Biosaintifika 16 (1) (2024): 155-165 p-ISSN 2085-191X | e-ISSN 2338-7610
Journal of Biology & Biology Education https://journal.unnes.ac.id/nju/index.php/biosaintifika

Topical Ointment *Anredera cordifolia* Leaves Ethanolic Extract-Loaded Nanochitosan Promotes Wound Healing in Hyperglycemic Rat

Zalfa Alfatinnisa[1], Mohammad Wahyu Andriyan[2], Muhammad Ragil Saputra[3], Endah Puji Astuti[3], Sunarno Sunarno[1*], Sri Isdadiyanto[1], Agus Subagio[4], La OdeIrman Jaya[5]

[1)] Department of Biology, Faculty of Science and Mathematics, Diponegoro University, Jl. Prof.Jacob Rais, Kampus UNDIP Tembalang, Semarang, Central Java, Indonesia – 50275
[2)]Department of Chemical and Materials, Faculty of Metallurgy and Environment, Central SouthUniversity, Hunan, China – 410017
[3)]Department of Chemistry, Faculty of Science and Mathematics, Diponegoro University, Jl. Prof. Jacob Rais, Kampus UNDIP Tembalang, Semarang, Central Java, Indonesia – 50275 [4)]Department of Physics, Faculty of Science and Mathematics, Diponegoro University, Jl. Prof.
Jacob Rais, Kampus UNDIP Tembalang, Semarang, Central Java, Indonesia – 50275 [5)]Medical Science, Departement Regenerative Medicine, Medical University of Warsaw,Poland

*Corresponding Author: sunarno@lecturer.undip.ac.id

Submitted: 2024-01-12. Revised: 2024-03-15. Accepted: 2024-04-17

Abstract. Wound healing in hyperglycemia patients is still a challenge in the medical field. Bioactive compounds of binahong leaf extract can support the wound healing process. Nanoencapsulation of the extract can avoid oxidation and optimize drug delivery to target tissues. This study aimed to analyze the effect of nanochitosan encapsulated binahong extract ointment (NEBE/Oint) on the percentage of wound healing, angiogenesis, collagen density, and epithelial thickness in hyperglycemic rats. This study used 80 mg/kg streptozotocin-induced hyperglycemia rats injured in the back area. Rats were divided into P0 (0,9% NaCl), P1 (10% povidone-iodine ointment (PI/Oint)), P2 (10% NEBE/Oint), P3 (20% NEBE/Oint), P4 (30% NEBE/Oint). Phytochemical screening of binahong leaves extract showed positive results for flavonoids, alkaloids, saponins, and tannins. NEBE particle size was 169 nm with a size distribution of 0.2 and a zeta potential value of -40,2 mV. The results showed NEBE ointment had a significant effect when compared with negative control on wound healing hyperglycemic rats. The conclusion is that nanochitosan drug delivery has the potential as an alternative wound treatment. The novelty of this study is the use of nanochitosan to accelerate wound healing in hyperglycemic rats. The results of this study are expected to become a reference for new wound-healing methods in the medical field.

Keywords: binahong (*Anredera cordifolia*), hyperglycemia, nanoencapsulation, wound healing

How to cite : Alfatinnisa, Z., Andriyan, M. W., Saputra, M. R., Astuti, E. P., Sunarno, S., Isdadiyanto, S., Subagio, A., & Jaya, L. O. I. (2024). Topical Ointment Anredera cordifolia Leaves Ethanolic Extract-Loaded Nanochitosan Promotes Wound Healing in Hyperglicemic Rat. *Biosaintifika: Journal of Biology & Biology Education, 16(1)*, 155-165.

DOI: http://dx.doi.org/10.15294/biosaintifika.v15i1.1842

INTRODUCTION

Diabetic ulcers are one of the most threatening complications for people with diabetes because they are the leading cause of amputation (Perez-Favila et al., 2019). High blood sugar levels can result in chronic wounds that are difficult to heal due to persistent infections as a result of cellular dysfunction, microvascular disorders, high oxidative stress, and hypoxia (Qin et al., 2022). Current methods of treating diabetic wounds include taking antidiabetics, antibiotics, debridement, dressings, administering exogenous growth factors, and stem cell therapy (Spampinato et al., 2020; Tekleyes et al., 2015). Currently, available methods still have shortcomings such as high therapy costs, side effects, and the risk of strain resistance, and cannot guarantee the success of effective healing (Spampinato et al., 2020; Pereira & Bartolo, 2016). Therefore the development development of alternative treatments needs to be carried out.

Binahong (*Anredera cordifolia* (Ten.) Steenis) is a medicinal plant containing secondary metabolite compounds such as flavonoids, alkaloids, tannins, saponins, and steroids

(Dwitiyanti et al., 2019). These compounds have mechanisms of action that can help wound healing, including antibacterial, antioxidant, anti-inflammatory, and analgesic (Yuniarti & Lukiswanto, 2017). Several studies have used an extract of binahong to heal wounds. Situmorang et al., (2020) proved that 10% ethanol extract of binahong leaves effectively reduced wound diameter. Anggraeni et al., (2017) observed microscopically the effect of ethanol binahong leaf extract in reduced infiltration of inflammatory cells in injured tissue. Nuroini et al. (2021), reported that 25% and 50% ethanol extract of binahong leaves can stimulate the formation of collagen on *S. aureus*-infected wounds.

Nanoparticle-based technology currently can be utilized to increase the effectiveness of pharmaceutical products by increasing drug stability and solubility, controlling drug release, minimizing toxicity, and providing high therapeutic effects (Hamimed et al., 2022). One example of the most widely used nano drug delivery system is chitosan polymer. This is because chitosan is non-toxic, biocompatible, mucoadhesive, biodegradable, antibacterial, cheap, and easily modified to nano size (Yalcin & Cankaya, 2022). Several in vivo studies have shown that nano-delivered bioactive compounds accelerate wound healing through the modulation of several growth factors, including tumor necrosis factor-alpha (TNF-alpha), interleukin-10 (IL-10), endothelial growth factor (EGF) and transforming growth factor-beta (TGF-beta), resulting in decreased inflammatory cell infiltration, increased collagen deposition, and re-epithelialization (Choudary et al., 2020; Hou et al., 2020; Menne et al., 2021). There is not much information regarding the use of nanochitosan combined with binahong leaf extract to heal wounds. Therefore, this research was carried out to reveal the effect of binahong extract encapsulated with nanochitosan on wound healing with a case study in this research of hyperglycemic rats that induced streptozotocin. The novelty of this research is the use of nanochitosan as an encapsulation of binahong extract for wound care. This research data can provide information and knowledge regarding the effects of nanochitosan as a drug delivery agent on the wound healing process in hyperglycemia sufferers. It is hoped that this research can be an initial study for further drug development, especially for sufferers of wounds caused by hyperglycemia.

