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Research Article

Antibacterial Activity and Wound Healing of Excision Gel of Ethanol Extract of Sintrong (*Crassochepallum crepidioides*)(Benth.) S. Moore.) Leaves in Mice Infected Staphylococcus aureus

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ABSTRACT

Crassochepallum crepidioides (Benth.) S. Moore.) is a plant demonstrated to be effective in wound treatment, empirically and experimentally. contain secondary metabolites, which contribute to the wound healing process. Further research is required to scientifically validate the antibacterial wound healing properties of sintrong leaf ethanol gel. This study aimed to empirically validate the antibacterial efficacy of a gel formulation derived from the ethanol extract of sintrong leaves. Excision wound healing assessments were conducted using the sintrong leaf ethanol extract gel on excision wounds infected *Staphylococcus aureus*. Phytochemical screening, the formulation of ethanol extract gel concentrations 10%, 20%, 30%, antibacterial activity testing of the ethanol extract gel. Excision wound healing activity with a depth 1 mm awidth of 2 cm. The wound became infected treatment was administered for 15 days to monitor the decrease in wound diameter on days 0, 3, 5, 10,15 indicated that the ethanol could be formulated and satisfied the preparation evaluation criteria. Ethanol extract gel has antibacterial properties against *Staphylococcus aureus*. The study on excision wound healing indicated that an increased concentration of extract gel correlateswith a greater percentage of healing. On day 10 the healing percentages of the 30% gel and Bioplacenton were similar at 80%. On day 15 the percentages of 30% gel and Bioplacenton were almost equal 30% gel being 98.50%. Bioplacenton being 99.50% and histopathological assay revealed elevated collagen, fibroblast, and angiogenesis levels corresponding to the increasing concentration of the preparation.

INTRODUCTION

Wounds are physical, chemical, or thermal injuries that compromise the normal continuity of the skin or underlying tissues, compromising their anatomical and functional integrity, potentially resulting in the loss or destruction of bodily tissues. This may result from severe or blunt trauma, temperature fluctuations, chemical exposure, explosions, electric shock, or animal attacks (Tsala et al., 2013; Sjamsuhidajat, 2010).

Excision wounds are wounds caused by sharp scratches that cause tissue damage. According to Singer and Dagum (2008) and Priyandari and Maulidah (2015) the skin surface and underlying layers will clipped the excision wound until the depth varies and the wound edges are regular. During this time, skin infections are more common. *Staphylococcus aureus* is the bacterium that most commonly causes skin wounds (Onggowaluyo and Samidjo, 2003). According to Soedarto (2014) *Staphylococcus aureus* is a gram-positive bacterium found in the axillary, inguinal, perineal, and anterior nostril areas. Its cocci are widespread in nature and are normal human flora.

Staphylococcus, Micrococcus, Staphylococcus aureus, and Streptococcus are

classified as cutaneous pathogenic bacteria, necessitating ability to adhere, proliferate, and penetrate the host to be deemed pathogenic. Staphylococcus aureus is the predominant etiological agent of systemic and cutaneous infections.

Infections caused by *Staphylococcus aureus* can be extremely perilous, potentially resulting in mortality, irrespective of their antibiotic resistance profiles. In 2019, *Staphylococcus aureus* was responsible for 30.9% of 7.7 million deaths attributable to infections worldwide. *Staphylococcus aureus*was the predominant bacterial cause of mortality in 135 countries, accounting for 1,105,000 fatalities in 2019 (Linz *et al*., 2023).

Akinpelu et al., (2019) indicated that the aqueous extract of sintrong leaves have anti-inflammatory properties. Oluwasesan *et al*., (2019) reported that the methanol extract of sintrong leaves exhibits antioxidant and anti-inflammatory properties via the inhibition of lipoxygenase. According to research by Can & Thao (2020) the hydroethanolic extract of sintrong leaves exhibits wound healing properties, demonstrating antioxidant, anti-inflammatory, fibroblast proliferation, contraction, and angiogenesis activities. Kusdianti *et al*., (2008) identified that sintrong leaves possess secondary metabolites such as saponins, flavonoids, and polyphenols, while Adjatin *et al*., (2013) reported the presence of tannins, flavonoids, and steroid chemicals in sintrong leaves as well. This work aims to further investigate the antibacterial efficacy and wound healing properties of ethanol gel derived from sintrong leaves, specifically against *Staphylococcus aureus* infected excision wounds. To enhance the efficacy of herbal medicines and improve their usability it is essential to formulate herbal medicines into pharmaceutical preparations. Researchers will develop gel formulations from the ethanol extract of sintrong leaves andevaluate their antibacterial efficacy and excision wound healing properties in mice infected with *Staphylococcus aureus*.

Gels are semi-solid systems comprising a suspension of small inorganic particles or big organicmolecules that allow liquid permeability. Gels were selected due to their numerous advantages, such asexcellent spreadability on the skin, lack of physiological inhibition on hair, a cooling sensation, and ease of removal with water.

