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Burn wound healing activity of ethanol extract gel of Green Algae (*Ulva lactuca* L) in mice

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ABSTRACT

Recently, burn injuries have become a major cause of morbidity and mortality in low-middle-income countries. Burn injuries are tissue loss caused by contact with heat sources such as hot water, fire, chemicals, electricity, and radiation. According to the WHO Global Burden Disease, in 2017 an estimated 180.000 people died from burn injuries. Ethanol extract of green algae (EEGA) contains compounds that potentially heal burn injuries. The research was conducted to obtain a gel formulation from EEGA and test its burn wound healing properties in mice (*Mus musculus*). Forty-five mice weredivided into five groups: I (negative control), II (treated with gel base), III (positive control, Bioplacenton gel), IV (5% EEGA gel), and V (10% EEGA gel). Wound diameters and description scores were observed every fourth, seventh, and fourteenth day, and the derived data were analyzed in the SPSS program with the one-way analysis of variance (ANOVA) and least significance difference (LSD) test. The results indicate that EEGA can be formulated into gels with physical properties compliant with the requirements of the dosage form. Further, it was found that Groups IV and V showed significant reductions in wound description scores and diameters ($p<0.05$). In conclusion, gels containing 5% and 10% EEGA possess burn wound healing properties.

Keywords: green algae, burn wounds, ethanol extract gel of green algae

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INTRODUCTION

Burn injuries are health problems that account for around 180,000 deaths each year worldwide (WHO, 2018). In Indonesia, their prevalence is up to 1.3% (Kemenkes RI, 2018). Burns by heat, for instance, can occur following exposure to fire, hot liquids, hot steam, radiation, electricity, and chemicals (Jose, 2014). This type of traumatic injury can damage andalter various body systems. When not treated immediately, it can disrupt daily activities and cause complications, such as infection, bleeding, electrolyte imbalance, and shock (Handayani et al., 2019). Recently, treatment with natural ingredients has become increasingly popular thanks to its lower side effects than conventional preparations. One of them is green algae.

Green algae (*Ulva lactuca* L) have several active substances with the potential to heal burns. For instance, (Widyaningsih et al., 2016), using thin-layer chromatography, identified the presence of melatonin green algae extracts, while (Dewi, 2018) found vitamin C in this plant. Melatonin can suppress inflammatory responses and increase angiogenesis and collagen synthesis (Liu et al., 2020). Inaddition, vitamin C acts as an antioxidant and promotes collagen and elastin formation. For thesereasons, the melatonin and vitamin C in green algae can accelerate wound recovery (Kembuan et al., 2013).Wound healing is a complex process naturally transpiring in three phases: inflammatory, proliferative, and maturation or remodeling (Jose, 2014). The infiltration of neutrophilsand macrophages into the injured tissue characterizes the inflammatory phase. The proliferative phase starts when macrophage cells secrete inflammatory mediators and enzymes begin to form, as indicated by angiogenesis, collagen deposition, granuloma tissue formation, wound contraction, and epithelialization. The final stage is the maturation or remodeling phase (Murray et al., 2015).

A study by (Lalang, 2021) showed that the ethanol extract of green algae (EEGA) exhibited burn wound healing activities when applied at 5% and 10% concentrations. EEGA is formulated into a gel dosage form to increase absorption and comfort of use through topical application. Therefore, the current research aimed to obtain a gel formulation from EEGA and determine the extent to which 5% and 10% EEGA gels could heal burn injuries (Lalang, 2021).

MATERIALS AND METHOD

Materials

The main component used in this study was the green algae collected from Drini Beach in Gunungkidul, Yogyakarta, Indonesia, and determined at the Faculty of Biology, Ahmad Dahlan University (269/Lab.Bio/B/XI/2020). In addition to the green algae, the EEGA gel was formulated with Carbopol 940 (Bratachem, Indonesia), triethanolamine (Bratachem, Indonesia), methylparaben (Bratachem, Indonesia), and aqua dest (Bratachem, Indonesia). Other materials used during burn wound care were lidocaine ointment (EMLA, AstraZeneca Pharmaceuticals, Sweden), povidone-iodine (Betadine, Mundipharma Healthcare, Indonesia), placenta extract, neomycin sulfate (Bioplacenton, Kalbe Farma, Indonesia), and injectable ketamine (Hameln Pharmaceutical, Germany).

