Old Dominion University

Commented [JC1]: The summary of the revisions to my paper are on the last page. Page 22

Marine Invertebrates: An Untapped Pharmacopeia

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BIOL 405W: Biology Seminar

Dr. Christopher Osgood

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Abstract

Marine invertebrates are one of the major groups of organisms, which can be classified under the major taxonomic groups of Arthropoda, Cnidaria, Mollusca, Porifera, Echinodermata, and many other minor phyla. These animals have been used in a plethora of research and resulted in countless medical breakthroughs over the years. But, with marine invertebrates making up such a large portion of life in the ocean one would think even more breakthroughs should be possible, are marine invertebrates being underutilized? In this paper we will look at just how wide spread marine invertebrates are around the world and some of the environments the can live in along with secondary metabolites (natural products) produced from marine invertebrates along with how these useful natural products can be found and look at what medical insight these products have produced, what medicine and medical procedures these products are used in and future potentially interesting marine invertebrates to keep an eye on for potential breakthroughs and more medical insight. Marine invertebrates are incredible organisms in a multitude of ways and this papers aim is to look at marine invertebrates and the natural products they produce and see if marine invertebrates are being underutilized for potential medical incite and breakthroughs because one of these animals could lead to the next breakthrough in the medical field whether it be through anti-cancer drugs or stem cell research. The next big breakthrough is just waiting to happen, and it will lead to a better future for humanity.

Introduction

The marine environment serves as a significant reservoir of biodiversity with the richest source of primary and secondary metabolites (Jensen 2019). Marine invertebrates are a diverse group widely distributed throughout the ocean from the intertidal zone to deep ocean ecosystems. Marine invertebrates make up around 92% of all marine animal species and are classified into different taxonomic groups such as Annelida (marine worms), Cnidaria (corals, jellyfish), mollusks (oysters, squids, mussels, crabs, and crayfish), echinoderms (starfish, sea cucumbers and sea urchin), and Porifera (sponges). An invertebrate is any animal that lacks a spinal column. Marine invertebrates are incredibly diverse and come in many different shapes and sizes from microscopic Tardigrades to Giant Squids. Marine invertebrates are found almost everywhere there is water on the planet and can survive some of the most extreme conditions this planet has to offer. With marine invertebrates making up such a large proportion of marine life on Earth there are limitless possibilities of potential biological products that could be useful for potential medical incites and therapies. The 1970s were really the "golden age" of marine natural products. But marine invertebrates were used in medicines even before the times of the ancient Greeks (12^{th} – 9^{th} centuries BCE). The reason marine invertebrates are of so much use is because of secondary metabolism or the natural products that these marine invertebrates produce and the way these secondary metabolites are identified as useful or not is through the field of natural products chemistry which looks at living organisms and what molecules they can make outside of primary metabolism and these molecules are called secondary metabolites or natural products. Marine invertebrates and their natural products are used in a variety of ways within the medical field such as anticancer and antioxidants, antimicrobials, and for collagen and gelatin. Since the revolutionary Limulus Amebocyte Lysate (LAL) test was developed using horseshoe

crab blood in the 1970s, it seems little has been done to explore the vast potential invertebrate studies have for medical science.

How Widespread Marine Invertebrates are Around the World

Invertebrates are a paraphyletic group of animals that neither possess nor develop a vertebral column. Invertebrates make up about 95% of all animal species on Earth. Invertebrates can also survive some of the most extreme conditions Earth has to offer from extreme heat to extreme cold. The *tardigrades* also known as the water bear can even survive the vacuum of space which is the only known animal on Earth that can do so. Making up over 92% of life in our oceans are marine invertebrates and can inhabit every zone in the water column, with contributions ranging from ecosystem functioning to socioeconomic development. Marine invertebrates can be put into different taxonomic groups such as Annelida (marine worms), Cnidaria (corals, jellyfish), mollusks (oysters, squids, mussels, crabs, and crayfish), echinoderms (starfish, sea cucumbers and sea urchin), and Porifera (sponges).

