student

Seat no:22P0440022

COMPLETION CERTIFICATE

This is to certify that the dissertation report **"Studies on sea anemone of Goan coast – larvicidal potential of secondary metabolites"** is a bonafide work carried out by **MISS AKSHADA ANAND PRABHU DESAI** under my supervision in partial fulfilment of the requirements for the award of the degree of **M.Sc. Zoology** in the Zoology Discipline at the School of biological sciences and biotechnology, Goa University/College.

Dr Preeti Pereira
Signature and Name of Supervising Teacher

Date: 8/4/24

SBSB, M.Sc., Zoology
Taleigão Plateau, Goa 403206
GOA UNIVERSITY

School/dept stamp

Signature of Dean of the School/HoD of Dept

Dean of School of Biological Sciences & Biotechnology Goa University, Goa-403206

Date:

Place: Goa University

CONTENTS

FIGURES

Figure No.	Description	Page no.

ACKNOWLEDGMENT

I extend my sincere gratitude and indebtedness to my dissertation guide Dr Preeti Pereira for her constant guidance, valuable inputs and assurance without whom this project would not have been possible.

I would like to thank Prof. Bernard Rodrigues, Dean of school of biological sciences and biotechnology for giving me an opportunity to carry out my dissertation work.

My deepest gratitude to Dr Nitin Sawant, Programme director of zoology discipline for providing the necessary infrastructural facilities and permissions to proceed with my dissertation work. Thanks to Dr Minaal Desai Shirodkar, Dr Shanti Desai, Ms. Gandhita Kundaikar, Dr Shamshad Shaikh & Dr Avelyno D'Costa for their constant words of encouragement. I would also like to thank the non-teaching staff of the zoology discipline for their timely help in my laboratory requirements.

I extend thanks to head in charge Dr Ajith Kumar Mohanty National Institute of Malarial Research Campal, Panaji, goa for granting me permission to work there for performing bioassay. I would like to thank Mrs. Sushma Bhingi, Miss Samiksha Naik, Miss Vaishnavi Naik and Mr. Abhishek Govekar for their assistance during my study.

I would like to thank my friends and well-wishers for their constant boost and encouragement in times of difficulty.

I take this opportunity to express my utmost gratitude to my parents Mr. Anand Prabhu Desai & Mrs. Aparna Prabhu Desai and my brother Mast Yash Prabhu Desai for accompanying me to the study sites as well as uplifting my spirits.

ABSTRACT

Sea water constitutes more than half of the earth. Marine ecosystem is home to different life forms, ranging from single-celled bacteria to multi-cellular forms. Recently marine environment is of interest due to different compounds found in organisms like poriferans, cnidarians, bacteria which are of pharmaceutical use can be used in treating different diseases. *Anthopleura anjunae* and *Bunodosoma goanense* are the two species of sea anemones identified by squash preparation of tentacles and using morphological characteristics. An extract was prepared using the same by using different solvents like methanol, 70% ethanol and ethyl acetate. Crude extract was assayed against mosquito larvae *Culex, anopheles* and *aedes* checking for mortality after 24 hrs and 48 hrs of exposure period with different doses (250 µl,500 µl, 750 µl, 1000 µl, 1500 µl & 2000 µl) to assess the effectiveness of the extract against mosquito larvae, so that in future it can used to control the population of mosquito at the larval stage. Methanolic crude extract of *Bunodosoma goanense* and *Anthopleura anjunae* showed highest mortality of larvae. Ethanolic extract did not showed any activity against *Culex* larvae. Ethyl acetate crude extract showed highest activity against *Anopheles* larvae both in control and experimental setup.

KEYWORDS

Bioactive compounds, Ecosystem, Larval stage, Pharmaceuticals

1. INTRODUCTION

1.1 BACKGROUND

Marine ecosystems cover more than 70% of the earth's surface. Marine environments are one of the largest sources of bioactive molecules, due to the high degree of biodiversity and the innumerable ecological relationships established between macro and microorganisms found in the different ecosystems of these complex environments (Macedo et al., 2021). Marine organisms are of interest due to their capacity to produce biotechnologically useful compounds (Karthikeyan et al., 2022).

Natural products from marine sources are more effective than the terrestrial sources. Hence, marine environment is proved as unlimited biological resource for the therapeutic drugs production (Janardhan et al., 2018). They are known for antiviral, antibiotic, anticancer, anti-inflammatory, antifungal, anti-obesity, immuno protective activities, anti-bacterial, anti-microbial, larvicidal (Cör Andrejč et al., 2022).

Approximately, 24,662 new bioactive compounds have been isolated from the marine environment in the last 50 years (Blunt et al., 2015). Over 3000 marine natural products have been described from the phylum cnidaria alone most of them in the 21st century (Rocha et al., 2011). The number of new compounds reported from cnidarians in 2013 (281) has increased by 38% over the average for each of the previous 10 years Blunt et al. (2015).

Secondary metabolites are small organic biomolecules, obtained from various marine animals are divided based on their chemical structure, nature of compound and biological activity *viz.*, steroids, terpenoids, isoprenoids, non-isoprenoids, quinones, brominated compounds, nitrogen heterocyclics and nitrogen sulphur heterocyclics (Rinehart et al., 2007; Datta et al., 2015).

The marine environment is known as a rich source of chemical structures with numerous beneficial health effects beyond the nutritional value. Biological processes have several advantages compared to nonbiological processes, including the provision of extracts with high quality and bioactivity, as well as extracts that present low toxicity and environmental impact (Lemes et al., 2022).

Studies are focused on bacteria and fungi isolated from sediments, seawater, fish, algae, and most marine invertebrates such as sponges, corals, sea anemones, molluscs, tunicates, coelenterates, and crustaceans. Sea anemone, one such marine resource, is used in recent years to extract the bioactive compounds (Thangaraj et al., 2018).

