LARVICIDAL POTENTIAL OF SECONDARY METABOLITES EXTRACTED FROM MARINE SPONGES

Dissertation report submitted to Goa University in partial fulfillment of the requirement for the degree of Master's of Science in Zoology by

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Larvicidal Potential of Secondary Metabolites Extracted from Marine Sponges

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I hereby declare that the data presented in this Dissertation report entitled, "The Larvicidal Potential of Secondary Metabolites Extracted from Marine Sponges" is based on the results of investigations carried out by me in the Zoology at the School of Biological Sciences and Biotechnology Goa University, under the mentorship of Dr. Preeti Antonetta 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 internship report/work.

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COMPLETION CERTIFICATE

This is to certify that the Dissertation report "The Larvicidal Potential of Secondary Metabolites Extracted from Marine Sponges" is a bonafide work carried out by Ms. Chhaya Ashok Singh under my mentorship in partial fulfilment of the requirements for the award of the degree of Master's Degree in Zoology in the Discipline Zoology at the School of Biological Sciences and Biotechnology, Goa University.

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ABSTRACT

Diseases caused by mosquitoes are of great concern worldwide. The present study is aimed at evaluating the larvicidal properties of 3 different species of sponges for the control of mosquitoes. Larvicidal assays were conducted based on methods developed by World Health Organization. Twenty-five early third instar larvae of *Anopheles* and *Culex* were exposed to three different species of sponge extract at various concentration in the laboratory setup. Mortality counts were performed every 24 and 48 hours during each treatment. Data were analyzed by calculating % mortality rate. Among the three samples extracted from 3 species of sponges, the crude extract (*Callyspongia sp.*3) was found to be the most effective against *Culex* larvae. The bioassays demonstrated that *Callyspongia sp*. 3 species is effective in controlling mosquito larvae in the laboratory environment. The metabolites extracted from these species may prove to be a good and environmentally friendly larvicidal agent that can be used as an alternative method to control the disease.

Keywords: *Anopheles*, Bioactive compounds, *Callyspongia*, *Culex*, Larvicide, Porifera.

CHAPTER 1: INTRODUCTION

1.1 Background

The marine environment is a repository of bioactive components of important pharmaceuticals. Marine organisms often produce bioactive substances for protection, communication, reproduction, and disease, and competition (Rajendran, 2019). These natural compounds form the basis of new molecular products and are considered the most important drugs in different aspects. Sea sponges are harmless plants-like animals that, when young, deposit themselves on rocks, shells or the sea, where they spend their lives. There are estimated 9,063 species of sponges worldwide, which are found throughout tropical, temperate and polar regions (Emmett et al., 2019). Sponges are considered as an important component of pharmaceutical bioactive compounds among other marine sources (Rajendran, 2019).

Sponges are the ancient, multicellular organisms and show relatively little differentiation and tissue coordination. A sponge is a sessile, sedentary, filter-feeding primitive aquatic invertebrate animal which attaches itself to solid surfaces from intertidal zone to depths of 85000m or more, where they can get sufficient food to grow. Marine sponges represent a significant component of benthic communities throughout the world, in terms of both biomass and their potential to influence benthic or pelagic processes. Sponges are among the richest sources of bioactive natural products from marine habitats. Sponges have recently come into the spotlight for two important reasons: (i) they form relationships with many different types of organisms, and (ii) they are the most common secondary biological organisms (Ingole et al., 2019).

Sponges are an important part of coral reefs and have ecological and economic importance. They are the oldest living organisms, and their abundance is related to their adaptation to changes in environmental properties and competing biota (Bergquist, 1978; Muller, 2003).

These are also considered good filter materials, and some can also bioerode and strengthen coral reef structures (Hooper, 2000). A total of 69 Sponges have become an important area of biochemical research due to the presence of new drugs and bioactive secondary metabolites that can lead to treatment of various diseases, including cancer, (Varsha et al., 2020). Some sponges, such as *Aplysina fulva* and *Mycale microsigmatosa*, are able to prevent pollution by marine organisms (Pereira et al., 2002). According to World Porifera Database there are a total of 9,162 Porifera species, out of which 8,904 are marine and 270 are freshwater species. In India there are about 486 species of sponges and majority of them are marine (Soest et al., 2023). Large-scale surveys in currently unexplored areas could reveal many new species that have not been previously reported.

Sponges are filter-feeding organisms that live in various aquatic environments such as oceans, estuaries and freshwater ecosystems. They grow abundantly everywhere under water, such as logs, boulders, stones, boulders, trees or branches, bamboo or poles, large plants, mollusk shells. Sponges are often found in lakes, rivers, reservoirs, ponds, lagoons, streams, lagoons, lakes, caves, abandoned waters, and other residual water and water flow. It is considered an important part of aquatic ecosystems. Due to their early response to any change in the environment, they can serve as biological indicators of water pollution (Rajendran, 2019).

The marine environment may contain over 80% of world's plant and animal species. Approximately 10,000 metabolites have been isolated, many of which have pharmacodynamic properties, (Chakraborty et al., 2019). The rich diversity of marine organisms provides a great resource for the discovery of compounds with useful and biomedical potential, (Kamboj 1999). Despite the large number and diversity of bioactive compounds isolated from terrestrial microorganisms, since the penicillin era, new infectious diseases and resistant pathogens still represent a serious problem for human life (Cragg et al., 1997, Desriac et al., 2013). Therefore, the exploration of new and under explored sources becomes extremely important in finding compounds with interesting bioactivities that can be used as new antibiotics (Penesyan et al., 2011).

Organisms biosynthesize compounds into non-primary or secondary metabolites to protect themselves and maintain environmental homeostasis (Selvin, 2002). Some of these secondary metabolites provide opportunities for the development of effective drugs. The rapid growth in the chemistry of marine organisms over the last 15 years has led to the discovery of a large number of new structures, many of which have no precedence among structures of terrestrial origin and possess previously unknown pharmacological and toxicological properties. Retrospective of research in this field indicated that although a number of diverse biologically active compounds have been isolated from marine organisms, the number of compounds taken-up for the field trial/clinical use is scanty, (Joseph et al., 2004). Therefore, exploration of chemical ecology of secondary metabolites synthesis and development of drugs from sponge-associated microorganisms are becoming a promising venture, (Soniya, 2003). Successful development of drugs from the sea completely relies on the availability of source organism or the organism having same secondary metabolites. Therefore, understanding the habitat, site richness, seasonality and harvest of eco-organisms is important for the development of effective bioactive drugs.

Mosquitoes are the most important group of insects from a public health perspective. Mosquitoes not only cause problems when they bite, but they can also transmit deadly diseases. Of the approximately 4,000 known species of mosquitoes, less than 10% are considered effective vectors of infectious disease pathogens that have significant direct and indirect impacts on human well-being and health. Mosquito-borne diseases remain a leading cause of death worldwide, affecting more than 700 million people each year, (Taubes, 1997). However, no part of the world is free from vector-borne diseases, (Fradin et al., 2002). Mosquitoes transmit a variety of diseases, including Malaria, Dengue fever, Japanese encephalitis, etc. The incidence of dengue fever has quadrupled since 1970, and nearly half of the world's population is now at risk. In 1990, approximately 1.5 billion people lived in areas with a greater than 50% risk of dengue transmission, (Hales et al., 2002). An outbreak of chikungunya virus infection began in the southwestern Indian Ocean islands in 2005 and spread to India, resulting in a sustained outbreak involving more than 1.5 million patients, including travellers to the region (Taubitz et al., 2007).

