INDONESIAN JOURNAL OF NURSING PRACTICES OPEN ACCESS

Volume 8, Issue 1, June 2024, p. 1-11

Empowering Natural Medicine: Combining Brotowali Extract (T.Crispa) with Lampung Natural Zeolite to Improve Diabetic Wound Healing

Lia Mulyati*, Afni Wulandari, Fitria Kusuma Devi, Nurunnisa Rahayuningrat
Departement of Medical Surgical Nursing, STIKes Kuningan Garawangi, Indonesia

Corresponding Author: Lia Mulyati
Email: lia@pintarword.com

Article Info
Online : http://journal.umy.ac.id/index.php/ijnp
ISSN : 2548 4249 (Print)
: 2548 592X (Online)
DOI :10.18196/ijnp.v8i1.19586

Article History
Received : 20 August 2023
Revised : 22 April 2024
Accepted : 29 April 2024

Abstract

Background: Diabetic ulcers are a serious complication that often occurs in people with diabetes mellitus. The complication leads to amputation and causes disability in about 30% of patients.
Objective: The study aims to test the effectiveness of an ointment formulation containing brotowali extract with a combination of Lampung natural zeolite (ZAL) in the healing process of diabetic ulcers.
Methods: The research method used is quasi-experimental with a control group. The sample of this study used mice, with a total of 6 in each group. Before the intervention, blood sugar levels in the samples were increased by injecting streptozotocin. The study was conducted for 14 days. The Mann-Whitney test was used to analyze the data obtained.
Results: The results showed a significant difference in the percentage of wound closure between the control group and the intervention group with p=0.002.
Conclusion: Based on these findings, it can be concluded that an ointment formulation containing brotowali extract with a combination of ZAL effectively accelerates the wound healing process in diabetic mice. Brotowali extract ointment with a combination of Lampung natural zeolites can be used as an alternative in wound care for patients with diabetes mellitus based on herbs and local wisdom so that it will be more easily accepted in the community.

Keywords: brotowali extract; diabetes mellitus; diabetic ulcers; tinospora crispa; zeolite

INTRODUCTION

Diabetes Mellitus is a condition with increased blood glucose levels or hyperglycemia caused by metabolic abnormalities of the insulin hormone (Subandi & Sanjaya, 2020). According to a survey by the International Diabetes Federation (IDF) in 2021, it is estimated that there are around 19.46 million people in Indonesia who have diabetes (Sun et al., 2023). This number has increased by 81.8% compared to the number in 2019. Indonesia is also the country with the fifth-largest number of diabetics worldwide in 2021. A diabetic wound or diabetic ulcer is a major clinical complication of diabetes. Diabetic wounds are chronic, infected, and ulcerated wounds that occur simultaneously, characterized by high inflammatory cytokines, impaired angiogenesis, distal arterial obstruction, and inability to distribute oxygen, nutrients, and healing cells to the wound site (Liu et al., 2022). The hemostatic and inflammatory phases of diabetic wounds during healing can proceed normally, but certain internal causes, such as peripheral neuropathy, microcirculation dysfunction, impaired growth factor activity, and hypoxia complicate the transition of diabetic wounds from the inflammatory phase to the proliferative phase (Gastaldi et al., 2021).

The normal wound healing process includes four overlapping phases, namely homeostasis, inflammation, proliferation, and remodeling (Li & Wu, 2022) In diabetes mellitus, all four phases are obstructed, and the wound remains in a state of chronic inflammation and fails to heal in time. Hyperglycemic conditions that inhibit the work of neutrophils (A. K. Singh et al., 2016), blood

circulation disorders, and neuropathy (nerve damage) make it difficult for diabetic wounds to heal, and infection is very easy to cause gangrene, the bacteria most often found in gangrenous wounds of people with diabetes mellitus is staphylococcus aureus sp. (Millah, 2021). T.Crispa extract, commonly known as brotowali, has demonstrated potential for regulating blood glucose levels and accelerating ulcer healing in diabetic rats. In rats, this extract has also been shown to decrease blood glucose levels and shield the retina against diabetic retinopathy (Agrawal et al., 2012). According to research conducted by (Banerjee et al., 2020) T.Crispa extract protects against diabetes and prevents complications. These results imply that T.Crispa extract may be useful in managing and avoiding diabetic ulcers; however, more research is required to verify the product's efficacy and safety in people.

