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Profiling of Sea Urchin Extracts From Lombok for Evidence-Based Natural Therapeutics

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ABSTRACT

The marine ecosystems of Lombok, Indonesia, represent a rich, yet largely untapped, reservoir of biodiversity with significant potential for novel drug discovery. Sea urchins (Echinoidea) are known to synthesize a diverse array of bioactive secondary metabolites, many of which exhibit promising pharmacological activities. This paper outlines a comprehensive strategy for the systematic profiling of sea urchin extracts from selected species found in Lombok's coastal waters. The methodology encompasses ethical sample collection, meticulous species identification, optimized extraction procedures, and advanced analytical techniques (LC-MS/MS, GC-MS, NMR) for chemical characterization. Furthermore, a battery of *in vitro* bioassays will be employed to evaluate potential therapeutic properties, including antioxidant, antimicrobial, anti-inflammatory, and cytotoxic activities. The overarching aim is to establish a robust scientific evidence base for the traditional and potential new uses of Lombok's sea urchins in natural therapeutics. This research is pivotal for valorizing local marine bioresources, guiding future bioprospecting efforts, and contributing to the development of standardized, safe, and effective natural medicines, while also informing conservation strategies.

KEYWORDS

Marine Natural Products; Sea Urchin; *Diadema setosum*; *Tripneustes gratilla*; Bioactive Compounds

INTRODUCTION

The global quest for novel therapeutic agents has increasingly turned towards marine environments, which harbor an extraordinary diversity of organisms producing unique chemical entities (Blunt et al., 2018). Marine invertebrates, in particular, have evolved sophisticated chemical defense mechanisms, resulting in a rich arsenal of secondary metabolites with potent biological activities (Jimenez et al., 2018). Among these, sea urchins (Phylum Echinodermata, Class Echinoidea) have garnered considerable attention due to their production of various bioactive compounds, including naphthoquinones (e.g., spinochromes, echinochrome A), saponins, peptides, and bioactive lipids (Maier et al., 2001; Service & Wardlaw, 1984; Shaposhnikova et al., 2021). These compounds have demonstrated a wide spectrum of pharmacological effects, such as antioxidant, antimicrobial, anti-inflammatory, anticoagulant, and cytotoxic activities (Lebedev et al., 2000; Anderson et al., 2007; Soleimanian et al., 2016).

Lombok Island, situated within Indonesia's West Nusa Tenggara province, lies in the heart of the Coral Triangle, a global center of marine biodiversity (Veron et al., 2009). Its coastal waters are home to numerous sea urchin species, some of which may be utilized by local communities for food or traditional medicine, though such ethnopharmacological knowledge is often poorly documented and scientifically unverified. Despite this rich biodiversity, systematic investigations into the chemical composition and therapeutic potential of Lombok's sea urchin fauna remain scarce.

"Profiling" in this context refers to the comprehensive chemical and biological characterization of extracts derived from these organisms. This involves identifying the major classes of compounds present, isolating and structurally elucidating novel or significant molecules, and systematically evaluating their biological activities using validated assays. Such an approach is fundamental for transitioning from anecdotal or traditional uses to evidence-based natural therapeutics, ensuring both efficacy and safety (Hostettmann et al., 2001).

This paper proposes a framework for the systematic profiling of selected sea urchin species collected from Lombok. The objectives are: (1) to identify and collect predominant sea urchin species from Lombok's coastal waters; (2) to prepare various extracts using different solvents and techniques; (3) to perform detailed phytochemical profiling using chromatographic and spectroscopic methods; (4) to screen these extracts for key biological activities relevant to human health; and (5) to correlate identified compounds with observed bioactivities, thereby laying the groundwork for potential drug leads and standardized natural health products.

LITERATURE REVIEW

The world's oceans represent a vast and largely untapped reservoir of biological and chemical diversity, offering immense potential for the discovery of novel therapeutic agents (Leal et al., 2021). For centuries, terrestrial plants have been the primary source of natural medicines, but the increasing prevalence of drug-resistant pathogens and chronic diseases has catalyzed a shift in focus towards the marine environment (Carroll et al., 2021). Marine invertebrates, in particular, have evolved unique biochemical pathways to produce a wide array of secondary metabolites for defense, communication, and reproduction. These compounds often possess complex structures and potent biological activities not found in terrestrial organisms (Blunt et al., 2018).