The overall prevalence and health significance of malaria, lymphatic filariasis, and dengue fever have made them a top priority for global eradication and control programs, (Kyelem et al., 2008).

Effective malaria control in many countries is complicated by inadequate health infrastructure and generally poor socioeconomic conditions. The situation has become more complex over the past 50 years, with increasing resistance to antimalarials used to control infections and the development of insecticide resistance in the *Anopheles* mosquitoes that transmit them (Mangen et al., 2008). According to the information service, two million people are at risk from mosquito-borne diseases, mostly in tropical countries. Although mosquito-borne diseases now cause more health problems in tropical and subtropical climates, no part of the world is immune to this risk and mosquito diseases are becoming increasingly difficult to control.

Insect-borne diseases remain a major cause of illness and death. Mosquitoes are responsible for transmitting many medically important pathogens and parasites. These include viruses, bacteria, protozoa, and nematodes that cause serious diseases such as malaria, dengue fever, yellow fever, chikungunya fever, and onchocerciasis, (Kettle, 1995).

Dengue fever and chikungunya are prevalent across the world, and India is no exception. *Aedes aegypti* is commonly responsible for transmitting dengue fever, yellow fever, and is also a vector for Zika fever. However, dengue fever has become a significant public health problem as the number of reported cases continues to increase, especially for more severe forms of the disease, unusual manifestations such as dengue hemorrhagic fever and dengue shock syndrome, or central nervous system involvement (Pancharoen et al., 2002).

About 249 million people live in Vector borne disease risk around the world, and about 250 million people are infected with the disease every year and estimated to cause 608K deaths globally. The highest incidence of malaria is believed to be in Africa. 233 million cases, which accounted for 94% cases globally. Since the beginning of 2024, over 5 million dengue cases and over 2,000 dengue-related deaths have been reported globally. It is endemic in 109 countries, most of which are located in tropical regions of Africa, Asia, and Latin America. In India around 339K cases and 5K deaths has been reported. (WHO, 2023). Goa being a smallest state accounted for 1,346 cases with no death. (NCVBDC, 2023).

Mortality rates for both humans and livestock lead to serious health and economic problems in developing and underdeveloped countries, where they are a higher priority. The use of large amounts of chemical pesticides can have long-term residual effects on the environment, and the use of bacterial pesticides can have long-term environmental effects. Mosquito control has become difficult due to inconsistent use of pesticides causing damage to the environment. Pesticides are often harmful to humans and animals, and some are difficult to decompose and are toxic. Increased use of pesticides enters food sources and causes damage to the liver, kidneys, etc. can cause harm and also cause genetic changes that may not occur for many generations. The search for herbal preparations that do not produce any adverse effects in the non-target organisms and are easily biodegradable remains a top research issue for scientists associated with alternative vector control (Muhammad, 2022).

Mosquito control chemicals also cause many health problems for humans and non-target animals. With the understanding of the side effects of these drugs, people have started to show interest in these drugs. Biopesticides and herbal preparations are considered environmentally friendly. Nature offers many bioactive products against mosquitoes in the form of plants, microbial products and other biological products. To date, the insecticidal properties of various sponge species have been examined. (Mathivanan et al., 2019) However, research on mosquito control by marine organisms has been limited to sponges in Indian waters, and the larvicidal activity of marine sponges have not been investigated recently. Therefore, this study was designed to test the crude extract of sponge species for their larvicidal activities against mosquito vectors *Culex sp*. and *Anopheles sp.* mosquito.

Bioprospecting: (or biodiversity research) is the search and use of products found to be suitable for production purposes. Products discovered through bioresearch can be divided into: chemical (such as advanced pharmaceuticals, agrochemicals and cosmetics), genetic (such as recombinant pharmaceutical proteins, enzymes and agricultural biotechnology) and design (such as civil and mechanical engineering). Bioprospecting is a valuable way to directly add value to the harvest and use of natural resources such as logging, mining and oil extraction. Individuals often have little value, and it is their property that makes them economically valuable. (Hanssen, 2014).

Natural Products: Primary and Secondary Metabolites All organisms require the biosynthesis of various organic compounds into functional products to survive, grow and reproduce. A natural product is a product produced by living organisms: *i.e.,* animal, plant or microorganisms.

Primary Metabolites: Some natural products represent all organisms and are found (with variation) in all organisms. These include carbohydrates, proteins, fats and nucleic acids. Because of their insignificance and widespread distribution, they are called primary metabolites.

Secondary Metabolites: Other products are not biosynthesized by metabolic processes and have a more limited taxonomic distribution, usually restricted to certain species or genera.

The natural activity of secondary metabolites generally does not affect direct survival of the product and is not required for growth, respiration, storage and growth. Instead, they affect long-term survival by influencing the organism's interaction with its natural environment. Therefore, they are called secondary metabolites. Biosynthesis of secondary metabolites are the result of various environmental reactions. As many external factors are constantly changing, the expression of secondary metabolites also constantly changes with season, stress, nutrient availability, and disease growth. The building blocks of secondary metabolite biosynthesis are derived from primary metabolism. Hanssen, (2014)

1.2 Aim and Objectives

The objective of this study is:

- To extract and prepare crude extracts from the marine sponges.
- To evaluate larvicidal potential of Sponges against different mosquito species.
- To reduce transmission of disease of mosquitoes by targeting the immature stages of mosquito.

1.3 Hypothesis:

The compounds extracted from Sponges could exhibit larvicidal activity due to their diverse array of bioactive compounds. These metabolites may effectively kill Mosquito larvae, potentially providing a natural and environmentally friendly alternative for mosquito control. Investigation of diversity of sponges may have the potential to encounter previously unknown species of sponges in Goa.

1.4 Scope

Importance of the study recognised:

The metabolites can play a crucial role in controlling Mosquito larvae. Finding natural larvicidal agents can aid in developing environmentally friendly and effective mosquito control strategies. Exploring larvicidal potential contributes to our understanding of the chemical ecology of marine organisms. Studying larvicidal potential of secondary metabolites offers Opportunities for discovering novel bioactive compounds with potential pharmaceutical applications. It can also help us appreciate the biodiversity and ecological importance of these organisms, holding promise in disease control, drug discovery, and ecological conservation.

CHAPTER 2: LITERATURE REVIEW

Secondary metabolites refer to a diverse array of organic compounds produced by various marine organisms, such as algae, sponges, corals, and microorganisms, that are not directly involved in the organism's primary metabolic processes like growth, development, or reproduction. These compounds often serve ecological roles such as defence against predators, competition for space, or communication. (Peterson, 2020).

Additionally, they have garnered significant interest due to their potential pharmaceutical, agricultural, and industrial applications, including drug discovery, biotechnology, and environmental protection. Understanding and exploring the chemical diversity of marine secondary metabolites hold promise for uncovering new bioactive compounds with valuable properties for various fields. (Karthikeyan et al., 2022).

