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Cover Page Footnote

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Anti-inflammatory and Antioxidant Effects of Sea Urchin Spine Extract

Dina Magdy Mabrouk El Gamal¹

Abstract

This research investigates the anti-inflammatory and antioxidant activity of the Sea Urchin (*Diadema savignyi*) spine extract in an experimental setup using L929 cell line in vitro. The cell metabolic activity of L929 cells is tested through an MTT assay. The sea urchin spine extract is applied to the cells in two concentrations: 100 µg/ml (136% viability) and 200 µg/ml (95% viability). The bioactive components of the sea urchin spine are identified via GC-MS, and the antioxidant and anti-inflammatory activities are evaluated using catalase assay (CAT), glutathione (GSH), and nitric oxide (NO) tests. Results show that the GC-MS identified bioactive components including the anti-inflammatory and anti-irritant bisabolol oxide and the pro-inflammatory oleic acid. Additionally, GSH and NO tests indicate significant antioxidant actions of the sea urchin spine in both the 100 µg/ml and 200 µg/ml with roughly 0.098 and 0.103 mmol/L in GSH test, and 20.46 and 20.6 mmol/L in NO test, respectively. However, the CAT results were inconsistent with that of the GSH and NO, therefore a repetition of this test is recommended. Overall, the study concludes that the significant anti-inflammatory and antioxidant actions of the sea urchin spine qualify it as a potential candidate in wound healing ointments.

Keywords: *Diadema savignyi*; sea urchin spine; anti-inflammatory; antioxidant; wound healing

Various factors affect the wound healing process, including age, medications, infections, obesity, and chronic diseases such as diabetes (Guo and DiPietro, 2010). These factors reduce the wound healing efficiency, prolong its duration, and often result in the alteration of severe skin conditions. The mechanism of wound healing follows a set of phases. For the wound healing process to be successful, four highly planned phases have to be accomplished: hemostasis, inflammation, proliferation, and remodeling. Crucially, all four phases must occur in the correct order and time frame for a wound to heal properly (Eming, Martin, and Tomic, 2016). A 2018 retrospective analysis stated that around 8.2 million people had wounds with or without infections (Sen, 2019). Sen furtherly stated that skin wounds, if left untreated, are prone to endure infections which can consequently lead to morbidity. Therefore, it is crucial to treat the wounds immediately to prevent foreign objects from infecting the skin and causing further consequences.

Common treatments for wounds include antibiotics such as bacitracin, mupirocin, mafenide, neomycin, silver sulfadiazine, and metronidazole. Antibiotics are defined as agents that have the ability to eradicate microorganisms or inhibit their activity through specific cell targeting action; However, antibiotics can show adverse effects (Atoe, Alves, Sarandy, Santos, Novaes, and Goncalves, 2019). Unfortunately, antibiotics are associated with many side effects. Both bacitracin and mupirocin antibiotics can lead to burning, irritation, itching, or rash. Bacitracin causes hypersensitivity after systemic and topical application and can make skin sensitive to sunlight due to T cells activation and the release of pro-inflammatory cytokines, resulting in skin irritation and

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inflammation (Gupta, 2017; Kimmel et al., 2015). Meanwhile, mupirocin was considered unsuitable for patients with renal impairment because its absorbance is high and can severely affect the kidney (Atoe et al., 2019). Moreover, Atoe et al. illustrated that mafenide antibiotics involve a cytotoxic mechanism that causes inhibition of synthesis pathways, a change in the concentration of hormones, and an alteration of folic receptor expression and pH (Punjataewakupt et al., 2018). Neomycin was also found to cause rashes and severe effects on kidneys. Whereas, silver sulfadiazine attributes to an alteration of cytokine expression, production of argyria, and stimulation of hemolysis in patients with glucose-6-phosphate dehydrogenase deficiency (Atoe et al., 2019). In addition, antibiotics do not only cause side effects in the human body but they can also cause mutations and the development of new bacterial strains (Gbotolorun et al., 2016; Punjataewakupt et al., 2018; Atoe et al., 2019). Therefore, antibiotics cannot be safely used on a long-term basis.

Consequently, this raised a demand of an alternative therapy for wounds recovery with a safe, yet with an effective use on the long term. Hereinafter, many research investigated marine creatures, including sea urchins, due to its high rate of injury trauma, high susceptibility of acute wounds, and frequent loss of different body parts; however, they have an extraordinary natural capacity to heal it safely and effectively (Abubakar, Mwangi, Uku, and Ndirangu, 2012). This emphasizes the successful machinery and anti-inflammatory pathways of marine organisms that protect them against severe infections. Studies have shown that sea urchin spines in specific can be used to treat a variety of wounds, including burns, diabetic ulcers, and surgical incisions. Studies stated that sea urchin spine extract can promote the migration of human skin cells (Jiang et al., 2020), which is an important step in wound healing. Accordingly, this research investigates the following questions: what are the anti-inflammatory and antioxidant effects of Sea Urchins spine extract? And what are their mechanisms in the wound healing process?

