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Porifera: Biology and Medicinal Properties

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Porifera:
Biology and Medicinal Properties

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Abstract

The focus of this thesis is the possibility of oceanic medicine through understanding the general biology of poriferans, their symbionts, medicinal properties, and how we could harvest sponges conservatively. It was necessary to understand the basic biology of poriferans regarding their structures, differences in their classes, and how they obtain nutrition, develop, and reproduce. The ecology of the poriferans was also researched including their larval and sessile stages and how their environment determines where they settle. Additionally, ecology was examined including how poriferans interact with symbionts and other organisms. This then was utilized to seek whether poriferans had useful medical properties for human diseases. These medicinal properties included antibacterial, anticancer, antiviral, antifungal, antimalarial, and anthelminthic properties and more. Lastly, current medicines already on the market with sponge-based chemical compound analogs were researched. Next, sustainability in using poriferan for medicinal aspects was investigated. This included ways by which poriferan could be harvested most efficiently including which environments they thrived in for the best cultivation of larvae.

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Introduction

Humans have been able to travel all over the earth's terrestrial habitats, yet despite earnest efforts, many aspects of the ocean have yet to be explored and understood. Therefore, when it comes to seeking new natural products, there is much undisclosed potential for oceanic medicine. In fact, if 71% of the Earth's surface is covered in water and the oceans contain 96.5% of the Earth's water (*U.S. Geological Survey*, 2018), how many medications have yet to be developed? This thesis explores the possibilities of oceanic medicine and some freshwater through understanding the general biology of Porifera, their symbionts, medicinal properties, and how we could harvest Porifera conservatively.

What is Porifera?

Porifera are otherwise known as sponges. A sponge is defined as a "sedentary, filter-feeding metazoan which utilizes a single layer of flagellated cells (choanocytes) to pump a unidirectional water current through its body" (Bergquist, 1978). Porifera are invertebrates and live in aquatic environments, which can be freshwater or saltwater. The phylum Porifera has four primary classes: Demospongiae, Hexactinellida, Calcarea, and Homoscleromorpha.

1: Basic Biology of Porifera

1.1 Porifera Classes

As Porifera are so diverse, intense studies have been conducted in various fields. One of the necessary tasks to do during these studies is to characterize and identify the sponge being studied. This can be completed for most sponges through the study of spicules, which are mineral elements that provide support to the sponge like a skeleton. Being able to organize the different spicule shapes can allow for basic sponge systematics and taxonomy to occur. Therefore, understanding how to identify spicules will enable sponge classification. Spicule names are usually described with the word actine, which is simply the ray of the spicules and, therefore, the prefix of each of these forms is the number of rays present. (Łukowiak et al., 2022). This is the primary way that Porifera are identified, which will be revealed through the following Porifera class descriptions.

1.1.1 Calcarea

The three classes of Porifera each have distinct characteristics. Calcarea, which only consists of marine sponges, has a skeleton of spicules made of calcium carbonate (Bergquist, 1978). They also contain no spongin (*OpenStax*, 2018). Calcarea spicules have three basic forms: diactines, triactines, and tetraactines. What makes the spicules of the class Calcarea unique is that their shape can be conical or cylindrical and their tips can be round, blunt, or sharp (Łukowiak et al., 2022).

1.1.2 Homoscleromorpha

Homoscleromorpha were initially thought to be a family or suborder of the subclass Tetractinellida, which is part of the class Demospongiae. Then later it was thought to be its own subclass in Demospongiae. Now it is considered its own class based on its position in

phylogenetic analyses which support that poriferans are monophyletic. If it was decided that poriferans were instead paraphyletic this again may change (Gazave et al., 2011). One of the reasons that Homoescleromorpha being a part of the class Demospongiae was challenged was because phylogenomic studies suggested a sister-group relation between Homoscleromorpha and Calcarea. Moreover, morphology and development are different among Homoscleromorpha and Desmospongiae. For instance, Homoscleromorpha has a true epithelium, including a basement membrane, whereas Desmospongiae and other sponge groups do not possess this. Moreover, a flagellated exopinacoderm is not shown in any of the other three classes either (Gazave et al., 2011). Therefore, this is a separate class because of distinguishable attributes not found in the other classes, which is made possible due to more data being available for a comparative analysis.

The homoscleromorph class has siliceous spicules if spicules are present; however, some of their families do not have a skeleton. For example, Oscarellidae have no skeleton and cytological traits are relied upon for identification. Therefore, spicule observations are not the only form of identification (Łukowiak et al., 2022) This class is therefore also distinguished by their massive and encrusting form (*28.1A: Phylum Porifera*, 2018).

1.1.3 Hexactinellida

Hexactinellids, which are also only marine sponges, are usually in deep water. They have a siliceous skeleton of spicules (Bergquist, 1978). These siliceous spicules may be loose or fused. The fused spicules can result in a framework that is rigid and choanosomal (Łukowiak et al., 2022). The spicules are classified by a hexactine spicule structure (1978) and can have hexactine or triaxonic symmetry (2022). The hexactine structure means that they have six rayed spicules, or six points on the spicules (*OpenStax*, 2018). This structure is why Hexactinellids are

also called glass sponges due to the hexactine spicules making their glass-like appearance (Wörheide et al., 2012).

1.1.4 Demospongiae

Then Demospongiae, the largest group of sponges, found in fresh and saltwater; they are classified by siliceous spicules and an organic skeleton of collagen which can be secreted as spongin (Bergquist, 1978). Using these spongin fibers, demosponges can also produce opaline spicules (Łukowiak et al., 2022). However, some demosponges do not have any spicules and just have spongin or chitin (Łukowiak et al., 2022). Some of the subclasses in demosponges include siliceous spicules and an organic collagen skeleton that also have living tissue supported by a calcareous skeleton (Bergquist, 1978). Demospongiae are the most widely known sponge as it is used in bath sponges, which lack spicules (*OpenStax*, 2018).

Determining the classes of Porifera is complex. Therefore, there are many shortcomings to morphological taxonomy. Phylogenomic studies are another way classes are distinguished (Van Soest et al., 2012). This involves genome-scale samples being taken to develop the evolutionary history of life on earth. The goals of phylogenomic studies include making inferences of phylogenetic relationships between taxa, understanding the mechanisms of their molecular evolution, and comparing phylogenetics of different species to infer supposed functions of DNA and protein sequences (Young & Gillung, 2019).

1.1.5 Monophyletic or Paraphyletic?

There are still some discrepancies that need to be resolved because Porifera has been considered to be paraphyletic in some prior studies. Support for monophyly of sponges is present when only morphological characteristics are considered. For instance, three demosponges were grouped as monophyletic and basal to Calcarea and Eumetazoa clade. However, when other

aspects were examined in a phylogenetic analysis it was found that two calcisponges were grouped with eumetazoans with 78% precision and poriferans were deemed not monophyletic. Characteristics analyzed included 2,039 amino acids from seven housekeeping genes, 228 amino acids from cytochrome oxidase I gene, 1,747 nucleotides from the 18S rDNA gene, and 150 morphological characters coded for the genus. Although, it was determined from this evidence that Porifera are paraphyletic instead of monophyletic, this conclusion is not yet strongly supported (Peterson & Butterfield, 2005). Therefore, there has been a continued pursuance to resolve this discrepancy.

In more recent studies phylogenomic studies have suggested solutions for decades of differing classifications by continuing to support the idea that Porifera are monophyletic (Van Soest et al., 2012). Monophyletic means a group of organisms or taxon that share the same recent ancestor (Biologydictionary.net Editors, 2017). Classification is also being settled through DNA barcoding, considered the Sponge Barcoding Project. Efforts have also been made to make the database for all Porifera across the globe to be complete. The number of species were recorded in each of the 232 marine ecoregions of the world (Van Soest et al., 2012).

1.2 Porifera shapes

There are three main shapes of Porifera. The least complex structure of the Porifera is asconoid. This structure is smaller and round with only one larger external opening. Due to the lack of folding, there is a limitation on size. This is because the more folds, the more canals, the more surface area, and the more complex the internal structure. The syconoid shape structure of has more folds. Lastly, the leuconid sponge has the most folds and because of this, it is often seen as more evolved (Goudie & Finn, 2013).

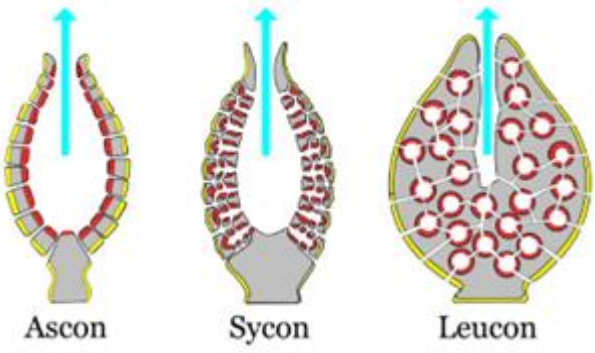


Figure 1 Picture of Porifera Shapes (Calcarea, 2020)

The increase in folds allows for Porifera to pump up to ten times their own volume every hour. Moreover, they can become much larger with more openings due to this increase in folding. There are also various growth forms, such as thinly encrusting, erect and branching, and massive without a defined shape. Porifera that live in high currents tend to have low encrusting forms whereas species that live lower currents might have more branches (Goudie & Finn, 2013). This is because branches are less likely to be able to withstand stronger currents so massive and encrusting sponges tend to be more dominant in those regions (Silvia et al., 2021).