Sea anemones are brightly colored organisms, classified under the phylum Cnidaria, class Anthozoa, subclass Hexacorallia and order Actinaria. Cnidaria is a large diverse ecologically important phylum of marine invertebrates which includes corals sea fans sea anemones and jellyfishes. It contains over 11000 species, 7500 of them belonging to the class Anthozoa with 68% of the members belonging to the class. Essentially laminar organisms' two-dimensional epithelial construction has shaped both behavioral and physiological responses and has led to great diversity in sea anemones as evidenced by their presence in all marine habitats, from the intertidal zone to deep sea hydrothermal vents and whale falls (Rodriguez et al., 2010). The distinguishing feature of Cnidarians is nematocysts that are specialized stinging cells that the animals use mainly for capturing prey and protecting themselves from predators (Thangaraj et al., 2019). Sea anemones show symbiotic partnership with the marine ornamental fishes, especially with clowns. Some of these fish species possess considerable resistance to sea anemones but appear to be mainly protected by a mucous coat which prevents discharge of the nematocyst (Mohsenipour et al., 2015). They are distributed in intertidal to deep oceans throughout the world. They live attached to rocks, sea floor, shells and some species burrow in the mud or sand. They are radially symmetric with two layers of cells ectoderm and endoderm, mesoglea a non-cellular matrix is present between the two layers. They have columnar body and have a single body opening, mouth which is surrounded by tentacles. The tentacles can be retracted inside the body cavity or expanded to catch passing prey. They are armed with cnidocytes (stinging cells). However, body shape of the sea anemones is often related to the habitat in which they live. There are over 1,100 recorded species. Sea anemones form a symbiotic partnership with photosynthetic algae based on an exchange of nutrients: the host receives sugar from the algae, the algae receive nutrients and consistent exposure to sunlight from the host (Muller- Parker et al., 2001). Marine ecosystems are marked by hostile conditions for growth and development of microorganisms, plant and animal species (Molinski et al., 2009).

Researchers focusing on bioactive molecules have discovered a wide range of sources of organic compounds from marine ecosystems. Few of these compounds have been reported to have industrial and toxicological applications however most of them have not been extensively studied. Thus, sea anemones have attracted great interest because they produce various biologically active polypeptides and exhibit multifarious properties against harmful diseases (Abinaya et al., 2019).

Recently extraction of biomedical compounds from marine organisms to treat various diseases has been increased due to the improvement in the modern laboratory techniques. There are 13 different species of sea anemones (*Anthopleura anjuna, Anthopleura nigrescens ,Anthopleura panikkarii, Anemonia indica, Bunodosoma goanense, Bunodosoma granuliferum, Cribrinopsis robertii, Metapeachia tropica, Nevadne glauca, Paracondylactis sps, Pelocoetes sps, Phytocoetes gangeticus, Stichodactyla haddoni)* reported from Goan coast by Anushma et al. (2022).

Mosquitos are the most important blood sucking arthropods capable of transmitting pathogens of malaria, dengue, yellow fever, encephalitis, filariasis, etc. Many millions of people all over the world suffer from mosquito transmitted diseases. Synthetic products have created a number of ecological problems, such as the development of insect resistant strains, ecological imbalance and harm to mammals. In recent years mosquito control programs have been suffering from failures because of the ever-increasing insecticide resistance, so using natural products to reduce the hazards to humans and other organisms by minimizing the accumulation of harmful residues in the environment (Maheswaran et al., 2008). Globally in 2022, there were an estimated 249 million malaria cases and 608000 malaria deaths in 85 countries (WHO, 2022).

In view of this mosquito control is an important step towards the prevention of such harmful infections. The selective pressure of conventional insecticides is enhancing resistance of mosquito populations at an alarming rate. There are limitations to mosquito control program due to rising cost of petroleum-based insecticides and also the problem of environmental hazards. In this context, the use of natural products to control mosquito population holds great promise (Thakur et al., 2004).

1.2 OBJECTIVES

- To prepare crude extract from sea anemones using different solvents.
- To study evaluate the larvicidal effect of bioactive compounds extracted from sea anemones on mosquito larvae.

1.3 HYPOTHESES

Bioactive compounds from sea anemone have a larvicidal potential on the mosquito larvae.

1.4 SCOPE

Bioactive compounds can be characterized from crude extract.

Can be used as a mosquito repellant.

2. LITERATURE REVIEW

Sea anemones are a group of marine invertebrates constituting the order Actiniaria. They are named after the Anemone, a terrestrial flowering plant, because of their colourful appearance. Sea anemones are classified in the phylum Cnidaria, class Anthozoa, subclass Hexacorallia. Initially, five species of Actiniaria (Cnidaria: Anthozoa) from the west coast of India were described *viz., Anthopleura anjunae, Bunodosoma goanense, Synantheopsis parulekari, Paracondylactis sinensis* and *Stichodactyla haddoni* (Den Hartog et al., 1993). Taxonomy and distribution of sea anemones (Cnidaria: Actiniaria, Corallimorpharia) from deep water of the Northeastern Pacific was studied by Eash-Loucks et al. (2010). Diversity and distribution of sea anemones in India with special reference to Andaman and Nicobar Islands was carried out by Raghunathan et al. (2014).

Bioactive compounds from marine invertebrates have an immense potential for medicines with examples of cytotoxic and antihistaminic compounds (Riguera, 1997; Datta et al., 2015). The larvicidal potential of two marine organisms Prawn and Sea Cucumber was investigated by testing their non polar petroleum ether extract and polar organic methanol extracts against mosquito *Culex pipiens fatigans* (Thakur et al., 2003). Larvicidal activity of a plant, *Leucas aspera* was studied against the mosquito larvae of *Culex quinquefasciatus* and *Aedes aegypti* by Maheswaran et al. (2008). Larvicidal activity of *Cymbopogon citratus* against larvae of *Anopheles sps.* was studied by (Karunamoorthi et al., 2010)

Drug development from marine natural products Molinski et al. (2009). Marine organisms are a rich source of biologically active peptides Macedo et al. (2021). Promising bioactive compounds from the marine environment and their potential effects on various diseases including anti-oxidant, anti neuro inflammatory, cholinesterase inhibitory activity and neuronal death inhibition and biological activities and neuroprotective effects of marine algae was studied by Karthikeyan et al. (2022).