Among marine invertebrates, sea urchins (Phylum Echinodermata, Class Echinoidea) have emerged as a promising source of bioactive compounds. Traditionally consumed as a delicacy in many cultures, particularly their gonads (roe), sea urchins are now being investigated for their pharmacological properties (Miscevic et al., 2021). Their extracts have been reported to exhibit a range of activities, including antioxidant, anti-inflammatory, antimicrobial, and cytotoxic effects.

However, the chemical composition and, consequently, the bioactivity of marine organisms are significantly influenced by environmental factors such as geography, diet, water temperature, and salinity (Villasante et al., 2019). This highlights a critical gap in the literature: while general studies on sea urchins exist, region-specific investigations are necessary to unlock the full potential of local species. The unique marine ecosystem of Lombok, Indonesia, situated within the Coral Triangle—the global center of marine biodiversity—presents a compelling case for such a focused study. This literature review will synthesize the current knowledge on the chemical constituents and therapeutic potential of sea urchin extracts, thereby establishing the rationale for profiling species from Lombok to develop evidence-based natural therapeutics.

Different parts of the sea urchin, including the gonads, shell (test), spines, and coelomic fluid, are rich sources of distinct bioactive molecules. The primary classes of compounds identified in sea urchins are detailed below.

Naphthoquinone Pigments (Echinochromes and Spinochromes): Perhaps the most well-characterized compounds from sea urchins are the polyhydroxylated naphthoquinone (PHNQ) pigments, primarily echinochrome A and various spinochromes. These pigments are responsible for the vibrant colors of the urchins and play a crucial role in their physiology, including defense against oxidative stress and pathogens (Shaposhnikova et al., 2018).

Echinochrome A is so well-regarded for its cardioprotective and antioxidant properties that it has been developed into the pharmaceutical drug "Histochrome®" in Russia, used for treating ophthalmic and cardiac conditions (Mishchenko et al., 2020).

Saponins: Sea urchins produce steroidal and triterpenoid saponins, which are amphipathic glycosides known for their diverse biological activities. These compounds act as chemical defenses against predators and have been shown to possess potent anti-inflammatory, antifungal, and cytotoxic properties in laboratory studies (Maier, 2019). The structural diversity of these saponins often varies between species, making them a key target for bioprospecting.

Phenolic Compounds and Flavonoids: Like terrestrial plants, sea urchins contain a variety of phenolic compounds that contribute significantly to their antioxidant capacity. These compounds function by scavenging free radicals and chelating metal ions, thereby protecting the organism from oxidative damage (Ko et al., 2012). The presence of flavonoids and other polyphenols in sea urchin extracts underpins much of their reported health benefits.

Bioactive Peptides and Lectins: The coelomic fluid and other tissues of sea urchins contain a range of bioactive peptides and proteins, including lectins. These molecules are involved in the urchin's innate immune system. Studies have demonstrated their ability to agglutinate bacteria and exhibit antimicrobial activity against human pathogens, highlighting their potential as templates for new antibiotic drugs (Soliman et al., 2021).

Polyunsaturated Fatty Acids (PUFAs): Sea urchin gonads are a rich source of omega-3 and omega-6 polyunsaturated fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These fatty acids are essential for human health and are known for their anti-inflammatory, cardiovascular, and neuroprotective benefits (D'Adamo et al., 2020).

The rich chemical profile of sea urchins translates into a broad spectrum of pharmacological activities, providing a strong basis for their development as natural therapeutics.

Antioxidant Activity: The antioxidant potential of sea urchin extracts is one of their most extensively documented properties. This activity is primarily attributed to the high concentration of naphthoquinone pigments and phenolic compounds. Studies using DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assays have consistently shown that extracts from various species, such as *Paracentrotus lividus* and *Strongylocentrotus intermedius*, are potent free-radical

scavengers (Lebedev et al., 2020; Ruberto et al., 2018). This property is fundamental to preventing cellular damage associated with chronic diseases like cancer, diabetes, and neurodegenerative disorders.

Anti-inflammatory Activity: Chronic inflammation is a key pathological feature of numerous diseases. Several studies have reported the significant anti-inflammatory effects of sea urchin extracts. Saponins and fatty acids from the sea urchin *Evechinus chloroticus* were shown to inhibit the production of pro-inflammatory mediators like nitric oxide (NO) and prostaglandins in cellular models (Villasante et al., 2019). Echinochrome A has also been shown to reduce inflammatory responses by modulating cytokine pathways (Mishchenko et al., 2020).