For instance, a structural analysis of *Cinachyrella australiensis* revealed a spherical shape when in a higher velocity of water where there was an increase in the presence of dangerously larger sediment particle sizes that could cause damage. In contrast, there was a more flattened shape when in calmer conditions. This relationship is linked to structural support by increased rigidity from spicule reinforcement in areas with higher water flow. An increase in spicule rigidity leads to an increase in tissue area for the tensile load to be distributed making the sponge more spherical and stronger (McDonald et al., 2002). A benefit to being in high flow environments involves current-induced flow within the sponge, which is possible in thin-walled sponges with large diameter osculum. Passive flow allows for energy gain and greater food intake when in lower food concentrations (Leys et al., 2011).

1.3 Porifera Body Layers and Cell Types

The sponge has three body layers. The basic anatomy consists of a choanoderm and pinacoderm, with the mesohyl in between. (Bergquist, 1978) The outer layer or pinacoderm is a single layer made of pinacocytes. Pinacocytes are flat "skin-like" cells (Van Soest et al., 2012) and are considered the epidermis of the sponge (*OpenStax*, 2018). The middle layer is called mesohyl, which has collagen and skeletal materials like organic spongin fibers and inorganic spicules. It is considered an extracellular matrix that houses specialized and pluripotent sponge cells and symbionts (Łukowiak et al., 2022). Specifically, mesohyl contains amoebocytes and sclerocytes. Amoebocytes are considered the stem cells of Porifera and scelerocytes are what secrete the skeletal material of spicules (*OpenStax*, 2018). The skeletal structure of the spicules allows for support (Łukowiak et al., 2022) as well as protection against predators (Turon et al., 2018). Spicules also help larvae stay buoyant while in plankton and enable Porifera to reach the bottom of the ocean at settlement to catch prey and be able to reproduce (Uriz et al., 2003). Lastly, the most inner layer is the choanoderm and it has choanocytes that line the canals (Goudie & Finn, 2013).

It is important to add to the discussion of body layers that Porifera does not have actual tissue like other animal groups such as insects and mammals. This is because they do not have an actual gastrula and cannot create an actual endoderm or ectoderm. Therefore, they do not have true tissues and instead function using specialized cells, most of which were mentioned above (*OpenStax*, 2018).

1.4 Porifera Canal System

Due to the folding and specialized cells, Porifera can have a system of water canals and chambers that flow only in one direction. The sponge uses ostia, which are pores, to allow the

water, the dissolved nutrients, and other material that it carries to enter the canals (Goudie & Finn, 2013). Then there is a current created by choanocytes that line the canals/chambers to create a current within the sponge and allow for absorption to occur (Goudie & Finn, 2013; *OpenStax, 2018)*. The excreted waste and reproductive material are then able to flow out with the excess water through the oscula, which is the larger external opening. The location of these channels and pores is critical to prevent contamination from substances in the seawater. (Goudie & Finn, 2013). What exactly will be filtered will be described in further detail in the filter feeding section.

1.5 Porifera Reproduction

Understanding and determining reproductive strategies is needed to understand Porifera ecology, life history, and community dynamics (Idan et al., 2020). Modes of reproduction include both asexual and sexual reproduction and these modes of reproduction can vary across the different sponge classes and can compete. For example, when asexual reproduction occurs in Demospongiae, there is a consistent alternation of sexual and asexual phases. This is due to competition from archeocytes during specific seasons (Ereskovsky et al., 2017). Archeocytes are embryonic cells that are believed to be undifferentiated and can develop into haploid cells, which are gamete cells (*Merriam-Webster Dictionary*, 2023). Other factors of whether a sponge undergoes sexual or asexual reproduction include exogenous influences that affect reproductive strategies, such as food availability, population density, and temperatures. It was suggested that temperature and food were the most crucial factors for sexual and asexual reproduction (Ereskovsky et al., 2017).

1.5.1 Porifera Sexes

Porifera can either be hermaphroditic or gonochoric (Ereskovsky et al., 2017). Hermaphroditic means that the sex of the organisms can change from male to female, female to male, or possess both male and female parts simultaneously. Gonochoric means that the sexes are separated and are either male or female (*Merriam-Webster Dictionary*, 2023). Many Porifera are called sequential hermaphrodites, which means that they can produce egg and sperms cells at various times so that cross-fertilization can occur when those of the same species are present. (Goudie & Finn, 2013). Not only does this allow for cross-fertilization, but sequential hermaphroditic species allow for an increase in genetic diversity (*OpenStax*, 2018).

1.5.2 Asexual Reproduction

Asexual reproduction is a necessary form of reproduction for sessile organisms like the sponge. This is especially true for lower populations of sessile Porifera as they may not be able to sexually reproduce with the opposite sex if they are gonochoric. Additionally, if the sponge were unable to find a mate the only way they could sexually reproduce is if the sponge were hermaphroditic and self-fertilizing. Ways by which Porifera can proceed with asexual reproduction include fragmentation, gemmulogenesis, and budding (Ereskovsky et al., 2017).

One version of fragmentation can be demonstrated when tissue is shredded artificially. The fragments can reorganize into balls so that the internal epithelia and choanocyte chambers dissociate and specialized cells regress so that the cells can become more archeocyte-like. If fragments lay on the substratum they can reattach and become functional (Maldonado & Uriz, 1999). Fragmentation can also occur when damage has occurred after physical trauma, such as a storm (Goudie & Finn, 2013).

Budding can occur when small reproductive bodies form on the external portion of the poriferans body that eventually detach and grow into new sponges (Goudie & Finn, 2013). This almost seems like a purposeful form of fragmentation where the fragments are prepared to develop into new Porifera. Porifera can bud and undergo fragmentation when sexual reproduction is not an option, making them exceptionally flexible creatures.

Another mechanism that allows Porifera to continue their existence during difficult periods is gemmulogenesis (Goudie & Finn, 2013). An example of the hostile environmental conditions that may cause a sponge to revert to this state include changes in water temperature (*OpenStax*, 2018). When the sponge produces a gemmule, this simply means that the sponge is in a resting phase. This can last up to two years where the sponge can remain in a dried-out spore state (Goudie & Finn, 2013). Gemmules can withstand harsh environments and can be resistant to environmental trauma. This is advantageous because once condition improves, the gemmule can then allow for colonization of the sessile organism to occur (*OpenStax*, 2018).

1.5.3 Sexual Reproduction

Regarding sexual reproduction, male and female reproduction takes place through the development of the gametes by both spermatogenesis and oogenesis (Ereskivsky et al., 2017). Fertilization of the egg and sperm of Porifera can occur in the water column but usually occurs within the adult sponge (Goudie & Finn, 2013), which can include the temporary brood chambers that later form into aquiferous system canals. (Ereskivsky et al., 2017).

A sponge's egg or oocytes come from the differentiation of the amboecytes and are held in the spongocoel. The sperm is stated to come from the differentiation of the choanocytes, which are ejected through the osculum (*OpenStax*, 2018). The choanocytes also are used to capture the sperm to transfer it to the egg cells (Goudie & Finn, 2013). This could be achieved in

the same way that choanocytes capture food by creating water currents with their flagellated projections for filter feeding.

1.6 Larval and Sessile Stages

Sponge can reproduce by viviparity, oviparity, and ovoviviparity. Viviparity is considered an ancestral reproductive mode of reproduction in Porifera and is otherwise known as brooding (Ereskivsky et al., 2017). When one is viviparous, this means that they are born living young instead of eggs. In contrast, oviparity is when one can produce eggs that develop and hatch without being in the maternal body (*Merriam-Webster Dictionary*, 2023). Porifera who are oviparous are in the class Demospongiae (Ereskivsky et al., 2017). Ovoviviparous means that the sponge can produce eggs that develop in the maternal body but hatch inside or right after being expelled from the mother (*Merriam-Webster Dictionary*, 2023).

Porifera have an ancient lineage and have a basal position on the animal tree of life and their development is diverse. However, all types of larval development are finished when the metamorphosis of the swimming larva turns into a settled sponge. The larval ciliated cells leave the larval epithelium and migrate to the amoeboid cells to differentiate into the choanocytes of the juvenile sponge. This happens through signaling mechanisms that sponge larvae use to develop into adult Porifera. The role of signaling in the metamorphosis of Porifera is suggested to occur through epithelial-mesenchymal transition (EMT), which allows for intercellular communication (Borisenko et al., 2019).