Marine invertebrates can be found basically anywhere there is water from freshwater streams all the way to the deep ocean and can survive some of the most extreme conditions Earth has to offer. Some invertebrates (*Tardigrades*) can even survive the vacuum of space. With all this diversity in species the potential for medical breakthroughs is endless.

Natural Products Chemistry and Secondary Metabolism

The marine world, due to its phenomenal biodiversity, is a rich natural resource of many biologically active compounds such as polyunsaturated fatty acids (PUFAs), sterols, proteins, polysaccharides, antioxidants, and pigments (Senthilkumar 2013). Primary metabolites include amino acids, simple sugars, nucleic acids, and lipids. Secondary metabolites, such as alkaloids, terpenoids, and other compounds, have known bioactivities and biological functions. Thus, marine invertebrate-derived compounds may be useful as an alternative medicine for various diseases (Senthilkumer 2013). Figure 1 provides an overview of this review.

Natural products chemistry in basic terms is the study of substances produced by living organisms. The research done by natural products chemistry can help improve the understanding of biological processes and can be used to identify compounds that might lead to the development of new drugs. Natural products chemistry looks at secondary metabolites produce by organisms to find potentially useful products being produced through secondary metabolism.

Secondary metabolites are compounds that are not required for the growth or reproduction of an organism but are produced to confer a selective advantage to the organism (Sanchez 2011). Secondary metabolites are derived from primary metabolites. Secondary metabolites are built by taking intermediates from primary metabolism and incorporating them into much more complex molecules. So, nature has learned to steal these smaller molecules from primary metabolism and use these intermediates to build them into complex structures using enzymatic machinery. Secondary metabolites are produced by marine invertebrates. The functions of these products are ecological in nature and can be used for a variety of responses such as defense, nutrient acquisition, settlement cues, predator/prey interactions, food selection, mate recognition, and symbiosis (Jensen 2019). These natural products produced from secondary metabolites are what

have become nature's pharmacopeia and can be used in a variety of ways. See figure 2 for an illustration showing some secondary metabolites derived from marine animals.

Current Uses of Secondary Metabolites Derived from Marine Invertebrates

Systematic searches for new drugs have shown that marine invertebrates produce more antibiotic, anti-cancer, and anti-inflammatory substances than any group of terrestrial organisms. Starting in the early 1970s is when scientists really began going out and collecting marine invertebrates and thinking about what kinds of molecules do these marine organisms make and do these molecules have the potential to function as new medical drugs. Researchers will collect different types of marine invertebrates and extract these organisms and examine the extracts to figure out if any of the extracts have the potential for medical use.

Secondary metabolites have been found to have interesting applications other than just their well-known medical uses, such as antimicrobials. These alternative applications include antitumor, cholesterol-lowering, immunosuppressant, antiprotozoal, antihelminth, antiviral and anti-ageing activities.

The most well-known marine invertebrate derived product is the Limulus Amebocyte Lysate (LAL) test which was developed using horseshoe crab blood in the 1970s. The *Limulus* test is a test for detecting nano gram of bacterial endotoxins and it was invented by Levin and Bang based on their finding that a trace amount of endotoxin coagulates hemocyte lysate of the horseshoe crab, *Limulus polyphemus* (Iwanaga 2007). The gelation reaction has been widely employed as a simple and very sensitive assay method for the detection of bacterial endotoxins. The LAL test is the most widespread endotoxin test. The way this test works is by a reaction between LPS, and a substance (clottable protein) contained within amoebocyte cells derived from the blood of the horseshoe crab, as illustrated in Figure 3 (of which *Limulus polyphemus* is the most commonly used species, although other species, such as *Carcinoscorpius* and *Tachypleus* demonstrate the same effect) (Sandle 2016). The reaction of the horseshoe crab to

endotoxin (the formation of a clot) has been known since the 1950s. LAL is an aqueous extract obtained after lysis of blood cells (amoebocytes).