Literature Review

Sea urchins contain compounds known as glycosaminoglycans (GAGs) in their spines that have been found to have anti-inflammatory and antioxidant properties, as well as promote tissue repair and regeneration. In a study conducted by Lee et al. (2020), the effects of sea urchin spine extract on wound healing were investigated in a rat model of burn injuries. When compared to a control cream, the researchers discovered that a cream containing sea urchin spine extract significantly enhanced the wound healing process, as indicated by more responsiveness, quicker wound closure, and less inflammation. Another study by Al-Naggar and colleagues (2019) discovered that sea urchin spine extract promotes tissue regeneration and wound healing in rats by activating cellular and molecular pathways that contribute to wound healing. Similarly, a study by Alshahrani and colleagues (2020) documented that sea urchin spine extract reduced oxidative stress and inflammation in diabetic rats thus contributing to the diabetic rats' wound healing.

Moreover, Sea urchin is rich in vitamin A, vitamin B complex, omega 3, and omega-6 fatty acids in addition to other bioactive components that function as anti-inflammatory agents against bacteria, yeast, and fungi. Among these agents are the acyclic thiosulfinates (1,2- Dithiolane) that are characterized by inflammatory, antiparasitic, and antitumor action (El-Sayed, Elshaer, Ibrahim, and El-Metwaly, 2020). It also contains xanthophylls that protect skin against phototoxic and UV damage, including hyperplasia (Li, Turner, and Brautigan, 2015). Furthermore, prior research discovered that Sea Urchins have several secondary metabolites that can promote wound healing by 50% and the inflammation was reduced by 80% (Kresnamurti, 2021).

Regulatory authorities (FDA/EMA) and clinical trials are now approving an increasing number of Sea-Urchin-based products (Lindequist, 2016). Examples are Clarins Multi-Active (Gamay company) and Natural Little Urchin (Hello natural living). These products are now widely distributed into markets as antimicrobial and antioxidant reagents for skincare and tissue treatment.

Materials and Methods

This work aims to identify the antimicrobial and antioxidant effects of Sea Urchins extract on wound healing *in vitro*. For this purpose, consecutive steps were executed.

First, Sea Urchin were collected and brought to the lab and spines were extracted and stored. Second, the phytochemical composition of the extract was identified through Gas chromatography-mass spectrometry test (GC-MS). In order to understand the possible health advantages of the extract and establish the right dosage for therapeutic usage, this test was carried out to identify and quantify the bioactive chemicals that were present in the extract (Scalbert and Williamson, 2000). Generally, phytochemicals such as alkaloids, flavonoids, and polyphenols have been shown to possess anti-inflammatory, antioxidant, and anticancer properties (Kaur and Arora, 2009). Since identifying the phytochemical profile of an extract aids in forecasting its potential biological action, it enables the development of new medications or functional foods. Not only that, but the the phytochemical composition can also serve as a benchmark for the standardization and quality control of dietary supplements or medicinal products. (Wang and Chung, 2001).

Third, cell viability and proliferation of the extract was assessed via the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, also scientifically known as MTT assay. For a variety of biological and biomedical research purposes, the MTT assay is a typical colorimetric experiment that assesses cell viability and proliferation. It is based on the reduction of MTT by mitochondrial enzymes in living cells, which results in the formation of a spectrophotometrically measureable blue-purple formazan dye. The amount of live cells in the culture directly correlates with the dye's intensity (Mosmann, 1983). The MTT test is frequently employed because of its affordability, versatility, and relative simplicity.

Forth, the cell lysate preparation was used to analyse the analysis of proteins or nucleic acids in cells. Cell lysate, scientifically known as lysate, is a solution that contains all of the proteins, nucleic acids, and other biomolecules that are released when cells are broken open. It acts as an investigative tool for examining the molecular mechanisms underlying various biological processes and diseases (Soni et al., 2020). Cell lysate serves as a source of nucleic acids, such as DNA and RNA, for multiple assays, including PCR, qPCR, and RNA sequencing, as well as proteins for biochemical assays, such as Western blotting, enzyme activity assays, and protein-protein interaction studies (Kumar et al., 2016; Soni et al., 2020). Cell lysate conveys biomarkers, whose detection can provide insights into diagnostic and therapeutic targets or into pathophysiology of a disease (Soni et al., 2020), and assays involved in drug discovery by detecting the compounds that affect the activity of particular proteins or pathways (Zhu et al., 2018).

Fifth, oxidative stress tests were conducted since its levels indication evaluates disease risk, progression, and treatment response. Oxidative stress, in definition, is a condition that develops when there is an imbalance between the generation of reactive oxygen species (ROS) and antioxidant defense mechanisms (Halliwell and Gutteridge, 2015). One of the commonly used approaches for oxidative stress measurement is the catalase assay (CAT). It is an enzyme that catalyzes the decomposition of hydrogen peroxide into water and oxygen, preventing oxidative

damage to cells. The CAT assay measures the activity of this enzyme thus indicates oxidative stress levels (Nikbakht et al., 2017). Another frequently used oxidative stress test is the assessment of glutathione reduced (GSH) levels. GSH scavenges ROS, thus is considered as a potent antioxidant that guards cells from oxidative damage. Conversely, a decrease in GSH levels indicates oxidative stress levels (Dringen et al., 2000). Also, the nitric oxide (NO) assay assesses oxidative stress levels. NO is a fundamental key free radical for several physiological processes, including vasodilation and neurotransmission. However, excessive production of NO causes oxidative stress and subsequent cell damage. The NO assay indicates cellular oxidative stress by measuring the levels of NO (Bhattacharyya et al., 2014).