The details on how each larvae settle and develop vary based on the sponge larvae type. For instance, the calcareous sponge *Leucosolenia laxa* has free-swimming hollow larvae. They are called coeloblastulae, which is a major sponge larvae type. The coeloblastula has flagellated cells that produce glutinous granules which are used as markers during metamorphosis and

settlement triggers metamorphosis where the larvae dedifferentiate their cells. This can include the surface of the larvae turning into pinacocytes and the cells below are scleroblasts to make spicules. Then the inner layer of cells differentiates into choanocytes to form a gastral cavity. This reveals that the flagellated cells are multipotent in the coeloblastula (Amano & Hori, 2001). Other types of sponge larvae include calciblastula, cintoblastula, and amphiblastula, which all have a cavity, and parenchymella, hoplitomella, and trichimella, which all lack a cavity (Ereskovsky, 2019).

2: Ecology of Porifera

2.1 Porifera Habitats

There is a large amount of global diversity in Porifera. This can be attributed to Porifera being the oldest group of animals still living on the planet. In fact, if the discovered identical vermiform microstructure from ancient reefs that only lived in reefs built by cyanobacteria and lived in microniches where calcimicrobes could not was truly fossilized tissue of keratose sponges, then the sponge represents the oldest body-fossil evidence of animals known. This is only if sponges are truly metazoans or animals, which is implied based on molecular phylogeny (Turner, 2021). Either way, the sponges' ability to continue their species from such an early time, indicates that they are clearly a highly adaptable organism living in numerous aquatic habitats. This includes species in both marine and freshwater habitats (Van Soest et al., 2012).

One of the ways a habitat choice is made for marine invertebrates includes the sessile larval phase. Chemical cues are thought to signal optimal habitat whereas physical settlement cue effects have had less effort to be studied and established. However, in a study completed by Whalan et al. (2015), a test was administered to determine if surface microtopography, which pertains to physical and not chemical cues, contributed to the settlement of larvae of coral reef invertebrates. When three species of coral reef sponges and some coral species were analyzed, it was found that physical cues also have a key role in larval settlement (Whalan et al., 2015). Therefore, when larvae attach to a substrate, there are not only chemical cues but also physical cues.

2.2 Filter-Feeding

Porifera feed off other organisms by filtering their surroundings (Tsubaki & Kato, 2014). The direction that the water passes through the sponge begins with the ostia and end withs the

osculum (*OpenStax*, 2018). Most filtering occurs where the food is trapped by the choanocyte when the water passes through their aquiferous systems. When food particles are too large, pinococytes can phagocytize them. However, the food particles cannot be bigger than the cell because all digestion is intracellular (*OpenStax*, 2018). This can be compared to when humans take a bite of food, the bite itself cannot be more than what their mouth can hold. Amebocytes also assist in digestion by taking food particles to cells that have yet to ingest anything to ensure an even distribution of nourishment (*OpenStax, 2*018). There are some species that do not have an aquiferous system. For example, certain deep-sea sponges instead use a sticky outer surface to capture small prey (Van Soest et al., 2012).

A benefit to filter feeding includes allowing Porifera to serve as a bioremediators. This involves removing/detoxifying pollutants in an environment. They can do this because of their renowned filtering capacity: they can filter their overlying water column in 24 hours. This includes filtering bacteria such as *Escherichia coli*, and filtering 14 liters of water in an hour. Some are also exposed to metal pollutants and hyper-accumulate these metals. Additionally, since they can store halogenated biomolecules, it is likely they are able to break down organic pollutants (Gifford et al., 2006).

2.3 Symbionts

2.3.1 Microbial symbionts

Filtering rates also impact symbiosis, as Porifera use their vast canal systems and provide distinctive substrates for diverse symbiotic organisms (Tsubaki & Kato, 2014). Symbionts of marine sponges include diverse microbial communities, and this has notable ecological and biotechnological benefits. In fact, some of these microbes have yet to be recorded in any other environment. These microbes consist of bacteria, archaea, fungi, and microalgae and can take up

almost half of the volume of the poriferan. Many of these symbionts are exclusive to this phylum (Webster & Taylor, 2011).

These symbionts have several essential functions in relation to the sponge which can include denitrification and anaerobic ammonium oxidation, shown by analyzing the sponge's nitrogen cycle. To protect the symbionts so sponges can continue to have these essential functions, an abundance of ankyrin repeat proteins may allow for the sponge to know the difference between the symbiont and food. This is understood through whole-genome amplification (Webster & Taylor, 2011).

Some ways these symbionts benefit sponges include detoxifying the sponge and feeding. For instance, certain *Deltaproteobacteria* can remove toxins and antibiotics and has a part in nutrient transport. Also, regarding nutrients, some symbionts with gene clusters help metabolize vitamin B12 (Webster & Taylor, 2011). In contrast, symbionts such as microalgae, algae, and cyanobacteria have been able to benefit from sponges by continuing photosynthesis even under low light intensities. This is due to a light transmission system in the sponge spicules. For example, in the sponge species *Tethya aurantium*, the spicules produce an electrical signal of photo-active radiation into the living sponge. The light then reaches the deep tissues of the pigmented sponges where the symbionts are located (Brümmer et al., 2008).

2.3.2 Non-microbial Symbionts

A non-microbial example of a sponge symbiont is demonstrated through the *Spongia* sp. This is a massive sponge that is inhabited by the host-specific endosymbiotic bivalve *Vulsella vulsella*. This organism benefits from the sponge by receiving protection. Also, bivalves such as *Vusella vulsella* exhale filtered water when inside the sponge instead of out to the ambient environment. It is suggested that the sponge uses this already filtered water to better circulate

water around its body. Observations of the water currents and the sponge's aquiferous structure revealed that the sponge could inhale the water exhaled by the bivalve. This, therefore, can be considered "filter mutualism," which promotes mutual filtering rates. These species do not tend to directly compete because some sponges are known to have different food particle size preferences that are less than a couple micrometers. Bivalves prefer to have particles with a broader size range and have less retention of particle sizes less than a micrometer. Therefore, sponges can still receive many small particles from the bivalves excurrent water (Tsubaki et al., 2014).

2.4 Porifera Defenses

When an organism is sessile, defense is important as they simply cannot run away from harm (Helber et al., 2018). There is evolutionary pressure from competitors which can involve threats by overgrowth, toxicity, infection, and predation. This makes chemical defense necessary (Thakur & Müller, 2004) as well as physical defense through spicules (Turon et al., 2018). The diverse types of competition have revealed the diversity of chemical compounds produced by sponges, the potential functions of the metabolites produced, and this has allowed for suggested and implemented strategies for human medications (Thakur & Müller, 2004).

2.4.1 Microbial Defense

The main competition of sponges involves microbial attacks, causing the sponge to have to defend their surface from over-colonization using antimicrobial properties. They are exposed even more due to filter-feeding activities where the microbes freely enter in with the water being pumped. Thankfully, sponges have over 5,300 described secondary metabolites and they use their metabolites to inhibit disease-causing microbes (Helber et al., 2018).

2.4.2 Animal Defense

These metabolites also allow for competition between other sea-bottom dwelling organisms and deter predators. This includes the two most bioactive sponges *Haliclona atra* and *Pseudoceratina* sp., which can defend against fish predators. Due to their successful defense mechanisms, these two species and others have high abundance at certain ocean depths and reefs (Helber et al., 2018).

In a study completed at Fairfield University, Connecticut, four temperate sponge species from Long Island Sound were tested for their anti-predator defenses. The sponges were placed with a hermit crab called *Pagurus longicarpus.* The only sponge that was unable to deter the crab feeding from it was the *Haliclona loosanoffi* sponge. The other species reduced the feeding rates, however, the method used was different for each species. For instance, the species *Microciona prolifera*, a combination of its spicules and crude extract was used to reduce the feeding rate of the crab by 53% compared to the control. The species *Cliona celata* reduced the feeding rate by 32% when using structural materials as deterrents instead. Structural defense materials can develop using spicules where the spicule concentration increases when “attacks” were induced experimentally (Hill et al., 2005).

3: Medicinal Properties of Porifera

3.1 Animals Using Poriferan Benefits

Dolphins rub their bodies on sponge and coral to self-medicate. This includes gorgonian coral *Rumphella aggregata*, the leather coral *Sarcophyton*, and the sponge *Ircinia*. Uses from these invertebrates were suggested to include antibacterial -properties, balancing of hormonal status, and the homeostasis of the dolphin's skin. These medicinal benefits are activated when the dolphin repeatedly rubs the sponge or coral to activate the metabolites. (Morlock et al., 2022). Due to animals such as dolphins using sponges and other invertebrates as medication, humans may be able, too. Although, it may be important to examine both human and sponge genomes first.