METHODS

Ethical Approval

All research procedures adhere to animal experimental ethics and have been approved by the Health Research Ethics Committee of the Faculty of Medicine, Diponegoro University (No.62/EC-H/KEPK/FK-UNDIP/VI/2023).

Plant Material

Binahong leaves were obtained from mature and healthy plants in July 2022 from Panggung Lor Village, North Semarang District, Semarang City (-6.9581667, 110.4009411). Determination of binahong plants was carried out at the Ecology Laboratory, Department of Biology, Faculty of Science and Mathematics, Diponegoro University.

Preparation of Extracts Binahong

The harvested binahong leaves are then sorted, washed with water, and drained. Next, the leaves are dried in an oven at 40^{o}C until completely dry. The dried leaves are then crushed and sifted using a 40-mesh sieve to produce a uniform size of simplicial powder (Rusli et al., 2020).

The extraction of bioactive compounds was carried out using the maceration method. Simplicia powder was soaked in 96% ethanol for 5 days with a ratio of 1:5, and the soak was stirred occasionally. The maceration results are then filtered to separate the pellet from the filtrate. The pellets were then macerated again with the same ratio and the procedure was done in 2 days. The filtrate from the first and second maceration is filtered again to eliminate impurities. The impurity-free filtrate is then evaporated using a vacuum evaporator at a temperature of 60^{o}C and heated using a water bath at a temperature of 60^{o}C to obtain a condensed extract (Kintoko & Desmayanti, 2016; Yuniarti & Lukiswanto, 2017). The condensed extract obtained was then subjected toa qualitative phytochemical screening to determine the content of bioactive compounds in it.

Nanoencapsulation Preparation and Characterization

Nanoencapsulation binahong leaf extract was synthesized using the ionic gelation method. 2 g of condensed binahong leaf extract was dissolved in 50 mL of 70% ethanol. This solution was mixed with 100 mL 0.2% chitosan and stirred using a magnetic stirrer. 0.2% Sodium tripolyphosphate (STTP) solution with a volume of 25 ml was added

to this solution gradually and homogenized using a magnetic stirrer for 30 minutes until a nanoencapsulation suspension of binahong leaves was formed (Utari et al., 2022; Winarti et al., 2023). The nanoencapsulation properties of binahong leaf extract, including particle size, polydispersity index (PI), and zeta potential, were characterized using the Malvern zeta sizer nano instrument (Soleymanfallah et al., 2022).

Preparation of Topical Formulations

The ointment is made with the essential ingredients of adeps lanae and vaseline album in a ratio of 1:5. Adeps lanae was melted in a sterilized mortar and heated using an oven at 50^{o}C. Vaseline album is added to the mortar and stirred until homogeneous. Nanochitosan encapsulated binahong extract (NEBE) was added to the ointment base at 10%, 20%, and 30%, respectively, and stirred again until homogeneous. NEBE at each concentration was made into an ointment of 30 grams (NEBE/Oint) (Dewi & Fatonah, 2019).

Excision Wound Making on Rats

Male Wistar rats measuring 180-230 g were used in this study. Acclimatization was carried out for a week in a standard cage with room temperature, 25 ± 2^{o}C, 65% relative humidity, and a 12:12 hour light-dark cycle, and food and water were given ad libitum.

Acclimatized animals were injected with streptozotocin (80 mg/kg, i.p.) and given 20% glucose solution orally. 2 days after induction, blood glucose levels were measured (Kamar et al., 2019). Rat confirmed to have fasting blood glucose levels above 200 mg/dl will be used in this study (Brodjonegoro et al., 2021). The excision wound was made using ketamine anesthesia (50mg/kg, i.m.), and the hair of the back area was shaved. An excision wound with an area of 36 mm^2 was created using punch biopsy (Kintoko et al., 2017; Nagar et al., 2016).

Wound Treatments on The Rats

The total test animals used were 25 rats randomly grouped into 5 groups with 5 replications. P0 (control negative): 0.9% NaCl, P1(control positive): 10% povidone-iodine ointment (PI/Oint),P2: 10% NEBE/Oint, P3: 20% NEBE/Oint, P4: 30% NEBE/Oint. Topical medication is applied once a day for 14 days of treatment.

Measurement of Percent Wound Closure

Excision wounds on days 0, 3, 7, 10, and 14 were documented as material for calculating the size of the wound area using software (ImageJ, NIH). The results of the wound area analysis are processed into percentages using a calculation formula (Choudary et al., 2020).

$$\text{Wound contraction (\%)} = \frac{wound\ area\ on\ day\ 0 - wound\ area\ on\ day\ observation}{wound\ area\ on\ day\ 0}\ x\ 100$$

Histopathological Preparation and Analysis

Rats were terminated with excessive doses of ketamine. A biopsy of the healing wound was carried out on the last day of the experiment to continue making histology preparations. Tissues were made into paraffin blocks, cut at a thickness of 5 µm, and stained with Hematoxylin Eosin. The parameters observed at the tissue level are angiogenesis, epithelial thickness, and collagen density. The tissue was observed under a light microscope connected to an Optilab at 400x magnification.

Statistical Analysis

Quantitative data were summarized as mean ± SD. Ratio data including percentage of wound healing, epithelial thickness, and angiogenesis amount, were tested by ANOVA followed by Tukey's advanced test. Ordinal data for collagen density was tested by Kruskal-Wallis andfollowed by the Mann-Whitney test. Statistical tests were carried out using SPSS version 29.0 software with a significance level of P≤0.05.

RESULTS AND DISCUSSION

Phytochemical screening

Binahong leaf simplicia macerated with 96% ethanol solvent produced an extract yield of 16.75%. Phytochemical screening of binahong leaf extract was carried out to determine the compound content in the extract so that it could be linked to its biological activity. Qualitative phytochemical test results show that binahong leaf extract contains alkaloids, flavonoids, saponins, and tannins (table 1.). These various bioactive compounds have pharmacological activities as antibacterial, antioxidant, analgesic, and anti-inflammatory agents which support the wound healing process (Yuniarti & Lukiswanto, 2017).

