Journal of Tropical Pharmacy and Chemistry

Journal homepage: https://jtpc.farmasi.unmul.ac.id

Effectiveness of Sea Urchin Extract *(Echinometra matthaei)* for Wound Healing on Deep Second-Degree in White Rats (*Rattus norvegicus*) Wistar

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Abstract

Burns is a form of tissue damage caused by high temperatures. *Echinometra matthaei* sea urchins have several secondary metabolites that can potentially help in the healing process of burns. In this study, 70% *E. matthaei* ethanol extract was formulated in the form of O / W (oil in water) type cream preparations which were applied topically. This study aims to determine the effectiveness of *E. matthaei* ethanol extract cream preparations on the healing of second-degree burns in Wistar strain rats. In this study preparations were made in 3 formulations, namely Formulation 1 (extract concentration of 1%), Formulation 2 (extract concentration of 3%), and Formulation 3 (extract concentration of 5%). This research was conducted for 7 days with the method used is the post-test only control group design. Experiments were given induction of burns using a hot plate with a diameter of 20 mm at a temperature of ± 200ºC for 15 seconds. Wound healing is observed periodically by observing macroscopically healing inflammation of the inflammatory phase and observing tissue growth in the proliferation phase. The average percentage of inflammation healing showed improvement in the F2: 100%, F1: 80%, F3: 71% results were better than the wound group: 50% as evidenced by the value $\alpha = 0.012$ (<0.05) means there are significant differences. The conclusion of this study shows that the ethanol extract of *E. matthaei* made in cream preparations has the effectiveness of healing of second-degree burns with a formulation of 3% is best formulation.

Keywords: *Echinometra matthaei*, burns, silversulfadiazine, wound healing, napthoquinone

Submitted: 01 October 2020 **Accepted**: 08 January 2021 **DOI**: https://doi.org/10.25026/jtpc.v5i3.281

1. Introduction

Echinometra matthaei is often used by coastal communities as food, the body parts that are often consumed are the gonads or eggs. Sea urchin gonad can be used as an alternative food source because it contains 28 kinds of amino acids, B complex vitamins, vitamin A, minerals, omega-3 and omega-6 fatty acids, while the shells have potential as anticancer, anti-tumor, and antimicrobial properties [1]. As an antimicrobial, sea urchin shell contains bioactive compounds, including

serotonin, glycosides, steroids, cholinergic substances, and bradykinin-like substances [2]. Bioactive compounds from methanol extract of sea urchins shells of the type *Salmacis virgulate*, namely *polyhydroxy naphthoquinone*, are known to have potential as antibacterial and antioxidant properties [3]. Sea urchin gonads also contain saponin compounds that can stimulate the formation of collagen, a structural protein that plays a role in the wound healing process.

Burns can cause damage and increased capillary permeability, damage to skin tissue, in severe cases can cause serious damage to the lungs, kidneys, and liver [4]. Burns in the healing process has four healing phases, including hemostasis, inflammation, proliferation, and remodeling [5]. In this study, induced burns were included in second-degree burns. It is observed in second-degree burns because at this degree a complex wound healing process occurs by involving fibroblasts in the collagen synthesis process to reduce scar formation and hypertrophy. This is by the aim of the study, namely to test the effectiveness of ethanol extract 70% *E. matthaei* which contains several metabolites with the potential to accelerate the healing process of burns.

The extract of sea urchins is made by extracting sea urchins using 70% ethanol solvent by maceration. After being extracted, the sea urchin extract was made semisolid preparations (in the form of cream) which were used as topical applications with various concentrations of 1%, 3%, and 5% of these concentrations following research conducted by Patel *et al* [6] and Jagtap *et al* [7]. *E. matthaei* extract cream was applied to the skin burns of white rats *(Rattus norvegicus)* as much as ± 100 mg once a day. This study used a comparison, namely 1% Silver sulfadiazine cream.

2. Experimental section

In this research, the tools used include glassware (Pyrex), shavers, analytical scales (Ohauss), ovens, rotary evaporators, mortars and stampers, hot plates, cream containers, soxhlet, digital scales, pipettes, syringes. injection, the hot plate 20 mm in diameter, spatula, parchment paper, horn spoon, scissors, tweezers, gauze, tweezers, a *Buchner funnel.*

The materials used in this study include the ethanol extract of 70% *Echinometra matthaei* from the island of Weh, Sabang city, male white rat *(Rattus norvegicus)* Wistar strain, ketamine-HCl, cetyl alcohol, liquid paraffin, span 80, stearic acid, propylene glycol, tween 80, aqua destilata.