Sponges have been recognised as the most promising source of new bioactive products for drug discovery. (Karthikeyan et al., 2022). This has led to many works being carried out on marine natural products isolated from sponges (Rao et al. 2008; Boobathy 2009; Feby and Nair 2010; Mohan et al., 2016; Lekshmi et al., 2020; Baig et al., 2021; Mote et al., 2021). These bioactive compounds have attracted attention for drug discovery and development efforts. Researchers are exploring their potential as leads for developing new pharmaceuticals or as molecular scaffolds for designing synthetic analogs with improved therapeutic properties.

Globally

Marine organisms, like other groups of marine organisms, produce novel compounds with unique structures (Boobathy et al., 2009; Gram et al., 2010). They inhabit different ecological niches, either dormant in the ocean or living in habitat-associated plankton or sediments (Jayanth et al., 2002). Approximately 230 properties of bioactive marine natural products were reported between 2009 and 2011; 102 of these have anti-inflammatory properties (Mayer et al., 2013).

Today, many of the newly developed bioactive drugs, along with antibiotics and anti-inflammatory drugs, have failed in clinical trials due to lack of efficacy to the needs or expectations (Kola et al., 2000; Pollock et al., 2001).

Marine bioactive compounds or Marine natural products (MNPs) offer avenues for developing cost-effective, safe and potent novel drugs and other useful products (Lipton, 2003). MNPs are organic compounds produced by microbes, sponges, seaweeds, and other marine organisms. The host organism biosynthesizes these compounds as non-primary or secondary metabolites to protect themselves and to maintain homeostasis in their environment.

Viruses present in *Brevibacterium* sp., *Moruxella* sp., and *Corynebacterium sp*. from Red Sea marine sediments in the Gulf of Aqaba were found to have biological activity in at least one test. Crude extract contains potent antioxidant and antibacterial activity (Zereini 2014).

Evaluation of the acute larval toxicity and repellent effect of solvent extracts of the big blue octopus on the mosquito, *Culex pipiens* indicated remarkable effects on both acute larval toxicity and repellent activities (Hussein 2018).

Larvicidal potential of sea cucumbers crude extracts on third instar of wild Aedes larvae of mosquito was examined and effective larvicidal activity was observed. (Anielle et al., 2019).

Perez et al. (2020) investigated the use of chitosan derived from crab shell waste as an insecticide against *Aedes aegypti* mosquitoes, and suggested that all acidified chitosan treatments have increasing mortality rate against *Aedes aegypti*.

National

Over the last 25 years, seaweeds, invertebrates and microorganisms have provided important models and compounds that reveal their potential in many fields, especially in new drugs for many diseases. Interest in this field is reflected in the scientific publications, the variety of new models, and the diversity of organisms studied (Faulkner 1996). A review (Bongiorni & Pietra 1996) noted that human health, health food, and cosmetics now account for over 80% of applications, including many different patents filed for seafood in the last 25 years.

Marine sponges have received increasing attention as a source of different processes and potential bioactive secondary metabolites, leading to the discovery of bioactive pharmaceuticals. Although thousands of chemicals obtained from sponges have been published in the literature, only some of them have been described in medicine. Many studies have shown that sponge-derived metabolites can be used as models of bioactive lead compounds for direct therapy or to create more potent and less toxic analogues. Many research groups (Miethke et al., 2021; Muteeb et al., 2023; Yamin et al., 2023; Quinn and Dyson, 2024) are working to identify new antibiotics, and marine organisms represent a large number of bioactive compounds with medicinal uses. Some ecological studies show that it contains many bioactive constituents produced from sponges, which often functions as to protect against environmental threats such as, microbial diseases, predation, competition for space or the growth of harmful bacteria. Sea sponges are objects of interest to chemists due to the number of metabolites they produce, the novelty of the samples encountered and their therapeutic potential. Scientists working and researching in the field of natural chemistry have determined that sponges have the potential to provide medicine for many diseases in the future (Ingole et al., 2019).

larvicidal potential of nonpolar to polar organic methanol extracts from body wall of sea cucumber and shrimps, which contained steroids and saponins, was tested against *Culex sp*. and were found to be effective (Thakur et al*.,* 2004).

Five sponge extracts from Gulf of Mannar showed the presence of significant novel bioactive compounds for both larvicidal (*Aedes aegypti*) and insecticidal assays *(Musca domestica*) (Rao *et al.,* 2008). The antimicrobial, antifungal, and antibacterial properties were observed from marine sponge extract by (Boobathy et al., 2009).

Examination of the secondary metabolites of five species of sponges *(Acanthella elongata, Axinella donnani, Callyspongia,diffusa, Callyspongia subarmigera, and Echinodictyum gorgonoides)* suggested that *A. elongata* extract has the potential for developing novel bioactive larvicidal agent. (Annie & Lipton 2012).

Indumathi et al*. (*2016) evaluated the in vitro antibacterial, antifungal and larvicidal properties of various tissue extracts of puffer fish *Takifugu oblongus*. The larvicidal activity of tissue extracts was evaluated by examining the mortality rate of all tested larvae.

Further study investigated the purpose and mechanism of action of two ethanol compounds 1 and 2 with antibodies isolated and identified from the blood vessels of the *Cribrochalina* sponge. Genetic analysis showed that insulin-like growth factor receptor (IGF-IR) signaling helps regulate the anti-cancer effects of compounds in non-small cell lung cancer minor (NSCLC). (Zovko et al., 2016).

Different mechanisms of antimicrobial activity of marine polysaccharides and their potential for clinical use were also noted. (Wang et al., 2012)

Evaluation of the secondary metabolites of the marine sponge *Haliciona exigua* collected from the Gulf of Mannar showed the presence of antibacterial, antifungal, anti-inflammatory and antibacterial properties (Bhimba et al., 2013)

Clara Grosso et al. (2014) elucidated the potential of marine invertebrate-derived medicine in the field of neuroscience.

Bioactive compounds from sponges have been reported to have various activities, including anti-inflammatory, anti-inflammatory, anti-inflammatory, anti-bacterial, anti-inflammatory pain and antibiotics. (Mehbub et al., 2014) The coasts of the Indian subcontinent are a hotspot for diverse marine floral and animal communities, primarily sponges, sea anemones, sea cucumbers, sea urchins, soft corals and more seagrass species (Sathiyanarayanan et al., 2014).

Additionally, sponge lectins exhibit various biological activities including modulation of the inflammatory response, antibacterial and cytotoxic activity as well as anticancer and neuromodulator activities (Garderes et al. 2015).

Costantini et al. (2015) tested the different effects of a methanol extract of the marine sponge, *Geodia cydium* on human mammary epithelial cells (MCF-10A) and the same human breast cancer cell (MCF-7).

Kamalakkannan (2015) conducted the study on marine sponges a good source of bioactive compounds in anticancer agents and found 87 natural products (marine) showing anti-tumor, anti-inflammatory, neurosuppressive, anti-malarial, antibiotic and cytotoxic, properties in marine sponges.

The methanolic extracts of two sponges, *Sigmadocia carnosa* and *Spongia officinalis var, Ceylonensis* were investigated and the results showed that both sponges contained alkaloids, phenols, steroids, triterpenoids, reducing sugars and aromatic acids, and also showed that both sponges has antibacterial, antifungal, insecticidal and larvicidal and antioxidant properties (Krishnan et al. 2016).

According to Bertrand et al. (1987) approximately 38% of sponges consist of bacteria. Various secondary metabolites have been isolated from sponges associated with antibacterial, antifungal, antibacterial, antiviral, antiviral, HIV protease inhibition, HIV reverse transcriptase inhibition, immunosuppressive and cytotoxic activities.