Brotowali (T.Crispa) is a plant that has long been used in traditional medicine in various countries, including Indonesia. Diseases often treated with this plant are liver disease, urinary system disorders, rheumatism, diabetes mellitus, heart disease, helminthiasis, allergies, and malaria (Sharma et al., 2019). T.Crispa contains alkaloids, flavonoids, flavonoid glycosides, triterpenoids, diterpenoids, and diterpene glycosides, cis clerodanetype furanoditerpenoids, lactones, sterols, lignans, and nucleosides and berberine, which has anti-inflammatory, antioxidant, and tissue regenerative and antimicrobial (Vijendren et al., 2017).

According to Fernandez et al. (2021) the study's findings, brotowali (T. crispa) can recover from wounds. A previous study by Arundina et al. (2017) showed that the number of lymphocytes in diabetic Wistar rats' traumatic ulcer healing on days 3, 5, and 7 was impacted by the stem extract of brotowali (T. crispa). A crucial part of the wound-healing process is played by lymphocyte cells during the inflammatory phase. T. crispa contains flavonoids and terpenoids, which can help control blood glucose levels and accelerate wound healing (Arundina et al., 2017). Another study shows that T. crispa has an effect on increasing initial re-epithelialization, wound tissue strength, granulation tissue, and collagen content and has wound healing activity through increasing antioxidant enzymes and influencing the inflammatory phase (Fernandez et al., 2021). Similar to T. Crispa extract, Ziolite Alam Lampung (ZAL) is a material with potential use in the medical field.

Zeolite Alam Lampung (ZAL), particularly, has been of interest to researchers due to its unique quality and mineral content. ZAL is a natural mineral with a porous structure that can absorb and bind certain substances. In a previous study, it was found that the use of ZAL as an additional ingredient in ointments can increase the effectiveness of wound healing (Soesilowati, 2021) as a stabilizer capable of maintaining the ability of the antibacterial activity of the extract formula (Mariani et al., 2023, Nasseri & Sharifi, 2022). Because of its possible health benefits, research combining ZAL with Brotowali (T. crispa) is needed. It is an interesting field for further studies because combining these two natural chemicals may increase the benefits of each. Nurses can contribute significantly to this research by caring for patients and keeping them informed on how the combined therapy affects people's health, particularly for wound care diabetic ulcers.

This study aims to test the effectiveness of an ointment formulation containing Brotowali stem extract with a combination of ZAL in the healing process of diabetic ulcers. It is hoped that this ointment formulation will provide significant benefits in accelerating wound healing, reducing the risk of infection, and improving the health condition of diabetic ulcer sufferers. In addition, the results of this study are expected to be the basis for the development of effective ointment products in the treatment of diabetic ulcers. They can provide benefits for people with diabetes mellitus in improving the quality of life of patients with diabetes mellitus.

METHOD

Preparation of the Extract

Fresh brotowali stems are collected from the Kuningan, Jawa Barat, and then dried using the oven method for 24–48 hours (simplisia). Furthermore, extraction was carried out by maceration for three days using 1.2 L of 70% ethanol as a solvent. The macerate is then separated from the dregs and evaporated using a rotary evaporator to obtain 100 ml of extract. Then, it is formulated with Lampung

natural zeolite, which removes water and organic molecules by baking at 100 °C for 2 hours. The following is a Brotowali Extract Ointment Formulation with a ZAL Combination

Ointment Making Procedure

Cera alba is melted over a water bath, and white vaseline is added, stirred until homogeneous in a liquid state, then mixed in a mortar containing brotowali stem extract and zeolite, then stirred until homogeneous. Then, the ointment is packaged in containers. Furthermore, the ointment is tested at several stages, including:

Ointment Physical Stability Test

The method used for stability testing is the freeze-thaw cycle. In this research, the formulation of brotowali extract ointment and brotowali extract ointment with a combination of Lampung natural zeolite was tested for the physical stability of the ointment preparations. It aims to prove that the natural zeolite of Lampung is an ointment stabilizer. Both ointment preparations were stored at 4 °C for 24 hours (freeze process); after that, the ointment was stored at 40 °C for 24 hours (thaw process). These two processes were counted as one cycle. After one cycle is completed, the physical properties of the ointment are tested, and this test is carried out for six cycles.