2.1. Experimental Animal

The type of experimental animal used was a male white rat Wistar strain with a bodyweight of about 150-250 grams aged 10-12 weeks. The number of mice used in this study was 30 rats, consisting of 6 groups and 5 rats used in each group. The experimental animal population is confirmed to be in good health and there are no visible abnormalities in its body parts, which are obtained from animal farms managed by veterinarians and there is a certification of healthy animals by certificate No. 04 / VIII / 2019.

2.2. Experimental Animal Grouping

A total of 30 rats were divided into:

Healthy group : Rats in good health who were not given induction burns and applied a cream base.
Burn group : Mice induced burns and applied a cream base.
SSD group : Mice induced burns and smeared with Silver sulfadiazine cream for comparison.
F1% group : Mice that were burn-induced and given sea urchin cream with a concentration of 1%.
Group F3% : Rats induced by burns and given sea urchin cream with a concentration of 3%.
Group F5% : Rats induced by burns and given sea urchin cream with a concentration of 5%.

2.3. Animal Preparation

Before use, the experimental animals are acclimatized for 1 week in the animal pen to adapt the animals to the new environment and are given standard food and drinking ad libitum. At this stage, observations are made of the general condition of the tested animal, including body weight and physical condition. Healthy mice are characterized by clean hair, non-standing hair, and clear glowing eyes, with weight that does not gain or decrease each day. Rats that were declared healthy were randomly assigned to the number of five for each group. To reduce the factors that affect the results of the study, test animals with the same strain, environment and food were used.

2.4. Extract Preparation

The extraction method used in this study is the cold extraction method, namely maceration, using 70% v / v ethanol solvent. Put 500 g of sea urchin powder (*Echinometra matthaei*) into a vessel then dissolved with 70% v / v ethanol solvent with a ratio of 1:10, which is 5 L, covered and left for 3 days protected from light and stirred occasionally. The maceration process was replicated 3 times, after which it was filtered to take the filtrate and concentrated using a rotary evaporator, while the ethanol was transferred to a beaker glass and covered with aluminum foil.

2.5. Cream Formulation

In this study, a dose of sea urchin extract was formulated for topical preparations in the form of a cream with the following stratified doses; 1%; 3%; and 5%. The ethanol extract 70% *Echinometra matthaei* cream preparation formulation can be seen in table 1.

Table 1. Formulation of 70% Ethanol Extract of Sea Urchins *(Echinometra Matthaei)*. The formulation is made in 100 gram preparations

Ingredient	Function	Percentage of usage	Formula 1	Formula 2	Formula 3
Extract sea urchin	Active ingredients	-	1	3	5
Paraffin liquid	Cream base (oil phase/emollient)	1-32%	25	25	25
Cetyl alcohol	Cream base (oil phase/emollient)	2-5%	5	5	5
Stearic acid	Cream base	1-20%	10	10	10
Tween 80	Surfactants / Emulsifier	1-10%	1,5	1,5	1,5
Span 80		(used 2%)	0,49	0,49	0,49
Propylenglycol	Co-solvent	5-80%	20	20	20
Aqua destilata	Solvent	-	Ad 100	Ad 100	Ad 100

The method of making sea urchin extract cream used the oil in water (O / W) method. This method is made by making the oil phase first, the oil from the base of liquid paraffin, cetyl alcohol, and stearic acid is melted in a cup at a water bath with a temperature of 60-70 ºC, and mix span 80 and dissolve it until it is homogeneous. The oil phase is set aside into the mortar first.

After making the oil phase then prepare the water phase. The water phase was prepared by mixing the *E. matthaei* extract with propylene glycol which was treated with a hot plate at a temperature of 60-70 °C. After that, Tween 80 was dissolved with water in a beaker glass. Then mix the water phase components at a temperature of ± 50 °C until homogeneous.

After each phase has been formed and is still warm, the oil phase is dissolved in the water phase and crushed until homogeneous in a warm mortar. Preparation of cream in a cream pot and let it cool and solidify to form a creamy mass.