3.2 Human Relation to Porifera for Medicinal Purposes

Genomic studies imply genes linked to human disease are present in species distantly related to humans. Studying these genes in simpler models may provide insight into their basic functions. For instance, Porifera are likely the simplest group of animals separated from metazoans; however, their genomes are complex. Not only this, but their genome has a lot of similarities to vertebrate homologs even though they are invertebrates (Ćetković et al., 2018).

For example, protein kinases are studied using Porifera to benefit humans (Cetković et al., 2018). A protein kinase is what allows for phosphorylation of the C terminal domain of RNA polymerase II. This phosphorylation is important to allow for additional proteins to be able to bind to the RNA polymerase II such as elongation factors so elongation can occur in translation. (M. Rowe, personal communication, February 22, 2023). Therefore, this protein is essential for translational processes so that other proteins can be made to be able to perform necessary functions within the body.

Protein kinase C has been analyzed in the sponge *Geodia cydonium* and this revealed that it contains 13 exons and 12 introns. An exon is the coding part of the gene that can translate into a protein and the intron is the noncoding part of the gene. This gene translated into metazoan-like protein kinase C in Porifera but contained the same promotor in heterologous mammalian cells (Ćetković et al., 2018). Therefore, this sponge gene could serve as a model to understand this specific protein. Another example is Bruton's tyrosine kinase, this protein causes X-linked agammaglobulinemia in humans and can be studied using a Bruton's tyrosine kinase-related protein in the sponge species *Suberites domuncula* (Ćetković et al., 2018). X-linked agammaglobulinemia is an inherited immune disorder that inhibits one's ability to produce antibodies and can lead to serious infections that can enter the bloodstream or central nervous system (*X-Linked Agammaglobulinemia (XLA)*, 2019).

3.3 Medicinal Potential

The potential for marine sponges regarding antimicrobial drugs is promising. There are 5,300 assorted products currently known to come from Porifera and their associated microorganisms. Additionally, over 200 new metabolites are reported annually. As drug resistance continues to develop, marine sponges provide leads against bacterial, viral, fungal, and parasitic diseases (Laport & Muricy, 2009).

3.3.1 Antimicrobial Activity

The improper use of antibiotics has allowed for "multidrug-resistant microorganisms" to reduce their effectiveness in treatment (Anteneh et al., 2021, p. 1). Therefore, other antimicrobials are being sought in marine sponges. A 2021 study in South Australia screened for bioactive metabolites from bacteria in the sponges collected. 12 samples from South Australia were found and a total of 1234 bacterial isolates were identified. 70 of these bacteria were found

to be active against one or more tested bacterial and fungal pathogens. 37% revealed antimicrobial activity against *Staphylococcus aureus* and 21% revealed antifungal activity. These results revealed the plentiful variety of bacteria in sponges that produce metabolites with antimicrobial activity against human-infecting bacteria and fungi (Anteneh et al., 2021).

3.3.1.1 Antibacterial Activity

As of 2013, over 800 antibiotic properties have been present and 5000 chemical compounds have been isolated (Renard et al., 2013). However, there continues to be more isolated compounds discovered, some of which possess medicinal properties such as antibacterial activity. A recent study to isolate known and unknown compounds from the Red Sea sponge *Spongia* sp. analyzed their compounds for medicinal uses such as cytotoxicity and antibacterial properties. It was found that 8 (7,7,7-trichloro-3-hydroxy-2,2,6-trimethyl-4-heptanoic acid methyl ester) had weak cytotoxicity and a significant amount of bacterial inhibition for *Staphylococcus aureus.* In contrast, compound 5 (10-hydroxykahukuene B) was inactive but had been previously reported to have antibacterial activity for *Staphylococcus aureus* and *Escherichia coli.* One of the positive controls consisted of the antibiotic tetracycline, which was used on the same plates as the tested compounds (Chi-Jen et al., 2022). Therefore, due to the ability for Porifera to inhibit bacteria, it is possible for antibiotic alternatives to be developed to combat antibiotic resistance.

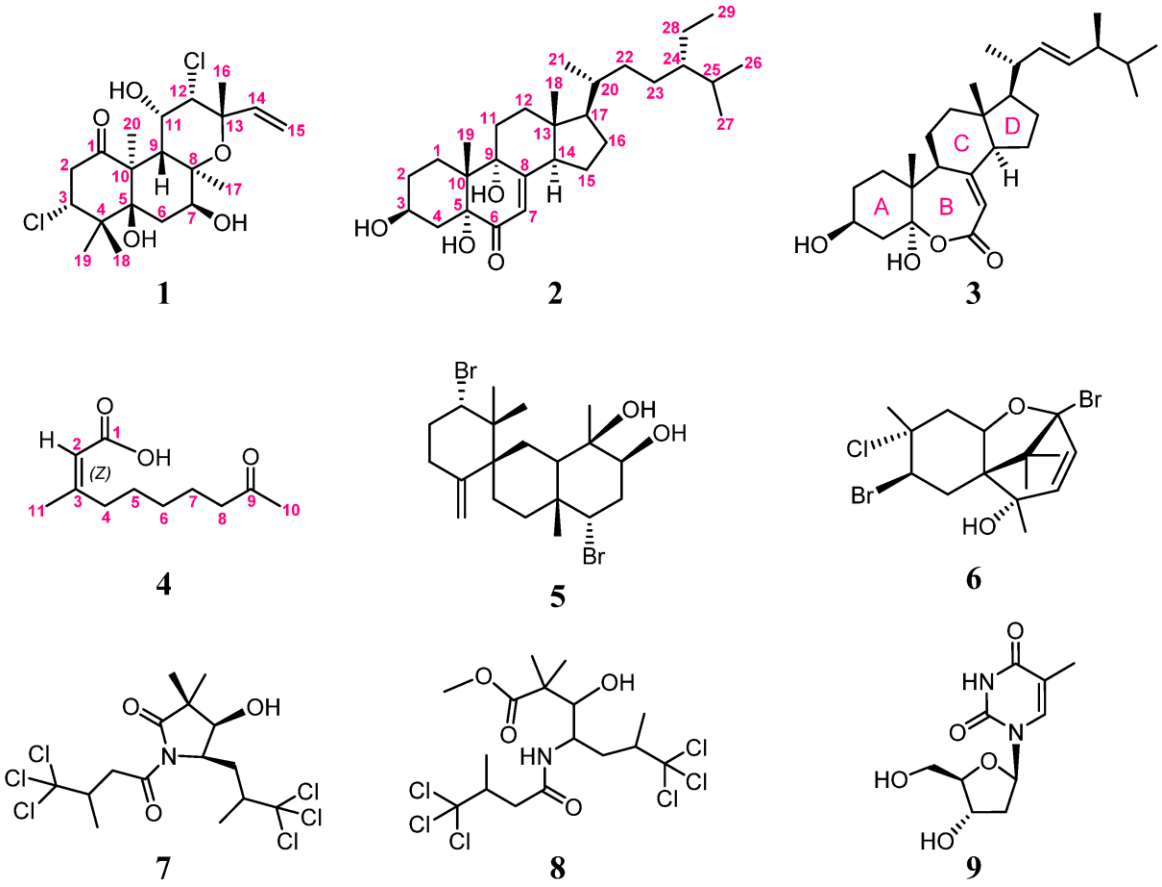


Figure 2. Chemical structures of some compounds of the Red Sea Sponge Spongia sp. (Chi-Jen et al., 2022).

Another example of Porifera being able to inhibit bacterial growth included two irciniid sponge species, *Sarcotragus spinulus* and *Ircinia variabilis*, which were studied for their biotechnological potential. Two hundred and seventy nine. isolates were found from 13 specimens that had patterns of dependency within the bacterial genomes upon the sponge species; 12% were effective against *Staphylococcus aureus* and 28% were active against *Escherichia coli*. The more active isolates included *Vibrio*, where 84% were active against *Escherichia coli* and 38% were active against *Staphylococcus aureus*.

These findings suggested a favorable source of metabolites from these genomes and metabolomes for antibacterial activity (Esteves et al., 2013). It was concluded that metabolomic

studies could be used to understand changes in the metabolic pathways due to natural compounds. This will help detect metabolites when there are small quantities present so that unknown metabolites can be discovered to be used for bioinformatic tools. Metabolite production then can be optimized for bioactive compound extraction and enhance yields (Esposito et al., 2022). This leads to yields that could make future medications easier to produce.