Deep-sea anemones as a prospective source of antimicrobial, hemolytic, enzyme inhibitory activities and cytotoxic activities of tentacle extracts from five species was studied by Kyetkina et al. (2021).

Biological approaches for extraction of bioactive compounds from agro-industrial by-products due to the provision of extracts with high quality and bioactivity as well as extracts that present low toxicity and environmental impact was studied by (Lemes et al., 2022).

Antiviral and larvicidal properties of novel bioactive compounds produced from marine actinomycetes were tested against the Newcastle disease virus and infectious Brucellosis disease virus and the larvae of *Aedes aegypti, Culex quinquefasciatus* and *Anopheles stephensi* (Janardhan et al., 2018).

The antibacterial and antibiofilm activity of sea anemone against antibiotic-resistant bacteria, also characterization and identification of bioactive metabolites from (*Stichodactyla haddoni*) was studied by Hamayeli et al. (2019). The crude extract of the sea anemone was obtained by methanol solvent.

Marine organisms are novel sources for biologically active compounds which are potentially valuable materials in biomedical research. The bioactive compounds were isolated from the sea anemone *Heteractis aurora* from Mandapam, Southeast coast India (Thangaraj et al., 2019). Additionally, Thangaraj et al. (2011) also evaluated *S. mertensii* and *S. gigantea* for antimicrobial activity against bacterial and fungal pathogens.

Extraction and characterization of sea anemone compounds and its anti-bacterial and hemolytic studies from two species of sea anemones namely, *Heteractis magnifica* and *Stichodactyla haddoni* studied by Kumar et al. (2020).

Assays against *Aedes aegypti* larvae with extracts to study marine natural products in the battle against Dengue, Zika, and Chikungunya Arboviruses isolated from different groups of marine organisms such as bacteria, fungi, seaweeds, and mangrove plants, sponges, crinoids, annelids, corals, anemones, ascidians, zoanthids, fishes, crabs were performed by (Fagundes et al., 2023).

Bioactive compounds of sea anemones are widely used in the field of anti-microbial, anti-tumor, anti-cancer drug discovery research, larvicidal and many such fields (Thangaraj et al., 2018). Sayono et al. (2020) studied Larvicidal Activity of Ethyl Acetate Extract of *Derris elliptica* Root against the Third-Instar Larvae of Cypermethrin-Resistant *Aedes aegypti* Offspring. Additionally, Ramanathan et al. (2022) studied Larvicidal and pupicidal activity of crude ethyl acetate extract fraction-7a of *Cymodocea serrulata* on *Culex quinquefasciatus*.

3.METHODOLOGY

3.1 FOR IDENTIFICATION OF SEA ANEMONE

Sea anemones were collected from the Goan coast from the intertidal regions, and preserved in 70% ethanol. Undischarged cnidae were measured from squash preparations of tissue from tentacles at 100x using a compound light microscope (Den Hartog et al., 1993) and by using the external characters of the organism. the specimens were identified using standardised keys (Raghunathan et al., 2014).

3.2 PREPARATION OF CRUDE EXTRACT

The samples were washed with sterile distilled water to remove adherent sediments and other foreign particles. The samples were chopped into small pieces and weighed. For extraction of biomolecules, sea anemones were freeze-dried. sea anemone immersed into a polar solution with methanol/ethanol/ethyl acetate for 48 h. Then, the obtained extracts were filtrated and concentrated. Each extract passed through Whatman No. 1 filter paper. The filtrates placed into an incubator at 40 ± 1°C for 24 h to remove residual of a solvent. This crude extract transferred into air tight bottles and stored at 4°C till further use. The concentrated extracts were applied for larvicidal activity against different species of mosquito larvae (Hamayeli et al., 2019).

The extract was prepared as follows

S1: Combined Methanolic crude extract of *Bunodosoma goanense* and *Anthopleura anjunae*

S2: Methanolic crude extract of *Bunodosoma goanense*

S3: ethanolic crude extract of *Bunodosoma goanense*

S4: ethyl acetate crude extract of *Bunodosoma goanense*

3.3 EVALUATION OF LARVICIDAL POTENTIAL OF BIOACTIVE COMPOUNDS ON MOSQUITO LARVAE

Plastic containers with wide mouth were used with 100 ml filtered water with 25 numbers of late third instar larvae were exposed to various concentrations in each container. *Culex*, *Anopheles*, and *Aedes* larvae were used for the tests. Different doses were used to check for the mortality. Doses like 250 µl, 500 µl, 750 µl, 1000 µl, 1500 µl, and 2000 µl. The control experiments were also run parallel with each replicate. The larval mortality was calculated after 24 h and 48 hrs of the exposure period. The corrected percent of mortality was calculated by applying following Abbott's formula (Karunamoorthi et al., 2010).

$$\text{Corrected mortality} = \frac{\text{observed mortality (\%) - control mortality (\%)}}{\text{100-control mortality (\%)}} \times 100$$

4. ANALYSIS AND CONCLUSION

4.1 RESULTS

In the present study, solvents of different polarities such as methanol, ethanol and ethyl acetate were used for the preparation of extract from sea anemone to test their activity against mosquito larvae. Crude extracts of *Bunodosoma goanese* and *Anthopleura anjunae* showed activity against mosquito larvae *Culex, Anopheles* and *Aedes* with different doses (250µl, 500 µl, 750 µl, 1000 µl, 1500 µl, 2000 µl).

Methanolic crude extract of *Bunodosoma goanense* and *Anthopleura anjunae* with control and experimental setup showed highest activity of 75% at 48 hours against *Culex* larvae at 2000 µl followed by 40% at 1500 µl, 35% at 1000 µl and 25% at 500 µl.