METHODS

Instrumentation

Autoclave, Incubator (Incubator) Microscope (Olympus), Analytical scales (Ohaus), Electric oven (Binder), pH Meter (AZ Instrument), Rotary Evaporator Viscometer (AZ Instrument), Vortex (Biosan) Climatic Chamber (Memmert), Incubator (Binder) Laminar Air Flow (Esco) system are all used in this study. Materials: Leaves of the Sintrong plant were procured from Aceh Province, and plant identification was conducted at the Medanense Herbarium Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of North Sumatra Medan, number 924/MEDA/2023 Materials; Ethanol 96%, *Stahphylococcus aureus* (ATCC® 6538) Metil Paraben (Yokkaichi-shi MTE) Carbomer, (D-RHEO 940) Gliserin (DO Chemical Pacific), Propilenglikol, Propilparaben, Nutrient Agar (HIMEDIA) Muller Hinton Agar (HKM) Trietanolamin (DO Chemical Pacific) Kalium Iodide (Merck) Bismuth (II) (Merck) nitrat (Merck) asam asetat glasial (Merck) raksa (II) klorida (Merck) asam sulfat pekat (Merck) Asam Klorida, Besi (Merck) Klorida (Merck) Kloralhidrat (Merck) Timbal (II) (Merck) Klorida (Merck) Alpha Naftol (Merck) Asam Nitrat (Merck).

Ethical clearance

All rats used in this study were handled in accordance with animal husbandry guidelines, and this study was approved by the Chairman of the Animal Research Ethics Committee of the Faculty of Mathematics and Natural Sciences - University of North Sumatra after considering its relevance to human health guided by the principles of ethical animal research for health research using experimental animal subjects with No.0575/KEPH-FMIPA/20 on 28, September 2023.

Preparation of Ethanol Extract

Simplicia powder was placed in a lidded container to which 5 liters of 96% ethanol was added and agitated for the initial 6 hours. Subsequently, we allow it to stand for 18 hours while intermittently stirring, then filter and collect the filtrate. Repeat the extraction process on the residue using 2.5 L of 96% ethanol to yield maserat II. Merge the two macerates and evaporate the mixture using a Rotavapor at 40°C to yield a concentrated extract (Depkes RI, 2017).

Table 1. Gel formula of ethanol extract of sintrong leaves

Material	Gel Extract Ethanol (10%)	Gel Extract Ethanol (20%)	Gel Extract Ethanol (30%)	Control Negative (Base)
Extract ethanol sintrong leaft	10	20	30	-
Carbomer 940	0.5	0.5	0.5	0.5
Ethanol	2	2	2	2
Propylenglycol	4	4	4	4
Methyl Paraben	0.2	0.2	0.2	0.2
Propyl Paraben	0.02	0.02	0.02	0.02
Triethanolamine	0.5	0.5	0.5	0,5
Aquadest	ad 100	ad 100	ad 100	ad 100

(Nurman *et al*., 2019; Zulkarnain, 2019; Pranuti *et al*., 2018).

Gel Preparation

The ethanol extract of sintrong leaves was measured based on the concentrations specified in Table 1. The extract was pulverized with ethanol until uniformly blended. Carbomer 940 was formulated with 10 mL of distilled water and permitted to rest for 24 hours, Methyl paraben and propylparaben are solubilized in propylene glycol, included into the gel base while continuously agitated until a translucent gel is achieved, followed by the addition of the extract, which is mixed until homogeneous. Incorporate triethanolamine to modify the pH of the gel formulation and gradually introduce distilled water while grinding until a homogeneous mixture is achieved (Zulkarnain, 2019).

Physical Characteristics of Preparations

The attributes of gel formulations using ethanol extract encompass organoleptic assessment and the uniformity of organoleptic evaluations, including aroma, morphology, and hue. The homogeneity test seeks to verify that the gel preparation is uniform and devoid of coarse particles.

Organoleptic examination

The examination is done by visually looking at the shape, colour odour of the gel made. The gel is usually clear with semi-solid concentration (Ansel, 2014).

Homogeneity

1 gram of the prepared gel is put to a transparent glass surface and subsequently coated with another transparent glass layer. The preparation must exhibit uniformity, devoid of any discernible coarse particles. (Ditjen POM, 1985).

Stability test of the preparation

The stability assessment of the formulation encompasses evaluating spreadability, low- temperature storage, ambient temperature, elevated temperature, pH measurement, viscosity of the formulation, and cycling test.

Spreadability test

The test was conducted using a 20×20 cm glass with a millimeter block foundation. One gram positioned at the center of the glass resulted in a measured diameter of 60 seconds, coated with plastic mica. Weights of 0 g, 25 g, 50 g, 75 g, 100 g, and 125 g were maintained for a duration of 60 seconds. The preparation's diameter was then measured (Garg *et al.,* 2002).

Rejuvenation of stock culture

Staphylococcus aureus bacteria were acquired from a pure culture, inoculated by streaking on a nutrient agar medium, and incubated at 37ºC for 24 hours in a Laminar Air Flow, resulting in pure bacterial culture stock (Kamal, 2015).

Preparation of bacterial inoculum

Bacterial colonies of *Staphylococcus aureus* were extracted from the culture stock using a sterile ose needle, suspended in a test tube with 10 mL of 0.9% NaCl solution, and homogenized with a vortex until a standard turbidity of 0.5 McFarland (10^8 CFU/mL) was achieved. A dilution of the bacterial suspension was performed by transferring 0.1 mL of bacterial inoculum into a test tube containing 9.9 mL of 0.9% NaCl solution, which was vortexed until a homogenous concentration of 10^6 CFU/mL was achieved.