The LAL test has six protein components, designated coagulogen, proclotting enzyme, factor B, factor C, and factor G, all of which are closely associated with the endotoxin-mediated coagulation pathway, have been purified and biochemically characterized (Iwanaga 2007). The hemolymph clotting system in the horseshoe crab is similar to the blood coagulation system found in mammals, although the limulus clotting factors seem to be separate entities from the clotting factors in vertebrates. The sequential activation of the clotting cascade consists of at least four serine protease zymogens linked to gelation, which is comparable to that of the mammalian system. Furthermore, each activation of the zymogens mediated by LPS or (1,3)-β-D-glucan proceeds by limited proteolysis, releasing an activation peptide. The sensitive assay of pico to nano gram of bacterial endotoxins depends on the amplification system consisting of the sequential activation of the limulus clotting factors. This biochemical principle appears to be a basis for the Limulus test (Iwanaga 2007). The LAL test is revolutionary breakthrough and plays a crucial role in pharmaceutical microbiology.

Kunitz domain proteins are ubiquitous protease inhibitors and can be used in the development of pharmaceutical drugs. The first natural Kunitz domain protein (Conkunitzin-S1), which has only two disulfide bonds, was identified in the venom of the cone snail (*C. striatus*) which interacts with voltage gated potassium channels and acts as a neurotoxin (Ranasinghe 2013). This protein acts as a potent trypsin inhibitor as well as being a weak potassium channel blocker (Ranasinghe 2013). Isolated compounds from marine invertebrates have pharmacological activities and are helpful for the invention and discovery of bioactive

compounds, primarily for deadly diseases like cancer, acquired immunodeficiency syndrome (AIDS), and osteoporosis (Senthilkumar 2013).

Cell lysates are experimental tools used in laboratories for a plethora of applications in drug development, translational research, and even synthetic biology. As reagents, cell lysates are inexpensive, rarely interfere with detection assays, and are comprised of a set of components which can be defined in detail if experimentally necessary. Lysates of marine invertebrates including tiger prawn, lobster, and mud crab exhibited antibacterial effects against MRSA and E. coli K1 (Figure 4). This shows antibacterial uses for marine invertebrates and the products produced by them.

Potentially Interesting Marine Invertebrates

The *Turritopsis dohrnii* also known as the immortal jellyfish is a hydrozoan and is an animal about 4.5 millimetres wide and tall (making it smaller than most people's nail on their little finger) and was first discovered in the 1800's. It was 100 years later, in the 1980s, that their immortality was accidentally discovered. While aging affects most living organisms, the hydrozoan *Turritopsis dohrnii* is the only species able to rejuvenate repeatedly after sexual reproduction, becoming biologically immortal (Pascual-Torner 2022). *Turritopsis dohrnii* actives its immortality through a process known as transdifferentiation. The process of transdifferentiation occur when the jellyfish is physically damaged or experiences stress such as starvation. The transdifferentiation process is as follows; the medusa cells and polyp cells are different - some cells and organs only occur in the polyp stage, others only in the adult jellyfish. Transdifferentiation reprograms the medusa's specialized cells to become specialized polyp cells, allowing the jellyfish to regrow themselves in an entirely different body plan to the free-swimming jellyfish they had recently been before transdifferentiation had occurred. They can then mature again from there as normal, producing new, genetically identical medusae. So, in basic terms the process of transdifferentiation is when an adult cell, one that is specialized for a particular tissue, can become an entirely different type of specialized cell. This process can occur continuously under the right conditions making the jellyfish essentially immortal. If this process could be replicated within human cells it could lead to further developments in stem cell research and could be revolutionary for the medical field. See figure 5 for an illustration of the transdifferentiation process in the *Turritopsis dohrnii*.

Conclusion / discussion

Research on marine invertebrates have already benefited humanity in so many ways whether it be anti-cancer drugs, antibiotics, or just overall human health and it will only continue to improve overall human health, but more could be done. The possibilities of medical research derived from marine invertebrates are endless for this field of study and will benefit humanity in a myriad of ways. The future of marine invertebrate research within the medical field is endless and we have only scratched the surface of what is possible with marine invertebrates and the natural products obtained from them. With marine invertebrates encompassing such a large portion of life found in the ocean and the extreme diversity found within this group of animals, it is only natural that they would be “nature’s pharmacopeia”.