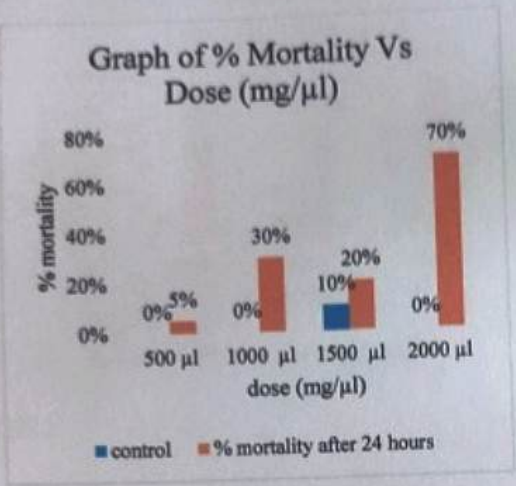
Methanolic crude extract of *Bunodosoma goanense* and *Anthopleura anjunae* with 3 replicates setup showed 51% of mortality after 48 hours against *Culex* larvae at 750 µl followed by 35% & 48 % at 500 µl and 250 µl.

Methanolic crude extract of *Bunodosoma goanense* and *Anthopleura anjunae* also showed 100% mortality against *Anopheles* & 60% mortality against *Aedes* larvae.

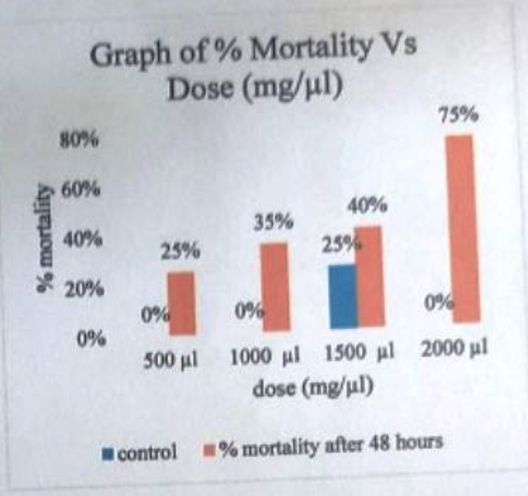
Methanolic crude extract of *Bunodosoma goanense* showed less activity of 30 % at 1500 µl and 15% at 500 µl against culex larvae in control and experimental setup. Whereas the same extract also showed less mortality (21%, 29% and 26%) at 1000 µl, 750 µl and 500 µl doses with 3 replicates setup.

Ethanolic extract did not showed any activity against *Culex* larvae.

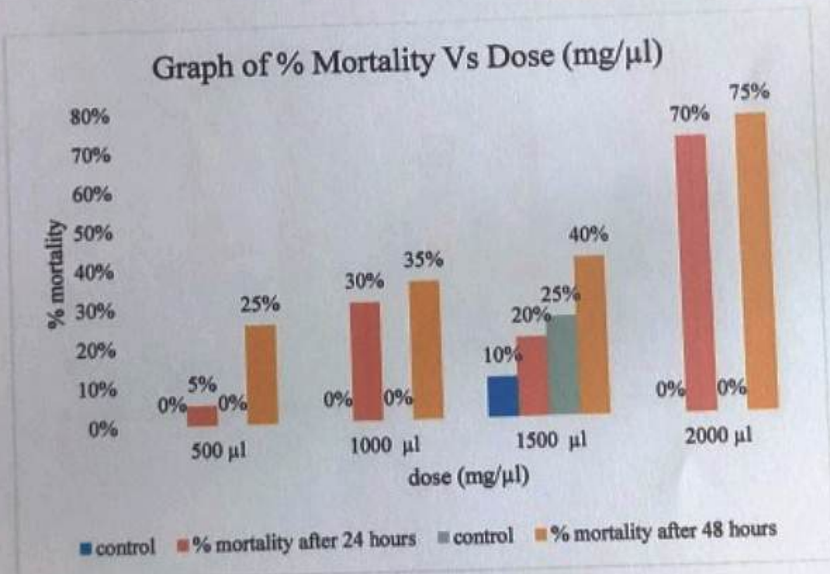
Ethyl acetate crude extract showed highest activity against *Anopheles* larvae both in control and experimental setup.



A



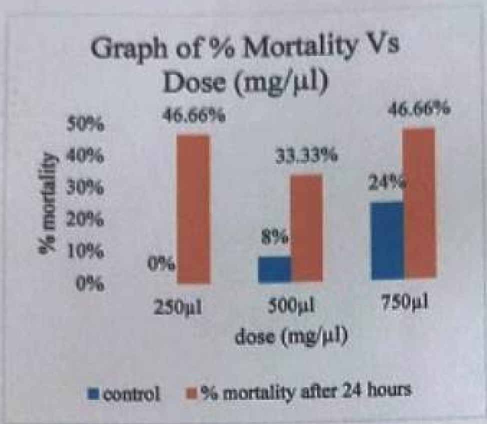
B



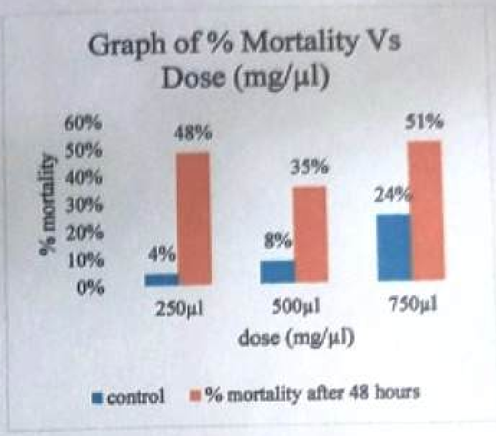
c

Figure 4 .1.1shows the graphs of % moratlity Vs Dose (mg/µl) of S1 extract with control & experimental setup with *Culex* larvae.