Table 1. Results of phytochemical screening of ethanol extract of binahong leaves

Types of Secondary Metabolites	Test Method	Description	Test Result
Flavonoid	Wilstater	Orange color	Positive
Saponin	Forth	Formed foam	Positive
Alkaloid	Wagner	Light brown precipitated	Positive
Tanin	$FeCl_3$ 1%	Blackish green color	Positive
Steroid	Liebermann-Bouchard	Didn't change	Negative

Characterization of Synthesized Nanoparticles

Nanoparticles were used in this research as drug delivery to increase the stability and efficiency of bioactive compounds absorption into the skin compartment. Things that need to be considered are the characteristics of size and PI value. Size characteristics are used to estimate the in-vivo distribution, toxicity levels, and the ability of materials to reach target cells (Hosyar et al., 2016; Egbuna et al. 2021). The average size of NEBE was 171.8 ± 2.43 nm. In this research, the NEBE produced had a particle size that met the requirements for a nano drug delivery system, from 50 to 300 nm (Sabdoningrum et al., 2021). The uniformity of nanoparticles is indicated by PI (Afifah et al., 2021). The PI value ranges from 0.0 (homogeneous particle size distribution) to 1.0 (particle size distribution with a high degree of heterogeneity) (Danaei et al., 2018). The results of the size uniformity analysis show a value of 0.207 ± 0.01, which indicates a homogeneous particle size.

Optimization of NEBE material as drug delivery is closely related to the zeta potential characteristics. The results of this character analysis show the level of stability of a particle. Afifah et al., (2021) stated that a stable particle is indicated by a zeta potential value of more than ± 30 mV. The value obtained from the zeta potential analysis of the NEBE material is -40.2 mV. This value indicates that NEBE material is stable or the particles are difficult to aggregate.

Wound Contraction in Rats Treated with Different Topical Formulations

Observations of wound healing in hyperglycemic white rats were divided into five treatment groups P0, P1, P2, P3, and P4 which were observed macroscopically for 14 days (Figure 1). The size of the wound area obtained from macroscopic observations is then converted into a percentage of wound contraction. Wound contraction data were analyzed by ANOVA. The ANOVA results showed that the treatment had a significant effect on the percentage of wound healing on days 3, 7, and 10 between the P0 group as a negative control and the treatment groups were given topical medication (P1 as control positive, P2, P3, and P4). P4 gave the highest percentage of observations on days 3, 7, and 10. Meanwhile, on day 14, there was no significant effect between all treatment groups ($p > 0.05$) (Table 1).

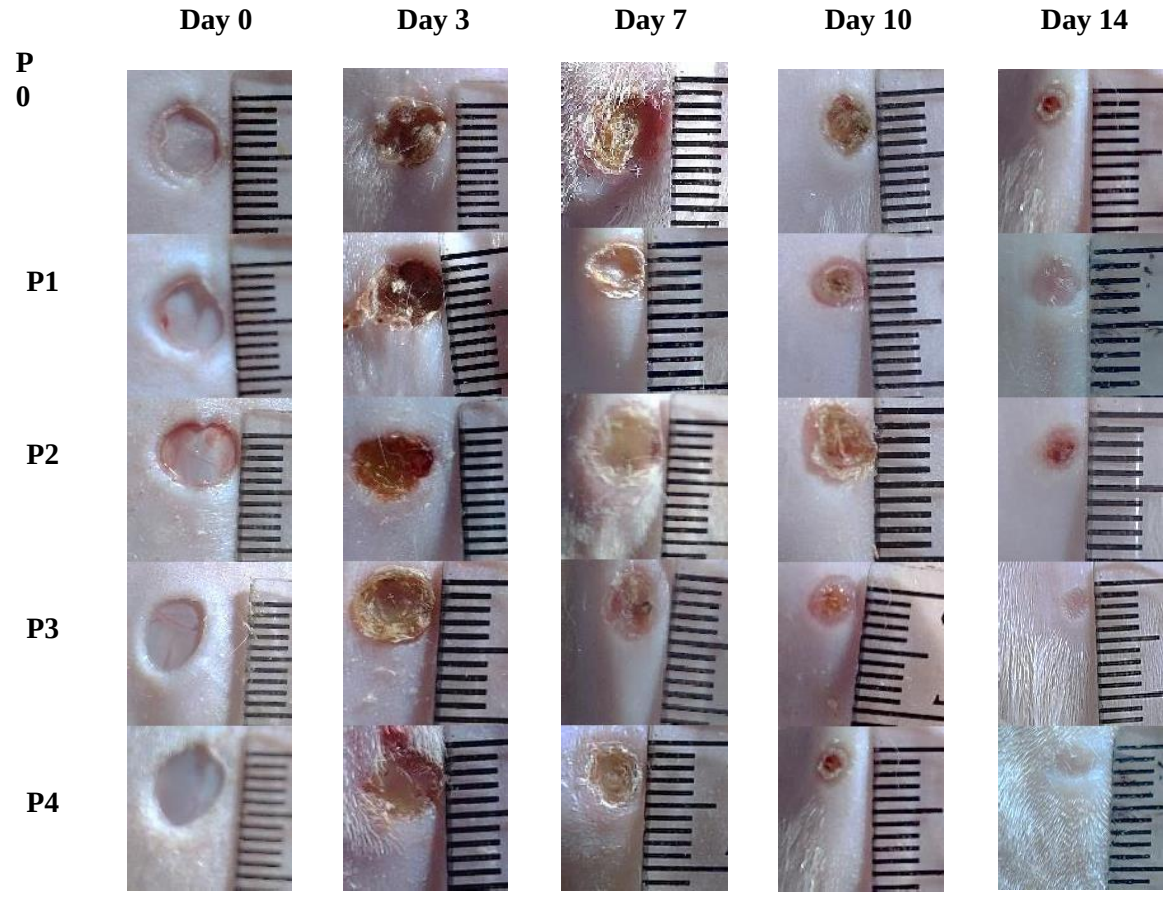

Figure 1. Documentation of macroscopic observations of wounds treated differently.