Though there are enormous works done on the various aspects of sponges worldwide, potential larvicidal activity from marine sponges are very few and unexplored in many parts of the world including India which may produce results that are beneficial for the health and well-being of the human beings.

CHAPTER 3: METHODOLOGY

Sponge collection:

The sponges were collected from Cabo de Rama beach located at the end of Cola village in Canacona-Goa, with 15.08806°N; 73.91991°E. Three species of sponges were collected in a clean polyethylene bag, one sample per bag. Samples were photographed as soon as they arrived at the laboratory.

Morphological Identification:

Morphological characteristics of each specimen, including size, shape, color, texture, were recorded. The samples were then preserved individually in 70% methanol for species identification. For identification, samples from different sponge samples were carefully removed using a fine razor blade and digested separately using concentrated nitric acid to extract spicules. Microscopic examination and measurement of spicules were performed using image analysis software and Compound Microscope (Infinix Trinocular Microscope) These specimens were identified using the taxonomic keys (Ackers et al.,2007, Van Soest & Hooper, 2002; WoRMS, 2024).

Sample preparation and extraction:

Sponges were washed carefully with distilled water to remove sand, dirt and symbionts and drained off the excess water on a blotting sheet. The sponges were cut into small pieces and dried in the shade. The sponge was then finely homogenized and weighed and soaked in methanol for 48 hours to prepare crude extracts.

To collect the crude extract, the extract was filtered using whatman filter paper and was concentrated using Rotary evaporator. Different solutions of different concentrations depending upon the weight of the sponge were prepared separately for all three species of sponges and used in larvicide bioanalysis. Extract was stored in sterile solvent glass vials and stored at 4°C until use (Annie & Lipton 2012).

Larvicidal Bioassay Procedure:

The experiment was carried out in the laboratory of National Institute of Malaria Research, Panaji-Goa. Adult male mosquitoes are supported using a 10% sugar solution and female mosquitoes were fed on the blood of Rabbit/Chicken. Larvae were placed in metal trays. Experimental setups were performed in triplicate using plastic containers.

Larvicidal activity of crude sponge extracts was evaluated according to the method approved by the World Health Organization (2016).

In order to conduct bioassays for larvicidal activity of crude methanol extract of sponge, late third instar and early fourth instar larvae of mosquitoes (*Culex sp.*) were used. 20 individuals of early third instar larvae were placed in 100 ml of water with the appropriate control.

Bioassays were performed at room temperature at 27°C in replicates per concentration. A series of doses were used to carry out the bioassays in plastic containers holding 100 ml of distilled water and 20 larvae each. Appropriate volume of extract was diluted in 100 ml of water in the plastic container to obtain the target dose. Replicates were set for each dosage and the same number of controls were set simultaneously using distilled water. Mortality was recorded at 24 and 48 hours of intervals after exposure; No food was provided to the larvae.

Dead and dying larvae were collected and larval mortality was calculated for each concentration. Mortality was converted to percent mortality and corrected mortality was calculated using Abbot's formula.

$$Corrected\ mortality\ (\%) = \frac{mortality\ rate\ in\ test\ (\%) -\ mortality\ rate\ in\ control (\%)}{100\ -\ mortality\ rate\ in\ control\ (\%)} \times 100$$

CHAPTER 4: ANALYSIS AND CONCLUSIONS

4.1 RESULT:

In the present study three species of marine sponges (*Callyspongia sp*.) were collected from marine environment, to study the larvicidal potential of secondary metabolites extracted from it.

The identification of sponges is very difficult due to their unique morphological traits and intraspecific variability in shape and colour. Identification of sponges was carried out by examining their morphological characteristics such as shape, size, color, surface texture and skeletal structures and by proper identification of sponges was done by collection and microscopic examination of their spicules by using fuild guide and key specific to marine sponges. (Van Soest & Hooper, 2002; WoRMS, 2024).

Figure 4.1 below represents the all three species of live sponges collectes from the study area for the extraction of secondary metabolites.and Figure 4.2 represents the sectioning of all three species of sponges for the purpose of their identification. And through diagnosis and by taking careful meaurements of skeleton (spicules/fibres) it was identified as *Callyspongia sp*. (Faundez &Valentine 1936).

Figure 4.1 Sponges before and after collection

A. *Callyspongia sp. 1*

B. *Callyspongia sp. 1*

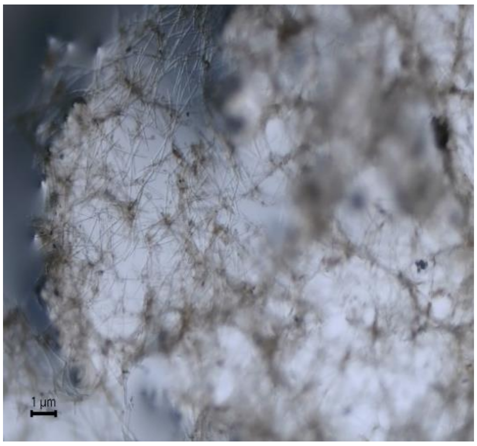
C. *Callyspongia sp. 2*

D. *Callyspongia sp. 2*

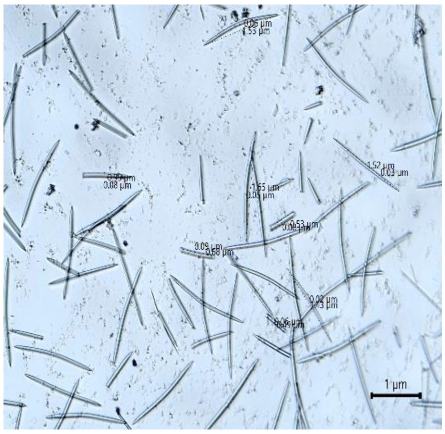
E. *Callyspongia sp. 1*

F. *Callyspongia sp. 1*

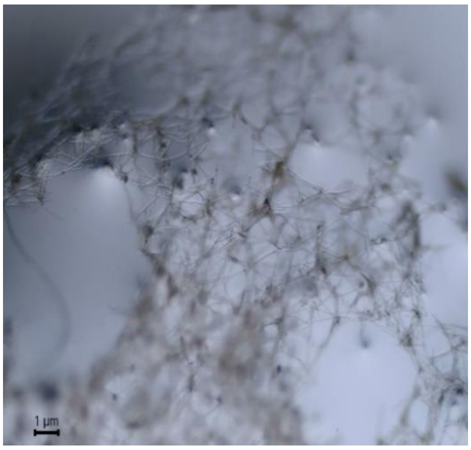
<u>Figure 4.2 Skeletal structure of three species of *Callyspongia*.</u>



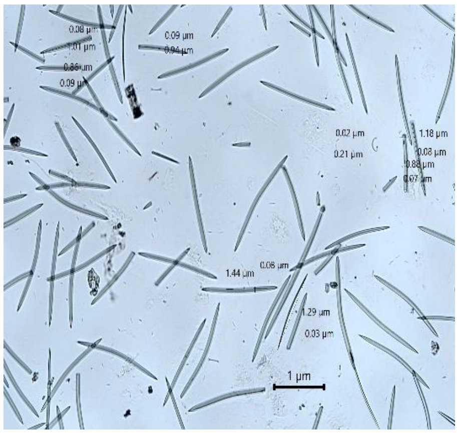
A. Cross section of *Callyspongia sp*. 1