2.6. Treatment of Experimental Animals

The animals had their hair shaved on their back in an area about 3 cm below the auricular of the rats to facilitate the observation of burns during the healing process. Before shaving, the animals were given an intramuscular injection of Ketamine-HCl at a dose of 0.1 mL / 100 multiplied the bodyweight of the animals. This is to facilitate handling and reduce the pain that will arise during treatment. Making burns was carried out according to the method of Akhoondinasab *et al* [8] by heating a circular iron plate with a diameter of 20 mm in boiling water at a temperature of 200 °C then placing it on the skin of the rats' backs for 15 seconds. Each test animal was given cream in the morning as much as ± 100 mg according to their respective groups. Observation of the wound was carried out every day to see the physical changes that occurred in the injured area for 7 days.

3. Results and Discussion

3.1. Yield Extract of *Echinometra matthaei*

The extraction method chosen in this study is the maceration method. The maceration method was chosen because it has certain advantages and is the right method in the sea urchin extraction process. The maceration method was chosen because this method is suitable for materials that are not heat-resistant so that it can avoid damage to efficacious compound components and in this method can minimize the number of raw materials used. The solvent used in the extraction process is

70% ethanol. The choice of ethanol solvent is based on the nature of ethanol which has selective properties, can mix with water, is economical, is able to extract most of the chemical compounds contained in the specimen. 70% ethanol solvent is a solvent that can be used to extract most secondary metabolites with the consideration that the more polar a solvent can only dissolve polar compounds so that 70% ethanol is able to extract a higher amount of secondary metabolites.

The amount of raw material for sea urchin powder used is 500.01 grams with 70% ethanol as a solvent used with a ratio of 1:10. The macerated filtrate obtained is evaporated using a vacuum rotary evaporator with the aim of removing solvents so that a thick extract is obtained, the thick extract obtained is then dried in a vacuum oven with a temperature of 51.8 ° C and a pressure of ± 17 mmHg for approximately 7 days to reduce water content and solvent residues in the extract. The result of the viscous extract obtained by re-macerate three times, namely 104.7753 grams of a viscous extract with the % yield obtained was 20.95%.

3.2. Optimized *Echinometra matthaei* Cream

The cream making of sea urchin *E. matthaei* extract was carried out with several optimization steps which then obtained a stable formulation. Optimization of cream making was carried out to obtain a stable cream preparation. The finished cream preparation is stored for 7 days to see the stability of the cream. The stability of the cream preparations was assessed from several parameters, namely cream color, cream consistency, and cream odor. The optimization of this cream preparation produces a cream that is stable within 7 days. The optimization results of making sea urchin extract cream can be seen in figure 1.

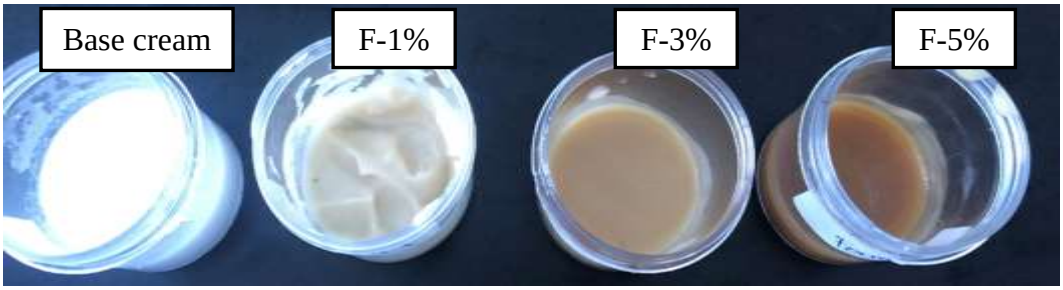

Figure 1. Optimization of Echinometra matthaei extract cream

The preparation is chosen in the form of cream because the cream has several advantages, namely, it is not sticky and easily spreads evenly. The O / W cream type is made by dispersing the water component into the oil component. Cream type O / W is difficult to wash with water but spreads better and has a longer contact time on the skin [9]. Creams can also soothe the inflamed area, can reduce itching and pain [10]. The type of O / W cream preparation was chosen because this type of preparation allows the oil phase to be outside so that it can make the cream preparation penetrate the skin longer and increase the effectiveness of the cream.