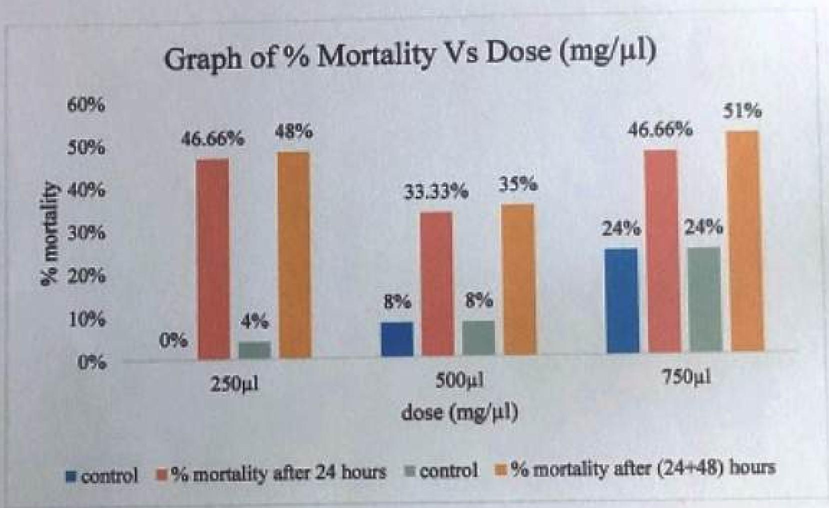
a:after 24 hours, b: after 48 hours, c:after (24+48) hours.



A



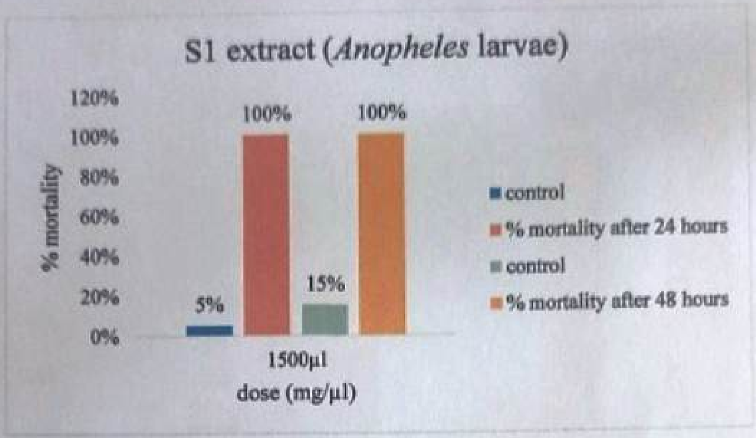
B



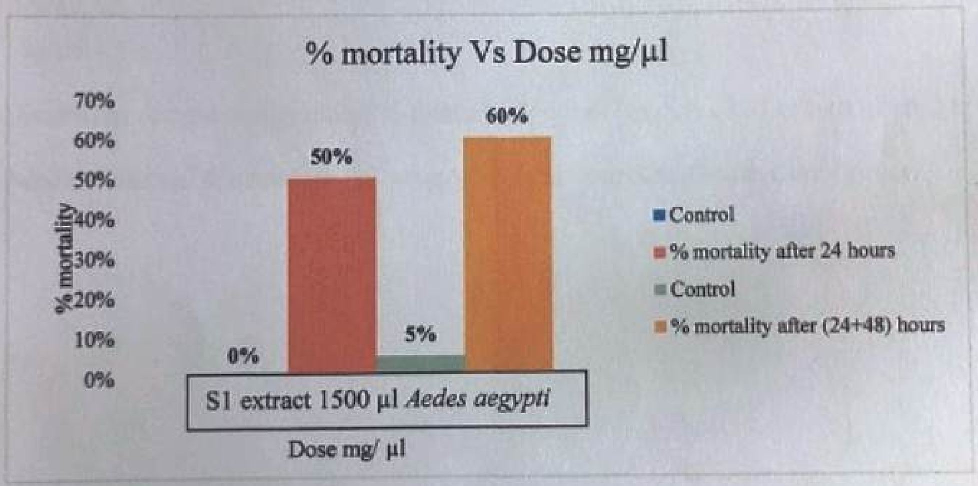
C

figure 4.1.2

shows the graphs of % moratlity Vs Dose (mg/µl) of S1 extract with 3 replicates with *Culex* larvae .a:after 24 hours, b:after 48 hours, c:after(24+48) hours



A



b

figure 4.1.3

shows the combined graph of % mortality Vs dose (mg/µl) of S1 extract of control & experimental setup.

a: *Anopheles* larvae, b: *Aedes* larvae

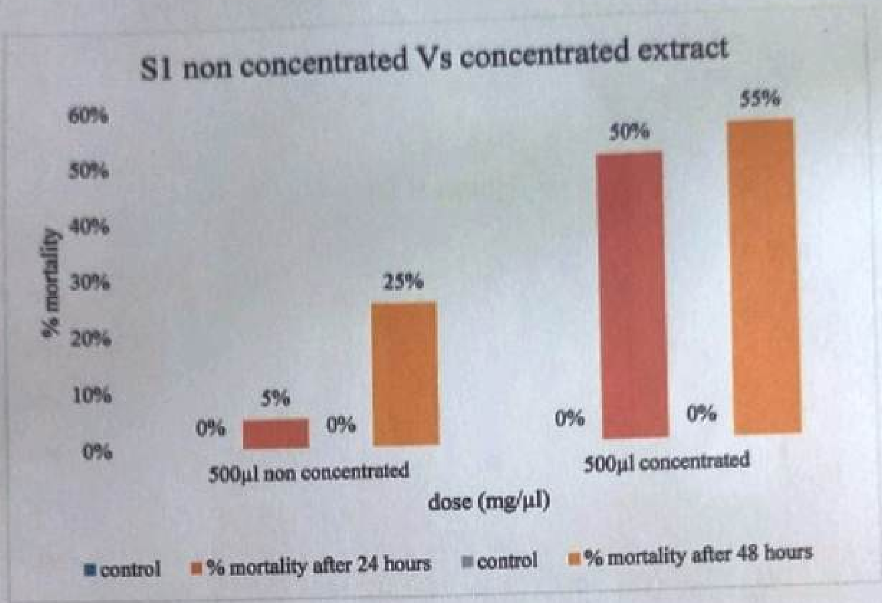


Figure 4.1.4

Shows the comparison graph of % mortality Vs dose (mg/µl) of S1 extract after (24+48) hours of control & experimental setup with same dose 500 µl with *Culex* larvae.