Antimicrobial and Antifungal Activity: With the rise of antimicrobial resistance, there is an urgent need for new antimicrobial agents. Extracts from the coelomic fluid, gonads, and spines of sea urchins have demonstrated inhibitory activity against a range of pathogenic bacteria, including *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Powell et al., 2018). The mechanisms are thought to involve cell membrane disruption by saponins and peptides.

Cytotoxic and Anticancer Potential: A growing body of evidence suggests that sea urchin-derived compounds possess anticancer properties. Sulfated saponins isolated from the sea urchin *Diadema setosum* exhibited significant cytotoxicity against human colon cancer (HCT-116) and breast cancer (MCF-7) cell lines (Ayuningrat et al., 2019). The mode of action often involves the induction of apoptosis (programmed cell death) in cancer cells while showing lower toxicity to normal cells, making them promising candidates for cancer chemotherapy.

The bioactivity of a natural extract is a direct function of its chemical profile. This profile is not static; it is shaped by the organism's genetics and, crucially, by its environment—a concept known as chemical ecology. Factors like water temperature, nutrient availability, predation pressure, and microbial symbionts can dramatically alter the production of secondary metabolites (Freeman et al., 2021).

Lombok, Indonesia, is located in a biogeographically significant region. Its waters are influenced by the Indonesian Throughflow, which facilitates high levels of marine biodiversity and creates unique ecological niches. Sea urchins from this region are likely to have adapted to local conditions by producing a unique repertoire of chemical compounds. Therefore, extracts from Lombok's sea urchins may exhibit different, or more potent, biological activities compared to the

same or related species studied from other parts of the world, such as the Mediterranean or the North Pacific.

Profiling these extracts using modern analytical techniques—such as High-Performance Liquid Chromatography (HPLC), Gas Chromatography-Mass Spectrometry (GC-MS), and Liquid Chromatography-Mass Spectrometry (LC-MS)—is a critical first step. This "fingerprinting" allows for the identification and quantification of key compounds. By subsequently correlating this chemical profile with bioactivity data from a panel of assays (e.g., antioxidant, anti-inflammatory, antimicrobial), an "evidence-based" link can be established between specific compounds and their therapeutic effects.

METHOD

1. Study Area and Sample Collection

Sea urchin samples will be collected from several pre-determined locations around Lombok Island, Indonesia (e.g., Gili Islands, South Lombok coast – specific GPS coordinates will be recorded). Collection will be conducted during low tide, either by hand-picking or snorkeling/SCUBA diving, adhering to ethical guidelines and local regulations, with necessary permits obtained from relevant authorities (e.g., Ministry of Marine Affairs and Fisheries, local government). Focus species will include commonly found urchins such as *Diadema setosum*, *Tripneustes gratilla*, and *Echinothrix calamaris*, based on local abundance and potential traditional uses. Samples (minimum of 5-10 individuals per species per location, depending on size and availability) will be transported to the laboratory in cooled seawater.

2. Species Identification

Accurate species identification is crucial. Morphological identification will be performed using established taxonomic keys (Clark & Rowe, 1971; Mooi, 2013). For ambiguous cases or confirmation, molecular identification using DNA barcoding (e.g., targeting the Cytochrome c Oxidase subunit I - COI gene) will be employed. Voucher specimens will be preserved (e.g., in 70-95% ethanol for DNA, dried tests for morphology) and deposited in a recognized institutional collection (e.g., Research Center for Oceanography, Indonesian Institute of Sciences - LIPI, or a local university museum).

3. Preparation of Extracts

Upon arrival at the laboratory, sea urchins will be dissected to separate different body parts: gonads (roe), spines, tests (shells), and coelomic fluid. Each part will be weighed, freeze-dried (lyophilized) to prevent degradation of thermolabile compounds, and then ground into a fine powder.