The oil phases used in the manufacture of cream preparations are stearic acid, cetyl alcohol, and liquid paraffin. Stearic acid is shaped like a crystalline solid, is white or slightly yellow, shiny, practically insoluble in water, and functions as an emulsifying agent [11]. Stearic acid was chosen as the base for the oil phase cream because stearic acid was able to increase the consistency of the cream. The higher the consistency of preparation, the higher the stickiness, so that the use of stearic acid can increase the stickiness and the cream will last longer when used. The use of stearic acid as surfactants in topical preparations will form a thick base [11]. Cetyl alcohol is formed as wax, in the form of granules or flakes, white in color, practically insoluble in water, functions as a stiffening agent. Cetyl alcohol can also function as an emollient, water-absorptive, and emulsifying agent [11]. The choice of cetyl alcohol as a cream base in the oil phase is because cetyl alcohol can be an emulsifying agent that can increase the stability of the cream preparations by producing a monomolecular and solid barrier on the interface layer of an emulsion to reduce droplet coalescence

[11][12]. Liquid paraffin functions as an emollient in a cream base as well as a solvent, the concentration used is 25% [11]. The water phase in the cream preparation used an excipient capable of dissolving sea urchin extract, namely propylene glycol. Propylene glycol is a clear, colorless liquid that has a chewy, odorless, sweet taste. Propylene glycol can be used as a humectant, it can also be used as a solvent, extractant, preservative, solvent disinfectant, stabilizing agent, water-soluble cosolvent, and antimicrobial. Propylene glycol as a humectant will bind to water and form hydrogen bonds so that it can bind water. Propylene glycol as a humectant can also affect the viscosity of cream preparations [11]. The combination of methylparaben and propylparaben that is used functions as a preservative and antimicrobial which can inhibit the growth of fungi and bacteria and helps stabilize the preparation because the cream preparations are used repeatedly.

In this study, the comparator used was Silver sulfadiazine cream (SSD) which is the gold standard therapy treatment in the form of a 1% topical cream containing silver nitrate and sodium sulfadiazine [13]. SSD is a broad-spectrum antimicrobial agent that is effective against various types of bacteria, fungi, and viruses [14]. SSD in clinical practice is used when there are severe burns accompanied by sepsis conditions. Silver sulfadiazine cream containing silver nitrate can absorb exudate in burns and cause structural changes resulting in the weakening of the bacterial cell walls which will cause distortion and destruction of the bacterial cell walls [15]. Resistance to antimicrobial agents is a major problem at this time so that the effect it causes can slow down the wound healing process, therefore SSD creams have begun to be abandoned.

3.3. Percentage of Healing on Phase Inflammation

The percentage of inflammation healing is based on several parameters in the inflammatory phase, which is seen from the color of the wound, the condition of the bruise on the wound, the presence or absence of irritation in the wound, and filling of pus in the wound area. The results of the percentage of inflammation healing in the inflammatory phase can be seen in table 2.

Table 2. Average Percentage of Inflammation Healing in the Inflammatory Phase

Group	Inflammation Healing Time (Days)							Average Percentage of Inflammation Healing
	1	2	3	4	5	6	7	
Healthy group	0%	0%	0%	0%	0%	0%	0%	0%
Burn group	16,67%	16,67%	33,33%	50%	50%	83,33%	100%	50%
SSD group	80%	80%	80%	80%	80%	100%	100%	86%
Formulation 1%	60%	60%	60%	80%	100%	100%	100%	80%
Formulation 3%	100%	100%	100%	100%	100%	100%	100%	100%
Formulation 5%	40%	40%	40%	80%	100%	100%	100%	71%

The percentage results obtained from the wound control group and the control group test formulation 1%, formulation 3%, and formulation 5% were tested using statistics to see whether there were significant differences in each group in the inflammatory phase. The results of the percentage of inflammation healing were tested for normality and the value of $\alpha = 0.004$ (<0.05) was obtained, which indicates that the results were not normally distributed. The next test is a homogeneity test to see whether the results are homogenous or heterogeneous. The homogeneity test result $\alpha = 0.001$ (<0.05) indicates that the results are not homogeneous. The results of the normality and homogeneity tests indicated that the results were not normally distributed and heterogeneous so that the next test carried out was the Kruskal-Wallis test. The Kruskal-Wallis test results obtained $\alpha = 0.012$ (<0.05), which means that there were significant differences in the wound group and the formulation test group 1%, 3%, and 5%. The next test performed was the Mann-Whitney test to see the differences in each group. The Mann-Whitney test results obtained a significance value of $\alpha = 0.004$ in the wound group compared to the 3% formulation test group.