Organoleptic Test

This test can be conducted visually by looking at the ointment's color, smell, and form (Purwaningsih et al., 2020).

Homogeneity Test

An analysis of the homogeneity of the ointment was carried out after the ointment mixture was distributed evenly on the glass plate. The sample test results show a homogeneous composition if they are marked by the absence of coarse grains in the preparation that is smeared on the glass.

PH test

The tool used to measure the pH value of the ointment is a pH meter dipped in 1 gram of ointment, which is diluted with 10 ml of distilled water. A good pH value is 4.5–6.5, according to the skin's pH value (Nurhayati, 2022).

Spreadability test

The spreadability test results confirmed that the ointment spread evenly and smoothly across the glass surface. The weight of the second glass increased slightly, indicating that a small amount of the ointment had successfully transferred onto it. These findings demonstrate that the ointment has excellent spreadability and can be effortlessly applied to the skin. The spreadability test was carried out by placing 0.5 g of ointment in the middle of a round glass and then covering it with another glass that had been weighed and left for 1 minute. Then, the diameter of the spread of the ointment was measured. After that, the load was added to 50 gr and left for 1 minute, then the diameter of the ointment spread was measured. Thus, on until the load is added until it reaches 250g (Purwaningsih et al., 2020).

Preparation of Experimental Animals

The sample used in this study was mice (Mus Musculus), with a total of 12, consisting of 6 in the control group and 6 in the intervention group. Mice were selected based on the criteria of body weight between 20 and 40 grams and age between 2 and 3 months. Each mouse was kept in its husk. The mice were given a standard meal of 20 g/head/day and unlimited water. Prior to the experiment, the mice were adapted for seven days to adjust and get used to the new environment and standard diet. Researchers set animal cages, ventilation, safety, hygiene, and food/water supply standards to ensure animal welfare. Mice welfare is regularly monitored.

Diabetes mellitus induction

Before the intervention, the mice underwent a 10-day quarantine period to adapt to their new environment. To create hyperglycemia conditions, mice were fasted for 16 hours and then induced with streptozotocin at a dose of 50 mg/kg BW. Blood glucose levels were measured on the 2nd to 4th days after induction using the NESCO® tool. The average blood sugar level examined in all mice was 135.9 mg/dL. Diabetes mellitus is diagnosed if the fasting blood glucose level in plasma is ≥ 126 mg/dL. To keep the mice in a state of hyperglycemia, the researcher made modifications by providing food in the form of rice and limiting the activity of mice by placing mice in cages without movement facilities.

Wound Incision

Wounds were made on the third day after streptozocin induction. Diabetic mice had wounds on the chest skin that were shaved on the dorsal side after being cleaned topically with alcohol. The wound was circular with a diameter of 1 cm on the back of the mice. Before making a wound, zoletil anesthetic was given to rats in 0.1 milliliters per kilogram of body weight.

Wound Evaluation

The wound is physically inspected daily to confirm the presence of exudate, swelling, color, consistency, and size of the surrounding tissue. The following formula is used to calculate wound closure:

$$\% \text{ wound closure} = \frac{\text{Initial wound area-final wound area}}{\text{Initial wound size}} \times 100$$

Research process

This research is an experiment using a control group. The research was conducted in three stages: the preparation of ointment formulations and the preparation of experimental animals; interventions for diabetic wound care in control mice and the intervention group; and evaluation of the results of the wound healing process. In the intervention group, the wound was treated using brotowali extract ointment with a combination of ZAL, while in the control group, it was given standard wound care using a 9% NaCl solution. Wound care is carried out every day with a duration of 14 days. Wound care is carried out every 09.00 WIB.