I. Marine animal extraction

An inclusion criteria was set to specify specific parameters by which the Sea Urchins were collected to serve the research focus. Nevertheless, the collection was done carefully with respect to acclimation protocols in animal research. The Sea Urchin (*Diadema savignyi*) were collected from the beach of Al Ain Al Sokhna, Red Sea, Egypt in summer 2021 during low tides. Only the adult individuals of *Diadema savignyi* species with a diameter of 6-8 cm with black or dark brown spines of up to ten cm in length were collected. The average weight of the collected specimen's was 31-45 grams each. Afterward, they were immediately transported in seawater-filled coolers within two hours of collection to the AUC laboratory where the extraction process took place. Before the beginning of the experiment, the collected sea urchins were acclimated to the laboratory conditions for 24 hours. After collection and acclimation of the sea urchin to the experimental setup, the spine of the Sea Urchin was first extracted and isolated from the soft tissues. Second, the spine extract was dried at 40°C then crushed and stored at -20°C (Vasileva et al., 2016). The fine powder was suspended in a mixed aqueous and ethanol with a ratio of (weight: volume = 500 ml: 500 g = 1:1), 30%: 70%, respectively. The extract was subsequently centrifuged at 8000 rpm at 4°C for 15 minutes then the resultant supernatant was concentrated in the rotary evaporator at 50°C under vacuum. Finally, the extract was stored at -20°C until further use.

II. GC-MS

Gas chromatography-mass spectrometry (GC-MS) was used to identify the phytochemical composition of the extract, using the GC-ISQ mass spectrometer with a direct capillary column TG-5MS (30 m x 0.25 mm x 0.25 µm film thickness) (Thermo Scientific, Austin, TX, USA). Indeed, this test was executed in an Egyptian Laboratory in New Cairo since AUC laboratories does provide GC-MS tests. Nevertheless, according to the International Journal of Analytical Mass Spectrometry and Chromatography, the GC-MS test was performed as follows. Initially, the temperature of the column oven was set at 55°C with a 5°C elevation each minute till it reached 250°C. A hold was applied for two minutes, then the temperature was furtherly increased to 300°C with 25°C/minute. Meanwhile, the temperature of the injector was ultimately set at 270°C with a continuous flow rate of 1 ml/min of helium as the carrier gas. A 4-minutes solvent delay was performed before the automatic injection of the diluted samples of 1 µl using the Autosampler AS3000 in split mode with GC. Next, in full scan mode, the electron ionization (EI) mass spectra were assembled at an ionization voltage of 70 eV with an average of 50–650 m/z. The temperature of the ion source and transfer line were set at 200 and 280 degrees Celsius, respectively. The

compounds were detected by comparing their retention durations and mass spectra to the mass spectral databases WILEY 09 and NIST14.

III. MTT assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay assesses cell viability and proliferation. The activity of the cells' mitochondria is indicated through the formation of formazan crystals from the tetrazolium salt MTT. After this conversion, the number of viable cells is determined by measuring the formazan optical density (OD) at 570-720 nanometers. By this methodology, the MTT test is frequently utilized in cell lines and primary cells to determine drug sensitivity (van Meerloo, Kaspers, & Cloos, 2011).

In the current study, the MTT assay was executed on L929 fibroblast cells. The cells were plated in 96-well plates, with 1% penicillin-streptomycin antibiotic and 5% fetal bovine serum in Dulbecco's Modified Eagle Medium (DMEM), and were incubated for 24 hours in Thermo Scientific™ Heracell™ VIOS with 5% CO₂ incubator at 37°C. After the medium was removed, the control, blank, and extract were added to the cells. After the second 24 hours-incubation, the media was discarded and the MTT reagent was added and incubated along with the sample for four hours. The spine extract was applied in two concentrations: 100 µg/ml and 200 µg/ml. Eventually, the MTT has discarded then Dimethyl sulfoxide (DMSO) was added instead to the wells of the plates. Afterward, SPECTROstar® Nano plate reader was used to measure the optical density (OD) at 570 nanometers (Ozdemir, Yilmaz H. & Yilmaz S., 2009)

IV. Cell Lysate

Cell lysis was applied after the MTT assay for purification to break open the cells and to prevent delicate proteins and DNA from denaturing or degradation by shear stress. This method was executed through four consecutive steps as identified by the biodiagnostic protocol for sample preparation (Biodiagnostic protocol, 2022). First, the cells were collected by centrifugation at 1500 rpm, as the speed determined as safe in the cell lysate protocol, for ten minutes at 4°C. Afterward, a sterilized inoculation loop was used to harvest the adherent cells instead of Trypsin. This is because trypsin is a proteolytic enzyme that affects the activity of the oxidative stress assay which was used after the cell lysate. Second, the cell pellet was homogenized in a cold buffer of 50 mM potassium phosphate and 2 mM EDTA at a pH of 7.5. Third, the sample was centrifuged at 4,000 rpm at 4°C for 15 minutes. Fourth, after centrifugation, the supernatant was removed for assay and was stored on ice. Nevertheless, an extra supernatant was prepared via the same steps but was saved in the freezer at -80°C as a stock for emergency use. After the cell lysate was accomplished, sonication was applied on each sample for one minute run then ten second break in between then an additional one minute run then washing afterward with distilled water + ethanol for 30 seconds in between samples.

V. Oxidative Stress Tests

Oxidative stress analysis was carried out to assess the overall status of the oxidative stress and the antioxidant reserve in the rats' bodies. This is to evaluate the antioxidant and anti-inflammatory effects of each compound of the sea urchin extract. This evaluation was achieved via three tests; catalase test, GSH, and NO.