Primary Data

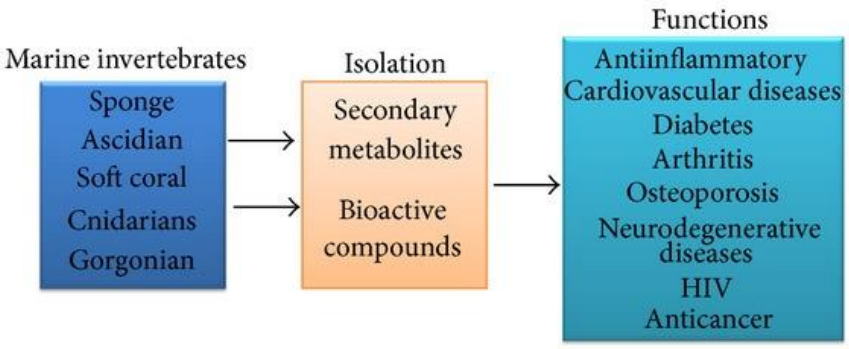

Figure 1: Summary of marine invertebrate natural products with anti-inflammatory and some chronic diseases (Senthilkumar 2013).

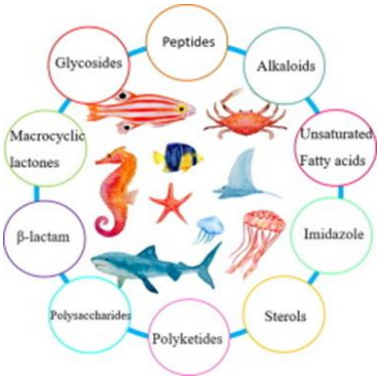

Figure 2: An illustration showing some secondary metabolites derived from marine animals (Lu 2021).

Figure 3: Image of the *Limulus* "horseshoe crab." (Sandle 2016)

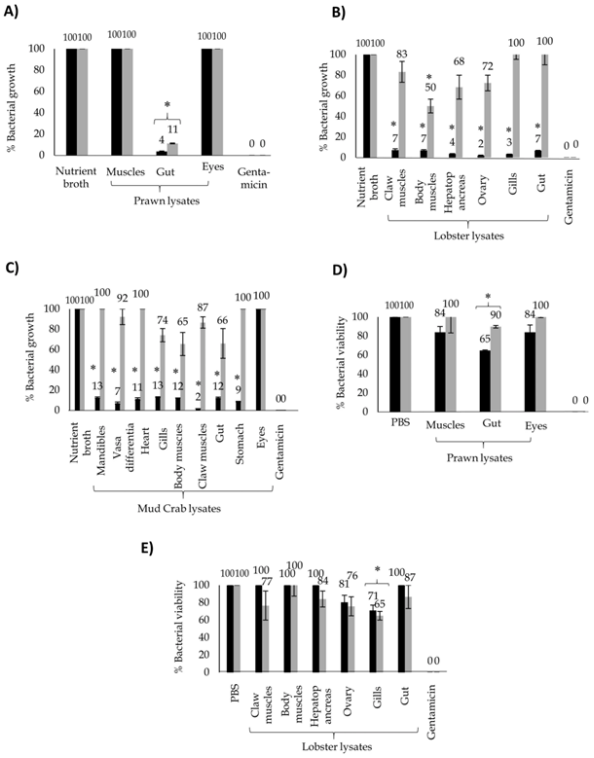

Figure 4: Lysates of marine invertebrates including tiger prawn, lobster, and mud crab exhibited antibacterial effects against MRSA (black bar) and E. coli K1 (grey bar). Briefly, bacteria were incubated with extracts and tested for antibacterial effects as described in "Materials and methods." * is $P \leq 0.05$ using Student's T-test, two-tailed distribution. A) Prawn gut extracts showed nearly 90% growth inhibitory effects against both bacteria. B) All the extracts of American lobster exhibited significant growth inhibitory activities particularly against MRSA, except muscle extract which exhibited significant activity against both bacteria. C) Mud crab extracts such as muscles, gut, stomach, gills, heart, and mouth part extracts possess nearly 90% growth inhibitory activity against MRSA; however, only gills, muscles, and gut extracts