Data Analysis

Data were analyzed using the central tendency for univariate and bivariate analysis using an alternative test, the Mann-Whitney test because the data were not normally distributed.

RESULT

In the presentation of the results, the results of laboratory tests to see the content and feasibility of brotowali extract with a combination of ZAL and the results of testing the effectiveness of the ointment on healing diabetic wounds were explained.

Extract ointment

Table 1. Brotowali Extract Ointment Formulation with ZAL Combination

Component	Usability	Total(gr)
Brotowali stem extract	Active substance	9
Zeolite	Active substance	2
Cera Alba	Ointment base	5
Vaselin Album add	Basis salep	100

Ointment formulations are pharmaceutical preparations in the form of creams or pastes that are applied to the skin. One of the ointment formulations that can be developed is brotowali extract ointment with a combination of ZAL. This formulation uses several main raw materials: brotowali stem extract, Lampung natural zeolite, and Cera alba (beeswax).

Phytochemical Test

The thick brotowali stem extract was subjected to a phytochemical test to identify the chemical compounds in the ingredients used.

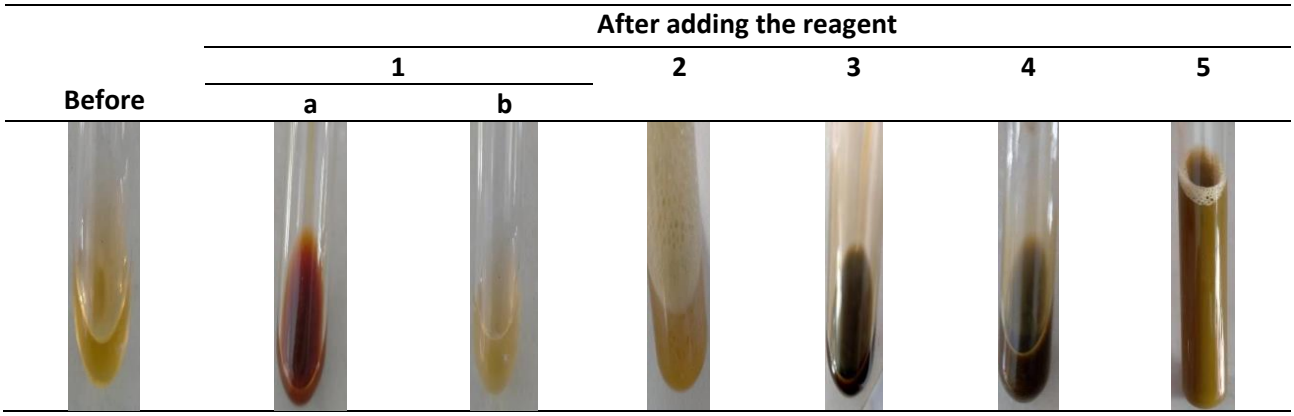


Figure 1. Phytochemical test process

Table 2. Phytochemical test results

No	Secondary Metabolites	Test Method	Test Results
1	Alkaloid	a. Dragendorf reagent	+
		b. Mayer's reagent	+
2	Flavonoid	Pereaksi HCl pekat + Mg	+
3	Tanin	FeCl Reagent 10%	+
4	Steroid dan Triterpenoid	Reactants Concentrated H2SO4 + anhydrous CH3COOH	+
5	Saponin	Heated Saponins	-

Ointment stability test

Table 3. Ointment Stability Test Results

Observational Formulas		Observation				
		Form	Odor	Color	Homogeneity	pH
Ointment	0	Semi Solid	Typical	Green	+	6
	1	Semi Solid	Typical	Green	+	6
	2	Semi Solid	Typical	Green	+	5.79
	3	Semi Solid	Typical	Green	+	5.39
	4	Semi Solid	Typical	Green	+	5.60
	5	Semi Solid	Typical	Green	+	5.24
	6	Semi Solid	Typical	Green	+	5