In the next stage, the test was carried out between the SSD group and the 3% formulation test group to see the best results between the SSD and the 3% formulation. The results of the

normality test obtained $\alpha = 0.000$ (<0.05) which states that the result is not normally distributed. The next test was to see the homogeneity between the SSD group and the 3% formulation group and the results obtained were $\alpha = 0.000$ (<0.05), so that the result was not homogeneous. The test performed was Kruskal-Wallis to see whether there was a significant difference between the SSD group and the 3% formulation test group and obtained $\alpha = 0.007$ (<0.05). The Kruskal-Wallis test results indicated that there was a significant difference between the SSD and the 3% formulation.

In this study, the inflammatory phase that occurred in the wound group was longer with the appearance of signs of inflammation that was more significant than the SSD group and the formulation group. This can be proven by the results of statistical tests between the wound group and the formulation groups of 1%, 3%, and 5% on the percentage of inflammatory healing in the inflammatory phase. The Kruskal-Wallis test results obtained $\alpha = 0.012$ (<0.05), which means that there were significant differences in the wound group and the formulation test group 1%, 3%, and 5%. The wound group showed signs of inflammation on the skin such as edema or swelling in the wound area, while the SSD group showed no signs of edema or blue swelling in the wound area. In the 1% formulation test group, there was also edema, but not all mice experienced it, only one rat had blue swelling, just as with the 5% formulation there was also one mouse that had inflammation in the wound area, while for the SSD group and formulation 3% does not occur in the wound area.

Burn healing involves several cascade pathways of events characterized by the presence of biological processes in a specific sequence and over a period of time. The healing stages are a series of overlapping ones, namely starting the process without waiting for the previous process to be completed [16]. The wound healing process is a process of restructuring the damaged tissue in an effort to restore normal tissue condition. The wound healing process in the inflammatory phase involves homeostasis and swelling, which starts shortly after the injury occurs until the 4^{th} to 6^{th} day [17]. Within 24 hours, neutrophils are seen at the margin of the incision, migrating toward the fibrin clot. The basal cells at the wound end of the epidermis begin to show increased mitotic activation. Within 24 to 48 hours, epithelial cells from both sides have started to migrate and develop along the dermis, storing basement membrane components as they develop. The cells meet in the midline below the surface of the wound area, producing a thin, interlocking epithelial layer between the two halves. By day 3, the neutrophils were largely replaced by macrophages, and there was an increase in granulation tissue in the wound area. Collagen fibers begin to appear at the wound margins, but these are oriented vertically and do not bridge the incision.

Assessed based on the percentage of inflammatory healing in the inflammatory phase, it can be concluded that the wound group that was only given a cream base alone could not help in the wound healing process, while the 1%, 3%, and 5% formulation test groups had similar percentage values to the SSD group which it can be interpreted that *E. matthaei* extract cream has effectiveness in the healing process of inflammatory phase burns.

This is because the extract of sea urchin *E. matthaei* contains polyhydroxy napthoquinone which has the potential as an antibacterial and antioxidant which is useful for preventing infection in the inflammatory phase. Polyhydroxy napthoquinone has antibacterial activity through the biocascade cycle of unstable free radicals by reacting with other molecules on free radicals and binding with oxygen to produce Reactive Oxygen Species (ROS) such as superoxide, hydroxyl radicals, and hydrogen peroxide. This cascade cycle is catalyzed by NADPH cytochrome P450 reductase and other flavoproteins which can damage cell components such as DNA, lipids and proteins causing tissue damage in microbial cells [18][19].

In the inflammatory phase, the best formulation that can help the process of healing burns is a 3% *E. mathaei* extract cream with a 100% percentage. These results were obtained from statistical tests conducted on the SSD group and the 3% formulation test group with a value of $\alpha = 0.007$ (<0.05). The Kruskal-Wallis test results indicated that there was a significant difference between the SSD group and the 3% formulation. The results of the 3% formulation were stated to be better than the 5% formulation. This may occur due to the effect of the depo, where in the skin structure there is a depo area and from that place the active substance will be released slowly [20]. The factors that can cause this to occur are the result of the time when cleaning the wound from the remaining 5% sea urchin extract cream is not completely clean because the 5% cream formulation is more concentrated and difficult to clean so that

absorption occurs and causes the active substance to be absorbed slowly.