Table 1. Effect of Various treatment on Wound Contraction Percentage

Treatment Groups	Day 3 (%)	Day 7 (%)	Day 10 (%)	Day 14 (%)
P0	$-1.06^{a} \pm 0.11$	$12.40^{a} \pm 0.19$	$38.92^{a} \pm 0.06$	66.20 ± 0.20
P1	$25.27^{b} \pm 0.15$	$40.18^{ab} \pm 0.15$	$75.96^{b} \pm 0.19$	97.00 ± 0.03
P2	$13.02^{ab} \pm 0.05$	$25.38^{ab} \pm 0.13$	$66.30^{ab} \pm 0.22$	89.00 ± 0.08
P3	$24.73^{b} \pm 0.13$	$48.59^{b} \pm 0.13$	$76.51^{b} \pm 0.15$	97.80 ± 0.03
P4	$25.64^{b} \pm 0.10$	$53.73^{b} \pm 0.09$	$78.72^{b} \pm 0.06$	98.40 ± 0.01

Note: Data are expressed as mean ± standard deviation (n=5). Numbers followed by the sameletters indicated that they were not significantly different at $p<0.05$.

Macroscopic observations of wound healing were carried out on days 0, 3, 7, 10, and 14. Based on observations on day 3, all treatment groups had wounds that had dried out and were growing scabs. Observations on days 7 and 10 showed that all groups had thinner scabs and smaller wound diameters. Observations on the 14th day, treatments P1, P3, and P4 showed that the scab had detached and the wound had closed, whereas in treatments P0 and P2 there were still scabs with a diameter that was getting smaller (Figure 1). The formation of a scab during the wound-healing process indicates that the wound has entered the proliferation phase of wound healing. This phase is characterized by the presence of fibroblasts, collagen, and capillaries that form granulation tissue. Scabs function to help wound hemostasis and prevent contamination by microorganisms. The scabs will detach when the wound has healed (Sudira et al., 2019).

Effect of Topical Application of Different Treatments on Angiogenesis, Collagen Density, and Epithelial Thickness

Microscopic observation of wound healing was carried out on the wound tissue after 14 days of treatment. The parameters observed microscopically are wound healing parameters in the proliferation phase, which include angiogenesis, collagen density level, and

epithelial thickness (Table 2).

Table 2. Results of microscopic observation data analysis.

Treatment Groups	Observation Parameters		
	Number of Capillaries	Collagen Density	Epithelial Thickness
P0	$2.44^{a} \pm 0.33$	$2.20^{a} \pm 0.00$	$12.18^{a} \pm 0.45$
P1	$7.00^{b} \pm 0.24$	$3.00^{b} \pm 0.00$	$23.95^{d} \pm 0.74$
P2	$6.53^{b} \pm 0.31$	$3.00^{bc} \pm 0.00$	$14.65^{b} \pm 0.71$
P3	$6.40^{b} \pm 0.65$	$3.04^{bc} \pm 0.22$	$16.02^{b} \pm 0.55$
P4	$7.00^{b} \pm 0.24$	$3.24^{c} \pm 0.17$	$21.73^{c} \pm 1.42$

Note: P0 (control negative): 0.9% NaCl, P1(control positive): 10% povidone-iodine ointment (PI/Oint), P2: 10% NEBE/Oint, P3: 20% NEBE/Oint, P4: 30% NEBE/Oint.

Angiogenesis is the process of forming new blood vessels. This process is induced by hypoxia tissueand the presence of VEGF signals produced by macrophages. This situation activates endothelial cells in the wound area, then endothelial cells proliferate and migrate around to form new capillary branches (Spampinato et al., 2020). Capillaries growing in the excision wound area were observed microscopically (Figure 2). Data on the number of capillaries indicates the existence of an angiogenesis process (Table 1). The results of the ANOVA analysis showed that the treatment given produced a significant difference in the number of capillaries ($p<0.05$). The difference in the number of capillaries in treatment groups was then tested by Tukey. Based on Tukey test, group P0 was different from groups P1, P2, P3, and P4 ($p<0.05$). The highest mean number of capillaries was found in groups P1 and P4 (7.00 ± 0.24).

The results of this study showed that P1, P2, P3, and P4 had a significant effect on increasing angiogenesis compared to P0. Povidone iodine ointment 10% is one of the drugs most often used in the treatment of various types of wounds. This type of ointment functions as an antiseptic to prevent microbial contamination of damaged tissue (Bigliardi et al., 2017). Low levels of microbial infection will accelerate the inflammatory phase towards the wound healing phase (Holzer-Geissler et al., 2022).

The treatment group given NEBE ointment also showed capillary growth. Bioactive compounds in NEBE ointment induce the angiogenesis process. Apriasari et al., (2016) reported tannin extracted from banana stems can increase the expression of VEGF which affects increasing angiogenesis of oral cavity ulcers in Wistar rats. Tannins are known to increase the expression of proangiogenic growth factors (HIF1α and HSp90α). Flavonoid quercetin is also known to help the angiogenesis process by increasing the secretion of VEGF (Zhou et al., 2015).

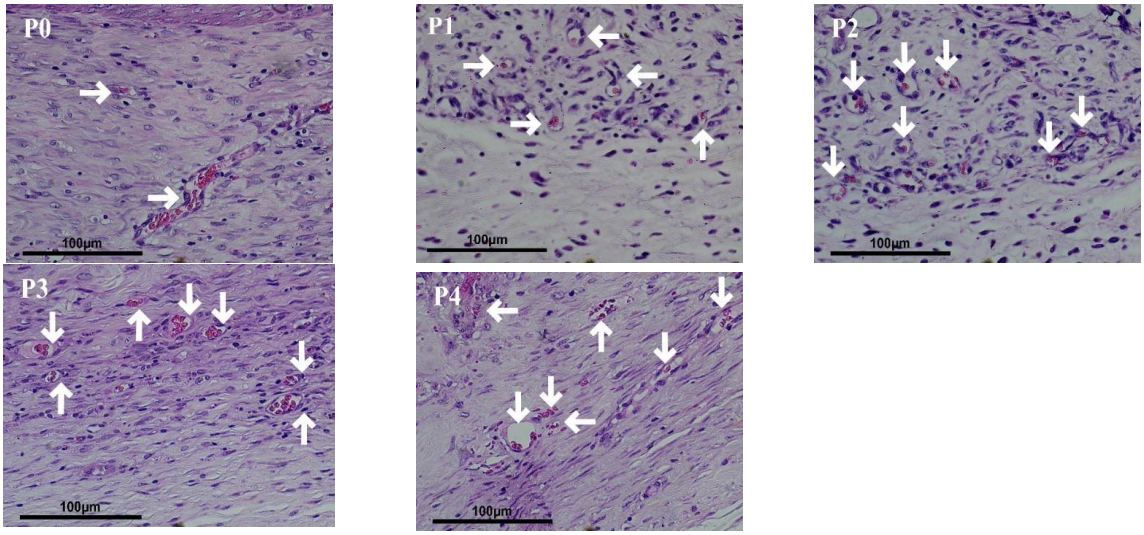

Figure 2. Histopathological image of angiogenesis (HE staining, 400x magnification). The white arrow indicates angiogenesis. P0 (control negative): 0.9% NaCl, P1(control positive): 10% povidone-iodine ointment (PI/Oint), P2: 10% NEBE/Oint, P3: 20% NEBE/Oint, P4: 30% NEBE/Oint.