Green algae extraction

The extraction method used was maceration (Widyaningsih et al., 2016). First, the raw green algae were ground into powder, then 500 g of the powder was weighed and soaked for three days in 1000 ml of 96% ethanol. Next, the macerate product was run through a Buchner funnel with a filter paper to obtain filtrate 1. The pulp retained was re-macerated twice using the same procedure and solvent, then the collected filtrate was evaporated in a rotary evaporator at 40°C until most ethanol was reduced. Finally, the extract thickening process was continued using a waterbath until a thick extract was obtained.

Gel formulation and preparation

The derived ethanol extract of green algae (EEGA) was made into gels using three formulations, as shown in Table 1 (Megawati et al., 2020).

Table 1. Gel formulations from 5% and 10% Green Algae extracts

Components	Gel Base	5% EEGA Gel	10% EEGA Gel
EEGA	0 g	5 g	10 g
Carbopol	1.5 g	1.5 g	1.5 g
Triethanolamine	1.76 mL	1.76 mL	1.76 mL
Methylparaben	0.2 g	0.2 g	0.2 g
Aquadest	100 mL	100 mL	100 mL

In the gel preparation, methylparaben was first dissolved in hot water and left to cool. Then, Carbopol was activated in a mortar by slowly adding warm water until it expanded. The addition of Carbopol serves to increase the viscosity (Allen, 2004). Afterward, the swelled Carbopol was added with methylparaben and triethanolamine solutions and stirred until a gel base was formed. Methylparaben was required in the formulation of gel preparation to prevent microbial contamination due to the high water content of the preparation (Rowe et al., 2006). Triethanolamine was chosen to provide an alkaline atmosphere to the carbomer so that the gel becomes thick and clear (Septiawan, 2012). Finally, the gel base was added with the EEGA gradually in small amounts while stirred until homogeneous and then placed in a gel container (Megawati et al., 2020).

Physical properties test of EEGA Gel

The results of the physical properties test were compared with the standards described in Indonesian Pharmacopoeia Edition VI. A gel preparation is deemed "good" when its physical characteristics correspond to these standards.

Organoleptic test

The organoleptic tests have observed the odor and color of the formulation gel (Kemenkes RI, 2020). It was to ensure that the gel had the smell and color of the active ingredients used.

Homogeneity testing

The test was performed by using visual observation from the upper, middle, and bottom sides of the amounts of gel. The sample gel was applied to a transparent glass three times from different sampling points and was observed after 24 hours. The preparation is required to demonstrate a homogeneous construction with no visible rough particles (Khairan et al., 2019; Nikam, 2017; Sugihartini & Wiradhika, 2017).

pH measurement

Gel pH was measured using a universal indicator. The pH paper was inserted into the gel and waited for about 1 minute then removed and adjusted based on the universal standard pH (Fara Azzahra et al., 2019). This procedure aimed to ensure that the gel preparation met the skin pH criterion, which is in the range of 4.5–7 (Firdausi et al., 2021).

Spreadability testing

The spreadability test was carried out by taking one gram of the gel preparation and placing it in the middle of a round glass that have been given a scale. Then covered with glass as the initial load and allowed to stand for 1 minute. After 1 minute the dispersion diameter was measured for each additional 50 g of weight up to 150 g. The gel satisfies the requirement if this diameter is in the range of 5–7 cm (Yusuf et al., 2017).

Adhesion testing

The gel was placed on a slide and covered with another, then a load weighing 1 kg was placed on top for 3 minutes. Adhesion was determined from the time it took for the two slides to come off. The standard requires that the said time should be longer than 4 seconds (Yusuf et al., 2017).