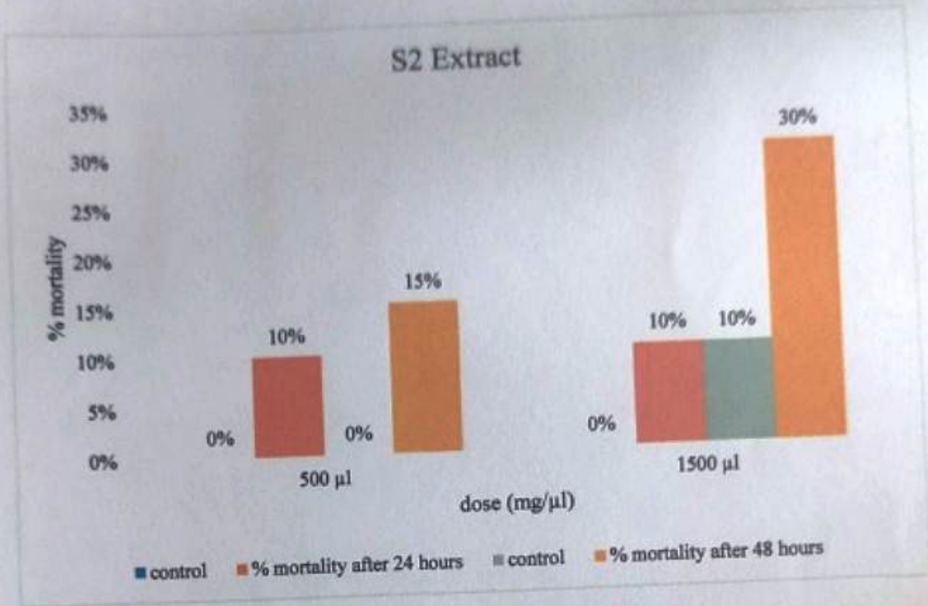
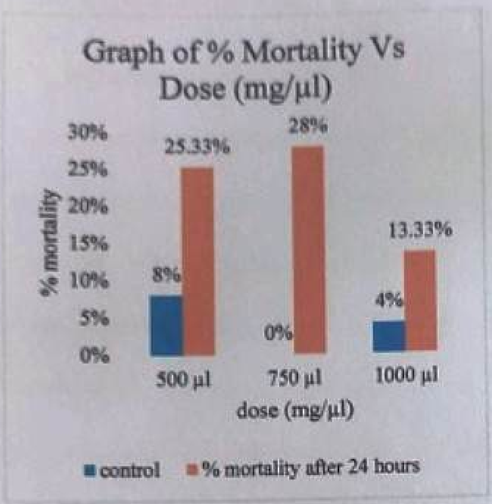
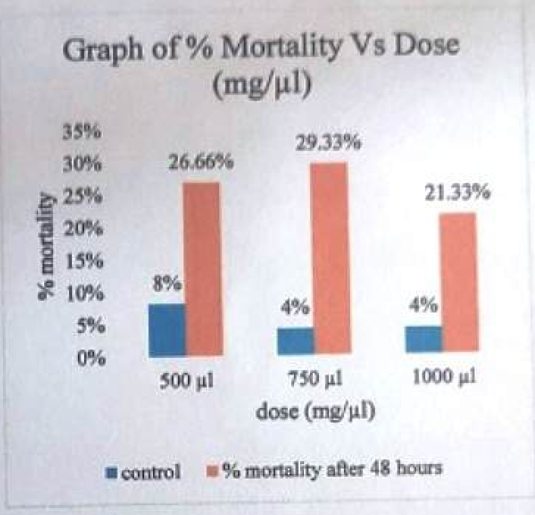


Figure 4.1.5

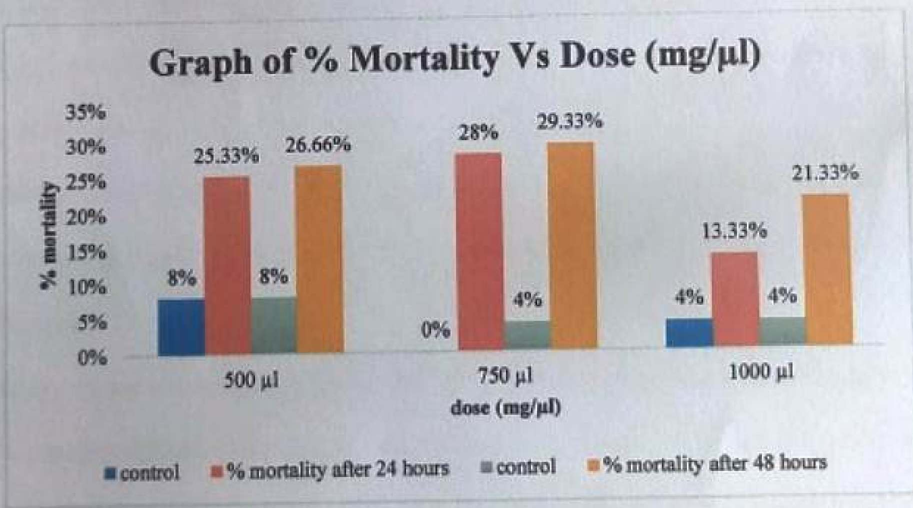
Shows the combined graph of % mortality Vs dose (mg/µl) of S2 extract after (24+48) hours of control & experimental setup with *Culex* larvae.



a



B



c

Figure 4.1.6

shows the graphs of % mortality Vs dose (mg/µl) of S2 extract 3 replicates with *Culex* larvae.

a: after 24 hours, b: after48 hours, c: after (24+48) hours.