3.4. Percentage of Proliferation Phase Skin Tissue Growth

The results of macroscopic observation of the percentage of skin tissue that occurred in second degree burns from day 1 to day 7 in the proliferation phase of all groups can be seen in table 3.

Based on table 3, it can be observed that the wound group has the lowest percentage of tissue growth than the SSD group, the 1% formulation test group, 3% formulation, and 5% formulation. The results on the percentage of tissue growth in the wound group and the formulation test group of 1%, 3%, and 5% were tested for normality by the One-Sample Kolmogorov-Smirnov test and obtained $\alpha = 0.200$ (> 0.05), which indicates that the results were normally distributed. The next test was carried out, namely the homogeneity test, the value of $\alpha = 0.465$ (> 0.05) was obtained, which means that the data was homogeneous. The data is normally distributed and homogeneous so that the Oneway-ANOVA statistical test can be performed. Oneway-ANOVA test results obtained $\alpha = 0.907$ (> 0.05) so that it means that there is no significant difference between the wound group and the formulation test group 1%, 3%, and 5%.

The next stage of the inflammatory phase is the proliferation phase which is estimated to begin on day 3 to day 14 where all groups begin to experience a wound healing process indicated by several parameters such as the presence of fibroblasts and the formation of granulation tissue. The formation of a scab indicates that the wound healing process enters an early stage of proliferation, wherein this phase the wound is filled with inflammatory cells, fibroblasts, collagen fibers, new capillaries forming a reddish tissue whose surface is uneven and called granulation tissue. In this study, the entire group began to show scab formation on day 3. The detachment of the scab in the SSD group and the 5% formulation test group could be observed on day 7, while the wound group and the 1% formulation group, and the 3% formulation group had no scab removal process. The speed at which the scab is formed and the removal of the scab from each group indicates the speed of wound healing, while the wound area that has poor vascularization, the healing process takes longer [21] [22].

Table 3. Average Percentage of Skin Tissue Growth in the Proliferation Phase

Group	Skin Tissue Growth Time (Days)							
	1	2	3	4	5	6	7	Average Percentage of Skin Tissue Growth
Healthy group	0%	0%	0%	0%	0%	0%	0%	0%
Burn group	0%	0%	9,26%	22,22%	24,07%	35,19%	50%	20%
SSD group	0%	0%	6,67%	24,44%	42,22%	53,33%	62,22%	27%
Formulation 1%	0%	0%	6,67%	17,78%	40%	51,11%	66,67%	26%
Formulation 3%	0%	0%	13,33%	26,67%	40%	55,56%	64,44%	29%
Formulation 5%	0%	0%	11,11%	24,44%	42,22%	57,78%	64,44%	29%

The wound group, the SSD group, and the formulation group experienced identified tissue growth with a percentage of tissue growth of 20% for the wound group, 27% for the SSD group, 26% for the 1% formulation group, and 29% for the 3% and 5% formulation groups. This percentage was assessed for 7 days from the start of wound induction to the 7th day so that the results obtained showed a percentage of <50%. However, based on the results of these percentages, it can be observed that the wound group that was only given a cream base alone could not stimulate tissue growth while the formulation group that was given 70% ethanol extract cream with a sea urchin *E. matthaei* concentration of 1%, 3%, and 5% results resembled the SSD group.

The saponin content in *Echinometra matthaei* extract plays a role in the wound healing process. Saponin content has the ability as a cleanser or antiseptic. Saponins can trigger vascular endothelial growth factor (VEGF) and increase the number of macrophages migrating to the wound area, thereby increasing the production of cytokines that activate fibroblasts in the wound tissue [23]. Fibroblasts are cells that produce collagen, reticulum, elastin, glycosaminoglycans,

and glycoproteins from amorphous intercellular substances. Mitosis will appear if the organism requires additional fibroblasts, that is if the connective tissue is injured. Fibroblasts will more actively synthesize matrix components in response to injury by proliferating and increasing fibrinogenesis [24].

In this study, the activity of 70% ethanol extract of sea urchin *Echinometra matthaei* in the healing process of second-degree burns has an activity that can increase the percentage of inflammation healing and the percentage of tissue growth. This shows that the ethanol extract of 70% sea urchin *Echinometra matthaei* has effectiveness in the healing process of second-degree burns in the inflammatory and proliferative phases.