exhibited nearly 25% of bacteriostatic activity against E. coli K1. D) Prawn gut extracts showed bactericidal activity against tested bacteria. E) Only lobster gills extract showed significant bactericidal activity against both bacteria (Ali 2021).

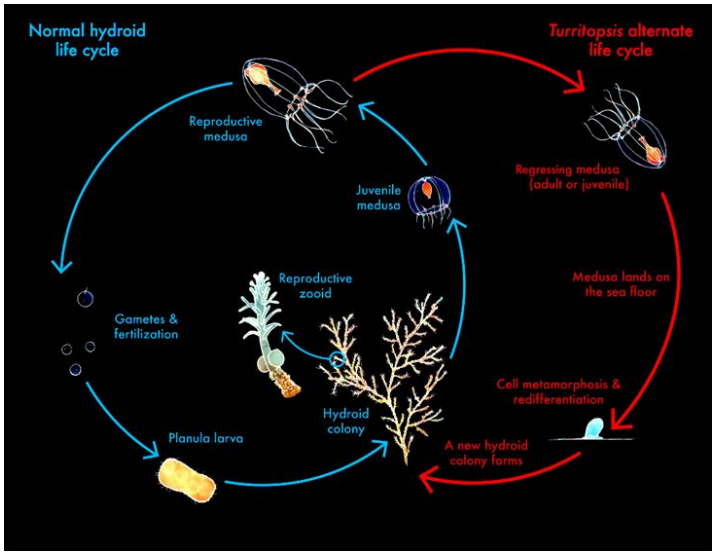

Figure 5: Shows an illustration of the transdifferentiation process in the *Turritopsis dohrnii* as compared to the normal life cycle of a hydrozoan (Miglietta 2022).

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The findings in the research demonstrated that invertebrates possess mechanisms to survive against pathogens in their natural habitat. This paper investigated 10 invertebrate species and found the most active ones were cockroach, centipedes, tarantulas, prawns, lobster, and mud crab. Future work is needed to determine antibacterial effects against a range of bacteria as well as in vivo testing of selected purified active molecule(s). Hence, future work will be an interesting aspect, which can act as a breakthrough in the development of potential antibiotic molecules from novel and unusual sources. (Ali 2021)

Almeida, M., Rui L. Reis, Tiago H. Silva, Marine invertebrates are a source of bioadhesives with biomimetic interest, Materials Science and Engineering: C, Volume 108, 110467, ISSN 0928-4931, (2020) https://doi.org/10.1016/j.msec.2019.110467. (https://www.sciencedirect.com/science/article/pii/S0928493119329753)

This article talks about bioadhesives possibilities in invertebrates. The composition and properties of adhesive secretions appears to be diverse and remains unexplored. To what the marine environment is concerned, it is therefore important to increase knowledge of biological adhesives by widening the object of study to include other marine invertebrates, namely the less familiar/studied groups. (Almeida 2020)

Abirami, R., Ganesan, Manoj Saravana, Guru Mohanram, Balamuralikrishnan Balasubramanian, In Ho Kim, Palaniappan Seedevi, Kannan Mohan, Sujatha Kanagasabai, Mariadhas Valan Arasu, Naif Abdullah Al-Dhabi, Savarimuthu Ignacimuthu, Marine invertebrates' proteins: A recent update on functional property, Journal of King Saud University - Science, Volume 32, Issue 2, Pages 1496-1502, ISSN 1018-3647, (2020) https://doi.org/10.1016/j.jksus.2019.12.003. (https://www.sciencedirect.com/science/article/pii/S1018364719318580)