Preparation for antimicrobial assessment

Evaluating the antibacterial efficacy of ethanol extract gel formulations derived from

sintrong gel utilizing the agar diffusion technique. 0.1 mL of bacterial inoculum into a Petri dish, thereafter add 20 mL of sterile Mueller Hinton agar medium, allow the temperature to stabilize at 45oC, homogenize, and permit the medium to solidify. Paper discs, saturated for approximately 15 minutes in test solutions with concentrations of 10%, 20%, and 30%, were positioned on the solidified medium. Incubation occurred at 36-37°C for 24 hours, after which the diameter of the inhibition zone (clear zone) was measured using a digital caliper, reported in millimeters. The test is conducted in duplicate.

Antibacterial activity assessment of gel preparation

Evaluate antibacterial efficacy using the agar diffusion method. 0.1 mL of bacterial inoculum was introduced into a sterile petri dish, followed by the addition of 15 mL of Mueller Hinton agar media at 45-50ºC. The media was homogenized with paper plates that had been immersed in the gel test solution for approximately 15 minutes, which contained varied concentrations. Dimethyl sulfoxide (negative control) and Bioplacenton (positive control) were incubated at 37°C for 24 hours, after which the diameter of the inhibitory zone was determined.

Animal testing and excision wound preparation

The determination of the sample size was carried out using the Federer formula as below (Maryanto and Fatimah, 2004).

(t-1) (n-1)	> 15
(10-1) (n-1)	> 15
9 n-5	> 15
6 n	> 20
n	> 3

Each group contained a minimum of 3 male Wistar rats that conformed to the established inclusion criteria obtained from the Pharmacology Laboratory. Rats were acclimatised for 7 days and then shaved on the back. Cleaned with 70% alcohol, anaesthetised rats with ketamine injection (intramuscular) made a circular wound with a diameter of ±2 cm depth of ±1 mm (Kour *et al*., 2021; Mispa *et al.,* 2022; Torabi, *et al.,* 2020).

The wound was cleaned with physiological solution (NaCl 0.9%) then dripped with 0.2 mL of *Staphylococcus aureus* bacterial suspension, then covered with sterile gauze and plastered after 24 hours of observation (Rajoo,2020). Infection is characterized by erythematous, edematous, and purulent lesions. The wound was cleaned with 0.9% sodium chloride solution. It is coated with gel 0.5 g and covered with sterile gauze, then bandaged and left for 24 hours (Kour *et al*., 2020).

Wound healing activity testing

Gel Extract Ethanol 10% : Infection wound addressed with 10% concentration gel Gel Extract Ethanol 20% : Infection wound addressed with 20% concentration gel Gel Extract Ethanol 30% : Infection wound addressed with 30% concentration gel
Control Negative (Base) : Infection wound managed with gel base (negative control) Control Positive (Bioplacenton) : Infectious wound managed with Bioplacenton (positive control) Without Treatment : Wound infection untreated

The wound area was evaluated on days 0, 3, 5, 10, and 15 by visually measuring the wound diameter and computing the percentage reduction in diameter. Wounds were assessed using a caliper, and the percentage of wound healing was calculated employing the formula shown below (Banna *et al*.,2000; Belachewet *et al*., 2020).

$$\% = \text{Diameter of wound area on day 0} - \frac{\text{Diameter of wound on day n}}{\text{Diameter on day 0}} \times 100\%$$

Data Examination

Data on the percentage of wound healing was analysed using the SPSS 25 program. using One- Way ANOVA with a p-value <0.05 if there is a significant difference then followed by a further test (Post Hoc Test) is Duncan.

RESULTS AND DISCUSSION

Plant identification

Plant identification was conducted at the Herbarium Medanense Laboratory (MEDA) within the Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), University of North Sumatra, under reference number 924/MEDA/2023, confirming that sintrong leaf plants belong to the species *Crassocephallum crepidioides* (Benth.) S. Moore.

The outcomes of ethanol extraction

The maceration of 1000 grams with 96% ethanol, followed by evaporation using a rotary evaporator at approximately ±40 °C, yielded a concentrated extract of 210 grams, resulting in a

yield of 21%. This yield represents the ratio of metabolites obtained post-extraction to the initial sample weight (Wardaningrum, 2019).

Gel formulation results of ethanol

The preparation formulation consists of carbopol 940, propylenglycol, methyl paraben, propyl paraben, triethanolamine, and distilled water. Ethanol extract of sintrong leaves as active substance, carbopol 940 base, propylenglycol humectant, methyl paraben and propyl paraben preservative, triethanolamine as a calculating agent to neutralise the acidity of carbopol. Triethanolamine neutralises the carboxylic group of the polymer and is ionised so that electrostatic repulsion occurs between negatively charged particles, thus increasing the development and thickening properties of the polymer (Rowe, 2009 ; Magbool *et al*., 2018)

Table 2. Organoleptic test results of sintrong leaf ethanol extract gel

Formula	Consistency	Smell	Colour
Gel Extract Ethanol 10%	Viscous	Sintrong leaf	Green-brown
Gel Extract Ethanol 20%	Viscous	Sintrong Leaf	Green-brown
Gel Extract Ethanol 30%	Viscous	Sintrong Leaf	Green-brown