Preparation and testing of EEGA gel on test animals

The treatment and testing of burn wounds in mice have been approved by the Research Ethics Committee of Ahmad Dahlan University with the number of approval 0120110. Deutsch Denken Yoken (DDY) mice aged one month and weighing ±32 g were used. Before starting the experiment, they were left to acclimate to their new surroundings for three days in the Pharmacology Laboratory. They were kept in cages with a 12/12 h light/dark cycle at 20–24°C and 45–65% humidity, fed 6 g of AD2 pellets per day, and given ad libitum access to drinking water.

Forty-five mice were divided into five groups: Group I as the pain control (no treatment), Group II as the negative control (gel-based), Group III as the positive control (Bioplacenton gel), Group IV (treated with 5% EEGA gel), and Group V (10% EEGA gel). The gel was prepared with Carbopol, triethanolamine, methylparaben, and aqua dest and then tested for its activity on the burn model made on the back skin of the test white mice. A metal plate measuring 2x2 cm^2 was heated with a blue-colored flame for three minutes and then placed on the back for five seconds. The gel was applied once a day at 09.00 for 14 days in the morning. The wound description, reduction in wound diameter (cm), and healing time were observed and analyzed on Days 4, 7, and 14 (Prasongko et al., 2020).

Wound description scoring system

The wound description scoring system (Sastrawan et al., 2016) included inflammation, swelling (edema), closure, and scarring, as seen in Table 2.

Table 2. Scoring system of burn wound description

Wound Description Parameters	Scores	Notes
Redness/inflammation (erythema)	3	Very red Slightly red
	2	Not red
	1	
Swelling	3	Very swollen
	2	Slightly swollen Not swollen
	1	
Wound closure	3	Not closed Slightly closed
	2	Closed
	1	
Scarring	3	Not yet dried Slightly dried
	2	Dried (Scar is formed)
	1	

Data Analysis

In addition, the scored parameters and wound diameters obtained on Days 4, 7, and 14 were analyzed statistically using One-way ANOVA in SPSS 25 software at a 95% confidence level, followed by an LSD test to determine differences between the experimental groups.

RESULTS AND DISCUSSION

Physical Properties of EEGA Gel

The formulated EEGA gel was evaluated organoleptically or from its sensory characteristics, including color, shape, and texture. Figure 1 depicts the organoleptic properties of the gel base and the gels containing 5% and 10% EEGA. The one with the highest concentration (10%) produced the darkest color. All gel formulations were soft, had a semisolid shape and texture, and felt cold to the skin.

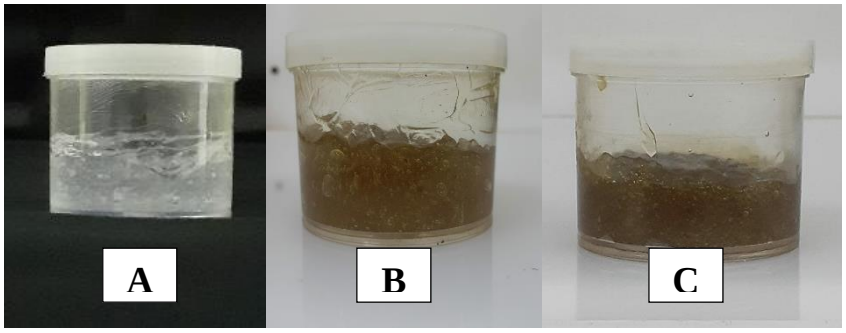

Figure 1. Formulation gel base (A), 5% EEGA Gel (B) and 10% EEGA gel (C)