4.2 DISCUSSION

The objective of the current study was to screen larvicidal potential of crude extract of sea anemone against mosquito larvae. Currently, studies on the activity of natural products from marine organisms against mosquito larvae has been receiving immense concern due to the fact that increasing cases of malaria, dengue are affecting thousands of lives. Marine environments are one of the largest sources of bioactive molecules, due to the high degree of biodiversity and the innumerable ecological relationships established between macro and microorganisms found in the different ecosystems of these complex environments (Macedo et al., 2021).

Methanolic crude extract of *Bunodosoma goanense* and *Anthopleura anjunae* with control and experimental setup showed highest activity at 48 hours against *Culex* larvae at 2000 µl followed by 1500 µl, 1000 µl and 500 µl.

Methanolic crude extract of *Bunodosoma goanense* and *Anthopleura anjunae* with 3 replicates setup showed 51% of mortality after 48 hours against *Culex* larvae followed by 750 µl, 500 µl and 250 µl.

Methanolic crude extract of *Bunodosoma goanense* & *Anthopleura anjunae* also showed 100% mortality against *Anopheles* & 60% mortality against *Aedes* larvae. Mortality of larvae increased directly proportional to the increase of extract concentration Sayono et al. (2020).

Methanolic crude extract of *Bunodosoma goanense* showed less activity 1500 µl and 500 µl against culex larvae in control and experimental setup. Whereas the same extract also showed less activity of 1000 µl, 750 µl and 500 µl doses with 3 replicates setup.

Hence, extract prepared using methanol with 2 species (*Bunodosoma goanense & Anthopleura anjunae)* is more lethal to the larvae than the extract prepared using single species of sea anemone *(Bunodosoma goanense).*

Ethanolic crude extract did not showed any activity against *Culex* larvae. As the solvent ethanol might not be lethal for killing the larvae.

Ethyl acetate crude extract showed highest activity against *Anopheles* larvae both in control and experimental setup. As ethyl acetate itself is toxic to the larvae as it has also killed the larvae from the control setup.

It was noted that the methanolic crude extract of sea anemone has larvicidal activity as compared to the other solvents ethyl acetate and ethanol. The methanolic crude extract of sea anemone showed activity against mosquito larvae at a different concentration. The larvicidal activity may be due to the bioactive metabolites which may destroy the cuticle layer of the larvae, leads to the destroy of the larvae. This type of work was reported by Ababutain et al. (2012).

4.3 CONCLUSION

In the present study, different solvents such as methanol, 70% ethanol and ethyl acetate were used for the preparation of the extract from sea anemone to test their activity against larvicidal potential. Different extracts show different mortality degrees with different doses against the mosquito larvae *Culex, Anopheles* and *Aedes*. Extract prepared using solvent methanol showed mortality of more than 50%. Ethanol used for the preparation of extract did not showed mortality of larvae whereas extract prepared using solvent ethyl acetate showed 100% mortality of larvae. Hence, can be concluded that methanol is a perfect solvent that can be used for preparing the extract that can be assayed against mosquito larvae checking for its effectiveness.

REFERENCES

Ababutain, I. M., Aziz, Z. K. A., & Al-Meshhen, N. A. (2012). Lincomycin Antibiotic Biosynthesis Produced by *Streptomyces* Sp. Isolated from Saudi Arabia soil: I- Taxonomical, antimicrobial and insecticidal studies on the producing organism. *Canadian Journal of Pure and Applied Sciences*, *6*(1), 1739-1748.

Abinaya, M., Rekha, R., Sivakumar, S., Govindarajan, M., Alharbi, N. S., Kadaikunnan, S.,Vaseeharan, B. (2019). Novel and Facile Synthesis of Sea Anemone Adhesive Protein-Coated ZnO Nanoparticles: Antioxidant, Antibiofilm, and Mosquito Larvicidal Activity Against Aedes aegypti. *Journal of Cluster Science*, *30*, 1393-1402.

Anushma, K., Prabhakaran, M. P., & Deepa, K. P. (2022). A Revised Checklist of Sea Anemones (Order: Actiniaria) from the Exclusive Economic Zone (EEZ) of India. *Journal of Aquatic Biology & Fisheries*, *10*(1), 15-26.

Blunt, J. W., Copp, B. R., Keyzers, R. A., Munro, M. H., & Prinsep, M. R. (2015). Marine natural products. *Natural Product Reports*, *32*(2), 116-211.

Cör Andrejč, D., Knez, Ž., & Knez Marevci, M. (2022). Antioxidant, antibacterial, antitumor, antifungal, antiviral, anti-inflammatory, and nevro-protective activity of Ganoderma lucidum: An overview. *Frontiers in pharmacology*, *13*, 934982.

Datta, D., Talapatra, S. N., & Swarnakar, S. (2015). Bioactive compounds from marine invertebrates for potential medicines-an overview. International Letters of Natural Sciences, (07).

Den Hartog, J. C., & Vennam, J. (1993). Some Actiniaria (Cnidaria: Anthozoa) from the west coast of India. *Zoologische mededelingen*, 67(42), 601-637.

Eash-Loucks, W. E. (2010). Taxonomy and distribution of sea anemones (Cnidaria: Actiniaria, Corallimorpharia) from deep water of the Northeastern Pacific (Doctoral dissertation, University of Kansas)

Fagundes, T. S., Vasconcelos, T. R., Santos Junior, F. M. D., Rajsfus, B. F., Allonso, D., Menezes, J.C., Campos, V. R. (2023). Marine Natural Products in the Battle against Dengue, Zika, and Chikungunya Arboviruses. *Journal of the Brazilian Chemical Society*, 34, 485-501.

Hamayeli, H., Hassanshahian, M., & Askari Hesni, M. (2019). The antibacterial and antibiofilm activity of sea anemone (Stichodactyla haddoni) against antibiotic-resistant bacteria and characterization of bioactive metabolites. *International Aquatic Research*, 11, 85-97.