Table 3. Mean results of gel spreadability diameter of ethanol extract of sintrong leaves

Added Load (g)	Mean Scatter Diameter Results (cm) (Mean ± SD)		
	Gel Extract Ethanol 10%	Gel Extract Ethanol 20%	Gel Extract Ethanol30%
0	5.30±0.05	5.25±0.05	5.20±0.05
25	5.50±0.15	5.40±0.10	5.35±0.10
50	5.70±0.10	5.50±0.05	6.40±0.10
75	6.23±0.12	6.20±0.14	6.55±0.05
100	6.40±0.10	6.30±0.10	6.70±0.10
125	6.75±0.10	6.60±0.05	6.90±0.10

*Numeric data presented as mean±SD, standard deviation.

Table 4. pH measurement data of extract gel at 12 weeks storage

Storage (Week)	Mean pH±SD		
	Gel Extract Ethanol 10%	Gel Extract Ethanol20%	Gel Extract Ethanol 30%
1	6.5±0.2	6.4±0.3	6.5±0.2
3	6.2±0.0	6.3±0.2	6.4±0.3
7	6.1±0.1	5.9±0.2	5.8±0.2
9	5.8±0.2	5.7±0.2	5.6±0.2
11	5.6±0.2	5.8±0.2	5.5±0.2
12	5.7±0.1	5.5±0.2	5.4±0.2

Table 5. Viscosity measurement data of sintrong leaf extract gel

Formula	Mean Viscosity Value (cPs) ±SD Storage Duration (Week)			
	0	4	8	12
Gel Extract Etanol 10%	3150±14.18	3142±11.01	3034±6.02	3014±5.03
Gel Extract Etanol 20%	3262±10.44	3243±11.26	3174±21.07	3029±21.57
Gel Extract Etanol 30%	3329±5.50	3281±1.52	3135±3.60	3048±8.08

Homogeneity test

The homogeneity test was conducted to ascertain the presence or absence of coarse grains and the uniform distribution of the ingredients in the mixture. The results indicated that the three gel formulations exhibited excellent uniformity, with no particles present on the glass surface.

Spreadability check

The gel's spreadability augmented with increasing load, although it consistently adhered to the semi-solid preparation spreadability standards of 5-7 cm (Garg *et al*., 2002). The outcomes of this spreadability test are presented in Table 3.

The assessment of spreadability seeks to evaluate the preparation's capacity to disseminate at the point of application during utilization. Spreadability is inversely related to viscosity; higher viscosity corresponds to reduced spreadability. The findings of the gel spreadability assessment indicated that it fell within the semi-solid preparation criteria, specifically 5-7 cm (Garg *et al*., 2002).

Stability of the preparation

Assessment of the stability of 10%, 20%, 30% ethanol extract gel by evaluating physical changesin the product over a storage duration of 12 weeks, physical stability assessment includes organoleptic analysis, pH measurement, and viscosity evaluation (Vadas, 2010).

Determination of the pH of the preparation

The pH measurements of the ethanol extract gel prepared from sintrong leaves indicated a weekly decline in pH until the twelfth week. The typical pH range for skin is 4.5 to 6.5. An excessively acidic pH in a formulation may lead to skin irritation, whereas an overly alkaline pH might result in dry skin (Afriani, et al., 2021). The pH of the ethanol extract gel formulation, according to measurements, falls within the required range approximate 4.5-6.5 for all of concentration.

Viscosity test measurement

The viscosity was assessed utilizing a Brookfield viscometer. The viscosity of an optimal gel formulation ranges from 2000 to 4000 cps (Garg et al., 2002). According to Tables 5 the viscosity of the preparation diminished from the first day to the twelfth week. This aligns with the findings of Ariani *et al*., (2021), which indicate that carbopol is hygroscopic, enabling it to absorb water vapor, hence reducing the viscosity of nanogels and gels.

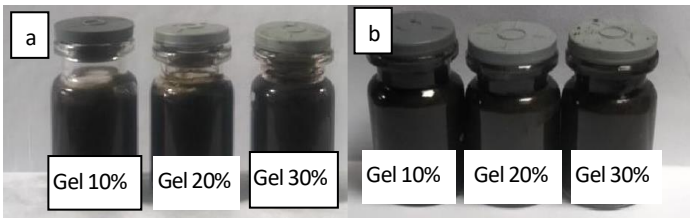

Figure 1. a) Gel preparation before *cycling test* and b) after *cycling test*

Table 6: Inhibition zone diameter results of sintrong leaf ethanol extract gel preparation

Concentration (%)	Diameter of inhibition zone against *Staphylococcus aureus* bacteria		
	Sintrong leaf ethanol extract gel		
	Logaritma Natural (Ln)	X.±SD	X^2
0	0	0	0
Gel Extract Ethanol 10%	2.302	7.26 ±0.11	52.7076
Gel Extract Ethanol 20%	2.995	8.13 ±0.05	66.0969
Gel Extract Ethanol 30%	3.401	9.00±0.2	81.00
Control positive (Bioplacenton)	-	21.02 ± 0.01	441.00