Table 3. Physical properties of the gel base, 5% EEGA gel, and 10% EEGA gel

	Organoleptic	Homogeneity	pH	Adhesion (sec)	Spreadability (cm)
Gel base	Translucent white	Homogenous	6.0±0.0	8.436 ± 0.109	5.616 ± 0.308
5% EEGA gel	Greenish	Homogenous	6.0±0.00	6.716 ± 0.120	5.220 ± 0.052
10% EEGA gel	Blackish green	Homogenous	6.0±0.00	5.343 ± 0.092	5.306 ± 0.183

Tests for homogeneity, pH, adhesion, and spreadability were intended to support the organoleptic test results, ensuring that the formulated EEGA gels met the standards, and determining differences in their physical properties. Table 3 summarizes the observed and measured physical properties of these gels. Gel base, 5% EEGA gel, and 10% EEGA gel were homogenous, as indicated by the absence of coarse grains. That is probably due to the use of Carbopol which can form a colloidal dispersion system and increase the viscosity. The suspended particles of the active substance would be trapped in the system or remain in its place and also not settle under gravity (Desmayanti, 2015). The pH test showed that all formulations produced gels with a pH of 6.0 or within the range of the skin pH, i.e., 4.5–7 (Firdausi et al., 2021). Further analyses revealed that all formulations also had good adhesion because it took longer than 4 seconds for the attached glass slides in the test to come off, which meets the requirement issued in the Indonesian Pharmacopeia Edition VI (Kemenkes RI, 2020).

Effectiveness of EEGA in healing burn wounds

Based on the literature review explained that the healing time of burn injuries for about 14-21 days (Moenadjat, 2009). Figure 2 shows the burn wound healing process of the five treatment groups in 14 days.

The prepared EEGA gels were able to accelerate burn recovery on the mouse's back skin. In this study, the healing process was scrutinized macroscopically to acquire wound closure description and reduction in wound diameter, as seen in Figure 2. There was a change in the condition of the wound from day 4 to day 14. The initial wound was still wide open and reddish in all groups. On day 7, the wound began to heal, but the diameter was still quite large. Then on day 14, the wound already healed

and the wound diameter was reduced by 5% and 10% EEGA Gel group while the other group was not completely healed.

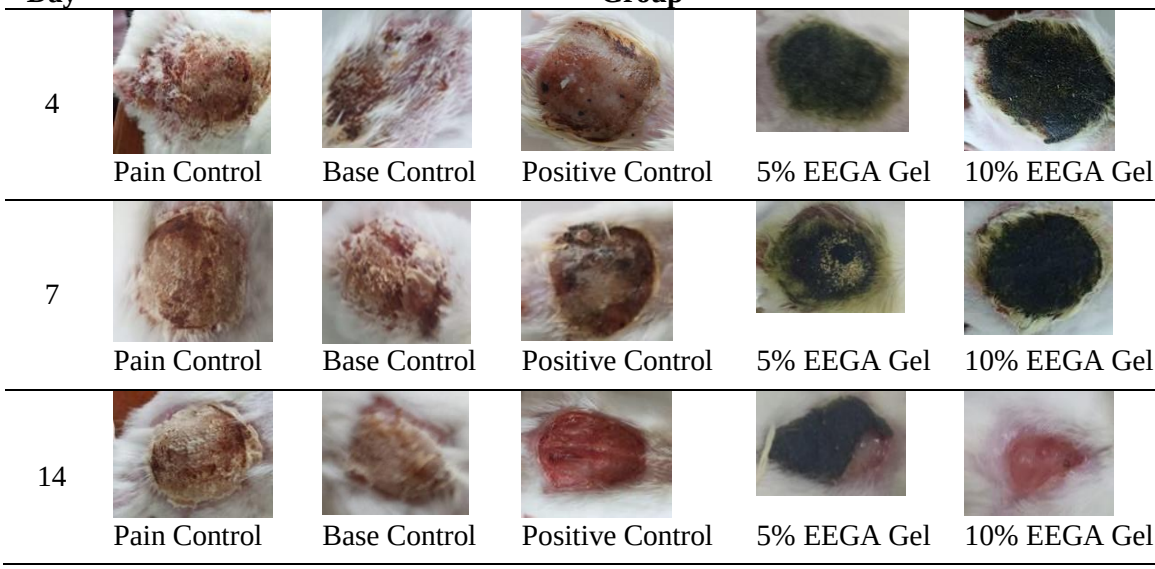

Figure 2. Macroscopic overview of the burn wounds on Days 4, 7, and 14 in the test mice groups