3.3.1.2 Anti-fungal Activity

Chemical modifications of compounds in Porifera can also serve as a tool for developing more effective antifungals. Sesquiterpenoids chemicals including (+)-curcuphenol and (+)-curcudiol were isolated from the Caribbean sponge *Didiscus oxeata.* These chemicals were then tested against *"Absidia ramosa, Aspergillus niger, Botrytis cinerea, Cladosporium cucumerinum, Fusarium oxysporum, Penicillium expansum, Rhizopus oryzae,* and *Trichoderma harzianum[...]"* (Gaspar et al., 2004, par. 5). It was found that the human pathogenic fungus *T. mentagrophytes* had inhibited growth from (+)-curcuphenol but it was less effective than commercial products. However, these commercial products have flaws including toxicity and resistant strains. The (+)-curcudiol was not as effective in comparison and this suggested that the extra double bond in the (+)-curcuphenol was needed for antifungal activity (Gaspar et al., 2004). Understanding these structure activity relationships will be beneficial to form more effective ways for developing a new antifungal medication.

3.3.2 Antitumor Activity

3.3.2.1 Breast Cancer

Antibiotic resistance is not the only type of resistance in medicine. For instance, even though there are advanced treatments options, drug resistance remains a problem in cancers such as breast cancer. Efforts have been made to find more effective drugs. Sponge species present

primarily in Indonesian territory have been examined for anti-tumor activity using the ethanol extract on breast cancer cells. The Porifera were collected by scuba diving 10 meters deep at Pramuka Island north of Jakarta, Indonesia and were identified visually and confirmed at the Department of Marine Science and Technology (Bashari et al., 2019).

The ethanol extract of these Porifera was tested for cytotoxic activity using a 3-2,5 diphenyl tetrazolium bromide (MTT) assay from Sigma-Aldrich, USA. The data revealed promising anti-tumor activity as the ethanol extract prompted breast cancer cell death that depended on the dosage. This study's extract also revealed that the more aggressive breast cancer cells were more sensitive to the extract (Bashari et al., 2019). This means that not only could these Indonesian sponges be used in breast cancer, but also other cancers such as leukemia and cervical cancer as well. Moreover, since the breast cancer cells that were aggressive were more sensitive to the treatment this could even be a better form of treatment than traditional forms. This extract also degenerates spheroids, induces apoptosis in aggressive cells, inhibits cell migration, and produces synergistic antitumor activity with conventional anti-breast cell agents such as doxorubicin and paclitaxel. Others have shown this species to have a cytotoxic effect on leukemic cells and cervical cancer. (Bashari et al., 2019).

Stylissa cateri used for anti-tumor activity (Bashari et al., 2019).

Additional studies conducted to find treatments for breast cancer using Porifera included a study using different concentrations of methanol extract from the marine sponge *Geodia cydonium.* These extracts were tested on human breast normal and cancer cells. The extract did not have a cytotoxic effect on either cell, but it did decrease levels of vascular endothelial growth factor and five proinflammatory cytokines. There also was NF-kB inactivation. This revealed an additional potential for future drug discovery for breast cancer (Costantini et al., 2015). The reason these results were beneficial was because NF-kB is a signaling pathway that can become excessively activate in tumor tissues (Xia et al., 2018).

3.3.2.2 Pancreatic and Colon Cancer

Other cancers that could be treated from using sponge biomaterial include pancreatic cancer and colorectal cancer. Since it was possible for the chemical Manzamine A, which is a sponge-derived beta-carboline-fused pentacyclic alkaloid, to have anticancer activity on cancerous pancreatic cells, colorectal cancer cells were investigated. This study examined reduced cell proliferation for colorectal cancer cell lines and gene expression with micro assays. The results revealed that induced cell cycle arrest at G0/G1 phase occurred by inhibition of

cyclin-dependent kinases using p53/p21/p27 and this activated apoptotic cell death through mitochondrial membrane potential depletion (Lin et al., 2018). G0 is the phase in the cell cycle that is considered the resting state or gap phase and G1 is when cells prepared for cell division otherwise known as interphase (Giglio & Gilbert, 2014). Therefore, since the cell cycle can be arrested prior to cell division, it can prevent uncontrolled cell replication of the mutated cancer cells and prevent tumors from forming.

Manzamine A also abolished epithelial-mesenchymal transition processes. Therefore, Manzamine A may serve as an anticancer drug treatment for metastatic colorectal cancer as well (Lin et al., 2018). The reason abolishing epithelial-mesenchymal transition processes is important is because it is what causes cancers to become more aggressive since it allows for motility of the cells and causes therapeutic resistance (Caja & Tan, 2018).

3.3.3 Antiviral Activity

Antiretroviral therapy is the current treatment for viruses like the human immunodeficiency virus-1. It uses drugs that decrease the viral load and increase $CD4^+$ T cell count in patients (Serna-Arbeláez et al., 2021). A $CD4^+$ T cell is a helper T cell that is a white blood cell that activates the other immune system cells such as B cells, which create antibodies (Britannica, 2023). However, when people cannot adhere to this treatment this can increase viral resistance to antiretroviral drugs and increase drug-resistant strains that are transmitted. Therefore, alternatives are necessary and are being sought in natural products (Serna-Arbeláez et al., 2021).

Antiviral natural products include zidovudine, which is an arabino nucleoside derivative of the Caribbean marine sponge called *Tectitethya crypta,* which prevents reverse transcriptase of the virus (Sagar et al., 2010). Reverse transcriptase is an enzyme that encodes retrovirus RNA

which can cause diseases in humans including the human immunodeficiency virus (Morier, 2023). Another notable antiviral from this sponge is Ara-A (vidarabine). The antiviral allows for DNA polymerase synthesis of herpes, vaccinia, and varicella zoster viruses to be inhibited (Sagar et al., 2010). It was not understood whether these products performed better compared to the current medications, yet they do provide an alternative for the drug-resistant strains.

Tectitethya crypta used for antiviral activity (TSG: Tectitethya crypta, 2023).

3.3.4 Anthelminthic Activity

A study on Porifera from Australian waters has revealed anthelminthic activity. In this study, sponge extracts were made as well as chordate and coral extracts. There was an analysis of chemicals that revealed the Porifera had a rich source of natural compounds with nematocidal and nematostatic properties. For example, the sponge species *Phyllospongia* was potent in the early larval stages of *H. contortus,* which is a nematode. It was not known whether this was due to simple molecules of the extracts or their microbial symbionts. Since Porifera specifically were the most noted, it was suggested that a more in-depth chemical investigation was needed as well as their cytotoxic tendencies in mammalian cells (Taki et al., 2021).

3.3.5 Hepatitis C Activity

Two known polybrominated diphenyl ethers were isolated from the Indonesian marine sponge *Lamellodysidea herbacea.* A range of bioactivities from organic crude extracts were found. This included a strong deterrent against the pufferfish *Canthigaster solandri*, potent inhibition of human pathogens like fungi and bacteria, and inhibition of the hepatitis C virus. There also was a higher fish deterrent in the second ether compared to the first due to a bromine atom and the second ether only had potent inhibition against gram-negative bacteria *Rhodotorula glutinis.* The first ether had greater inhibition towards more human pathogenic bacteria and fungi. The deterrence against bacteria and fish Porifera may indicate why Porifera can live in shallow water and be present throughout Indonesia and the Indo-Pacific. Some controls in this study included drug controls, positive controls such as gentamicin, and control of fish pellets to determine chemical defenses (Faisal et al., 2021).

3.3.6 Immunosuppressive Activity

Immunosuppressive properties are present in the Caribbean sponge *Plakortis simplex.* These properties were suggested to be used for immunosuppressive drugs. This is because marine sponges are a rich source of unique glycolipids that are active in animal immune systems. The new glycolipids simplexides have been isolated from this species and their structure has been determined using spectroscopic data as well as microgram-scale chemical degradation. These simplexides are long-chain secondary alcohols that are glycosylated by a saccharide chain. Simplexides inhibit the proliferation of activated T-cells using a non-cytotoxic mechanism. Therefore, this simple model of molecules can be used to design immunosuppressive drugs (Constantino et al., 1999).

3.3.7 Anti-inflammatory Activity

Fractions and methanol extracts of the sponge *Haliclona cratera w*ere used to test for antioxidant and anti-inflammatory activity. This species was used because it belongs to a genus known for having a reliable source of secondary metabolites, known to have antimicrobial, anti-inflammatory, and cytotoxic compounds. This extract was also used to check for cytotoxicity. An enzyme-linked immunosorbent assay was utilized to investigate the inflammatory mediator's levels. High performance liquid chromatography mass spectrometry was also utilized for the fractions of the sponge compound. The extracts had good bovine serum albumin denaturization inhibition but not good antioxidant activity. The viability of the sponge extract was good as well, which revealed it had cytotoxic nature. The fractionated compounds lowered inflammation that was induced by carrageenan, but this was more notable for the in vitro group (Kumar, 2022). Therefore, these compounds have not been developed to the point where they can be used in humans or animals. However, in the future the fractions of sponge that were cytotoxic in nature may be able to be used for anticancer agents and the anti-inflammatory activity may be beneficial to diseases that have chronic inflammation (Kumar, 2022).