V.I. Catalase Assay (CAT)

Catalase is an antioxidant enzyme with a defense mechanism that shields the body against strong oxidants; its assay provides investigations on oxidative stress that a body was exposed to (Nandi, Yan, Jana, and Das, 2019). In this assay, R2 1000 was instantly diluted with a diluent of (10 μ L + 10 ml distilled water) before use. For each well, the quantities for the Sample Blank, Sample, Standard Blank, and standard were as demonstrated in Table 1, after which an incubation of exactly one minute was applied at room temperature (25°C). Afterward, the procedures continued as demonstrated in Table 2, after which a 10-minutes incubation was applied at 37°C. The sample (A_{sample}) was set against sample blank, whereas the standard (A_{standard}) was set against Standard blank at 510 nm. Up till an hour after the procedures were performed, following the bio-diagnostic Catalase Assay Protocol, the color shall be fixed.

	Sample Blank (ml)	Sample (ml)	Standard Blank (ml)	Standard (ml)
Sample	0.05	0.05	-	-
D. H ₂ O	0.05	-	0.10	0.05
R1	0.50	0.50	0.50	0.50
R2	-	0.10	-	0.10

Table 1. Catalase Assay Procedure with Sample, D. H₂O, R1, & R2

	Sample Blank (ml)	Sample (ml)	Standard Blank (ml)	Standard (ml)
R3	0.20	0.20	0.20	0.20
R4	0.50	0.50	0.50	0.50

Table 2. Catalase Assay Procedure with R3 & R4

V.II. Glutathione Reduced (GSH)

Similar to the catalase, glutathione is an antioxidant; it protects vital biological components within the cell against reactive oxygen species (e.g., peroxides, lipid peroxides, heavy metals, and free radicals) (Wilson, 2018). The test's procedure included the quantities in Table 3 and Table 4. After the preparation of Table 3, the solutions were mixed well and left to rest for five minutes at room temperature, then it was centrifuged at 3000 rpm for 15 minutes. Afterward, the aliquots in Table 4 were mixed in their wells and the absorbance was measured after seven minutes at 405

nanometers of the sample (A_{sample}) against the blank. The linearity was 120 mg/dL (4 mmol/L) as specified in the bio-diagnostic Glutathione Reduced and sample preparation Protocol.

	Blood (ml)	Tissue (ml)	Blank (ml)
Sample	0.1	0.5	-
D. H₂O	0.5	-	0.5
Reagent 1	0.5	0.5	0.5

Table 3. Glutathione Reduced Procedure with Sample, D. H₂O, & Reagent 1

	Blood (ml)	Tissue (ml)	Blank (ml)
Supernate	0.5	0.5	0.5
Reagent 2	1.0	1.0	1.0
Reagent 3	0.1	0.1	0.1

Table 4. Glutathione Reduced Procedure with Supernate, Reagent 2, & Reagent 3

V.III. Nitric Oxide (NO) Assay

Nitric oxide tests employ a two-step procedure, in which nitrate reduction yield nitrite by vanadium (III) chloride, then nitrite is quantified using the Griess reaction. A nitric oxide assay was used in this research to research immunological responses and identify any possible oxidative damage in cells (Thermofisher, 2020). The procedure was implemented using the aliquots in Table 5. Sample, R1, and up till R2, the aliquots were thoroughly mixed and left to rest for five minutes, the time after which R3 was added. Then, it was mixed well in its well and allowed to stand for five minutes. The sample absorbance (A_{sample}) was set against the sample blank, whereas the standard absorbance (A_{standard}) was set against the Standard blank at 540 nm. The linearity was up to 200 $\mu\text{mol/L}$; the color shall be fixed after many hours after which the procedures were performed.

	Sample (ml)	Sample Blank (ml)	Standard (ml)	Standard Blank (ml)
Sample	0.1	0.1	-	-

R1	-	-	0.1	0.1
R2	1.0	1.0	1.0	1.0
R3	0.1	-	0.1	-

Table 5. Oxidative Stress Assay Procedure with Sample, R1, R2 & R3

Results

I. GC-MS

No.	RT	Compound Name	Conc %	Molecular Formula
1	11.96	Nizatidine	4.07	C ₁₂ H ₂₁ N ₅ O ₂ S ₂
2	12.24	15-methyltricyclo[6.5.2(13,14).0(7,15)]pentadeca-1,3,5,7,9,11,13-heptene	1.39	C ₁₆ H ₁₄
3	14.14	9-Eicosyne	1.98	C ₂₀ H ₃₈
4	14.89	Bisabolol oxide	27.88	C ₁₅ H ₂₆ O ₂
5	14.97	Patchouli alcohol	4.8	C ₁₅ H ₂₆ O
6	15.41	Bisabolone oxide	7.47	C ₁₅ H ₂₄ O ₂
7	16.03	8-(2-nitro-phenoxy)-octan-1-ol	2.99	C ₁₄ H ₂₁ NO ₄
8	19.62	Hexadecanoic acid, ethyl ester	10.12	C ₁₈ H ₃₆ O ₂