Janardhan, A., Kumar, A. P., Viswanath, B., Gopal, D. S., & Narasimha, G. (2018). Antiviral and larvicidal properties of novel bioactive compounds produced from marine actinomycetes. *Russian Journal of Marine Biology*, *44*, 424-428.

Karthikeyan, A., Joseph, A., & Nair, B. G. (2022). Promising bioactive compounds from the marine environment and their potential effects on various diseases. *Journal of Genetic Engineering and Biotechnology*, *20*(1), 14.

Karunamoorthi, K., & Ilango, K. (2010). Larvicidal activity of Cymbopogon citratus. *European review for medical and pharmacological sciences*, *14*, 57-62.

Kumar, A., Naveen, B., Abhilash, G., & Akila, C. R. (2020). Extraction and characterization of sea anemones compound and its Anti-bacterial and hemolytic studies. *International Journal of Review in Life Sciences*, *10*(4), 93-97.

Kvetkina, A., Kostina, E., Gladkikh, I., Chausova, V., Yurchenko, E., Bakunina, I., ... & Leychenko, E. (2021). Deep-sea anemones are prospective source of new antimicrobial and cytotoxic compounds. *Marine Drugs*, *19*(12), 654.

Lemes, A. C., Egea, M. B., Oliveira Filho, J. G. D., Gautério, G. V., Ribeiro, B. D., & Coelho, M. A. Z. (2022). Biological approaches for extraction of bioactive compounds from agro-industrial by-products: a review. *Frontiers in Bioengineering and Biotechnology*, *9*, 802543.

Macedo, M. W. F. S., Cunha, N. B. D., Carneiro, J. A., Costa, R. A. D., Alencar, S. A. D., Cardoso, M. H., Dias, S. C. (2021). Marine organisms as a rich source of biologically active peptides. *Frontiers in Marine Science*, *8*, 667764.

Maheswaran, R., Sathish, S., & Ignacimuthu, S. (2008). Larvicidal activity of Leucas aspera (Willd.) against the larvae of Culex quinquefasciatus Say. and Aedes aegypti L. *Int J Integr Biol*, *2*(3), 214-217.

Mohsenipour Z, Hassanshahian M (2015). The inhibitory effect of Thymus vulgaris extracts on the planktonic form and biofilm structures of six human pathogenic bacteria. Avicen J Phytomed 5(4):309–317.

Molinski, T. F., Dalisay, D. S., Lievens, S. L., & Saludes, J. P. (2009). Marine natural products. *Nature reviews Drug discovery*, *8*(1), 69-85.

Muller- Parker, G., & Davy, S. K. (2001). Temperate and tropical algal- sea anemone symbioses. *Invertebrate Biology*, *120*(2), 104-123.

Ramanathan, M., Sukumaran, M., Narayanan, M., Devarajan, N., Chinnathambi, A., Alharbi, S. A., ... & Brindhadevi, K. (2022). Larvicidal and pupicidal activity of crude ethyl acetate extract fraction-7a of Cymodocea serrulata on Culex quinquefasciatus. *Process Biochemistry*, *122*, 166-171.

Raghunathan, C., Raghuraman, R., Choudhury, S., & Venkataraman, K. (2014). Diversity and distribution of sea anemones in India with special reference to Andaman and Nicobar Islands. *Records of the Zoological Survey of India*, 269-294.

Riguera, R. (1997). Isolating bioactive compounds from marine organisms. *Journal of Marine Biotechnology*, 5, 187-193.

Rinehart, K. L. (2007, September). Secondary metabolites from marine organisms. In *Ciba Foundation Symposium 171- Secondary Metabolites: their Function and Evolution: Secondary Metabolites: Their Function and Evolution: Ciba Foundation Symposium 171* (pp. 236-254). Chichester, UK: John Wiley & Sons, Ltd.

Rocha, J., Calado, R., & Leal, M. (2015). Marine bioactive compounds from cnidarians. *Springer handbook of marine biotechnology*, 823-849.

Rodríguez, E., Barbeitos, M. S., Brugler, M. R., Crowley, L. M., Grajales, A., Gusmão, L., ... & Daly, M. (2014). Hidden among sea anemones: the first comprehensive phylogenetic reconstruction of the order Actiniaria (Cnidaria, Anthozoa, Hexacorallia) reveals a novel group of hexacorals. *PloS one*, *9*(5), e96998.

Sayono, S., Anwar, R., & Sumanto, D. (2020). Larvicidal Activity of Ethyl Acetate Extract of Derris elliptica Root against the Third-Instar Larvae of Cypermethrin-Resistant Aedes aegypti Offspring. *Journal of Arthropod-Borne Diseases, 14*(4), 391.

Thakur, N. L., Mainkar, S. P., Pandit, R. A., & Indap, M. M. (2004). Mosquito larvicidal potential of some extracts obtained from the marine organisms– prawn and sea cucumber.

Thangaraj S, Bragadeeswaran S, Suganthi K and Kumaran NS. 2011. Antimicrobial properties of sea anemone Stichodactyla mertensii and Stichodactyla gigantea from Mandapam coast of India. Asian Pac J Trop Biomed 1, S43-S46.

Thangaraj, S., Bragadeeswaran, S., & Gokula, V. (2019). Bioactive compounds of sea anemones: A review. *International journal of peptide research and therapeutics*, *25*, 1405-1416.

Thangaraj, S., Bragadeeswaran, S., & Gokula, V. (2018, November 21). Bioactive compounds of sea anemones: A review – International Journal of Peptide Research and therapeutics. Springer. https://link.springer.com/article/10.1007/s10989-018-

9786-6

World Health Organization. (2022). *WHO guidelines for malaria, 3 June 2022* (No. WHO/UCN/GMP/2022.01 Rev. 2). World Health Organization.

LIMITATIONS

- Unavailability of larvae at required time
- Larvae was never of uniform size, some too small and some about to become pupa.
- Temperature fluctuation can be one of the reasons for affecting mortality rate.
- Damage to the larvae while handling, transferring from one bowl to another can also be the reason that might have affected the mortality rate.