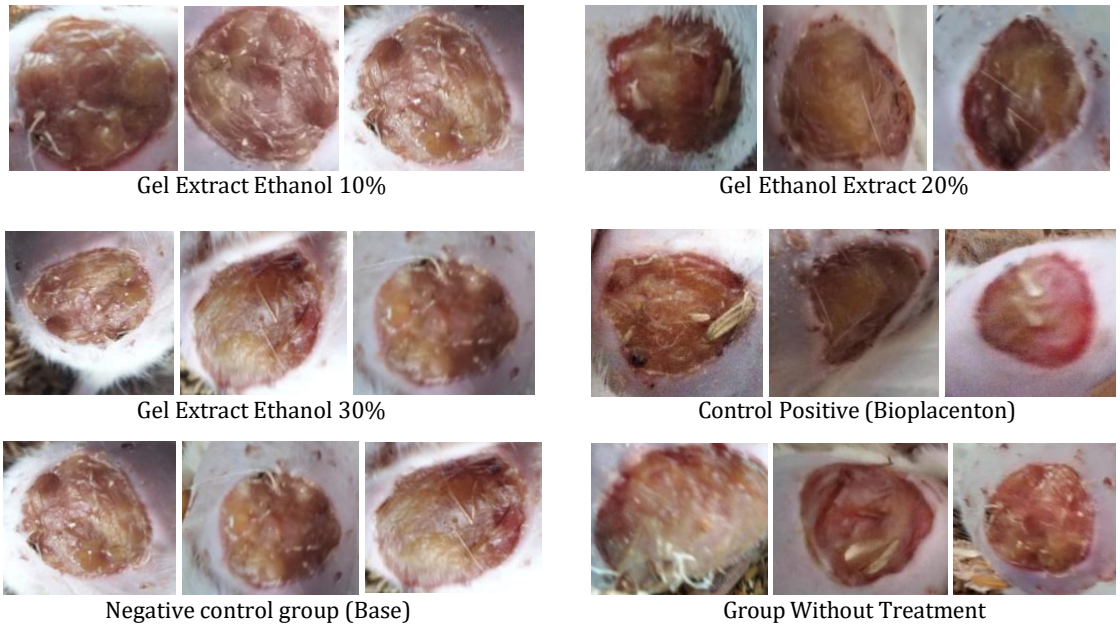

Figure 2. Wound photo of infected excised wound test animals on day 3

Cycling test

The *Cycling* Test is carried out to see the simulation of temperature changes every year and even every day of a preparation (Nisak, 2016). The results of the *cyling* test can be seen in Figure 1.

The samples were maintained at a temperature of 4±2ºC for 24 hours before being transferred to an oven set at 40±2ºC for a further 24 hours. The experiment was conducted across six cycles, during which phase separation was detected. The Cycling Test was performed to examine the simulation of temperature variations annually or even daily (Nisak, 2016). The observational results color, distinct odor, thick gel consistency, no particle clumping, and easy application showed no alteration before or after storage. This signifies that the ethanol extract preparation is stable when stored at both low and high temperatures.

Antibacterial activity test of leaf ethanol extract gel

The antibacterial activity test findings of sintrong leaf ethanol extract gel indicated that the maximum inhibition diameter occurred at a 30% extract concentration. The diameter of the inhibitory zone measured 9.00 mm, indicating (partially active category) a 10% ethanol extract gel produced a diameter of 7.26 mm (inactive category) and 20% gel extract resulted in an inhibitory zone diameter of 8.13 mm (inactive category). The concentration of the extract influences the diameter of the inhibition zone, a higher concentration results in a larger diameter of the inhibition zone. The potency of antibacterial action is categorized into four classes, less than 9 mm inactive category 9-12 mm (partially active category) 13-18 mm (active category) and more than 19 mm (very active category)(Alves et al., 2000).

Excisional wound healing activity test results

Male rats were utilized as test subjects and thereafter infected with 0.2 mL of *Staphylococcus aureus* bacterium (Septiari, 2012). Wound infection is characterized by erythema, edema, and purulence. Infection symptoms typically manifest 2 to 3 days post-treatment. Following the observation of test animals infected for 3 days, characterized by festering, swollen, and erythematous wounds, additional observations were conducted for 15 days post-infection (Kozier, 1995; Taylor, 199; Cuazitl *et a*l., 2014; Jamila, 2015; Arisanty, 2014).

Test subjects were inoculated with 0.2 mL of *Staphylococcus aureus* bacteria. Septiari (2012) states that wound infection is marked by erythema, edema, and purulence. Infection symptoms typically manifest 2-3 days post-treatment. Throughout the observation period, the test subjects exhibited infections after three days. The infection was marked by pustular, swollen, and erythematous lesions (Kozier, 1995; Taylor, 1997).