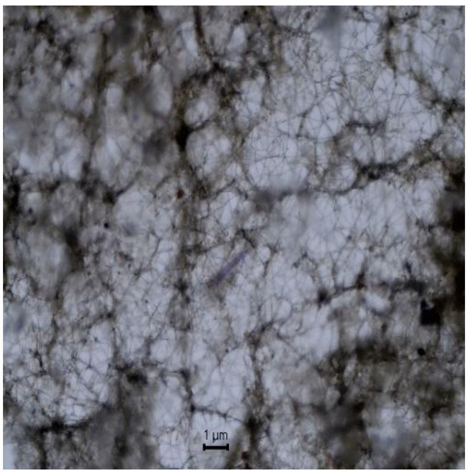
B. Styles of *Callyspongia sp.* 1



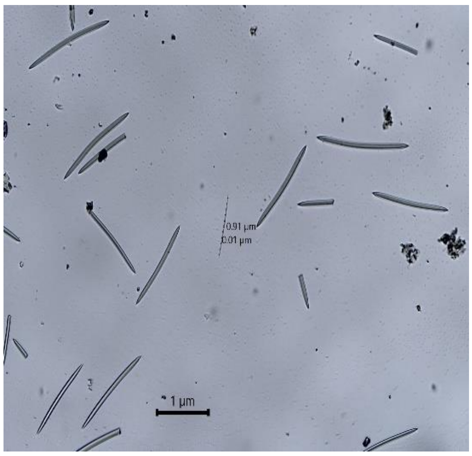
C. Cross section of *Callyspongia sp*. 2



D. Styles of *Callyspongia sp.* 2



E. Cross section of *Callyspongia sp*. 3



F. Styles of *Callyspongia sp.* 3

Figure 4.1.1 shows % mortality of culex with 3 species of *Callyspongia* after 24 hours and 48 hours at the concentration of 4000µl respectively. There was sudden increase in the mortality of sp.1and sp.3 with no change in the mortality rate of sp.2 after 48 hours of observation. methanolic extracts of three species of sponge exhibit larvicidal activity. It is clear from the results that methanolic extract of all three sponges (*Calllyspongia sp*.) has the larvicidal activity against *Culex sp.* larvae. Among all three species tested *Callyspongia sp.3* showed the highest mortality (28%) and *Callyspongia sp.*1 showed the least activity (12%).

The larval mortality rates and activity levels of 3 different species of *Callyspongia* were analysed across 2 larval species: *Anopheles* and *Culex sp*. at the concentration of 2000µl. Figure (4.1.2) illustrates the trends observed over the experimental period with a time interval of 24 hours and 48 hours respectively.

Culex sp. exhibited the highest mortality rate, reaching a peak of 28% after 48 hours of exposure to the experimental conditions. Conversely, *Anopheles sp.* demonstrated the lowest mortality rate, with only a marginal increase of (12%) observed throughout the experiment.

In terms of larval activity, *Callyspongia sp.* 3 and *Callyspongia sp.*2 displayed the most consistent and vigorous movement, for *Culex* and *Anopheles sp.* respectively. *Callyspongia sp.* 1 exhibited minimal activity throughout the experiment, with slight fluctuations observed but no significant peaks.

Among the two larval species of mosquito*; Anopheles sp.* and *Culex sp.* tested, to compare the mortality between both the species, *Culex sp*. showed the higher mortality as compared to

Anopheles sp. as shown in Figure 4.1.2. These findings suggests that larvicidal activity is species specific.

Figure 4.1.3 displays the relationship between the mortality of larvae and the dosage (25µl, 50µl, 100µl, 200µl & 500µl) of secondary metabolites of *Callyspongia sp.*3. As the dosage of secondary metabolites increases, the mortality of larvae exhibits a notable upward trend."

At lower dosages, the mortality remains relatively low; however, a sharp increase in mortality is observed beyond a dosage of 200(µl).

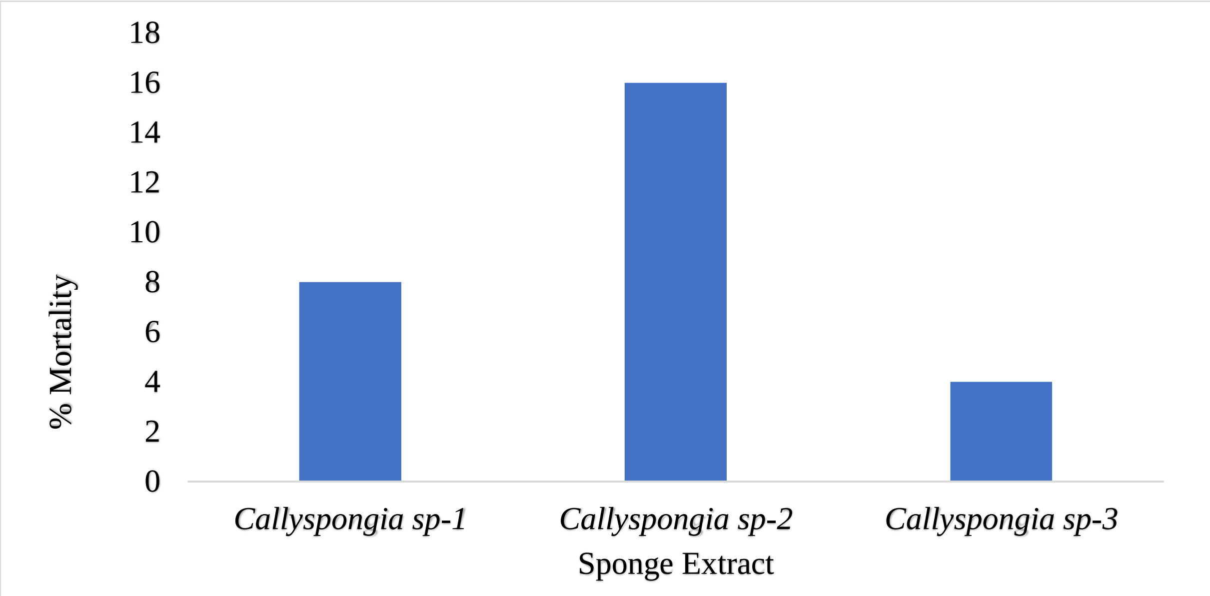
This result indicates that increasing doses of secondary metabolites lead to higher mortality rates in larvae, supporting the notion of their potential as insecticidal agents."

These findings suggest that the relationship between mortality of larvae and dosage of secondary metabolites follows a clear dose-response pattern."

In our study investigating the effect of dosage on larval mortality, we observed distinct trends in mortality rates at both 24-hour and 48-hour intervals. At 24 hours, lower dosages showed minimal impact on larval mortality, However, as dosage increased, mortality rates exhibited a sharp rise, reaching a peak of 100% mortality at the highest dosage tested, proving a significant larvicidal potential of marine sponges. These results suggest a clear dose-response relationship, indicating the importance of dosage in determining larval mortality over time.

FIGURES:

Figure 4.1: Mortality rate of mosquito larvae against marine sponge extract.