Collagen is a protein that makes up the extracellular matrix (Mathew et al., 2021). Collagen formation in the wound healing process is carried out from the proliferation to remodelingstages. The cells that play a role in the collagen deposition process are fibroblasts. TGF-β will induce fibroblasts to start synthesizing type III collagen. When entering the remodeling stage, fibroblasts will produce collagen metalloproteinase to degrade type III collagen and replace it with type I collagen which has thicker and stronger characteristics as a protective mechanism for the skin (Dorantes & Ayala, 2019). The collagen density was scored (Figure 3), and then statistically analyzed (Table 1).

NEBE ointment has a higher effect on collagen density compared to treatment with control, P0, and P1. This is because NEBE ointment contains bioactive compounds that can stimulate collagen formation. Zhang et al., (2022) stated that flavonoids play a role in TGF-β expression which induces fibroblast cell proliferation. An increase in activated fibroblast cells will increase the production of collagen. Gopalakrishnan et al., (2016) stated that quercetin is a type of flavonoid that is known to increase TGF-β expression in the early proliferation phase, thus supporting fibroblast activation, extracellular matrix deposition, and granulation tissue formation. Mastuti et al., (2017), saponin compounds can stimulate the formation of fibronectin by fibroblasts. The fibronectin that is formed will stimulate the migration of fibroblasts to the wound tissue, so more collagen is synthesized by the fibroblasts. Alkaloids are a type of bioactive compound besides flavonoids and saponins which are known to help heal wounds inthe proliferation phase. The results of in vitro research conducted by Azzazy et al., (2021) show that alkaloid compounds extracted from *Peganum harmala* can increase the number of fibroblasts and collagen.

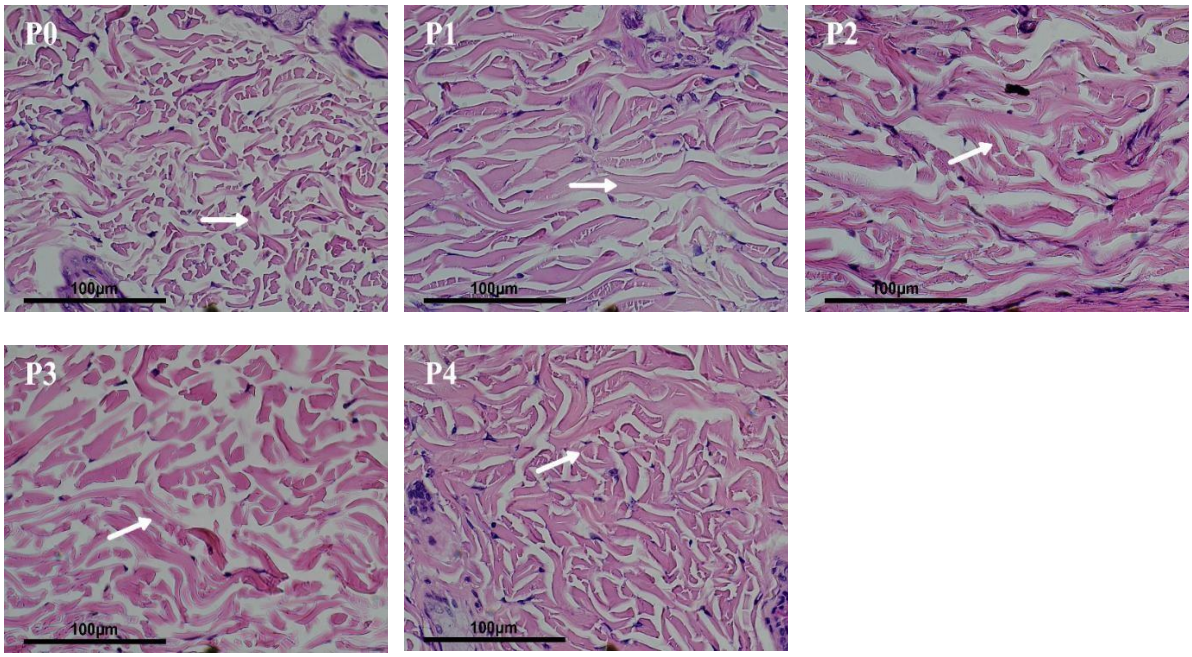

Figure 3. Histopathological image of collagen density (HE staining, 400x magnification). The white arrow indicates collagen fiber. P0 (control negative): 0.9% NaCl, P1(control positive): 10% povidone-iodine ointment (PI/Oint), P2: 10% NEBE/Oint, P3: 20% NEBE/Oint, P4: 30% NEBE/Oint.

Epithelialization is the process of forming new epithelial cells on the surface of damaged skin. This process involves the proliferation and migration of keratinocyte cells (Bartolo et al., 2022). Keratinocytes have junction structures in the form of desmosomes and hemidesmosomes which help connect keratinocyte cells. The initial process of reepithelialization is characterized by the degradation of desmosomes and hemidesmosomes to facilitate keratinocyte migration (Rakita et al., 2022). IL-1 is secreted autocrine by keratinocytes to increase the migration and proliferation of these cells. IL-1 also activates fibroblasts to increase KGF secretion thereby inducing keratinocyte migration and proliferation. TNF-α is also secreted autocrine

for keratinocyte migration and paracrine activates fibroblasts to increase the secretion of fibroblast growth factor (FGF) which has an impact on extracellular matrix deposition thereby increasing keratinocyte motility during re-epithelialization (Bartolo et al., 2022).