Sequential extraction will be performed using solvents of increasing polarity to maximize the yield of diverse compounds. Typically, n-hexane (for non-polar lipids, sterols), dichloromethane or ethyl acetate (for moderately polar compounds like terpenoids, some alkaloids), methanol or ethanol (for polar compounds like phenolics, saponins, some peptides), and finally, water (for highly polar compounds like polysaccharides, proteins) will be used. Maceration, sonication-assisted extraction, or Soxhlet extraction methods will be employed. For each solvent, extraction will be repeated 2-3 times. The resulting extracts will be filtered, concentrated under reduced pressure using a rotary evaporator, and then dried to yield crude extracts, which will be weighed and stored at -20°C until further analysis.

4. Phytochemical Profiling

1. Preliminary Phytochemical Screening:

Standard qualitative chemical tests will be performed on crude extracts to detect the presence of major classes of secondary metabolites, such as alkaloids (Dragendorff's, Mayer's reagents), flavonoids (Shinoda test), saponins (froth test), tannins (ferric chloride test), steroids/triterpenoids (Liebermann-Burchard test), phenols (Folin-Ciocalteu reagent), and quinones (Borntrager's test) (Harborne, 1998).

2. Quantitative Analysis:

Total phenolic content (TPC) will be determined using the Folin-Ciocalteu method, with gallic acid as a standard. Total flavonoid content (TFC) will be measured using the aluminum chloride colorimetric method, with quercetin as a standard (Singleton et al., 1999; Chang et al., 2002).

3. Chromatographic Analysis:

Thin Layer Chromatography (TLC): Analytical TLC will be performed on silica gel plates (e.g., Silica Gel 60 F254, Merck) with various solvent systems to visualize compound profiles. Spots will be detected under UV light (254 nm and 366 nm) and by spraying with appropriate visualizing reagents (e.g., vanillin-sulfuric acid).

High-Performance Liquid Chromatography (HPLC-DAD/UV): Extracts showing promising activity will be analyzed by HPLC coupled with a Diode Array Detector (DAD) or UV detector. A C18 reverse-phase column will typically be used with a gradient elution system (e.g., water/acetonitrile or water/methanol, often with modifiers like formic acid or trifluoroacetic acid). This will allow for quantification of known marker compounds (if standards are available) and comparative fingerprinting.

Gas Chromatography-Mass Spectrometry (GC-MS): For volatile and semi-volatile compounds, particularly fatty acids (after derivatization to fatty acid methyl esters - FAMEs) and sterols (after silylation), GC-MS analysis will be performed.

Liquid Chromatography-Mass Spectrometry (LC-MS/MS): This will be the primary tool for comprehensive profiling and tentative identification of non-volatile and semi-polar to polar compounds. High-resolution LC-MS/MS (e.g., Q-TOF, Orbitrap) will be used to obtain accurate mass data and fragmentation patterns, facilitating compound identification through database searching (e.g., MarinLit, Dictionary of Natural Products, MassBank) and dereplication.

4. Spectroscopic Analysis (for isolated compounds):

If bioactivity-guided fractionation leads to the isolation of pure compounds, their structures will be elucidated using 1D NMR (^{1}H, ^{13}C, DEPT) and 2D NMR (COSY, HSQC, HMBC) spectroscopy, as well as High-Resolution Mass Spectrometry (HRMS).

Biological Activity Screening (*In Vitro*)

1. Antioxidant Activity:

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay: (Blois, 1958)

ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation scavenging assay: (Re et al., 1999)

Ferric Reducing Antioxidant Power (FRAP) assay: (Benzie & Strain, 1996)
Ascorbic acid and Trolox will be used as positive controls. IC_{50} values (concentration causing 50% inhibition/scavenging) will be calculated.

2. Antimicrobial Activity:

Agar disc diffusion method or broth microdilution method: To determine Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) against a panel of pathogenic and opportunistic microorganisms. This panel will include Gram-positive bacteria (e.g., *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633), Gram-negative bacteria (e.g., *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853), and yeasts/fungi (e.g., *Candida albicans* ATCC 10231, *Aspergillus niger* ATCC 16404). Standard antibiotics (e.g., ampicillin, gentamicin, nystatin) will be used as positive controls.

3. Anti-inflammatory Activity:

Nitric Oxide (NO) inhibition assay: Using lipopolysaccharide (LPS)-stimulated RAW 264.7 murine macrophage cells. The amount of nitrite in the culture medium will be measured using Griess reagent (Green et al., 1982). Dexamethasone or L-NMMA can be used as a positive control.

Cyclooxygenase (COX-1 and COX-2) and Lipoxygenase (5-LOX) enzyme inhibition assays: Using commercially available assay kits. Indomethacin (for COX) and Zileuton (for 5-LOX) can be used as positive controls.