The results of the stability test of the ointment for six cycles showed that the ointment was stable. Furthermore, the ointment was evaluated by conducting organoleptic, homogeneity, and pH tests. The results of the sample test showed a homogeneous composition characterized by the absence of coarse grains in the preparation that was smeared on the glass, no lumps in the smearing results, an even structure, and a uniform color from the starting point to the end point of application. The results of the spreadability test of brotowali extract ointment with ZAL had an average diameter of 5 cm, which met the requirements for good spreadability, namely 5-7cm. The pH test results combination was 5. It indicated that the ointment had met the standards or was declared safe for skin (4.5–6.7). Stability tests were carried out to ensure that the ointment remained homogeneous and of good quality throughout its shelf life. A homogeneous ointment ensures that the components are mixed evenly so that each application provides a consistent dose and benefit. The stability test results showed that the brotowali extract ointment formulation with the combination of ZAL and Cera alba had stable characteristics over a certain period.

Table 4. Differences in the average percentage of wound closure in the intervention group and the control group

Group		D1	D3	D6	D9	D12	D14
Intervention	Mean	0	23.52	58.90	80.28	95.51	100
	Median	0	25.00	60.00	78.90	95.55	100
	Sd	0	8.86	7.52	7.86	4.14	0
	Min-max	0	11.1-33.3	50-66.7	70-90	90-100	100-100
Control	Mean	0	12.40	41.03	61.80	84.83	95.33
	Median	0	15.50	41.65	61.90	84.50	94.85
	Sd	0	6.61	14.75	14.44	3.36	1.94
	Min-max	0	0 – 16.7	20-60	40-82	80-90	93.3-98.3

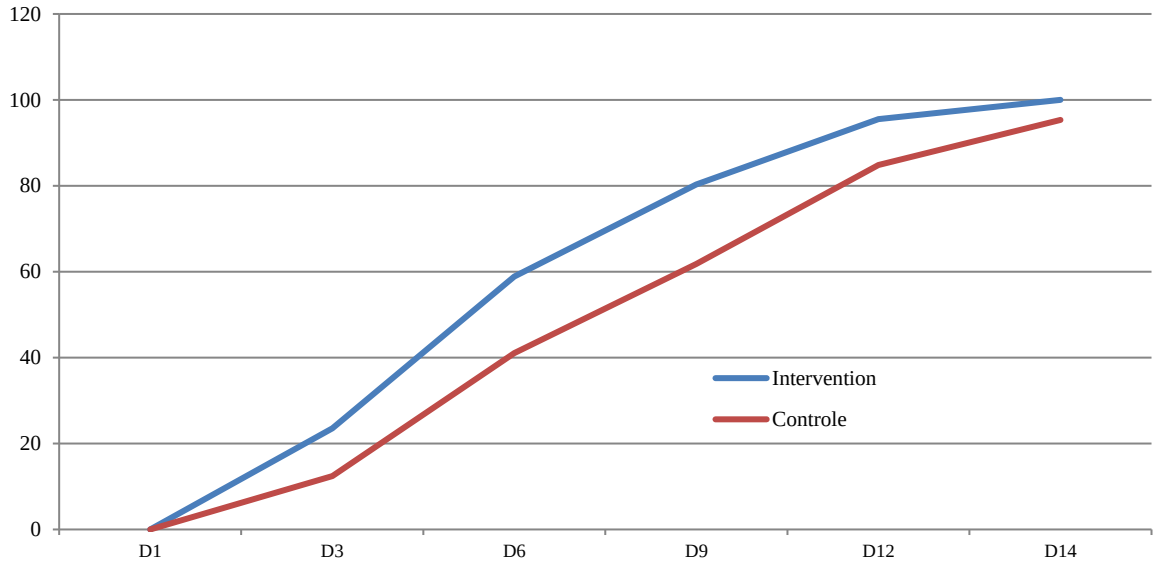


Figure 1. Graph of the percentage of wound closure in the intervention group and the control group