Measurement of epithelial thickness produces an average thickness for all treatment groups (Figure 4 & Table 2). ANOVA statistical analysis showed that the treatments given produced significant differences in epithelial thickness ($p<0.05$). Differences in epithelial thickness in the treatment groups were then tested by Tukey. Based on further tests, the negative control group was different from the positive control group and NEBE treatment. The highest value was obtained in P1 (positive control) followed by P4, P3, and P2.

P4 had the second highest effect on epithelial thickness (21,73 µm) after P1. The flavonoids contained in NEBE ointment are known to induce keratinocyte proliferation (Gallelli et al., 2020), and induce the secretion of TGF-β and FGF with increased collagen production which causes accelerated keratinocyte migration for re-epithelialization (Kartikaningtyas et al., 2015; Mi et al., 2022). Subramanian et al., (2023) stated that the ethyl acetate fraction of *D. viscosa* contains the

flavonoid type quercetin. The results of the western blot test stated that quercetin could increase FGF regulation in wound granulation tissue. This is also supported by in-vivo research results which prove that the extract is able to produce wound healing through the mechanism of forming collagen fibers with a high level of density, blood vessel formation, and optimal epithelialization (Subramanian et al., 2023).

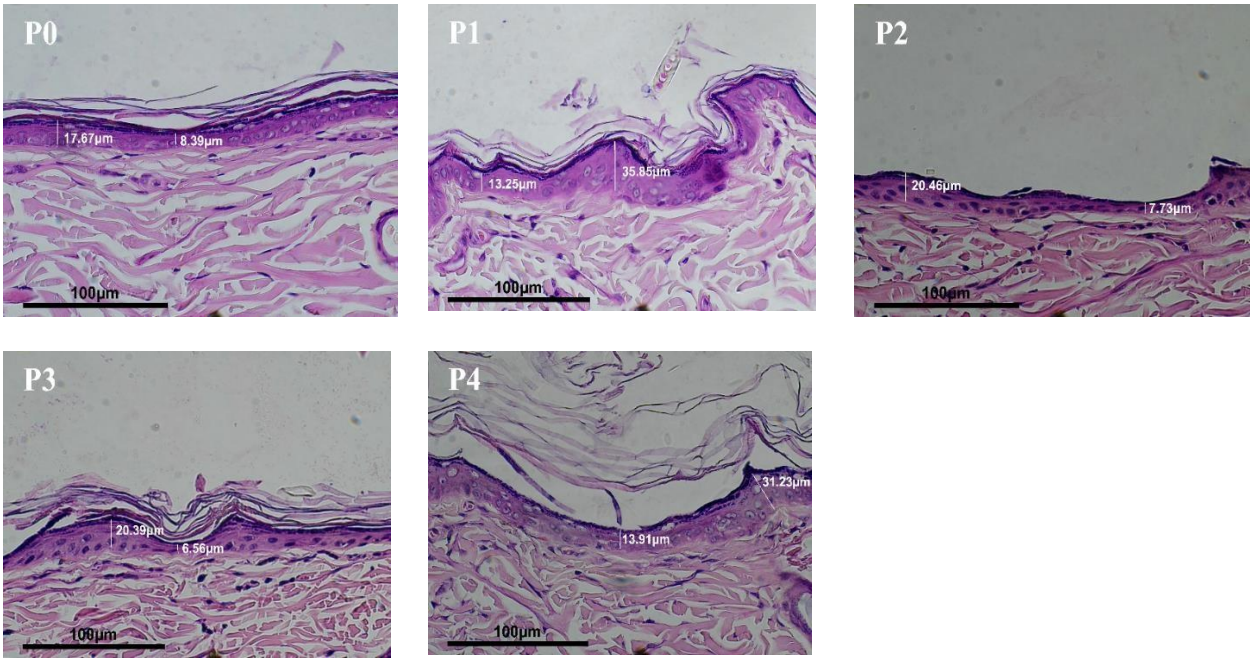

Figure 4. Histopathological image of epithelialization thickness (HE staining, 400x magnification). P0 (control negative): 0.9% NaCl, P1(control positive): 10% povidone-iodine ointment (PI/Oint), P2: 10% NEBE/Oint, P3: 20% NEBE/Oint, P4: 30% NEBE/Oint.

Based on the results found, this research can become a new reference in the medical world for wound healing cases. The innovation of combining natural ingredients, binahong extract encapsulated with nanochitosan as drug delivery, can be an alternative topical medicine for wounds, apart from the general use of povidone-iodine. The percentage value of wound closure and observation of the quality of tissue undergoing wound healing is considered good and can balance the value of wound tissue treated with povidone-iodine. It is hoped that this research willopen up further research opportunities to optimize wound healing using natural bioactive compounds with nano-delivery systems.

CONCLUSION

Nanochitosan has the potential as an alternative treatment in the form of drug delivery material for wound healing. In this study, wounds in hyperglycemia sufferers were demonstrated by

wound closure, angiogenesis, collagen deposition, and epithelialization.

Further research on specific bioactive compounds extracted from binahong leaves as well as immunohistochemical evaluation of growth factors in the wound healing process needs to be carried out so that the compounds and their molecular wound healing mechanisms can be identified with certainty. This can support more specific optimization of the wound healing process.

ACKNOWLEDGMENT

Thank you to the Bionano Laboratorium Terpadu UNDIP for providing a place and supporting all types of facilities to conduct this research. The authors also thank Heydar Ruffa Taufiq and Chaesbullah, who helped and were involved in discussions during the synthesis process in the laboratory, Sumardi and Taufiq for all their assistance during in vivo test data collection at Integrated Biomedical Laboratories FK UNISSULA, and dr. Sumarno gave much direction and help in histopathological observations

REFERENCES

Afifah, A. N., Dono, N. D., & Zuprizal. 2021. Optimizing Antioxidant Properties of Salam (*Syzygium polyanthum*) Leaf Extract through Nanoencapsulation Technology. *The International Conference on Smart and Innovative Agriculture, IOP Conf. Series: Earth and Environmental Science,* 686 012040.

Anggraeni, D., Airin, C. M., & Raharjo, S. 2017. The Effectiveness of Ethanol Extract of Binahong leaves on Diabetic Wound Healing. *Jurnal Kedokteran Hewan*, 11(4), 146-152.

Apriasari, M. L., Dachlan, Y. P., & Ernawati, S. D. 2016. Effect of *Musa acuminata* Stem by Immunohistochemistry Test in Ulcer. *Asia Journal of Biochemistry*, 11(3), 135-141.