9	20.22	Dibutyl phthalate	4.25	C ₁₆ H ₂₂ O ₄
10	22.50	9-octadecenoic acid (Z) (Oleic acid)	12.56	C ₁₈ H ₃₄ O ₂
11	22.81	Octadecanoic acid, methyl ester	2.56	C ₁₉ H ₃₈ O ₂
12	24.25	Cholestan-3-ol, 2-methylene-, (3 α ,5 α)-	2.82	C ₂₈ H ₄₈ O
13	28.67	Diisooctyl phthalate	4.07	C ₂₄ H ₃₈ O ₄
14	32.81	Cholesta-3,5-diene	3.57	C ₂₇ H ₄₄
15	35.37	Cholesterol	9.47	C ₂₇ H ₄₆ O

Table 6. GC-MS active compounds in sea urchin spine extract

Table 6 displays the active compounds, that were revealed from the GC-MS test, along with their concentration in percent (conc %), their molecular formula, and the retention time (RT) taken by each compound. RT shows the time that was taken by each compound in its column in both the stationary and mobile phases.

Analysis & Discussion

Mtt analysis was employed to test the cell viability after being introduced to the two treatment dosages (100 μ g/ml and 200 μ g/ml), along with a control of 100% cell viability, to assess if they have any toxic effects on the cells. As seen in figure 1, at dose 100 μ g/ml, the cell normally proliferated and the cell viability ordinarily increased, to around 136% viability meaning that sea urchin spine extract with concentration 100 μ g/ml can be safely applied on cells. On the other hand, in case of 200 μ g/ml concentration treatment, the cells could not tolerate the dose and their number was reduced lower past the control, referring to a cytotoxic activity of this dosage which resulted in cell death (Ghasemi, Turnbull, Sebastian & Kempson, 2021).

Moreover, the GC-MS chemical analysis, as displayed in table 6, revealed the presence of 15 active compounds in the sea urchin spine extract, with the bisabolol oxide being the major component with almost 28% concentration (RT= 14.89 minutes). Bisabolol oxide (conc %= 27.88) has an anti-inflammatory, anti-irritant, and anti-microbial effect (Kamatou & Viljeon, 2009). It soothes the irritated skin, protects it against free radical damage and is used to lighten the skin. Consequently, it is not only used for wound treatment as an anti-inflammatory agent but also as a scars corrector and a skin care product. Most importantly, it improves the percutaneous absorbance of skin-care chemicals therefore contributes to the absorbance of the other 14 compounds through the skin. This overall considerably enhances the improvement the skin's health condition. Another compound that comes second after bisabolol oxide, in terms of the concentration %, is 9-octadecenoic acid (Z), also known as Oleic acid. Oleic acid (conc %= 12.56, RT = 22.5) stimulates the synthesis of chemoattractant cytokine-induced neutrophil in inflammation 2 alpha/beta (CINC-

2alpha/beta) in addition to increasing the wound healing tissue mass. Consequently, the potential of oleic acid's pro-inflammatory effect was declared to speed up the wound healing process. Third most abundant compound identified through GC-MC were hexadecanoic acid- a fatty acid that is formed naturally in animals and plants and can also be synthesized in the laboratory- and ethyl ester (both with conc %= 10.12, RT = 19.62). Hexadecanoic acid serves in a wide range of applications, including cosmetics and personal care products due to its ability to smooth the skin surface, fix blemishes as well as acting as a surfactants that facilitates wound cleansing (ChemicalSafety, 2022). Moreover, ethyl ester protects the skin fibroblast as well as lymphoid cells in humans against the damaging radiation. Also, another functionality that it exhibits lies in its potential as a treatment for some skin disorders, e.g., skin hyperpigmentation (Sigma-Aldrich, 2006). Forth compound that was identified is Cholesterol (conc %= 9.47, RT = 35.37) which is generated in the liver, then released into the bloodstream, then directed to the regions that requires repair. Cholesterol is in fact responsible for damaged-tissue repair. While the non-extreme LDL deactivates the damaging activity of pathogenic bacteria that can affect an exposed surface of the skin, the topical application of moderate HDL is a successful therapeutic approach corrects impaired wound healing conditions (Stoler, 2018; Gordts, Muthuramu, Amin, Jacobs, Geest, 2014). Furthermore, other compounds were identified as well through the GC-MS test that were furtherly researched in terms of their activity and potential towards the wound healing process. Among these compounds is the Nizatidine (conc %= 4.07, RT = 11.96) which treats ulcer formation and prevents its recurrence (Medline Plus, NIH, 2020). Then, at RT = 14.97, Patchouli alcohol was detected with conc %=4.8 then was labeled as an anti-inflammatory, anti-ulcer, anti-viral, anti-cancer, and anti-aging agent (Lee J., Kong, & Lee S. H., 2020). As mentioned in the literature review section, obesity reduces the efficiency of the wound healing process, alters skin inflammation and prolongs its duration. Meanwhile, the administration of Patchouli alcohol in wound treatments was found to accelerate the healing process as well as ameliorating skin inflammation in mice that were put on a high fat diet (Kim, Pyun, Park, Lee, Abd El-Aty, Song, Shin, Jeong, & Jung 2021). Afterwards, at RT=24.25, cholestan-3-ol, 2-methylene-, (3 α ,5 α) compound- which has anti-inflammatory, antioxidant, antimicrobial, and anticancer properties (Kalaimagal, 2019) was detected. Cholesta-3,5-diene is a derivative of cholesterol that is formed via auto-oxidation. It is also an oxysterol meaning that it regulates cholesterol homeostasis; it modifies apoptosis, protein prenylation, platelet aggregation, and sphingolipid metabolism. Colesta-3,5-diene recruits fibroblasts in wound-gap closure thus promoting the process of wound healing (Al-Hassan, Hinek, Renno, Wang, Liu, Guan, Wen, Litvack, Lindenmaier, Afzal, Paul, Oommen, Nair, Kumar, Khan, Palaniyar and Pace-Asciak, 2020).