Regarding chronic inflammation, a study on the Red Sea marine sponge *Spongia* sp. revealed that inhibition was present toward the superoxide anion generated from the sponge's isolated compound 8 (7,7,7-trichloro-3-hydroxy-2,2,6-trimethyl-4-heptanoic acid methyl ester) (Chi-Jen et al., 2022). It is important to note that compound 8's ability to inhibit superoxide anion generation can allow a way to fight other prevalent diseases. This is because as knowledge begins to increase on this anion, its understood role in increasing the severity and progression of disease is also being unveiled. This includes chronic inflammation, but also cardiovascular disease, and cancer malignancy. This is because all these medical concerns have intracellular and

extracellular excess superoxide anions that prevent cellular homeostasis (Varela-Chinchilla & Farhana, 2022).

3.3.8 Antimalarial Activity

Manzamine A is a beta-carboline alkaloid in marine sponges that prevents the growth of *Plasmodium berghei* inside a rodent. All the asexual erythrocytic states were inhibited after injecting manzamine A into the infected mice. This allowed for 40% recovery two months after one injection. This suggests that Manzamine A may serve as a new antimalarial agent (Ang et al., 2000).

3.4 Synthesizing Chemicals Analogs of Porifera for Medicinal Properties

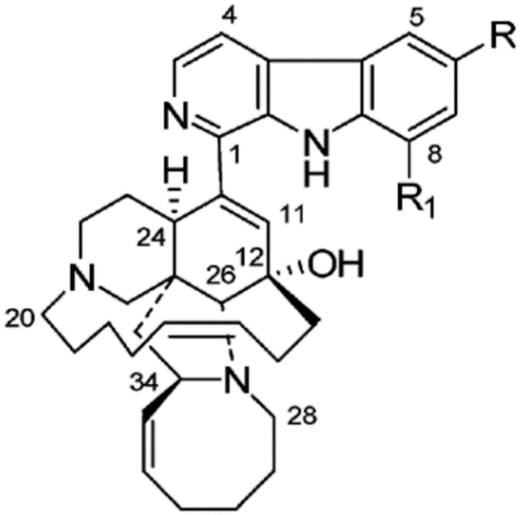
3.4.1 Manzamines

There are several different forms of manzamines, which are present in about 15 to 16 sponge species that primarily come from Indonesia. This includes the *Acanthostrongylophora* sponge and *Haliclona* sponge (Peng et al., 2009; Rao et al., 2006). They are characterized by a unique 5-, 6-, 6-, 8-, and 13-membered heterocyclic ring system coupled to a β-carboline moiety. They possess antimicrobial, antiparasitic, anti-neuroinflammatory, pesticidal, antimalarial, antifungal properties, and provide potential treatment for Alzheimer's disease (Peng et al., 2009; Rao et al., 2006; El Sayed et al., 2001).

To create a pharmaceutical drug with some of these benefits, analogs have been developed and the effect on activity based on the structure of the analogs are being evaluated. For example, when 8-hydroxymanzamine A underwent a reduction where the C-32 double bond in the eight-membered ring of 8-hydroxymanzamine was removed, activity decreased. However, when a reduction of the analog was completed and a double bond at C-15 was removed this increased activity. These results indicated that the double bond at C-32 is important for activity (Peng et

al., 2009). Therefore, the more modifications and comparisons of the effect on activity, the more therapeutic value that will potentially be added.

Other significant differences that were compared included 8-hydroxymanzamine A with oxa-derivatives. It was found that the oxa-derivatives have less activity. This indicates a role from either the C-12 hydroxyl group, the C-34 methine group, or the conformation of the lower aliphatic rings in the compounds that were not the oxa-derivatives. Moreover, the change of the hydroxyl substitution from the C-8 position of the β-carboline moiety to the C-6 position also decreases activity (Rao et al., 2006). These references to the carbon number and functional groups can be seen in the 8-hydroxymanzamine chemical structure below:



$R = H; R_1 = OH$

Figure 2. 8-hydroxymanzamine A (El Sayed et al., 2001)

A benefit to using manzamines for malaria is that they perform better than traditional medications, such as chloroquine and artemisinin. This is because they have more activity and less cytotoxicity. They also prevent the risk of drug resistance. This was demonstrated in mice

where one intraperitoneal dose of 100 micromoles per kilogram of 8-hydroxymanzamine A was used on the malarial parasite *Plasmodium berghei.* Intraperitoneal means that the dose was injected into the peritoneal cavity, which is in the umbilical region. The results revealed an increase in the number of days survived. With the manzamine dose the mice survived 9 to 12 days, with chloroquine the mice survived 6 days, and left untreated the mice only survived 2 to 3 days. The advantage to being able to provide only one dose to future patients with malaria is that there will be less of a risk for drug resistance since patients do not risk the option of being non-compliant with a one-time treatment. Three doses were also given using 50 micromoles of manzamine per kilogram and this allowed for the parasite to be completely eradicated, revealing that the doses were considered curative in the mice (El Sayed et al., 2001).

Another aspect to look at when analyzing chemical analogs for better treatment options is the enantiomeric ratio, otherwise known as the racemic mixture. Enantiomers are a way to describe two chemical analogs that are non-superimposable mirror images, which means that they are alike in every way except for their optical rotation. This can be illustrated with your left and right hands which are typically mirror images of each other. They are considered enantiomers as they are non-superimposable because when one places their hands on top of the other the thumbs, for instance, are on opposite sides (Jones, 2023). Knowing whether structures are enantiomers or not is important because one enantiomer of a structure may perform better, or a certain racemic ratio may increase the therapeutic effectiveness of a medication. This is because it can allow for the opportunity to separate the toxicity and efficacy in a medication (Williams & Lee, 1985).

When examining the structure-activity relationships of medicinal compounds from Porifera like manzamines it was found that the free base –(+)-8 hydroxymanzamine A and

Manzamine F were enantiomers and not diastereomers. This was found when these compounds were compared using nuclear magnetic resonance, optical rotation, melting point data, and column chromatography. Based on these test results the only difference between the two compounds was the optical rotation, which reveals that they were mirror images that were non-superimposable (El Sayed et al., 2001).

3.5 Medications in their Final Clinal Stages

Several products have reached the final stages of clinical trials such as ara-A (vidarabine), which is an anti-viral drug that works against herpes simplex encephalitis virus. Also, manzamine A, which works against malaria, tuberculosis, and human immunodeficiency virus, has been selected to reach the final stages of clinical trials. Other products in these stages include: lasonolides, which is an anti-fungal, and psammaplin A, which is antibacterial (Laport & Muricy, 2009). This reveals that futuristic treatments mentioned above not only are being pursued but are currently being developed for different illnesses in humans.

4: Porifera Collection

There is a concern that Porifera does not produce an industrial amount of these useful chemicals to create the drugs. Therefore, the main issue is that supplies are scarce to develop these new effective drugs, such as antivirals used in human immunodeficiency virus. However, Porifera farming, metagenomics, and microbial cultivation are being improved for more of these future drugs (Sagar et al., 2010). This involves developing methods for reaggregation, suitable environments for larval cultivation, and isolation of compounds to be used in the medications.

4.1 Porifera Cultivation

4.1.1 Porifera Aggregation

Cultivation systems for Porifera are being developed to produce valuable compounds for biotechnological processes. One of the ways this is implemented is through organotypic culture attempts, which mimic natural tissue structure. Mediterranean species *Chondrosia reniformis* has been used for sponge fragmentation for in vitro cultures. This is a common species in the Mediterranean that shows good cell reaggregation. After fragmentation, regeneration lasts several days with a result of an ovoid sponge body shape with a reduced aquiferous system. Cell proliferation was not shown until after it was cultured for 3 months. However, collagen production increased, and these fragments were cultured for 19 months (about 1 and ½ years). This can serve as a model system for other sponge cultures (Nickel & Brümmer, 2003).

Porifera are simple organisms and have an organization that is characterized by high plasticity of structure. One of the ways this is true is their plasticity allows sponge cells to reaggregate even after the tissue has become dissociated. A 2014 study analyzed the behavior of the dissociated cells in suspension and the mechanism and factors that allow for reaggregation. There also is a comparison of the rates and stages of reaggregation for different Porifera.

Additionally, there is a review of information on the histological structure of multicellular aggregated and how they undergo regenerative processes for the formation of normal Porifera (Lavrov & Kosevich, 2014).

4.1.2 Cultivation of Larvae

4.1.2.1 Laboratory Cultivation

A study was conducted on cultured sponge larvae with hopes that they would turn into juveniles. Efforts were made to enhance survival and development as well as to lower variations in the parameters of the cultures. The sponge larvae, for settlement success, needed to undergo morphological changes during metamorphosis, and each of the species underwent comparison when under the same conditions. Then the effects of water flow and food were tested when considering the survival and growth of the juveniles. Then as the concluding step, these juveniles were transplanted to the sea to compare results from the lab versus the sea. This study concluded that the sponge culture from larvae was a good method for sponge supply and that the laboratory culture using this method was preferred over sea cultures. This was to prevent biomass loss during early life stages (de Caralt et al., 2007).