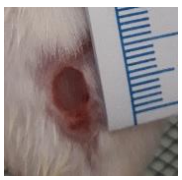
Observation	Days to-				
	1	3	6	11	14
Intervention group					
Control group					

Figure 2. Macroscopic picture of the development of wound closure in the intervention and control groups

In this study, an analysis of the differences in the percentage of wound closure between the intervention group and the control group was performed. The results showed that the average wound closure in the intervention group on D3 was 23.52 with a standard deviation of 8,86, while in the control group, it was 12.40 with a standard deviation of 6,61, and on D14, the intervention group showed 100% perfect wound closure, while the control group had an average wound closure rate of 95.33 with a standard deviation of 1,94. It shows that the group of mice treated for wounds using brotowali stem extract ointment with a combination of ZAL grew faster than those treated with conventional wound care.

Table 5. Description of wound reduction in the intervention group and the control group

Group	Reduction of Wound Area				N
	Mean Rank	Sum of rank	Z	Mann-Whitney U	
Intervention	9.50	57.00	-3.08	P value	6
Control	3.50	21.00		0.002	6

Furthermore, an analysis of the difference in average wound reduction between the two different groups was carried out. The normality test results showed that all data are not normally distributed, so the analysis used is the Mann-Whitney test. The results showed a z score of -3,08 with a p-value of 0,002, indicating there was a significant difference in average wound closure between the intervention and the control groups.

DISCUSSION

Based on Table 2, Brotowali stem (T. Crispa) contains a variety of phytochemicals that are beneficial to health, such as alkaloids, flavonoids, tannins, and saponins. Brotowali stem extract has the potential to be an active ingredient in ointment formulations with anti-inflammatory and antimicrobial benefits. One of the challenges in formulating this ointment is ensuring product consistency and stability. Using ZAL in ointment formulations can help improve physical stability and prevent phase separation in ointments. In their research, Susanti, L., et al. (2018) found that ZAL was able to stabilize and improve noni extract's performance in suppressing staphylococcal aureus bacteria growth. Meanwhile, cera alba (beeswax) is a natural wax material that is a thickening agent in ointments. Cera alba can maintain color stability and consistency, functions as an oil binder, and produces a homogeneous preparation mass (Salsabila et al., 2023).

The progress of wound closure from D0 to D14 in the intervention and control groups can be seen in Figure 1. Meanwhile, macroscopic wound closure can be seen in Figure 2. In both the intervention and control groups, wound healing progressed, and the intervention group progressed faster than the control group. From the observation results, there was no development of infection in either group.

Wound healing is a process consisting of four overlapping and continuous phases independent of one another. The four phases are coagulation, inflammation, granulation tissue formation, and scar tissue formation (Fernandez et al., 2021). For optimal wound healing, the phases must occur in a precise sequence for a specified duration of time under optimal conditions. These steps are intended to enhance the wound healing environment and prevent infection. Brotowali stem extract derived from the T.Crispa plant has long been used in traditional medicine because it is known to have antimicrobial, anti-inflammatory, and immunomodulatory properties (Ahmad et al., 2018).

The active compounds in brotowali extract, such as flavonoids and tannins, can inhibit the activity of inflammatory mediators and reduce the inflammatory response. By reducing inflammation, the wound healing process can run more efficiently (B. Singh et al., 2021). Based on the research results of Haque et al. (2011), brotowali stem extract has an anti-microbial effect with very significant toxicity. It also has activity as an anti-oxidant, which can help fight infection in wounds. Alkaloids have anti-inflammatory and anti-microbial effects, while flavonoids can act as protective enzymes because they can fight bacteria and viral infections (B. Singh

et al., 2021). By inhibiting infection, brotowali extract can create better conditions for wound healing.

In the context of wound healing, collagen, and epithelial cells play an important role in the healing process. Inflammatory, proliferative, and maturation phases are part of the wound-healing. Brotowali extract ointment with a combination of Lampung natural zeolite contains active substances such as flavonoids, steroids, triterpenoids, and alkaloids that have anti-inflammatory effects, improve blood circulation, prevent blood vessel blockage, and accelerate tissue regeneration. In addition, the tannins in the ointment also help stop bleeding and prevent bacterial infections (Ahmad et al., 2016).