Azzazy, H. M. E., Fahmy, S. A., Mahdy, N. K., Meselhy, M. R., & Bakowsky, U. 2021. Chitosan-Coated PLGA Nanopaticles Loaded with *Peganum harmala* Alkaloids with Promising Antibacterial and Wound Healing Activites. *Nanomaterials,* 11(9).

Bartolo, I., Reis, R. L., Marques, A. P., & Cerqueira, M. T. 2022. Keratinocyte Growth Factor-Based Strategies for Wound Re-epitheliazation. *Tissue Engineering*, 28(3), 665-676.

Bigliardi, P. L., Alsagoff, S. A. L., El-Kafrawi, H. Y., Pyon, Jai-Kyong, Wag, C. T. S., & Villah,

Brodjonegoro, S. R. Ghinorawa, T., Wonatorey, N. K. R., Zulfiqqar, A., Heriyanto, D. S. 2021.Effect of hyperglycemia on fertility in streptozotocin-induced diabetic male Wistar rats:focus on glucose transporters and oxidative stress. *Medical Journal of Indonesia*, 30, 177-181.

Choudary, A., Kant, V., Jangir, B. L., & Joshi, V. G. 2020. Quercetin Loaded Chitosan Tripolyphosphate Nanoparticles Accelerated Cutaneous Wound Healing in Wistar Rats. *European Journal of Pharmacology,* 880(173172).

Danaei, M., Dehghankhold, M., Ataei, S., Davarani, F. H., Javanmard, R., Dokhani, A., Khosrasani, S., & Mozafari, M. R. 2018. Impact of Particle Size and Polydispersity Index on the Clinical Applications of Lipidic Nanocarrier Systems. *Pharmaceutics* 10(57), 1-17.

Dewi, S. R. P. D.& Fatonah, A. 2019. The Effect of Betel Quid Extract on Wound Healing Process in Male Wistar Rats (*Rattus norvegicus* L.). Asian Journal of Applied Sciences, 7(6), 788-797.

Dorantes, L.C., & Ayala, M. C. 2019. Skin Acute Wound Healing: A Comprehensive Review.

Dwitiyanti, Harahap, Y., Elya, B., & Bahtiar, A. 2019. Impact of Solvent on the Characteristics of Standardized Binahong Leaf (*Anredera cordifolia* (Ten.) Steenis). *Pharmacogn J*, 11(6), 1463-1470.

Egbuna, C., Parmar, V. K., Jeevanandam, J., Ezzat, S. M., Patrick-Iwanyunwu, K. C., Adetunji, C. O., Khan, J., Onyeike, E. N., Uche, C. Z., Akram, M., Ibrahim, M. S., Mahdy, N. M. E., Awuchi, C. G., Saravanan, K., Tijjani, H., Odoh, U. E., Messaoudi, M., Ifemeje, J. C.,Ezeofor, N. J., Chikwendu, C. J., & Ibeabuchi, C. G. 2021. Toxicity of Nanoparticles in Biomedical Application: Nanotoxicology. *J Toxicol*, 9954443.

Gallelli, G., Cione, E., Serra, R., Leo, A., Citraro, R., Matricardi, P., Di, Meo, C., Bisceglia, F., Caroleo, M.C., Basile, S., & Gallelli, L. 2020. Nano-Hydrogel Embedded with Quercetin and Oleic Acid as A New Formulation in the Treatment of Diabetic Foot Ulcer: A Pilot Study. *Int Wound J*, 17(2), 485-490.

Gopalakrishnan, A., Ram, M., Kumawat, S., Tandan, S., & Kumar, D. 2016. Quercetin Accelerated Cutaneous Wound Healing in Rats by Increasing Level of VEGF and TGF- β1. *Ind. J. Exp. Biol*, 54(3), 187-195.

Hamimed, S. Jabberi, M., & Chatti, A. 2022.

Nanotechnology in drug and gene delivery. *Naunyn- Schmiedeberg's Archives of Pharmacology*, 395, 769–787.

Holzer-Geissler, J. C. J., Schwingenschuh, S., Zacharias, M., Einsiedler, J., Kainz, S., Reisengeer, P., Holecek, C., Hofmann, E., Wolff-Winiski, B., Fahrngruber, H., Birngruber, T., Kamolz, L., & Kotzbeck, P. 2022. The Impact of Prolonged Inflammation on Wound Healing. *Biomedicines*, 10(4), 856.

Hosyar, N., Gray, S., Han, H., & Bao, G. 2016. The effect of nanoparticle size on in vivo pharmacokinetics and cellular interaction. *Nanomedicine (Lond)*, 11(6), 673–692.

Hou, B., Qi, M., Sun, J., Ma, X., Cai, W., Zhou, Y., Ni, L., Hu, J., Xu, F., & Qiu, L. 2020.

International Journal of Inflammation, 3706315, 1-15.

Kamar, S. S., Kader, D. H. A., & Rashed, L. A. 2019. Beneficial Effect of Curcumin Nanoparticles-Hydrogel on Excisional Skin Wound Healing in Type-I Diabetic Rat: Histological and Immunohistochemical Studies. *Annals of Anatomy*, 222, 94-102.

Kintoko & Desmayanti, A. 2016. The effectivity of ethanolic extract of binahong leaves (anredera cordifolia (tenore) steen) gel in the management of diabetic wound healing in aloxan-induced rat models. *JKKI*, 7(5), 227-236.

Kintoko, Karimatulhajj, H., Elfasyari, T. Y., Ihsan, E. A., Putra, T. A., Hariadi, P., Ariani, C., Nurkhasanah. 2017. Effect of diabetes condition on topical treatment of binahong leaf fraction in wound healing process. *Trad. Med. J*, 22, 103-110.

M. A. 2017. Povidone Iodine in Wound Healing: A Review of Current Concepts and Practices. *International Journal of Surgery*, 44, 260-268.

Mastuti, S., Hadi, D. T., & Chodidjah. 2017. Nigella Sativa Seed Extract Ointment Increasing the Amount of Healing Fibroblast and Collagen on Balb/C Mice. *Sains Medika*, 8(1), 15-19.

Mathew-Steiner, S. S., Roy, S., & Sen, C. K. 2021. Collagen in Wound Healing. *Bioengineering (Basel)*, 8(5).

Menne, A. A., Arigela, B., Giduturi, A. K., Komaravolu, R. K., Manganmuri, U., & Poda, S. 2021. *Pterocarpus marsupium* Roxburgh Heartwood Extract/Chitosan Nanoparticles Loadade Hydrogel as an Innovative Wound Healing Agent in The Diabetic Rat Model. *Materials Today Communications*, 26(101916), 1-12.