4.1.2.2 Sea Cultivation

Regarding sea cultures, it was found that sea temperatures have started rising and due to this, there may potentially be effects on the dispersive larval phases of marine sponges affecting their adult population. For instance, planktonic larvae of a common Great Barrier Reef sponge called *Rhopaloeides odorabile* have been able to survive when exposed to temperatures over 9℃ over the annual maximum of 29℃. Normally planktonic larval duration is 54 hours (about 2 and a half days) at about 28 ℃ but this was reduced to 18 hours when these larvae were exposed to elevated temperatures at 32 ℃ to 36 ℃. Larvae survived up to 38 ℃ (Whalan et al., 2008). This

analysis of the larvae will be beneficial not only in controlled cultivation environments, but also to understand some of the effects of the rising sea temperatures on such valuable creatures as the sponge.

Other aspects of larval cultivation can include the pH of their aquatic environment. Increased carbon dioxide concentration in the atmosphere due to anthropogenic influences have led to ocean acidification. Ocean acidification has pronounced effects on sponges due to their low capacity for acid-base regulation. It was found in sponge larvae, which are considerably vulnerable, that exposure to a low pH of 5.8 to 6.5 affected the hatching rate. In addition, slight increases in pH resulted in larval developmental abnormalities. There was also a pH effect on larval settlement (Goodwin et al, 2013). Therefore, ocean acidification must be controlled to allow for greater cultivation of sponge larvae.

4.2 Isolation of Porifera Medicinal Compounds

One possible approach to the isolation of compounds in Porifera first involves collecting the sponges and then bleaching part of them. This digests the sponge material, and the spicule preparation is placed on a microscope slide. This was to identify them as stated previously. Then to extract the bioactive compound, the sponge's material was placed in a blender with ethyl acetate and dichloromethane: methanol (1: 1). This was agitated for 48 to 72 hours and was filtered using filter paper. Then the extract was concentrated using a rotary vacuum evaporator. The extracts were then tested for their antimicrobial activity using a standard agar well diffusion assay on the Muller Hinton Agar and was inoculated with *Pseudomonas aeruginosa* and *Clostridioides difficile*. Antibiotic disks were used for positive control (Kibungu et al., 2021).

To isolate other compounds from the same sponge is possible by placing them in a different solution. This allows for different compounds to be extracted. For instance, in the effort

to isolate manzamines a study developed four new analogs and reproduced thirteen known compounds. They did so by developing extracts of the sponge *Acanthostrongylophora* with acetone at room temperature and treating the concentrated aqueous solution with chloroform. Then this extract underwent silica gel vacuum liquid chromatography and was eluted using variations of hexane, hexane and acetone, chloroform and methanol, and methanol to collect 15 major fractions. The metabolites isolated were identified using methods such as thin line chromatography (Rao et al., 2006). Therefore, several new compounds could be isolated from the same species in the future as the chemicals used to elute the compounds are adjusted.

Adjustments to the isolation procedure can also allow for greater yields of the desired compound. For example, there is an isolation procedure for collagen of the marine sponge *Chondrosia reniformis* that was altered and now yields 30% of collagen. Dispersing of collagen was benefited when dilute basic mediums were utilized. These acid-base properties were investigated using titration. The sponge extracts were incorporated and compared to extract free analogues in a collagen skincare product based on effect to biophysical skin parameters. Neither preparation influenced the skin pH and hydration only slightly improved. However, all formulations of the extract had a significant increase of lipids (Swatschek et al., 2002). Although this isolation procedure of gathering the collagen from the sponge only improved hydration slightly compared to other collagen products this compound can still offer another outlet to gather collagen products where the yield is attempting to be increased from each extraction. Moreover, as the analogs from this compound continue to be analyzed, a new analog of the sponge collagen may perform better than traditional products.

4.3 Restoration and Conservation Efforts

Coastal ecosystems are affected by anthropogenic forces that degrade habitats and ecological functions. This lowers the availability of the products and services that humans rely on. In the past few decades, efforts have been made to restore these habitats. This includes restoration of the hard-bottom sponge communities in Florida Bay. During these efforts, insights were found into the basic sponge biology and ecology such as their growth rates and sedimentation rates (Butler et al., 2021).

With these insights, responsive actions have increased to reestablish these communities. Some of the degradation has been due to pollution, destruction, and resource overexploitation. One of the ways by which this was counteracted by sponge cutting and placing portions in degraded areas. It was found this method was successful since the transplanted sponges grew quickly due to less competition for the planktonic food resources (Butler et al., 2021).

5: Original Porifera Research Conducted

5.1 Introduction

At Gardner-Webb University, the 2022 study above inspired thesis advisor Dr. David Campbell and honors student Emma Rayfield to perform qualitative research on the antibacterial activity of Porifera. The purpose of this research was to see if there was any inhibition of bacterial growth from sponge and coral crude extracts.

5.2 Experiment

First the specimens were collected by Dr. David Campbell, and these included a sponge from Lake Waccamaw, coral (*Leptogorgia virgulata*) from Waties Island beach, and *Spongilla* ordered from Carolina Biological. Then these specimens were preserved. The sponge from Lake Waccamaw was preserved in ethanol and frozen. The coral from Waties Island beach was sun dried and kept at room temperature. Lastly, the sponge from Carolina Biological was preserved in ethanol. The specimens were preserved until they were to be used to create an extract.

The extracts were developed by taking each specimen and using a razor to cut smaller fragments. Then fragments were placed in tubes, which included one tube with a milliliter of water and another tube with a milliliter of ethanol. Therefore, there were two extracts to be made for each preservation method used for the specimens. Each tube was centrifuged for about 12 minutes. Then after a few days, they were recentrifuged and the top layer of liquid was pipetted out and placed into new tubes leaving behind the layer of visible fragments.

Once the extracts were made, they were kept at room temperature until they were to be tested for their zones of inhibition for bacteria. After the extracts were made, Mueller Hinton plates were inocculated with bacteria by streaking them. The nine Mueller Hinton plates were incubated with *Staphylococcus simulans* and *Staphylococcus epidermis* at 37 ℃ and

Enterobacter at 25 ℃. Each bacteria had three Mueller Hinton plates. Once there was a sheet of bacterial growth across the Mueller Hinton plates, each plate was divided into quadrants and labels with abbreviations for each of the extracts, which are defined in table 1.

Then a sterile disc six millimeters in diameter was placed in each of the extracts and placed with their corresponding labeled quadrant on the plates. Three sterile discs of positive controls were used too, including the antibiotics kanamycin, tetracycline, and chloramphenicol. A sterile disc was also dipped in ethanol to serve as a negative control. The plates were then incubated for a few days and then the zones of inhibition and notable zones of contamination were measured in millimeters. The results are shown in the appendix, including Table 1 and the images of the incubated Mueller Hinton plates with the extract.

5.3 Results and Discussion

The extracts that were able to perform better than both a positive control and a negative control included the *Spongilla* specimen that was extracted with ethanol. When measuring the zones of inhibition *Spongilla* was able to inhibit 16 millimeters, which was greater compared to the negative control ethanol of 13.5 millimeters inhibition and the positive control tetracycline of 15 millimeters inhibition for the bacteria *Enterobacter.* Moreover, the *Spongilla* that was extracted with ethanol, the coral that was extracted with ethanol, and the species from Lake Waccamaw, also performed better than the antibiotic tetracycline and negative control for the bacteria *Staphylococcus epidermis.*

There were also discs that appeared to allow the bacteria to thrive better when compared to the rest of the Mueller Hinton plates. This could have been due to contamination in the extracts, or the specimen having a property that allows microorganisms such as bacteria to thrive

better. This included the *Spongilla* extracted with water, the coral extracted with water, and the sponge from Lake Waccamaw extracted with water.