In addition to these compounds, T.Crispa also contains magnoflorin, which exhibits various pharmacological effects, including immunomodulatory, antioxidant, and neuropharmacological activities. T. crispa extract can also stimulate tissue regeneration in wounds. Several studies have shown that brotowali extract can accelerate the processes of cell proliferation and granulation tissue formation, which are important for optimal wound healing. In addition, T.Crispa extract can also accelerate epithelialization, which is the process of forming an epithelial layer on the wound surface (Philip et al., 2018).

Silver nanoparticles synthesized from Tinospora stems had excellent antibacterial activity against various drug-resistant Pseudomonas aeruginosa strains isolated from burn patients. Silver nanoparticles suggestively increase cell proliferation and stimulate the rate of wound closure, with less scar tissue and more fibroblasts (Qi et al., 2021). The flavonoids and berberine alkaloids contained in brotowali stem extract (T.Crispa) have antifungal properties and can easily destroy biofilms by reducing the biofilm's thickness and destroying its structure. Biofilm is a layer or polysaccharide membrane made by bacteria attached to the surface. Biofilms have been known to be responsible for establishing infections in humans. The most common pathogen causing biofilms is Pseudomonas aeruginosa (Hutomo et al., 2022). Riswanto (2022), in his research, found that biofilm-forming pathogens had a 0.24 times lower probability of wound repair than non-biofilm-forming pathogens within 14 days after definitive antibiotics (Riswanto, 2022).

Brotowali extract with ZAL combination is an optimal formulation, besides functioning as a stabilizer for the active substances in brotowali extract. Zeolite, a natural mineral with a hollow pore structure, can absorb and bind certain substances. ZAL is a type of zeolite found in Lampung, Indonesia. This zeolite has high adsorption and decontamination abilities, so it is often used in various applications, including wound care products. Lampung's natural zeolite can help reduce the risk of infection by absorbing bacteria and other harmful substances from wounds and improving environmental conditions that support healing (Kadja, G., & Ilmi, 2019).

The results of this study indicate that brotowali extract ointment with ZAL has the potential to accelerate the wound healing process in patients with diabetic ulcers. The combination of active ingredients from brotowali extract and ZAL has a positive effect on increasing the formation of granulation tissue and epithelialization, as well as reducing the risk of infection and inflammation. Other natural components that have the potential to improve the wound healing process in hyperglycemic rats are Plantago, a major leaf extract in Indonesian terms is Daun Sendok. This extract has active compounds, such as terpenoids, phenolic acids, flavonoids, alkaloids, and iridoids have been shown to increase wound closure and accelerate wound healing time in hyperglycemic mice (Kartini et al., 2021). Gallic acid contained in black tea is found to be antioxidants that can accelerate the migration of keratinocyte cells and fibroblasts under normal and hyperglycemic conditions (Yang et al., 2016). Overall, the results of this study indicate that wound care using brotowali extract ointment with a combination of ZAL has significant effectiveness in reducing wound area and accelerating the healing process of diabetic ulcers compared to conventional wound care. This ointment has the potential as an alternative therapy that can improve patients' quality of life with diabetic ulcers.

In this study, no morphological tests or observations were carried out for the development of bacteria. The fundamental response to injury is wound healing, which results in the restoration of tissue integrity.

IMPLICATION FOR NURSING PRACTICE

This research is fundamental in improving the quality of diabetic wound care. This study shows that combining brotowali extract and Lampung natural zeolite can accelerate wound healing in diabetic mice, which can be applied in nursing practice for human patients. With this approach, nurses can introduce natural remedies as an alternative or complement to conventional therapies, potentially reducing the cost of care and the side effects of synthetic drugs. In addition, the use of more affordable and readily available natural ingredients can increase the accessibility of effective wound care, especially in resource-limited areas. This also opens up opportunities for nurses to play a more active role in patient education regarding safe and effective natural treatment options, thereby improving the overall quality of life of diabetic patients.