Mi, Y., Zhong, L., Lu, S., Hu, P., Pan, Y., Ma, X., Yan, B., Wei, Z., & Yang, G. 2022. Quercetin Promotes Cutaneous Wound Healing in Mice Through Wnt/B-Catenin Signaling Pathway. *J. Ethnopharmacol*, 290(115066).

Nagar, H. K., Srivastava, A. K., Srivastava, R., Kurmi, M. L., Chandel, H. S., & Ranawat, M. S. 2016. Pharmacological Investigation of the Wound Healing Activity of *Cestrum nocturnum* (L.) Ointment in Wistar Albino Rats. *Journal of Pharmaceutics*, 9249040, 1- 8.

Nuroni, F., Ratnaningrum, K., Soejoto, M., R., A., Dewi, S. S., Putri, G. S. A. Binahong Leaf Extract Activity in The 8th Day of Wound Healing Infected with *Staphylococcus aureus* Towards Collagen Tissue. *Jurnal Teknologi Laboratorium*, 10(2), 124-130.

Pereira R. F., & Bártolo P. J. 2016. Traditional Therapies for Skin Wound Healing. *Adv Wound Care (New Rochelle)*, 5(5), 208-229.

Perez-Favila A., Martinez-Fierro M. L., Rodriguez-Lazalde J. G., Cid-Baez M. A., Zamudio-Osuna M. J., Martinez-Blanco M. D. R., Mollinedo-Montaño F. E., Rodriguez-Sanchez I.P., Castañeda-Miranda R., & Garza-Veloz I. 2019. Current Therapeutic Strategies in Diabetic Foot Ulcers. *Medicina (Kaunas)*, 5(11), 714.

Preparation, Characterization and Wound Healing Effect of Vaccarin-Chitosan Nanoparticles. *International Journal of Biological Macromolecules*, 165, 3169-3179.

Qin, W., Wu, Y., Liu, J., Yuan, X, & Gao, J. 2022. A comprehensive review of nanoparticles in diabetic wound healing: therapeutic potential and future perspective. *International Journal of Nanomedicine*, 17, 6007–6029.

Rakita, A., Nikolic, N., Midner, M., Matiasek, J., & Elbe-burger, A. 2020. Re-epithelialization and immune cell behaviour in an ex vivo human skin model. *Scientific Reports*, 10(1).

Rusli, Z., Sari, B. L., Utami, N. F., & Sabila. 2020. Optimization of Microwace-Assisted Extraction of Flavonoids from Binahong (*Anredera cordifolia*) Leaves Using Respon Surface Methodology. *Jurnal Fitofarmaka Indonesia*, 7(3), 10-19.

Sabdoningrum, E. K., Hidanah, S., Chusmiati, S., & Soeharsono. 2021. Characterization and Phytochemical Screening of Meniran (*Phyllanthus niruri Linn*) Extract's Nanoparticles Used Ball Mill Method. *Pharmacogn J*, 13(6), 1568-1572.

Situmorang, G. A., Yamamoto, Z., Ichwan, M., & Prayugo, B. 2020. *Anredera cordifolia* Leaves Extract Accelerates The Wound Healing of Normal and Hyperglycemic Rats.

Pharmaciana, 12(1), 39-48.

Solleymanfallah, S., Khoshkoo, Z., Hosseini, S. E., & Azizi, M. H. 2022. Preparation, Physical Properties, and Evaluation of Antioxidant Capacity of Aqueous Grape Extract Loaded in Chitosan-TPP Nanoparticles. *Food Sci Nutr*, 10, 3272-3281.

Spampinato S. F., Caruso G. I., De Pasquale R., Sortino M. A., & Merlo S. 2020. The Treatment of Impaired Wound Healing in Diabetes: Looking among Old Drugs. *Pharmaceuticals (Basel)*, 13(4), 60.

Subramanian, S. Duraipandian, C., Alsayari, A., Ramachawolran, G., Wong, L. S., Sekar, M., Gan, S. H., Subramaniyan, V., Seethalakshmi, S., Jeyabalan, S., Dhanasekaran, S., Chinni, S. V., Rani, N. N. I. M., & Wahab, S. 2023. Wound Healing Properties of A New Formulated Flavonoid-rich Fraction from *Dodonaea viscosa* Jacq. L. Extract. *Front. Pharmacol*, 14.

Tekleyes, B., Huluka, S. A., Wondu, K., & Wondmkun, Y. T. 2015. Wound Healing Activity of 80% Methanol Leaf Extract of *Zehneria scabra* (L.f) Sond in Mice. *Journal of Experimental Pharmacology*, 13, 537-544.

Utari, R. D., Saputra, B., Kristiana, R., Yunita, T., Kusumaningtyas, V. A., & Jasmansyah. 2022. Characteristics and Antioxidant Activity of Nanoparticles Chitosan Roselle (*Hibiscus sabdarifffa*). *J. Kartika Kimia*, 5(2), 157-162.

Winarti, L., Safitri, A. A., Az-Zahro, S., & Oktora, L. 2023. Formulation and in vitro study of nanoparticles loaded Anredera cordifolia leaf extract as anti-acne.Pharmacy Education, 23(2), 65 -70.

Yalcin, S., & Cankaya, N. 2022. Chitosan-Based Nanocomposites for Biological Applications. In (Ed.), Nanoclay - Recent Advances, New Perspectives and Applications [Working Title]. IntechOpen.

Yuniarti, W. M., & Lukiswanto, B. S. 2017. Effects of Herbal Ointment Containing the Leaf Extracts of Madeira Vine (*Anredera cordifolia* (Ten.) Steenis) For Burn Wound Healing Process on Albino Rats. *Vet World*, 10(7), 808-813.

Zhang, M., Chen, X., Zhang, Y., Zhao, X., Zhao, J., & Wang, X. 2022. The Potential of Functionalized dressing releasing flavonoids facilitates scar-free healing. *Front Med (Lausanne)*, 9(978120).

Zhou, Y., Wu, Y., Jiang, X., Zhang, X., Xia, L., Lin, K., & Xu, Y. 2015. The Effect of Quercetinon the Osteogenesic Differentiation and Angiogenic Factor Expression of Bone Marrow- Derived Mesenchymal Stem Cells. *PloS ONE*, 10(6).