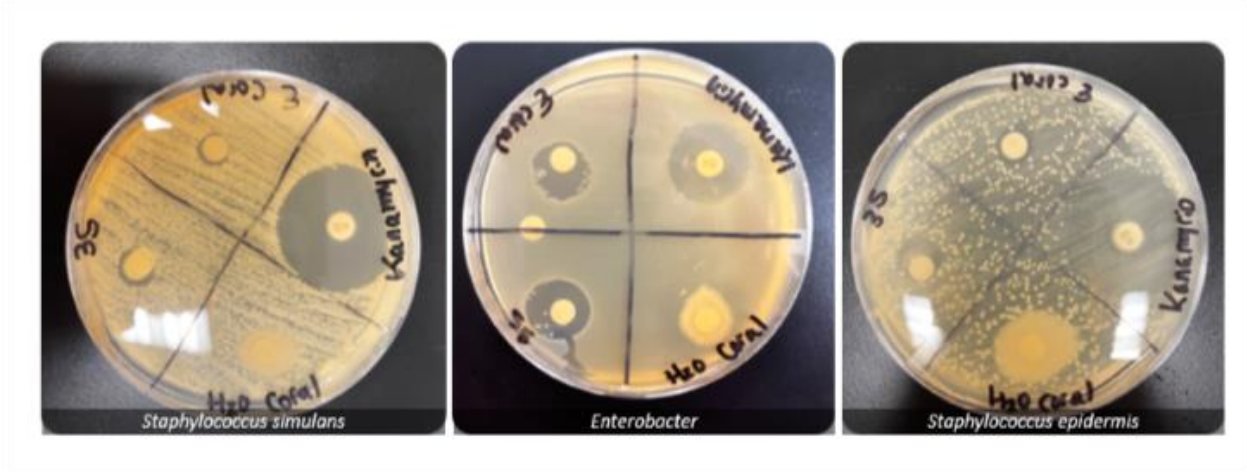
Sources of error could have included not removing all the excess condensation of the Mueller Hinton plates after incubation causing the liquid to cross contaminate the quadrants of the plates, contamination to the extracts during development, unsterile discs, inaccurate measurements of the zones of inhibition, and not allowing the *S epidermis* to finish incubating to create a full bacterial sheet to accurately measure zones of inhibition. In addition, there was not a statistical analysis completed for this experiment and, therefore, no statistically significant values were found. Repeating the experiment would provide greater accuracy.

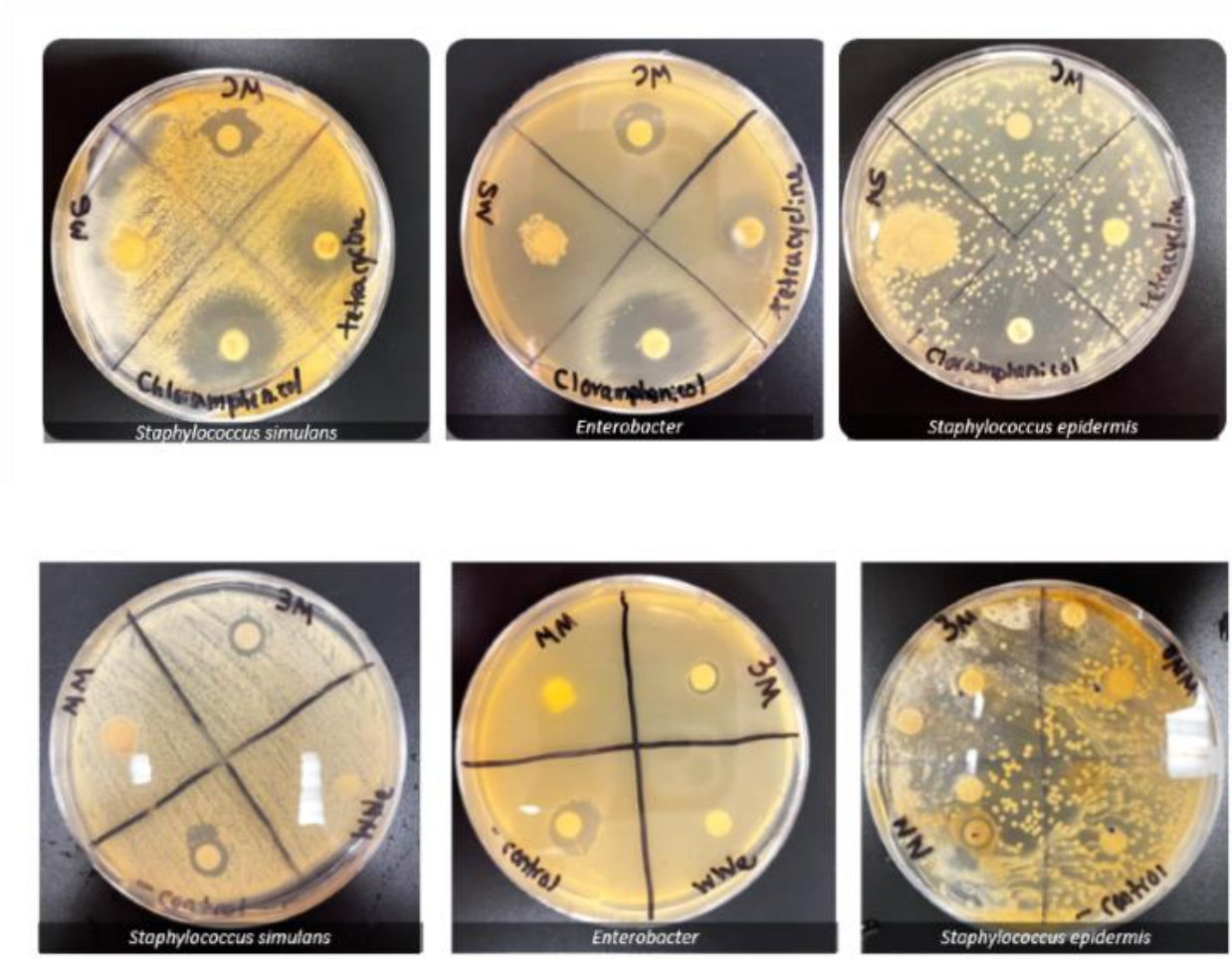
5.4 Conclusion

There were signs of inhibition in which the Porifera and coral performed better than the antibiotic tetracycline and the negative control of ethanol revealing the implication that these specimens were capable of inhibiting bacteria. However, this experiment would need to be repeated for statistical data.

5.5 Appendix

5.5.1 Mueller Hinton Plates After Crude Extract Incubation





5.5.2 Tables of Coral and Porifera Sources and Inhibition Data

Table 1: Coral and Porifera Sources

Sample Label On Mueller Hinton Plates	Specimen	Source	Preservation	Extracted Using
SE	Spongilla	Carolina Biological	Room Temperature Water	Ethanol
WE	Unknown Porifera	Lake Waccamaw	Room Temperature Ethanol	Water
WC	Unknown Porifera	Lake Waccamaw	Froze	Ethanol
H20 coral	Unknown Coral	Waties Island beach	Sun Dried	Water
e coral	Unknown Coral	Waties Island beach	Sun Dried	Ethanol
SW	Spongilla	Carolina biological	Room temperature Water	Water
WWe	Unknown Porifera	Lake Waccamaw	Room temperature Ethanol	Water
WW	Unknown Porifera	Lake Waccamaw	Froze	Water
kanamycin	Antibiotic	BBL Company through Carolina Biological	Chilled	
tetracycline	Antibiotic	BBL Company through Carolina Biological	Chilled	
chloramphenicol	Antibiotic	BBL Company through Carolina Biological	Chilled	
ethanol	Alcohol	Gardner-Webb University Lab	Room Temperature	

Table 2: Coral and Porifera Zones of Inhibition and Contamination

Sample Label on Mueller Hinton Plates	Zone of Inhibition (mm)			Notable Zones of Contamination (mm)		
	Enterobacter	*Staphylococcus epidermis*	*Staphylococcus simulans*	*Enterobacter*	*Staphylococcus epidermis*	*Staphylococcus simulans*
SE	16 mm	11 mm	8 mm			
WE	8.5 mm	error	9 mm			
WC	13 mm	9 mm	17 mm			
H20 coral	6 mm	6 mm	6 mm	15 mm	21 mm	15 mm
e coral	14 mm	9 mm	7 mm			
SW	6 mm	6 mm	6 mm	13 mm	20 mm	13.5 mm
WWe	6 mm	6 mm	6 mm		9 mm	
WW	6 mm	6 mm	6 mm	9 mm	13 mm	9.5 mm
kanamycin	22 mm	35 mm	31 mm			
tetracycline	15 mm	6 mm	15.5 mm			
chloramphenicol	25 mm	27 mm	25 mm			
ethanol	13.5 mm	6 mm	10 mm			

Final Remarks

Porifera, although simple organisms, are durable creatures that have global diversity as one of the oldest groups or organisms. Their ability to survive under harsh conditions such as in the ocean and ability to defend themselves against predators despite being sessile really reveals their undeniable potential. Porifera similarities with humans can be of benefit when seeking their medicinal uses. It is utterly amazing that sponges possess antimicrobial, antitumor, antimalarial properties and many other properties that allow for medications to be developed for diseases such as malaria and tuberculosis. Moreover, the ability for the sponge to reaggregate and chemists' ability to develop analogs of their chemical compounds reveal that conservation efforts can be maintained. This is because sponges and their chemicals can be reproduced without continuously going back to the ocean. Therefore, sponges are highly diverse, adaptable, possess lots of medicinal potential, and can be easily cultivated to sustain the earth, which makes them a wonderful candidate for futuristic treatments.

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