4. Conclusion

Echinometra Matthaei 70% ethanol extract cream has the effectiveness of healing second-degree burns. The best result from the ethanol extract cream formulation of 70% *E. Matthaei* is the cream with the 3% formulation.

References

[1] Aprilia H.A, Pringgenies D, & Yudiati E, 2012. Uji Toksisitas Ekstrak Kloroform Cangkang dan Duri Landak Laut (Diadema setosum) Terhadap Mortalitas Nauplius artemia sp, *Journal of Marine Research*, **1(1)**,75-83. https://dx.doi.org/10.14710/jmr.v1i1.890.

[2] Dahl W.J, Jebson P, & Louis D.S, 2010. Sea Urchin Injuries to The Hand: A Case Report and Review of The Literature, *The Iowa Orthopaedic Journal*, (30),153-156. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2958287/.

[3] Shankarlal S. K. Prabu, & E. Natarajan, 2011. Antimicrobial and Antioxidant Activity of Purple Sea Urchin Shell (Salmacis virgulata L. Agassiz and Desor 1846), *American-Eurasian Journal of Scientific Research* 6, (3): 178-181. https://www.semanticscholar.org/paper/Antimicrobial-and-Antioxidant-Activity-of-Purple-L.-Shankarlal-Prabu/aac22adb623a05281032c4583e2e7123e43175e4.

[4] Moenadjat Y, 2009. Masalah Luka Bakar dan Tata Laksana. Balai Penerbit, *FK UI*. Jakarta.

[5] Guo S, & L. A. DiPietro, 2010. Factors Affecting Wound Healing, Departement of Periodontics, *J Dent Res* 89(3):219-229, USA. DOI: 10.1177/0022034509359125.

[6] N. A. Patel et al, 2011. Formulation and Evaluation of Polyherbal Gel for Wound Healing, *International Research Journal of Pharmaceuticals*, **Vol.** 01, Issue 01, pp. 15-20. https://www.researchgate.net/publication/268422819_Formulation_and_Evaluation_of_Polyherbal_Gel_for_Wound_Healing.

[7] N. S. Jagtap *et al*, 2009. Development and Evaluation of Herbal Wound Healing Formulations. *International Journal of PharmTech Research*, **Vol**.1, No.4, pp 1104-1108. https://www.academia.edu/15139190/DEVELOPMENT_AND_EVALUATION_OF_HERBAL_WOUND_HEALING_FORMULATIONS

[8] Akhoondinasab Mohammad Reza, Motahhare Akhoondinasab, Mohsen Saberi, 2014. Comparison of Healing Effect of Aloe Vera Extract and Silversulfadiazine in Burn Injuries in Experimental Rat Model, *Original Article*, **Vol**. 3. No.1: 29-34. https://www.researchgate.net/publication/269413849_Comparison_of_Healing_Effect_of_Aloe_Vera_Extract_and_Silver_Sulfadiazine_in_Burn_Injuries_in_Experimental_Rat_Model

[9] Fatmawaty A, T Apolarosa R, Rhadia, & Michrun N, 2012. Formulasi, Evaluasi Fisik dan Permeasi Krim Pemutih Asam Kojat dengan Variasi Enhancer, *Majalah Farmasi dan Farmakologi*, **Vol.**16 No.3: 139-142. https://www.e-jurnal.com/2013/11/formulasi-evaluasi-fisik-dan-permeasi.html

[10] Indrayudha P, Dewi Rahmawati, Anita Sukmawati, 2010. Formulasi Krim Minyak Atsiri Rimpang Temu Giring (Curcuma heyneana Val & Zijp): Uji Sifat Fisik Dan Daya Antijamur Terhadap Candida albicans Secara in Vitro, *Majalah Obat Tradisional*, 15(2),56-63. https://www.researchgate.net/publication/260158609_FORMULASI_KRIM_MINYAK_ATSIRI_RIMPANG_TEMU_GIRING_Curcuma_heyneana_Val_Zijp_UJI_SIFAT_FISIK_DAN_DAYA_ANTIJAMUR_TERHADAP_Candida_albicans_SECARA_IN_VITRO_FORMULATION_CREAM_CONTAINING_ESSENTIAL_OIL_OF_Cu

[11] Rowe R. C, Sheskey P. J, Quinn M. E, 2009. Handbook of Pharmaceutical Excipients 6th edition, London; *Pharmaceutical Press*.