Similar to the data obtained in "Antimicrobial and Antioxidant Activity of Sea Urchin", Sea Urchin spine extract was found to be enriched with 2,2-diphenyl-1-picrylhydrazyl, commonly known as DPPH (Shankarlal, Prabu, and Natarajan, 2011). They represent 41% higher significance in the wound healing process than that of the ascorbic acid (Vitamin C) that assists in naturally producing collagen in the human body (Shankarlal, et al., 2011; Hou, Carne, McConell, Bekhit, Mros, Amagase, 2020). This supports the significance of the Sea Urchin spine extract compared to other alternatives in terms of their antioxidant activity.

An additional study with the title "Isolation and Characterization of novel dimeric antimicrobial peptides from Sea Urchin" was published in 2010. It reveals that the cells in the Sea Urchin spine contain two antimicrobial peptides (AMPs) that play a vital role in the invertebrate immune system. This study showed that the two antimicrobial peptides, named centrocins 1 (4.5kDa) and 2 (4.4kDa), are cationic and possess great activity against gram-negative and gram-

positive bacteria (Li, Haug, Moe, Styrvold, and Stensvag, 2010). Therefore, the peptides prevent any bacterial activity if tissue is injured and protect the tissue until being repaired. Not only does the Sea Urchin spine has an antimicrobial effect but it was also found to possess antioxidant activity. Polyhydroxylated naphthoquinone (PHNQ)- in the pigments of the Sea Urchin spine- contains several phenolic compounds, which exhibit strong antioxidant activities similar to the biological activities in edible plant phenolics which are widely used in biomedical applications as natural antioxidants. (Powell, Hughes, Kelly, Conner, and McDougall, 2014).

Furthermore, after identifying the active compounds of the extract, the antioxidant tests of GSH and catalase (antioxidant enzymes) was performed. Since these enzyme act as a defense mechanism that help the cells in fighting the oxidative stress, their presence in a treatment would be an indication of success of this treatment as a potential antioxidant therapy. Figure 2 shows the results of the catalase test (CAT). The results of the test of dosage 100 $\mu\text{g/ml}$ shows a decrease in the cells, which means that 100 $\mu\text{g/ml}$ extract treatment is not much effective as an antioxidant. Although the catalase result with dose 200 $\mu\text{g/ml}$ was higher than that of the 100 $\mu\text{g/ml}$, it was still lower than the control, meaning that the catalase enzyme of the treatment with both dosages is not significant (Nandi, Yan, Jana, and Das, 2019). However, the treatment's antioxidant activity was significant in the GSH test. The antioxidant activity of both dosages increased far past the control: spine 100 $\mu\text{g/ml}$ treatment scored an average of around 0.1 mmol/L; spine 200 $\mu\text{g/ml}$ treatment detected a GSH activity of around 0.098 mmol/L, whereas the control reached around 0.095 mmol/L on average (figure 3). This indeed supports the fact that the treatment was effective with a superior antioxidant activity towards oxygen species (e.g., peroxides, lipid peroxides, heavy metals, and free radicals) (Wilson, 2018). Nevertheless, further investigations are needed, and the CAT test would preferably be repeated to resolve the inconsistency between the results of catalase and the glutathione reduced tests.

Meanwhile, Nitric oxide assay assesses the oxidative stress that a cell was exposed to. The nitric oxide normally creates cell stress if found in abundant form. NO test results of the 100 $\mu\text{g/ml}$ treatment showed a significant increase in the nitric oxide (NO). Also, in the case of the 200 $\mu\text{g/ml}$ treatment, the NO levels was lower than the control, yet not as significant as the 100 $\mu\text{g/ml}$ treatment (figure 4). Given the fact that the moderately lower the NO is, the better and the higher competency of the treatment is (Wong & Lrner, 2015), and since the two dosages scored low NO results, they are declared as safe treatment for the cell in terms of oxidative stress.

Limitations and Future Directions

Alongside the contribution to research, this study encompassed some limitations to be considered across the interpretation of the results section. As priorly discussed, CAT results were inconsistent with other oxidative stress tests done to the same extract with the same concentration. Hence, CAT test would preferably be repeated, with replicas of at least three to ensure consistent and accurate data, to resolve the inconsistency between the results of catalase and the glutathione reduced tests. Moreover, since the tests were done on the cellular level in vitro, future research is needed to extend this study and test the efficacy of the extract in vivo on an animal model, with respect to the acclimation protocols in animal research.