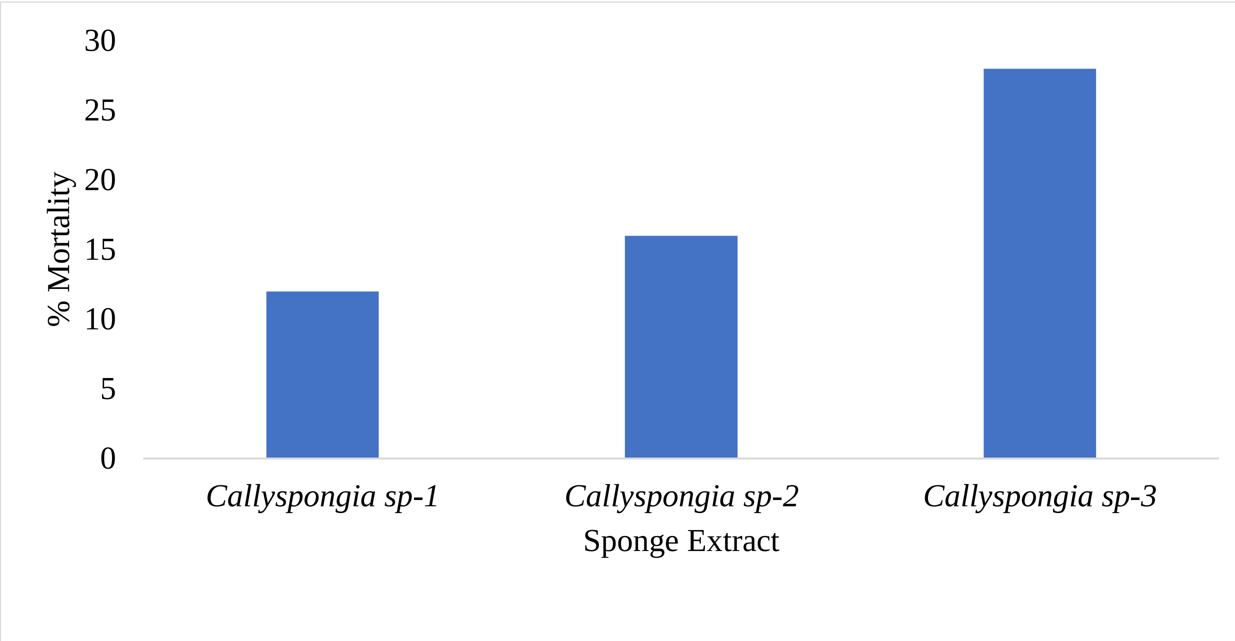
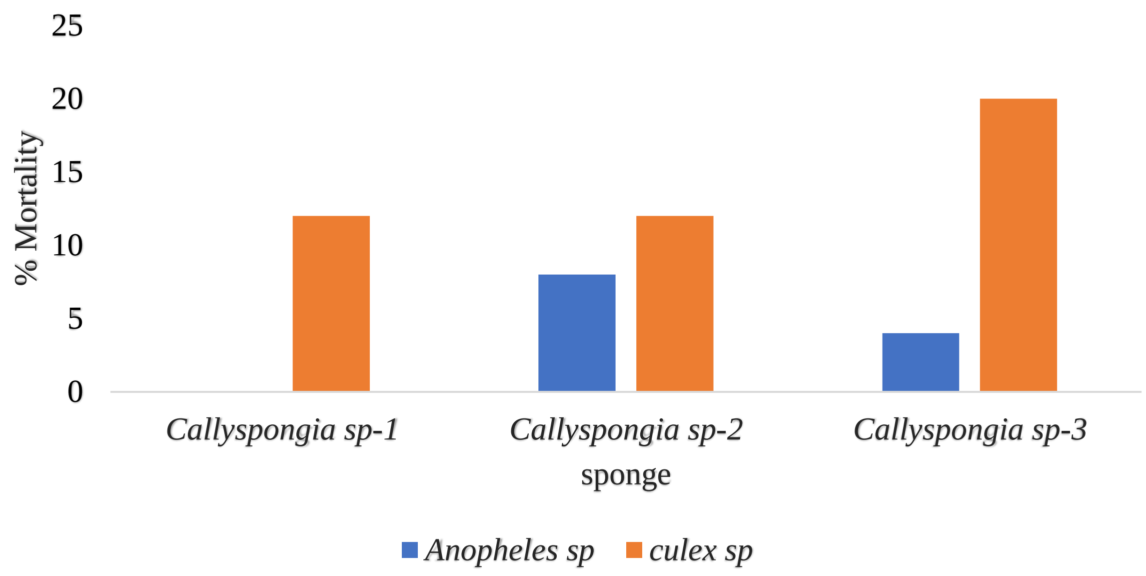


Figure 4.1.1: Larval mortality (%) observed in *Culex sp*. 3rd instar larvae tested with 3 different methanol extracts of marine sponges at same concentration.



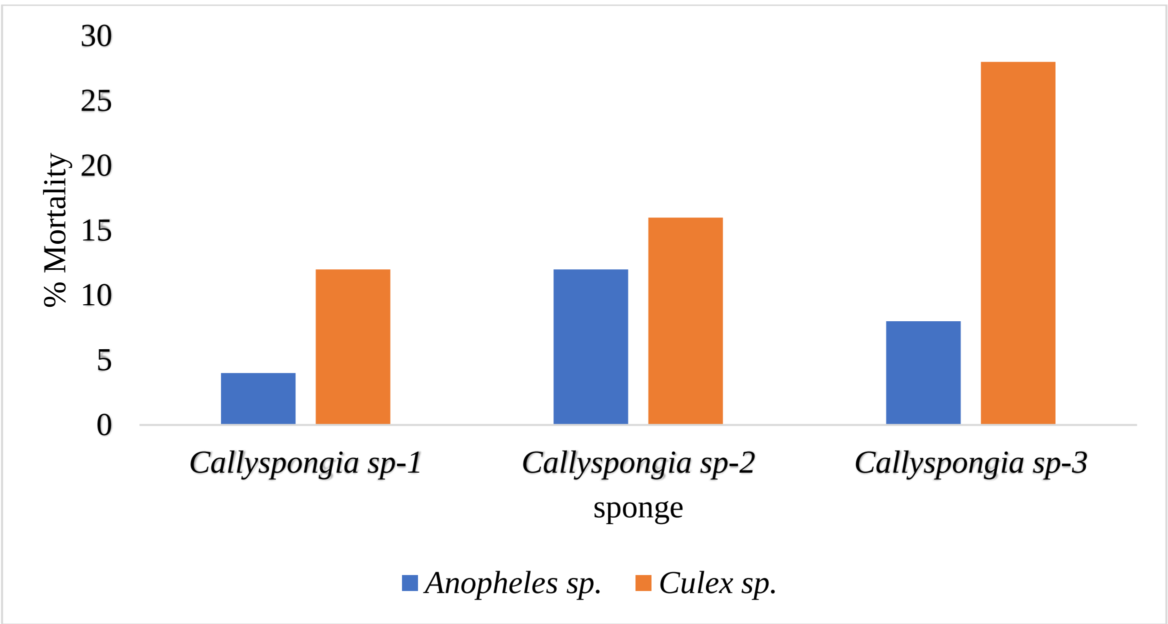
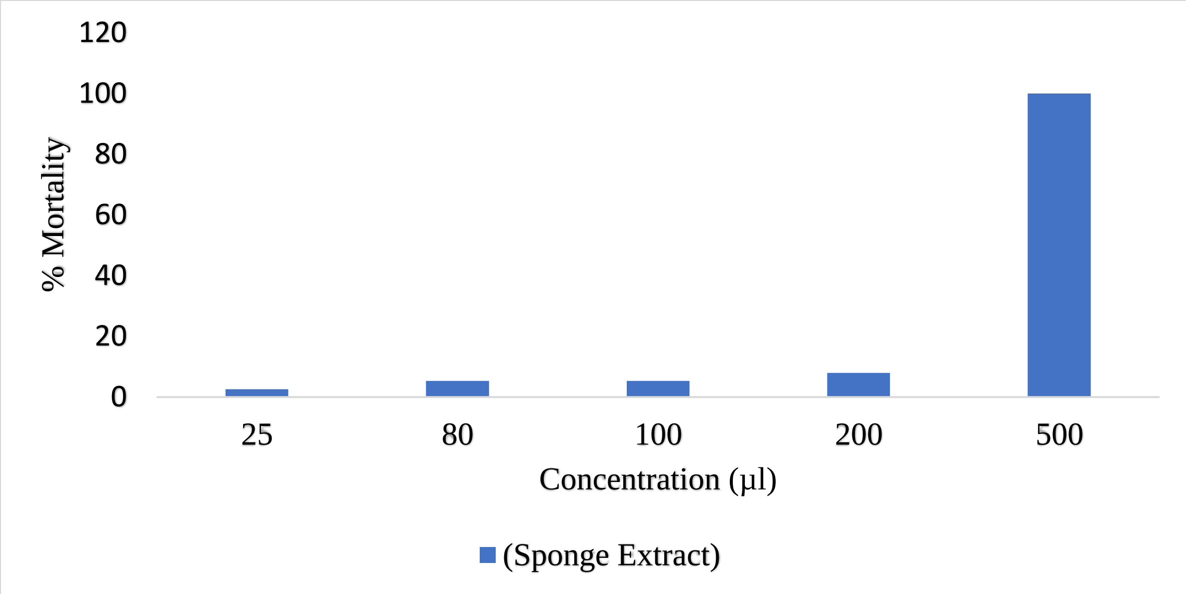


