THE AMERICAN UNIVERSITY IN CAIRO
100 YEARS | SCHOOL OF SCIENCES AND ENGINEERING

Anti-inflammatory and Antioxidant Effects of Sea Urchin Spine Extract

Dina Magdy Mabrouk El Gamal

A thesis proposal submitted to fulfilling undergraduate requirements of bachelor's degree

Under Dr. Ahmed Abdullatif's Supervision

Spring 2022

Thesis Proposal Approval

"Advisors Approval

As your thesis advisor, I have provided you with the research topic and discussed the overall approach. Thus, you have my approval."

Student Full Name : Dina Magdy Mabouk El Gamal

Student ID : 900182958

Thesis Title : Antiinflammatory and Antioxidant Effects of Sea Urchin Spine Extract

Thesis title and proposal have been approved:

Thesis Supervisor:

Name: : Dr. Ahmed Abdellatif

Title: : Assistant Professor and Thesis Advisor

Department: : Biology Department

Institution: : School of Sciences and Engineering

Signature : Ahmed Abdellatif

Acknowledgement

Throughout this experimental work, I have received great assistance and support. First and foremost, I want to express my gratitude to my professor and thesis advisor, Dr. Ahmed Abdellatif, whose expertise guided me in formulating the research methodology. His feedback pushed me to improve academically, practically, and intellectually.

I would also like to acknowledge the laboratory assistant and thesis mentor, Eman Khalil, for her invaluable guidance, aid, and support in the experimental work presented in this report.

Last but not least, I dedicate my thanks to the Undergraduate Research Program at The American University in Cairo which awarded me an academic grant that overall funded this project; the program provided me with the resources I needed to complete this experimental work effectively.

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Abstract

The therapeutic potency of natural products derived from aquatic life has been widely assessed in terms of wound treatment. This research investigates the anti-inflammatory and antioxidant activity of the Sea Urchin spine extract in an experimental setup that uses L929 cell line in vitro. Cell migration and proliferation of L929 cell lines are tested using an MTT assay to assess the cell metabolic activity where the sea urchin spine extract was applied to the cells in two concentrations: 100 µg/ml (with around 136% viability) and 200 µg/ml (around 95% viability). The active compounds of the sea urchin's spine were investigated via GC-MS; the antioxidant and antioxidant activities of the extract were evaluated by catalase test, GSH, and NO. As a result, GC-MS identified bioactive components including bisabolol oxide which has anti-inflammatory and anti-irritant, protects the skin from free radical damage, and improves the percutaneous absorbance of skin-care chemicals. Also, oleic acid, which exhibits a pro-inflammatory effect that is capable of speeding up the wound healing process was identified. Moreover, the oxidative stress tests: GSH and NO revealed the significant anti-inflammatory and 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 repetition of this test is recommended. Nevertheless, the identification of significant anti-inflammatory and antioxidant actions of the sea urchin spine qualifies it as a potential candidate in wound healing ointments.

Keywords: sea urchin spine, anti-inflammatory, antioxidant, wound healing

Literature Review

As defined in the Cambridge dictionary, a wound is a breach in tissue continuity caused by an injury or trauma. 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. Meanwhile, mupirocin was considered unsuitable for patients with renal impairment because its absorbance is high and can severely affect the kidney. Moreover, the authors 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.

Natural products found in the ocean have recently attracted a lot of attention as a worthwhile source of natural remedies with a great impact on human health. Marine creatures are characterized by rich biodiversity with a high potential for discovering medications with antiinflammatory effects to treat wounds. Marine creatures are exposed to a tough environment with challenging conditions compared to terrestrial organisms. They suffer from a high rate of injury traumas and frequent loss of different body parts compared to terrestrial organisms, yet they have an active regeneration and wound healing system (Abubakar, Mwangi, Uku, and Ndirangu, 2012). This emphasizes the successful machinery and anti-inflammatory pathways of marine organisms that protect them against severe infections. This consequently supports a great opportunity for the potential discovery of rich bioactive compounds for new anti-inflammatory treatments.

Indeed, previous works on the effect of Sea Urchin spine extract showed varying degrees of efficiency. Sea urchin is rich in vitamin A, vitamin B complex, omega 3, and omega-6 fatty acids in addition to other valuable 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, previous studies revealed that Sea Urchins have several secondary metabolites that can potentially improve wound treatment 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). Some of the products 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.

Objectives

This work aims to identify the antimicrobial and antioxidant effects of Sea Urchins extract in vitro.

Materials and Methods

I. Marine animal extraction

The Sea Urchin *(Diadema* savignyi) was collected from the beach of Al Ain Al Sokhna, Red Sea, Egypt. The specimen's average weight was 31-45 grams each. After collection, they were immediately transported to the laboratory where the extraction process was carried out. The spine extract of the Sea Urchin was first 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). 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 2 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% CO_2 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 4 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 10 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 1 minute run then 10 second break in between then an additional 1 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 µL + 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_2O	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 5 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 7 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. H_2O	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 5 minutes, the time after which R3 was added. Then, it was mixed well in its well and allowed to stand for 5 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 μmol/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	C12H21N5O2S2
2	12.24	15-methyltricyclo[6.5.2(13,14).0(7,15)]pentadeca-1,3,5,7,9,11,13-heptene	1.39	C16H14
3	14.14	9-Eicosyne	1.98	C20H38
4	14.89	Bisabolol oxide	27.88	C15H26O2
5	14.97	Patchouli alcohol	4.8	C15H26O
6	15.41	Bisabolone oxide	7.47	C15H24O2
7	16.03	8-(2-nitro-phenoxy)-octan-1-ol	2.99	C14H21NO4
8	19.62	Hexadecanoic acid, ethyl ester	10.12	C18H36O2
9	20.22	Dibutyl phthalate	4.25	C16H22O4
10	22.50	9-octadecenoic acid (Z) (Oleic acid)	12.56	C18H34O2
11	22.81	Octadecanoic acid, methyl ester	2.56	C19H38O2
12	24.25	Cholestan-3-ol, 2-methylene-, (3á,5à)-	2.82	C28H48O
13	28.67	Diisooctyl phthalate	4.07	C24H38O4
14	32.81	Cholesta-3,5-diene	3.57	C27H44
15	35.37	Cholesterol	9.47	C27H46O

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.