Conclusion

Based on the data presented and discussed in the above sections, the Sea Urchin spine (*Diadema savignyi*) is indeed a marine source that exhibits significant anti-inflammatory and antioxidant effects. It contains active compounds which have anti-inflammatory, antioxidant, and anti-irritant agents such as bisabolol oxide, oleic acid, and nizatidine. In particular, spine extract of 100 $\mu\text{g/ml}$ dosage has higher promising results in terms of cell viability, as detected by the MTT test, and has potential as an antioxidant agent and immunological stimulator as detected by GSH and NO tests, respectively. Though spine extract of 200 $\mu\text{g/ml}$ dosage had the highest results and competency as an antioxidant as determined by GSH. Therefore, this research paper could be furtherly used as a reference for further research on sea urchins as an anti-inflammatory and antioxidant therapeutic agent. Notably is that even though when skin directly touches the sea urchin spines, a wound would form from the sharp pointy ends of the spine, its spine extract has a great potential to be considered as an anti-inflammatory and an antioxidant drug that is employed and integrated within skincare products and wound healing ointments.

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Figures

II. MTT

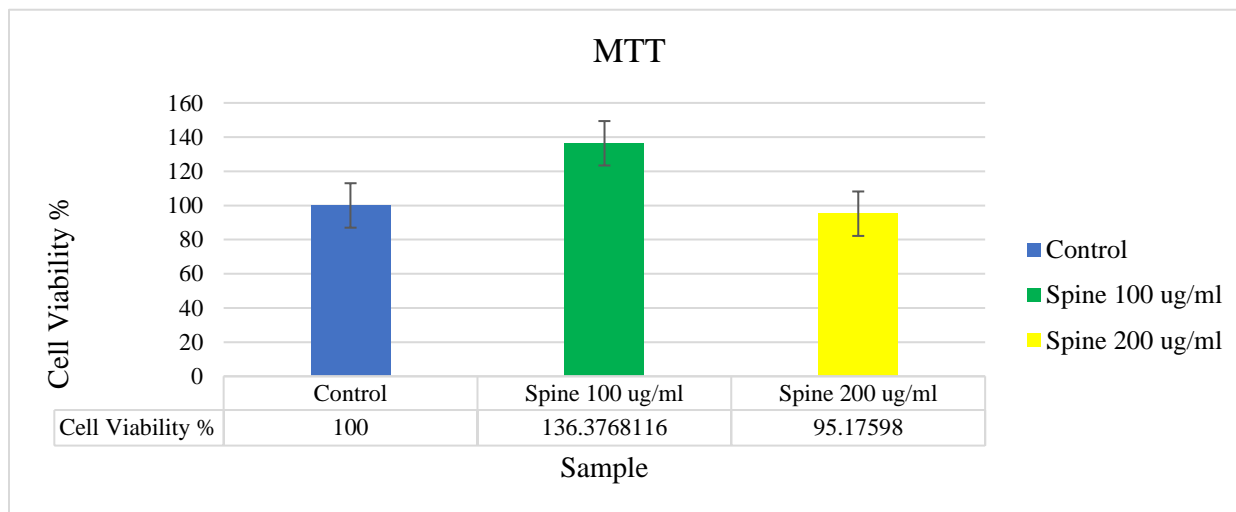


Figure 1. MTT Cell viability of spine extract of different concentrations

Figure 1 shows the results of the MTT test using different concentrations of sea urchin spine extract with respect to a control of 100% cell viability. The y-axis of cell viability in percentage exemplifies the percentage of cells that were declared as vital after the MTT test was carried out. The sample on the x-axis demonstrates the concentration status of the samples that the MTT was applied to; it includes sea urchin spine extract of 100 $\mu\text{g/ml}$ and 200 $\mu\text{g/ml}$ and a control sample.

III. Catalase

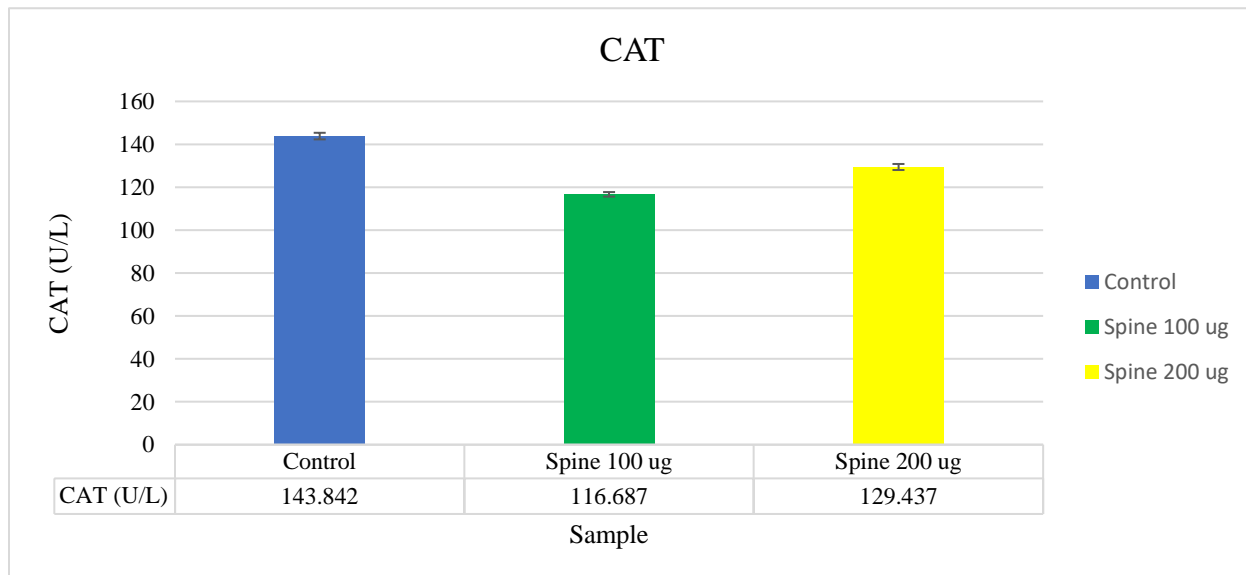


Figure 2. CAT result of spine extract of different concentrations

Figure 2 shows the results of the catalase test of different concentrations of sea urchin spine extract with respect to the control (untreated cells). The y-axis of CAT demonstrates the oxidative stress

that a body was exposed to, in units per liter, that corresponds to the treatment provided by each of the samples presented on the x-axis. The three samples on the x-axis are measured in μg .