Observation of wound diameter

According to Table 7. on day 3, the wound diameter has diminished in all group. By day 5, the wound diameter has diminished

considerably. On day five, the wound has progressed to the proliferative phase. The macrophage phase will secrete fibroblast growth factor (FGF) and angiogenesis growth factor (AGF). Fibroblast growth factor will induce fibroblasts to synthesize collagen and elastin, which are essential for wound closure. On days 10 and 15, the wound diameter shows a dramatic reduction, attributed to the continuous proliferation process, resulting in the initially deep wound attaining a flat surface (Febram, 2010). Bioplacenton demonstrated progressively accelerated wound healing, with the wound nearly fully closed by day 15, measuring a diameter of 0.01±0.00. Bioplacenton is a topical medication comprising 10% placental extract and 0.5% neomycin sulfate Placenta extract can enhance collagen synthesis, augment tissue protein, expedite angiogenesis, and promote epithelialization (MIMS, 2016 and Navadiya *et a*l., 2012).

The decrease in wound diameter with increasing concentration is ascribed to the influence of chemical compounds on wound healing. Flavonoids inhibit Cyclooxygenase and lipoxygenase, thereby reducing the migration of inflammatory cells to the wound tissue, which shortens the inflammatory reaction and allows the proliferation phase to commence promptly. Flavonoids function as antioxidants by diminishing reactive oxygen species. (Kusumawardhani et al., 2015; Kartikasari *et a*l., 2019).

Alkaloids exhibit antibacterial properties by altering the peptidoglycan structure of bacterial cells, resulting in incomplete cell wall formation and subsequent cell death. Saponins enhance fibroblast TGF-B receptors, facilitating the binding of TGF-B essential for collagen synthesis in fibroblasts. Saponins facilitate wound healing by initiating the formation of collagen, which is essential in the healing process. Stimulates fibroblasts to synthesize collagen and elastin, which contribute to wound closure by generating new ligaments and granulations that subsequently multiply, resulting in a wound that, originally deep, develops a flattened surface with the borders of the wound. Angiogenesis growth factor will promote the development of new blood vessels.

Table 7. Average data of wound diameter measurement results of sintrong leaf extract gel.

Preparations	Mean Wound Diameter (cm) ± SD (Days)				
	0	3	5	10	15
Gel Extract Ethanol 10%	2±0.00	1.83±0.00	1.65±0.25	0.79±0.18	0.70±0.00
Gel Extract Ethanol 20%	2±0.00	1.78±0.05	1.58±0.0	0.61±0.20	0.45±0.00
Gel Extract Ethanol 30%	2±0.00	1.64±0.00	1.50±0.00	0.40±0.05	0.03±0.00
Control Positive (Bioplacenton)	2 ± 0.00	1.58±0.23	1.30±0.18	0.39 ±0.00	0.01±0.00
Negative control (Base)	2 ± 0.00	1.89±0.00	1.72±0.00	1.29±0.00	1.0±0.00
Without treatment	2 ± 0.00	1.91±0.00	1.77±0.00	1.40±0.00	1.06±0.02

*Numeric data presented as mean±SD, standard deviation.

Table 8. Data on the percentage change in wound diameter of sintrong leaf ethanol extract gel

Preparations	Percentage cure ± SD(Days of Observation)				
	0	3	5	10	15
Gel Extract Ethanol 10%	0±0.00	8.50 ± 2.88^{a}	17.50 ± 13.2^{ab}	60.50 ± 9.25^{b}	$65.00\pm8,66^{b}$
Gel Extract Ethanol 20%	0±0.00	11.00 ± 2.55^{b}	21.00 ± 2.64^{c}	69.50 ± 10.3^{b}	77.50 ± 11.2^{b}
Gel Extract Ethanol 30%	0±0.00	18.00 ± 1.25^{bc}	25.00 ± 0.28^{c}	80.00 ± 2.50^{c}	98.50 ± 0.28^{c}
Control Positive (Bioplacenton)	0±0.00	21.00 ± 2.46^{c}	35.00 ± 12.3^{d}	80.50 ± 0.28^{c}	99.50 ± 0.86^{c}
Negative Control (Base)	0±0.00	5.5 ± 0.72^{a}	14.00 ± 0.28^{a}	35 ± 0.00^{a}	50.0 ± 5.00^{a}
Without Treatment	0±0.00	4.5 ± 0.5^{a}	11.50 ± 1.32^{a}	30 ± 2.78^{a}	47 ± 2.88^{a}

*Numeric data presented as mean±SD, standard deviation. Treatments with the same superscript are not significantly different, conversely treatments with different superscripts mean they are significantly different.

Table 9. Mean collagen density values

Treatment Group	Mean Collagen Density ± SD
Gel Extract Ethanol 10%	1.2±1.21 mm^{2a}
Gel Extract Ethanol 20%	1.6 ± 0.6 mm^{2abc}
Gel Extract Ethanol 30%	2.46±0.11 mm^{2bcd}
Control Positive (Bioplacenton)	2.86±0.23 mm^{2d}
Negative Control (Base)	1.2±0.34 mm^{2a}
Without Treatment	0.86±0.11 mm^{2a}

*Numeric data presented as mean±SD, standard deviation. Treatments with the same superscript are not significantly different, conversely treatments with different superscripts mean they are significantly different.