Figure 4.1.2: Comparative larval mortality (%) of 3 different extracts of marine sponges against 3rd instar larvae of *Culex sp. and Anopheles sp.*



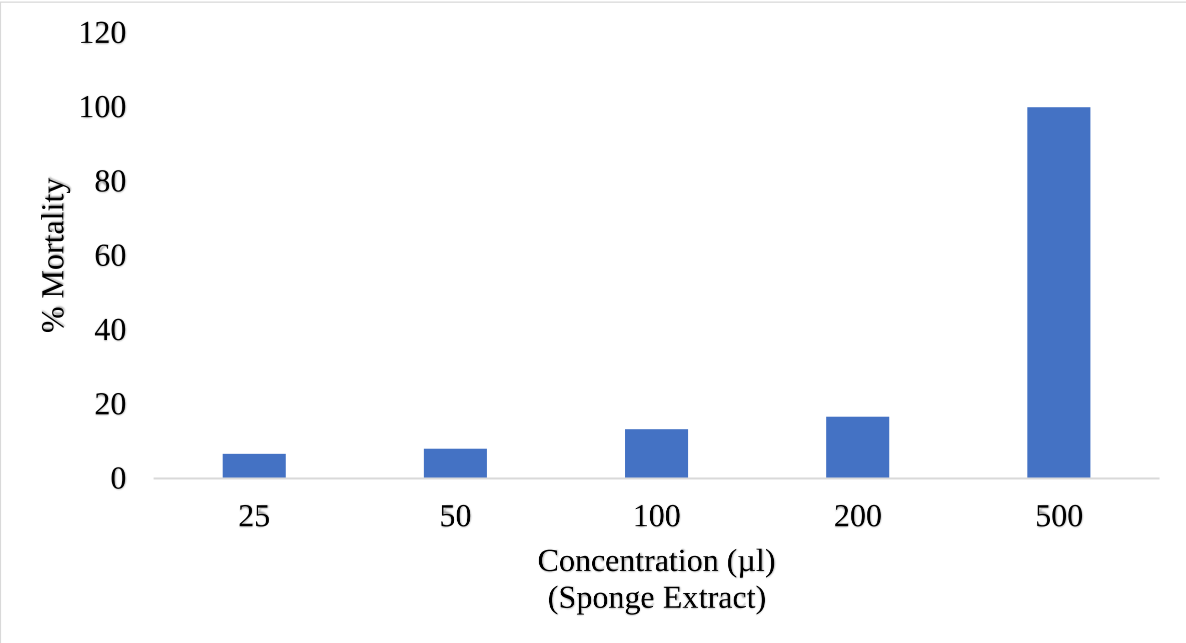


Figure 4.1.3: The difference of mortality rate of Culex sp. larvae of 3rd instar after 24 hours of exposure of a methanolic extract of *callyspongia sp*.1 at different concentrations.

All experimental methanol sponge extracts were found to have larvicidal activity. % mortality was recorded at the higher concentration of 500 µl with 100%, mortality.

Among the extracts examined *Callyspongia sp.*1, 2 and 3 all showed significant activity in larvicidal tests. The results obtained in this study show that the species of *Callyspongia* can be used to search for new pesticides from marine sources.

Larvae may damage the metabolism in endoscopic individuals with greater and longer exposure time. It is recommended to develop environmentally friendly and cheap, effective natural products such as larvicides. This study seeks to further investigate the isolalation of larvicidal compounds for potential biomedical applications.

<u>4.2 DISCUSSION</u>:

Marine sponges are a rich source of complex compounds, some of which have been shown to have diverse activities (De Rosa et al., 2003). The concentrations of many highly active compounds in marine invertebrates are generally small; in wet water it is less than 1 part per million (Proksch et al., 2002). Sponges are always in close association with many species of bacteria, and this symbiotic relationship provides a wealth of bioactive secondary metabolites. Resistance to pesticides and herbicides has increased rapidly in the last few years. Therefore, it is necessary to develop and find new pesticides from the natural environment.

Sponges have been recognised as the most promising source of new bioactive products for drug discovery. (Karthikeyan et al., 2022). The present study was aimed to investigate the larvicidal potential of secondary metabolites extracted from marine sponges. Findings reveal significant implications for both pest control strategies and the utilization of marine natural products.

Biopesticides are also safe, cheap and widely available worldwide and will therefore be suitable for other pesticides in the future. Today, mosquito control focuses on larvae and, when necessary, adults. This is because the fight against adult parasites is temporary, unpleasant and harmful to the environment, while further treatment of larvae in time and space leads to less danger. Because mosquitoes are less mobile, brood control can be an effective control tool, especially where primary breeding grounds are man-made and easily identified. “This study shows that sponge species extract acts as a good larvicidal agent against *culex* larvae”

Most mosquito control programs target the larval stage of the mosquito population with larvicides because adulticides only reduce the adult mosquito population. As mosquitoes are increasingly exposed to commercial pesticides (e.g., organochlorides, organophosphates, carbamates) as well as biopesticides, researchers are currently searching for a less harmful and easier-to-use way to non-target humans. (Sunmonu & Ekpunobi 2023).