Table 4. Average scores of the burn wound description in different treatment groups on Days4, 7, and 14

Group	Total average ± SD		
	Day 4	Day 7	Day 14
Pain control	2.80 ± 0.17	2.23 ± 0.08	2.1 ± 0.2
Base control	2.44 ±0.08	1.80 ± 0.00	1.13 ± 0.11
Positive control	1.80 ± 0.14	1.03 ± 0.15	0.00 ± 0.00
5% EEGA Gel	1.51 ± 0.22*	1.40 ± 0.00*	0.20 ± 0.00*
10% EEGA Gel	1.55 ± 0.16*	1.20 ± 0.00*	0.53 ± 0.11*

Notes: *significantly different from the pain control and the gel base control

Table 5. Mean diameter (cm) of the burn wounds on the mouse back skin

Treatment	Mean ± SD Diameter Wound on Day *i*-th		
	Day 4	Day 7	Day 14
Pain control	2.36 ± 0.08	2.16 ± 0.14	1.74 ± 0.16
Base control	2.22 ± 0.06	2.11 ± 0.15	1.83 ± 0.60
Positive control	2.22 ± 0.07	2.07 ± 0.11	1.89 ± 0.04
5% EEGA Gel	2.12 ± 0.05	1.80 ± 0.07*	1.35 ± 0.02*
10% EEGA Gel	2.12 ± 0.05	1.70 ± 0.02*	1.30 ± 0.07*

Notes: *significantly different from the pain control and the negative control ($p<0.05$)

Then, wound description parameters were scored to determine how the wound healing process on the back skin accelerated and compare the results between the experimental groups. In addition, wound diameter was measured on Days 4, 7, and 14 to support the parameter scores. Tables 4 and 5 show the average parameter scores and wound diameters, respectively. In all groups, the scores

generally lowered and the diameters shrank during the 14 days of observation, but the ones treated with 5% and 10% EEGA gels showed the most substantial reduction in both. These findings correspond to the statistical analysis results pointing out that the decrease in their wound description scores and diameters were significantly different from the pain control and negative control groups ($p<0.05$).

Based on observations, the 5% and 10% EEGA gel groups exhibited the best and fastest burn healing activities compared with the pain, positive, and negative control groups. Melatonin and vitamin C in the gel is believed to be responsible for its healing capacity. Melatonin relieves burn injuries associated with modulation of the anti-inflammatory balance and mitigation of lipid peroxidation (Bekyarova et al., 2017). Melatonin shortens the transition time from the inflammatory to the proliferative phase by increasing the M1 macrophage (pro-inflammatory)/M2 macrophage (anti-inflammatory) polarization ratio. Moreover, melatonin plays a part in increasing angiogenesis and collagen synthesis (Liu et al., 2020). Vitamin C (ascorbic acid) in green algae has an antioxidant activity that scavenges free radicals, thus inhibiting oxidation and stopping cell destruction. Aside from this antioxidant nature, it also acts as a cofactor in hydroxylation, activating prolyl hydroxylase to convert procollagen into collagen in collagen synthesis (Arief & Widodo, 2018; Kembuan et al., 2013).

CONCLUSION

Ethanol extract of green algae (EEGA) can be formulated into gel whose physical properties (i.e., organoleptic features, homogeneity, pH, adhesion, and spreadability) meet the requirements for a good topical dosage form at 5% and 10% concentrations. Both 5% and 10% EEGA gels effectively heal burn wounds. This is evident from the decrease in wound description scores and wound diameters throughout the observation period.

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