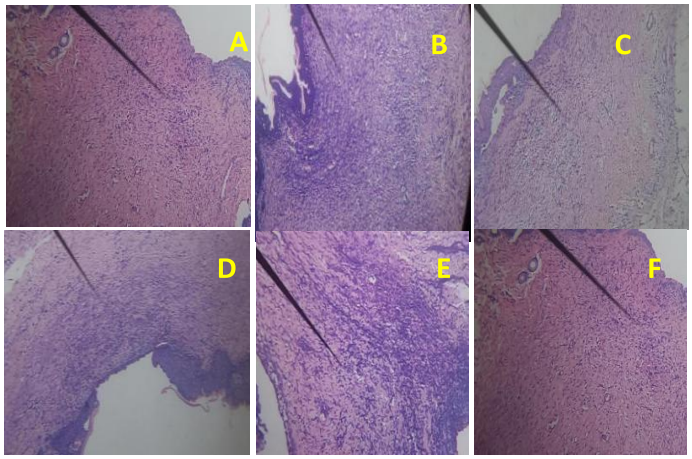

Figure 3. Histopathological features of fibroblast density (a) bioplacenton; (b) base; (c) untreated infected wound; (d) 10% sintrong leaf ethanol extract gel; (e) 20% sintrong leaf ethanol extract gel; (f) 30% sintrong leaf ethanol extract gel; (g) Collagen (→)

On days 10 and 15, the wound diameter diminishes due to cellular proliferation, resulting in a transition from a deep wound to a flat surface at the wound edges. (Masir *et al*., 2012; Nadira *et al*., 2021; Putrianirma *et al*., 2019; Ibrahim & Kuncoro, 2012; Febram, 2010).

Significantly, on day 10, both the 30% EEDS gel and Biplacenton exhibited a similar therapeutic effect, attaining an 80% recovery rate. On day 15, the percentage gel extract 30% to Bioplacenton improved significantly, attaining 98.50% for gel extract 30% and 99.50% for Bioplacenton (positive control). It means that gel extract is 30% effective for wound healing excision. Anti-inflammatory, antibacterial, antioxidant, analgesic, and astringent effects have been associated with secondary metabolites found in EEDS gel, such as alkaloid, flavonoid, tanin, saponin, and triterpenoid.

Histopathological observation results

Wound healing was evaluated by collagen density metrics in histological samples of skin from experimental animals. The density of collagen fibers generated during the wound healing process was quantified following HE staining and examined using an Olympus BX51 microscope. Olympus DP 20 camera has 10x10 magnification for a 5-degree field of view. Collagen pictures were acquired in pink hue and subsequently evaluated using a scoring algorithm.

According to Table 9. the negative control group (1.2±0.34 mm^2) shown no significant difference in collagen production compared to the 10% extract gel group (1.2±1.21 mm^{2a}) or the 20% extract gel group (1.6±0.6 mm^{2abc}). Nonetheless, it exhibited substantial divergence from the 30% extract gel group (2.46±0.11 mm^{2bcd}) and Bioplacenton (2.86±0.23 mm^{2d}). The 30% extract gel (2.46±0.11 mm^{2bcd}) exhibited no significant difference compared to Bioplacenton (2.86±0.23 mm^{2d}).
This indicates that the 30% extract gel concentration can enhance collagen synthesis to

levels akin to Bioplacenton. Collagen density serves as a crucial signal in the wound healing process. The collagen produced will create connective tissue that securely binds the wound edges, and the presence of collagen can enhance the connection between new tissues, so fortifying the healing region (Ermawati, 2021).

Fibroblast count results

Table 10 indicates that the negative control group (41.26±9.81ab) exhibited no significant difference compared to the untreated wound group (41.20±68.32a). Nonetheless, it markedly differed from the 10% extract gel group (91.26±68.32b), the 20% extract gel group (97.73±32.85c), and the 30% extract gel group (118.86±13.05c). Compared to the positive control group (Bioplacenton), the 10%, 20%, and 30% gel extracts yielded considerably fewer fibroblasts (165.60±0.80d). This indicates that the ethanol extract gel of Sintrong leaves is less effective in stimulating fibroblast formation compared to the negative control group (Bioplacenton).

The primary cell involved in the wound healing process is the fibroblast, which is responsible for synthesizing collagen fibers. Upon injury, fibroblasts promptly migrate to the wound site, proliferate, and generate a collagen matrix to repair connective tissue. They also synthesize various extracellular matrix components, stimulate collagen production, and initiate the closure and repair of damaged skin tissue (Sumbayak, 2015; Amita *et al*., 2017; Al-Fa'Izah *et al*., 2018; Dwita *et al*., 2020).

Results of total angiogenesis

Table 11. indicates that the negative control group (3.00±0.00a) exhibited no significant difference in angiogenesis production when compared to the untreated infected wound group (1.60±0.00^{a}), the 10% extract gel group (4.06±2.01^{a}), the 20% gel extract group (4.30±1.03^{ab}), and the 30% gel extract group (5.53±0.50^{ab}). whereas the 10%, 20%, and 30% gel extract groups exhibited a statistically

Table 10. Mean values of fibroblasts

Treatment Group	Fibroblast Mean Value ± SD
Gel Extract Ethanol 10%	91.26±68.32^{b}
Gel Extract Ethanol 20%	97.73±32.85^{c}
Gel Extract Ethanol 30%	118.86±13.05^{c}
Control Positive (Bioplacenton)	165.60± 0.80^{d}
Negative Control (Base)	41.26± 9.81^{ab}
Wound infection without treatment	41.20±68,32^{a}

*Numeric data presented as mean±SD, standard deviation. Treatments with the same superscript are not significantly different, conversely treatments with different superscripts mean they are significantly different.