Earlier five sponge extracts from Gulf of Mannar showed the presence of significant novel bioactive compounds for both larvicidal (*Aedes aegypti*) and insecticidal assays *(Musca domestica*) (Rao *et al.,* 2008). The antimicrobial, antifungal, and antibacterial properties were observed from marine sponge extract by (Boobathy et al., 2009). Examination of the secondary metabolites of five species of sponges *(Acanthella elongata, Axinella donnani, Callyspongia,diffusa, Callyspongia subarmigera, and Echinodictyum gorgonoides)* suggested that *A. elongata* extract has the potential for developing novel bioactive larvicidal agent. (Annie & Lipton 2012). The methanolic extracts of two sponges, *Sigmadocia carnosa* and *Spongia officinalis var, Ceylonensis* were investigated and the results showed that both sponges contained alkaloids, phenols, steroids, triterpenoids, reducing sugars and aromatic acids, and also showed that both sponges has antibacterial, antifungal, insecticidal and larvicidal and antioxidant properties (Krishnan et al. 2016).

The present results of this study demonstrated that secondary metabolites extracted from marine sponge *callyspongia sp.* exhibited promising larvicidal activity against 3rd instar larvae of *Culex sp*. This finding aligns with previous research indicating the bioactivity of marine-derived compounds against various pests and pathogens (Rao et al., 2008; Boobathy 2009; Feby and Nair 2010; Mohan et al., 2016; Lekshmi et al., 2020; Baig et al., 2021; Mote et al., 2021). The observed larvicidal activity suggests the potential of these secondary metabolites as alternative agents for pest management.

Previous studies have suggested that certain secondary metabolites possess neurotoxic or disruptive effects on insect larvae, leading to mortality (Bertrand et al., 1987; Garderes et al., 2015; Kamalakkannan, 2015).

Further investigation into the specific modes of action of these compounds is warranted to elucidate their potential applications in larval control strategies.

Despite the promising findings, it is important to acknowledge the limitations of this study. The scope of research was limited to a specific set of marine sponge species and larval organisms. Additionally, the bioactivity of the extracted metabolites may vary depending on factors such as extraction methods, habitat of sponges, and seasonal variations. Future research may be aimed to explore a broader range of marine sponge species and investigate the effects of different extraction techniques on the bioactivity of secondary metabolites.

4.3 CONCLUSION

Sea sponges naturally produce many bioactive substances and secondary metabolites and increase their defence mechanisms by combining with other species. These bioactive bacteria can be used as active metabolites in the treatment of many diseases, such as antibiotics, antibiotics, anti-inflammatory drugs, anti-inflammatory drugs, and can affect the disease of various diseases.

Marine invertebrates are one of the major groups of biological organisms that provide significant number of natural products and secondary metabolites with pharmacological properties and lead to formulation of novel drugs. The rich diversity in bioactive compounds from sponges has provided molecules that interfere with the pathogenesis of a disease at different points, which increase the chances of developing selective drugs against specific targets. Marine sponges have provided many examples of novel secondary metabolites that possess varied chemical status and potential larvicidal activity. Marine natural products provide a novel and rich source of chemical diversity that can contribute to design and development of new and potentially useful antibacterial agents. Unfortunately, these secondary metabolites are often abundant in natural products and are too small to support the development of effective and efficient drugs. The current metabolite research is a major investigation into the possibility of extracting the drug from marine sponges. Marine ecosystems are useful not only in the discovery of larvicidal drugs but also in identifying new targets for many diseases.

The implications of this findings extend beyond the field of pest control to the broader field of marine biotechnology. Marine sponges represent a rich source of bioactive compounds with diverse biological activities. The identification of larvicidal secondary metabolites highlights the potential for the sustainable utilization of marine resources in the development of novel pharmaceuticals and agrochemicals.

In conclusion, this study provides valuable insights into the larvicidal potential of secondary metabolites extracted from marine sponges. The observed activity underscores the importance of further research in this area to harness the full potential of marine biodiversity in addressing global challenges in pest management and drug discovery.

In summary, this study reports larvicidal activities of the marine sponge against culex Screening shows methanol extracts of sponge hold great promise for mosquito control. This is an environmentally friendly alternative to pesticides and shows promise in mosquito control.

4.4 LIMITATIONS OF THE STUDY:

Collection of marine sponges for research purposes can be challenging due to their remote habitats, depth, and accessibility.

Marine sponges produce a wide array of secondary metabolites with diverse chemical structures. Identifying and isolating specific compounds responsible for larvicidal activity can be complex and time-consuming.

It also raises ecological concerns, as these organisms play crucial roles in marine ecosystems. Sustainable collection methods must be employed to minimize environmental impact.

The bioavailability and stability of secondary metabolites in marine sponge extracts can be influenced by various factors, including extraction methods, storage conditions, and interactions with other compounds. Optimizing extraction and purification techniques is essential to ensure accurate assessment of larvicidal activity.

Contamination from other marine organisms or environmental pollutants can affect the purity and quality of extracted secondary metabolites, leading to inaccurate research results.

Obtaining sufficient numbers of mosquitoes for bioassay experiments may be difficult, especially when working with specific developmental stages.

The efficacy of the extract in producing the desired biological effect may be diminished due to its low concentration, if the extracts is not concentrated properly, leading to inconclusive or unreliable results. Dilute extracts may contain a high proportion of solvent, which can interfere with biological assays, affecting the accuracy and reproducibility of results.

4.5 FUTURE PROSPECTS:

Research on the larvicidal potential of secondary metabolites extracted from marine sponges holds promise for several reasons. Firstly, marine sponges are known to produce a wide variety of bioactive compounds, many of which have shown potential for insecticidal and larvicidal properties. Secondly, the search for environmentally friendly alternatives to chemical insecticides is becoming increasingly important due to concerns about resistance development and environmental impact. Thirdly, the unique chemical structures of marine sponge metabolites offer opportunities for novel modes of action against insect pests, potentially overcoming resistance issues. However, further research is needed to explore the efficacy, safety, and practical applications of these compounds before they can be utilized effectively as larvicides in pest control strategies.

Marine sponges are known to harbor a vast array of secondary metabolites, many of which possess bioactive properties. The immense biodiversity of marine sponges offers a rich source of compounds with diverse chemical structures and biological activities.

Utilizing secondary metabolites from marine sponges as larvicides offers a natural and eco-friendly alternative to synthetic chemical pesticides. These compounds are biodegradable and generally pose minimal risks to non-target organisms and the environment compared to conventional pesticides.

Many secondary metabolites exhibit selective toxicity towards insect larvae while sparing beneficial insects, such as pollinators, and other non-target organisms. This targeted action reduces the collateral damage often associated with broad-spectrum chemical insecticides.

Marine sponges inhabit unique ecological niches and produce compounds that have evolved as chemical defences against predators and competitors. Consequently, these organisms may harbor novel bioactive compounds with potent larvicidal properties that can be explored for pest control purposes.

In conclusion, the future prospects of harnessing secondary metabolites from marine sponges for larvicidal purposes offers a promising avenue for sustainable pest control and disease management. With further research, technological advancements, and collaborative efforts, these natural compounds have the potential to play a significant role in mitigating the global burden of vector-borne diseases while promoting environmental conservation.

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