This journal goes into some details on marine invertebrates and what they are used in and how there uses have increased over the years. Thus, marine peptides and proteins have vast biomedical applications like antioxidant, antimicrobial, anticancer, hepatoprotective, bone marrow regeneration and tissue regeneration properties. The market value of these proteins and peptides is increasing every year, and this shows the value in nutraceutical and biomedical industries. In future, these proteins and peptides from marine invertebrates could be a valuable ingredient in the food, feed and pharmaceutical industries. (Ganesan 2020)

Loko, L.E.Y., Medegan Fagla, S., Orobiyi, A. Traditional knowledge of invertebrates used for medicine and magical–religious purposes by traditional healers and indigenous populations in the Plateau Department, Republic of Benin. J Ethnobiology Ethnomedicine 15, 66 (2019). https://doi.org/10.1186/s13002-019-0344-x

This article has good background information for my research topic. Our results reveal that several invertebrate species play an important role in healing practices and magical–religious rituals in the Plateau Department. We suggest further studies to confirm the presence of any bioactive compounds on invertebrate species use in traditional medicine. In addition, this study highlights the need for ecological investigations of these species, in order to develop strategies for their conservation and sustainable use. (Loko 2019)

Ranasinghe, S., McManus, D. Structure and function of invertebrate Kunitz serine protease inhibitors, Developmental & Comparative Immunology, Volume 39, Issue 3, Pages 219-227, ISSN 0145-305X, (2013) https://doi.org/10.1016/j.dci.2012.10.005. (https://www.sciencedirect.com/science/article/pii/S0145305X12002169)

This paper talks about Kunitz type proteins and how they could be used in the medical field. With the increased publication of a large number of draft invertebrate genomes, more Kunitz-type inhibitor genes are likely to be identified by data mining and, with their subsequent biochemical characterization, more light will be shed on their biological function. In turn, this may lead to the identification of new vaccine and drug intervention targets against those invertebrate species that are parasitic. (Ranasinghe 2013)

Jensen, P. Marine natural products: From sea to pharmacy. UCTV, University of California Television. (2019) https://www.uctv.tv/shows/Marine-Natural-Products-From-Sea-to-Pharmacy-34636

This seminar talks about secondary metabolism and is quite interesting.

Pascual-Torner, M., Dido Carrero, José G. Pérez-Silva, Diana Álvarez-Puente, David Roiz-Valle, Gabriel Bretones, David Rodríguez, Daniel Maeso, Elena Mateo-González, Guillermo Mariño, José Luis Acuña, Víctor Quesada, and Carlos López-Otín. Comparative genomics of mortal and immortal cnidarians unveils novel keys behind rejuvenation, PNAS. Vol. 119 | No. 36, (2022) https://doi.org/10.1073/pnas.211876311

Talks about the Immortal jellyfish.

Senthilkumar, K., Kim SK. Marine invertebrate natural products for anti-inflammatory and chronic diseases. Evid Based Complement Alternat Med. (2013) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3893779/

On the basis of their bioactive properties, this review focuses on the potential use of marine invertebrate derived compounds on anti-inflammatory and some chronic diseases such as cardiovascular disease, osteoporosis, diabetes, HIV, and cancer. (Senthilkumar 2013)

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Book that talks about secondary metabolites.

Vaishnav, P., & Demain, A. L. Unexpected applications of secondary metabolites. Biotechnology advances, 29(2), 223–229. (2011) https://doi.org/10.1016/j.biotechadv.2010.11.006

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Book talks about the LAL test.

Summary of Revisions

I fixed the spelling mistake that you caught were I put incite instead of insight so that has been corrected. I italicized *C. striatus* where I had forgotten to italicize it in the paper which you had pointed out. I added some more details to the LAL test section of the paper like you had suggested and I also added two more figures one to the show the process of transdifferentiation and a figure to show some secondary metabolites derived from marine animals as suggested by you. Lastly, I fixed my figures numbering so, they should all be in the correct order as they show up in the paper now. These were all the revisions you told me to make to my paper.