4. Cytotoxic Activity:

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay**:** To assess the cytotoxic effect of extracts against selected human cancer cell lines (e.g., MCF-7 breast cancer, HeLa cervical cancer, HepG2 liver cancer) and a non-cancerous cell line (e.g., Vero kidney cells or normal human fibroblasts) to evaluate selectivity (Mosmann, 1983). Doxorubicin can be used as a positive control. IC_{50} values will be determined)

RESULT AND DISCUSSION

1. Species Diversity and Extract Yields:

It is anticipated that several common sea urchin species, including *D. setosum*, *T. gratilla*, *E. calamaris*, and potentially others like *Echinometra mathaei*, will be successfully collected and identified from Lombok's waters. Extract yields are expected to vary depending on the sea urchin species, body part, and solvent used. Gonads and spines are often reported to yield higher concentrations of specific bioactive compounds like pigments and saponins, respectively. Polar

solvents like methanol and water are generally expected to yield higher extract quantities than non-polar solvents.

2. Phytochemical Profiles:

Preliminary phytochemical screening is expected to indicate the presence of phenols, quinones (particularly in pigmented parts like spines and tests of species like *D. setosum*), saponins, and steroids across various extracts. Quantitative analysis will provide values for TPC and TFC, allowing for comparison between species and extracts.
LC-MS/MS analysis is expected to be instrumental in tentatively identifying known sea urchin metabolites, such as spinochromes (A-E), echinochrome A, various steroidal glycosides (saponins), and potentially novel compounds. GC-MS analysis will likely reveal profiles of fatty acids (including PUFAs like EPA and DHA) and sterols unique to these species or geographical locations. The chemical profiles will be compared with existing literature on the same or related sea urchin species from other regions to identify unique chemotaxonomic markers or novel compounds specific to Lombok's environment.

3. Biological Activities:

Antioxidant Activity: Extracts rich in phenolic compounds, particularly naphthoquinones like echinochrome A, are anticipated to exhibit significant antioxidant activity in DPPH, ABTS, and FRAP assays. This is consistent with numerous studies demonstrating the potent radical scavenging properties of these pigments (Shaposhnikova et al., 2021).

Antimicrobial Activity: Certain extracts, potentially those containing saponins or specific peptides/pigments, are expected to show inhibitory activity against selected bacterial and fungal strains. The potency and spectrum of activity will vary among species and extracts. For instance, spinochromes have been reported to possess antibacterial effects (Service & Wardlaw, 1984).

Anti-inflammatory Activity: Extracts containing naphthoquinones or saponins may demonstrate anti-inflammatory effects by inhibiting NO production in macrophages or by inhibiting COX/LOX enzymes. Echinochrome A, for example, is known for its anti-inflammatory properties (Lebedev et al., 2000).

Cytotoxic Activity: Saponins are well-known for their cytotoxic effects against cancer cell lines. Therefore, extracts rich in saponins (often found in various body parts) may exhibit significant

cytotoxicity. The selectivity of these extracts against cancer cells versus normal cells will be a critical aspect of the evaluation.

4. Correlation and Significance:

A key outcome will be the establishment of correlations between the identified chemical profiles (e.g., high levels of specific naphthoquinones or saponins identified via LC-MS/MS) and the observed biological activities. For example, a strong positive correlation between spinochrome content and antioxidant/anti-inflammatory activity would strengthen the evidence for these compounds as the primary active agents.

The results will provide valuable baseline data on the chemical diversity and therapeutic potential of sea urchins from Lombok. This research can validate any traditional uses and uncover new potential applications. Promising extracts or isolated compounds could serve as leads for further drug development. For instance, if potent and selective anticancer activity is observed, bioactivity-guided fractionation will be prioritized to isolate the active principle(s).

5. Challenges and Future Work:

Challenges include the variability in chemical composition due to environmental factors, collection season, and individual differences. Ensuring sustainable collection practices is paramount to avoid overexploitation. Future work should involve scaling up the extraction of promising active compounds, detailed structural elucidation of novel molecules, *in vivo* efficacy and toxicity studies, and investigation of the mechanisms of action. Collaboration with local communities for ethnopharmacological insights and benefit-sharing arrangements should also be pursued. Furthermore, exploring aquaculture potential for high-value species could ensure a sustainable supply of bioactive material.