IV. GSH

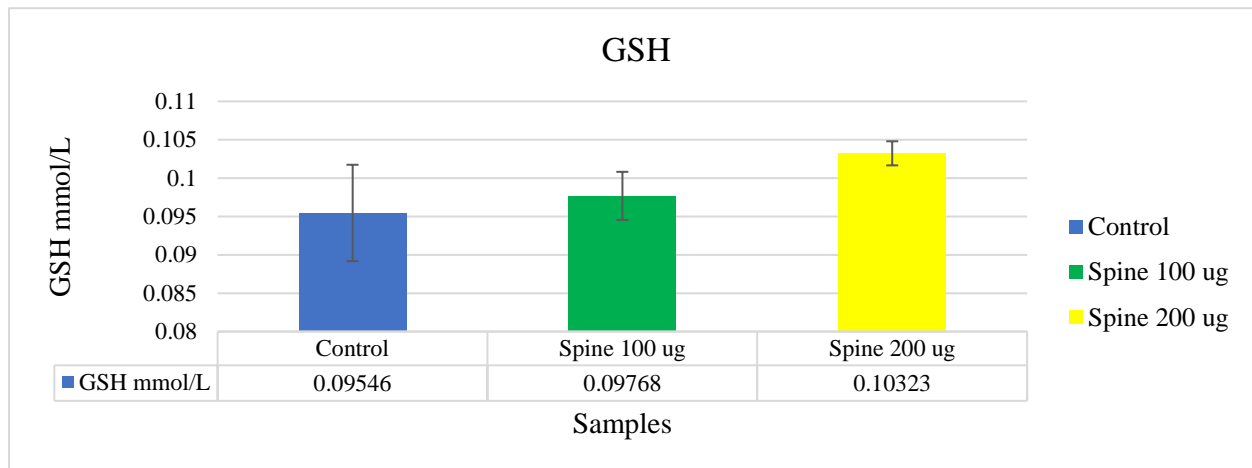


Figure 3. GSH result of spine extract of different concentrations

Figure 3 reveals the results of the Glutathione Reduced (GSH) test of different concentrations of sea urchin spine extract with respect to the control (untreated cells). The y-axis of GSH mmol/L indicates the level of glutathione, an antioxidant, in the blood of mice that were exposed to three samples on the x-axis. The x-axis exhibits the samples that underwent the GSH test.

V. NO

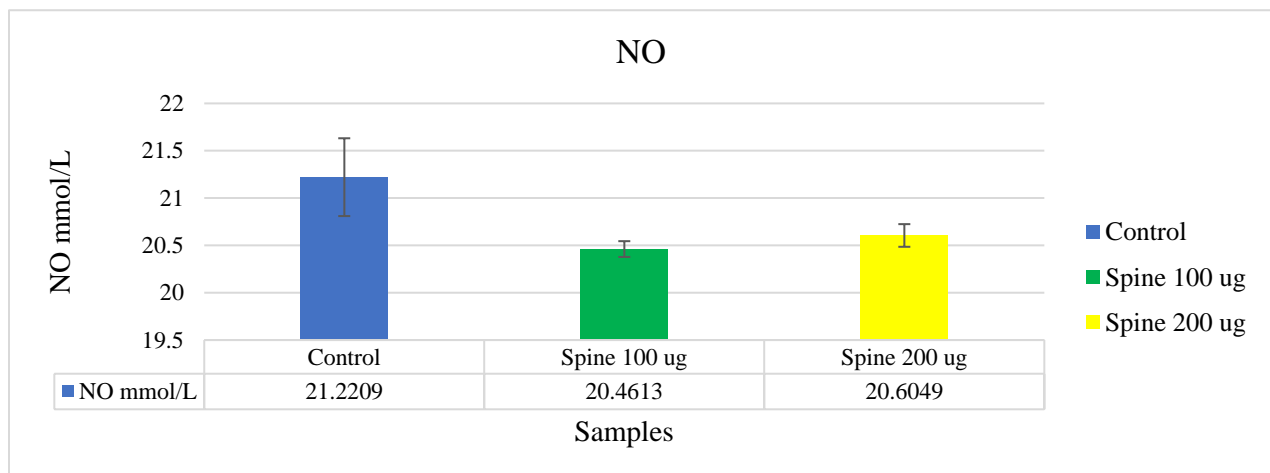


Figure 4. NO assay result of spine extract of different concentrations

Figure 4 shows the results of the Nitric oxide assay of different concentrations of sea urchin spine extract with respect to the control (untreated cells). The y-axis of NO mmol/L indicates the level of oxidative stress and immune response of the samples in millimoles per liter. On the x-axis, the samples that underwent the NO assay are demonstrated.