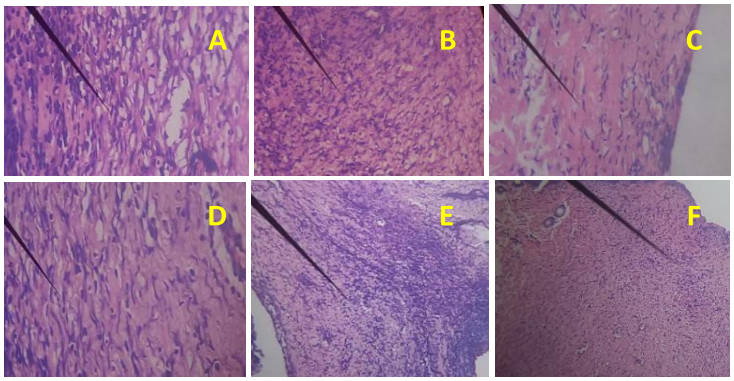

Figure 4. Histopathological features of fibroblast density (a) bioplacenton; (b) base; (c) untreated infected wound; (d) 10% sintrong leaf ethanol extract gel; (e) 20% sintrong leaf ethanol extract gel; (f) 30% sintrongleaf ethanol extract gel; (g) Collagen (⟶)

Table 11. Mean value of the number of angiogenesis

Treatment Group	Angiogenesis Mean Value ± SD
Sintrong leaf ethanol extract gel10%	4.06±2,01[a]
20% sintrong leaf ethanol extract gel	4.30±1.03[ab]
30% sintrong leaf ethanol extract gel	5.53±0.50[ab]
Bioplacenton	11.53±4,58c
Base (Negative Control)	3.00±0,00[a]
Wound infection without treatment	1.60±0,00[a]

*Numeric data presented as mean±SD, standard deviation. Treatments with the same superscript are notsignificantly different, conversely treatments with different superscripts mean they are significantly different

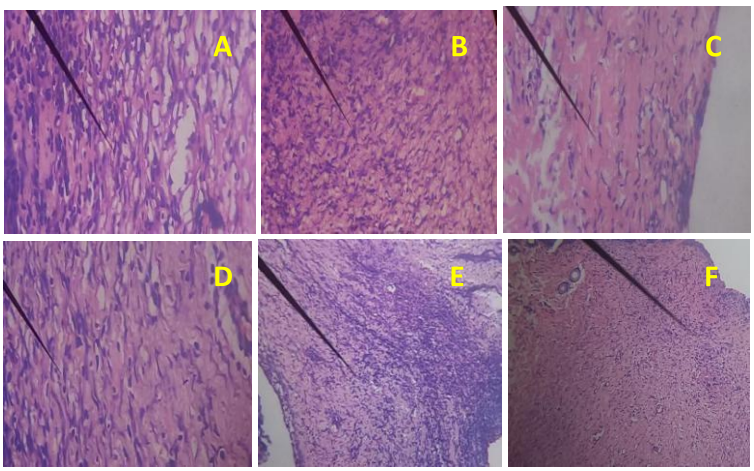

Figure 5. Histopathological features of angiogenesis density (a) bioplacenton; (b) base; (c) untreated infected wound; (d) 10% sintrong leaf ethanol extract gel; (e) 20% sintrong leaf ethanol extract gel; (f) 30% sintrong leaf ethanol extract gel; (g) Collagen (→)

significant difference in angiogenesis growth compared to the positive control group (Bioplacenton), which had a value of (11.53±4.58c). This suggests that ethanol gel extracts of sintrong leaves at concentrations of 10%, 20%, and 30% are less effective than the positive control group (Bioplacenton) in stimulating angiogenesis.

Angiogenesis, the formation of new blood vessels, is a crucial component of the healing process. Angiogenesis is the formation of new blood vessels from existing ones. Blood vessels in wounds serve as conduits for the delivery of nutrients and oxygen essential for cellular healing, the elimination of waste products, and the formation of granulation tissue. During wound healing, new capillaries proliferate rapidly within the wound, leading to a thick network of blood vessels that may be 2, 3, or even 10 times more concentrated than normal tissue (Figure 4) (Toyoda, 2001; Li et al., 2022).

CONCLUSIONS

Ethanol extract gel sintrong exhibit antibacterial properties. The antibacterial efficacy of sintrong leaf ethanol extract gel surpasses that of sintrong leaf nanoparticle gel against *Staphylococcus aureus* infection-causing bacteria. The ethanol extract of sintrong leaves and sintrong leaf nanoparticles can be formed into gel formulations, demonstrating stability for 12 weeks at room temperature, low temperature, and high temperature, as reported by Kusdianti *et al.*, (2008). The secondary metabolites included in sintrong leaves include tannins, flavonoids, polyphenols, and vitamin C, according to research conducted by Adjatin *et al.*, (2013). Sintrong leaves are comprised of tannins, flavonoids, and steroids, which are secondary metabolites that contribute to the enhancement of the healing process in excision wounds infected with *Staphylococcus aureus* in rats.

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CONFLICT OF INTEREST

No conflict of interest between all authors.

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