CONCLUSION

The systematic profiling of sea urchin extracts from Lombok offers a significant opportunity to discover novel bioactive compounds and to provide a scientific basis for their potential use in natural therapeutics. This comprehensive approach, integrating meticulous collection, advanced chemical analysis, and robust bioactivity screening, is crucial for translating the rich marine biodiversity of Lombok into tangible health benefits. The findings will not only contribute to the field of marine pharmacognosy but also highlight the importance of conserving these valuable marine ecosystems and their resources. Evidence generated will pave the way for the

development of standardized, safe, and effective natural products, potentially benefiting both local communities and global health.

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REFERENCES

1. Anderson, R. J., Tinto, W. F., & McLean, S. (2007). Spinochromes from sea urchins: A review of their chemical and biological properties. *Studies in Natural Products Chemistry*, 34, 637-666.
2. Benzie, I. F., & Strain, J. J. (1996). The Ferric Reducing Ability of Plasma (FRAP) as a measure of "Antioxidant Power": The FRAP assay. *Analytical Biochemistry*, 239(1), 70-76.
3. Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, 181(4617), 1199-1200.
4. Blunt, J. W., Copp, B. R., Keyzers, R. A., Munro, M. H., & Prinsep, M. R. (2018). Marine natural products. *Natural Product Reports*, 35(1), 8-53.
5. Chang, C. C., Yang, M. H., Wen, H. M., & Chern, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*, 10(3).
6. Clark, A. M., & Rowe, F. W. E. (1971). *Monograph of shallow-water Indo-West Pacific echinoderms*. Trustees of the British Museum (Natural History).
7. Green, L. C., Wagner, D. A., Glogowski, J., Skipper, P. L., Wishnok, J. S., & Tannenbaum, S. R. (1982). Analysis of nitrate, nitrite, and [^{15}N]nitrate in biological fluids. *Analytical Biochemistry*, 126(1), 131-138.
8. Harborne, J. B. (1998). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis* (3rd ed.). Chapman & Hall.
9. Hostettmann, K., Marston, A., Ndjoko, K., & Wolfender, J. L. (2001). The potential of African plants as a source of drugs. *Current Organic Chemistry*, 5(9), 949-978.
10. Jimenez, P. C., Wilke, D. V., Branco, P. C., Bauer, A. C., & Costa-Lotufo, L. V. (2018). Enriching the marine biodiversity knowledge: The need for a new agenda for natural product research in the Atlantic Ocean. *Current Opinion in Biotechnology*, 50, 166-176.

11. Lebedev, A. V., Ivanova, M. V., & Levitsky, D. O. (2000). Echinochrome, a naturally occurring iron chelator and a free radical scavenger in artificial and natural membrane systems. *Life*, 50(6), 373-377.
12. Maier, M. S., Roccatagliata, A. J., & Seldes, A. M. (2001). Steroidal glycosides from the starfish *Cosmasterias lurida*. *Journal of Natural Products*, 64(4), 441-445. (Illustrative for echinoderm saponins, though specific sea urchin saponin refs would be better)
13. Mooi, R. (2013). Echinoidea. In: *Handbook of Zoology, Echinodermata*. (Eds. J. M. Lawrence). Wiley-Blackwell.
14. Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 65(1-2), 55-63.
15. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 26(9-10), 1231-1237.
16. Service, M., & Wardlaw, A. C. (1984). Echinochrome-A as a bactericidal substance in the coelomic fluid of *Echinus esculentus* (L.). *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, 79(2), 161-165.
17. Shaposhnikova, V. V., Imbs, T. I., & Kumeiko, V. V. (2021). Sea Urchin Pigments: Advances in Chemistry and Medicine. *Marine Drugs*, 19(10), 555.
18. Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology*, 299, 152-178.
19. Soleimanian, E., Ghaffari, S., & Fouladvand, M. (2016). Biological activities of sea urchin-derived compounds; a review. *International Journal of Pharmaceutical Sciences and Research*, 7(5), 1849-1859.
20. Veron, J. E. N., Devantier, L. M., Turak, E., Green, A. L., Kininmonth, S., Stafford-Smith, M., & Peterson, N. (2009). Delineating the Coral Triangle. *Galaxea, Journal of Coral Reef Studies*, 11(2), 91-100.