II. MTT

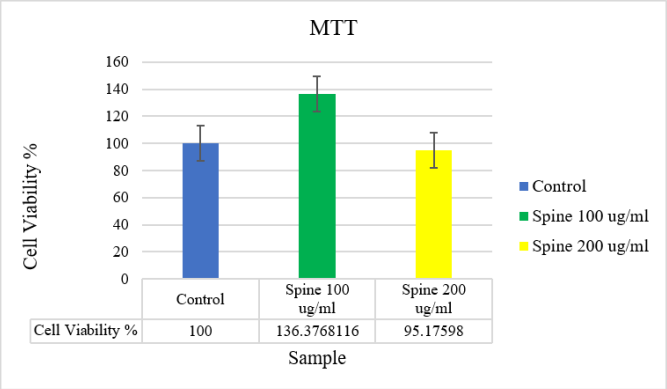

Figure 1. MTT Cell viability of spine extract of different concentrations

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 µg/ml and 200 µg/ml and a control sample.

III. Catalase

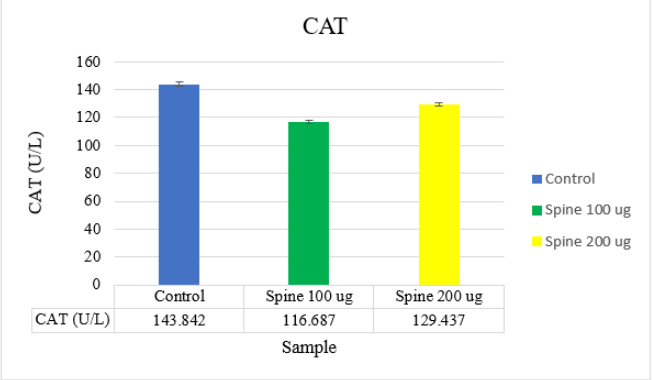

Figure 2. CAT result of spine extract of different concentrations

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 (in µg) presented on the x-axis.

IV. GSH

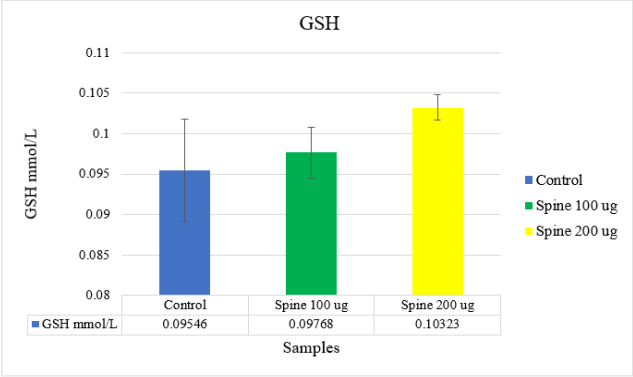

Figure 3. GSH result of spine extract of different concentrations

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

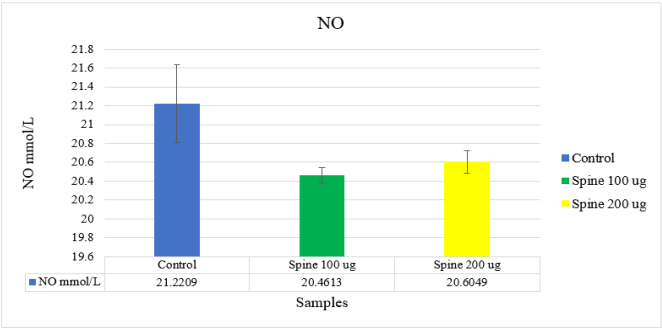

Figure 4. NO assay result of spine extract of different concentrations

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.

Discussion

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

Moreover, the GC-MS chemical analysis 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 cosematics 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 Cholestrol (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 therauptic 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-inflammotory, anti-ulcer, anrti-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-, (3á,5à) compound- which has antiinfalmamatory, 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-picryhydrazyl[1], 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[2] (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).

[1] DPPH: radicals used in model systems to investigate the scavenging activity of neutral phytocompounds.

[2] Ascorbic acid: also known as Vitamin C. It majorly functions in wound healing by assisting the formation of collagen, the most important protein of connective tissue.

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 defence 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. In the catalase test with dosage 100 µg/ml, the cells decreased, which means that 100 µg/ml extract treatment is not much effective as an antioxidant. Although the catalase result with dose 200 µg/ml was higher than that of the 100 µg/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 astonigly significant in the GSH test. In the GSH, the antioxidant activity of both dosages increased far past the control: spine 100 µg/ml treatment scored an average of around 0.1 mmol/L; spine 100 µg/ml treatment detected a GSH activity of around 0.098 mmol/L, whereas the control reached around 0.095 mmol/L on average. 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 invistigations are needed, and the CAT test would preferably be repeated to resolve the inconsistentcy between the results of catalase and the glutathtione 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. Based on the NO test results of the 100 µg/ml treatment, the nitric oxide was observed in a significant decrease. The 200 µg/ml treatment was lower than the control, yet not as significant as the 100 µg/ml treatment. 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.

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 µg/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 µg/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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