[12] Nurdianti Lusi, Ira Rahmiyani, 2016. Uji Aktivitas Antioksidan Krim Ekstrak Daun Mangga (Mangifera indica) Terhadap DPPH (1,1-diphenyl-2-picrylhydrazil), *Jurnal Kesehatan Bakti Tunas Husada*, **Vol.** 16,No.1.(Doi: http://dx.doi.org/10.36465/jkbth.v16i1.165).

[13] Syuhar MN, Windarti I, Kurniawati E, 2015. The Comparison of Second Degree Burns Healing Rate Between the Smear of Honey And The Collision of Binahong Leaves In Sprague Dawley Rats, *Journal Majority*, **Vol**.6 No.1, 2015:103–12.

https://docplayer.info/44711389-The-comparison-of-second-degree-burns-healing-rate-between-the-smear-of-honey-and-the-collision-of-binahong-leaves-in-sprague-dawley-rats.html

[14] Aziz, Zoriah, and Bassman Abdul Rasool Hasan, 2017. The Effect of Honey Compared to Silver Sulfadiazine for The Treatment of Burns: a Systematics Review of Randomized Controlled Trials, *Burns*, 43.1: 50-57. doi: 10.1016/j.burns.2016.07.004.

[15] Venkataraman M, & Nagarsenker M, 2013. Silver Sulfadiazine Nanosystems for Burn Therapy, *AAPS PharmSciTech*, 14 (1):254-64. doi: 10.1208/s12249-012-9914-0

[16] Mardiyantoro Fredy, Khusnul Munika, Viranda Sutanti, Miftakhul Cahyati, Ariyati Retno Pratiwi, 2018. Penyembuhan Luka Rongga Mulut. Malang; *UB Press*.

[17] Politis C, Schoenaers, Jacobs R and Agbaj J O, 2016. Wound Healing Problems in the Mouth, *Frontiers in Physiology* 7, Doi: 10.3389/fphys.2016.00507. doi: 10.3389/fphys.2016.00507

[18] Castro M. A, Gamito A. M, Tangarive-Castano V, Zapata B et al, 2013. Synthesis Antifungal Activity of Terpenyl-1,4 naphthoquinone and 1,4 Anthracenedione Derivatives, *Eur J Med Chem*, 67: 19-27. https://doi.org/10.1016/j.ejmech.2013.06.018

[19] Kumagai Y, Shinkai Y, Miura T & Cho A K, 2012. The Chemical Biology of Naphthoquinones and Its Environmental Implications, *Annu Rev Pharmacol Toxicol*, 52: 221-247. DOI: 10.1146/annurev-pharmtox-010611-134517

[20] M. T Simanjuntak, 2006. Biofarmasi Sediaan Yang Diberikan Melalui Kulit, *USU Repository*.

[21] Aponno, Jeanly V, Paulina V. Y. Yamlean, Hamidah S. S, 2014. Uji Efektivitas Sediaan Gel Ekstrak Etanol Daun Jambu Biji (Psidium guajava) Terhadap Penyembuhan Luka Yang Terinfeksi Bakteri Staphylococcus aureus Pada Kelinci (Oryctolagus cuniculus), *PHARMACON Jurnal Ilmiah Farmasi-UNSRAT* 3 (3): 2302-2493. DOI: https://doi.org/10.35799/pha.3.2014.5400

[22] Lostapa IWFW, Warditha AAGJ, Pemayun IGAGP, Sudimartini NLM, 2015. Kecepatan Kesembuhan Luka Insisi Yang Diberi Amoksisilin Dan Asam Mefenamat Pada Tikus Putih, *Bul. Vet. Udayana*, 8(2): 172-179. https://www.semanticscholar.org/paper/Kecepatan-Kesembuhan-Luka-Insisi-Yang-Diberi-Dan-Lostapa-Wardhita/83aea189c69e6aa8d2a0fd0a9180bf8d0cf715e7

[23] Kimura Y, Sumiyoshi M, Kawahira K, and Sakanaka M, 2006. Effects of Ginseng Saponins Isolated from Red Ginseng Roots on Burn Wound Healing in Mice, *British Journal of Pharmacology*, 148: 860-870. DOI: 10.1038/sj.bjp.0706794

[24] Junqueira L. C, Carneiro J, Contopoulos A. N, 2007. Basic Histology, 10th ed, *Lange Publications*, p.82, Los Altos.