CONCLUSION

Brotowali stem extract contains active compounds: alkaloids, flavonoids, tannins, steroids, and triterpenoids. These active compounds have beneficial properties in the wound healing process in reducing the inflammatory response. They are anti-microbial and increase vascularization and tissue regeneration, causing acceleration in the wound healing process.

Natural zeolite Lampung is also proven to have active compounds that function as stabilizers of active compounds of brotowali stem extract in ointments. Moreover, based on the results of organoleptic tests and spread tests, it was found that brotowali extract ointment with a combination of natural zeolites Lampung proved to be homogeneous (has good spreadability) and does not smell pungent with a pH level of 5. It can be concluded that the ointment is safe for the skin to use.

The experimental test results of applying brotowali extract ointment with a combination of natural zeolites Lampung on diabetes mellitus mice wounds proved effective (the wound healing process was faster in the intervention group compared to the control group).

REFERENCES

Agrawal, S. S., Naqvi, S., Gupta, S. K., & Srivastava, S. (2012). Prevention and management of diabetic retinopathy in STZ diabetic rats by Tinospora cordifolia and its molecular mechanisms. *Food and Chemical Toxicology*, *50*(9), 3126–3132. https://doi.org/10.1016/j.fct.2012.05.057

Ahmad, W., Jantan, I., & Bukhari, S. N. A. (2016). Tinospora crispa (L.) Hook. f. & Thomson: A review of its ethnobotanical, phytochemical, and pharmacological aspects. *Frontiers in Pharmacology*, *7*(MAR), 1–19. https://doi.org/10.3389/fphar.2016.00059

Ahmad, W., Jantan, I., Kumolosasi, E., Haque, M. A., & Bukhari, S. N. A. (2018). Immunomodulatory effects of Tinospora crispa extract and its major compounds on the immune functions of RAW 264.7 macrophages. *International Immunopharmacology*, *60*(April), 141–151. https://doi.org/10.1016/j.intimp.2018.04.046

Arundina, I., Diyatri, I., & Jit, F. Y. (2017). The effect of brotowali stem extract (Tinospora Crispa) towards increasing number of lymphocytes in the healing process of traumatic ulcer on diabetic wistar rat. *Journal of International Dental Medical Research*, *10*(3), 975–980.

Banerjee, A., Singh, S., Prasad, S. K., Kumar, S., Banerjee, O., Seal, T., Mukherjee, S., & Maji, B. K. (2020). Protective efficacy of Tinospora sinensis against streptozotocin induced pancreatic islet cell injuries of diabetic rats and its correlation to its phytochemical profiles. *Journal of Ethnopharmacology*, *248*, 112356. https://doi.org/10.1016/j.jep.2019.112356

Fernandez, M., Shivashekaregowda, N. K., & Yin, Y. (2021). The Potential Role Of Genus Tinospora In Wound Healing: A Review. *Science*, *International Journal of Pharmacy and Pharmaceutical Sciences, 21–29*. https://doi.org/10.22159/ijpps.2021v13i4.37980

Gastaldi, G., Pannier, F., Roztočil, K., Lugli, M., Mansilha, A., Haller, H., Rabe, E., & Van Rijn, M. J. (2021). Chronic venous disease and diabetic microangiopathy: pathophysiology and commonalities. *Int. Angiol*, *40*, 457–469.

https://doi.org/10.23736/S0392-9590.21.04664-2

Haque, A., Islam, A., & Shahriar, M. (2011). Antimicrobial, cytotoxicity and antioxidant activity of Tinospora crispa. *Journal of Pharmaceutical and Biomedical Sciences*, *12*(13), 1–4. https://www.academia.edu/download/37153609/JPBMS_1212_2011.pdf

Hutomo, S., Sooai, C. M., Merry, M. S., Larope, C. G., & Kristiyanto, H. D. (2022). The effect of brotowali (Tinospora crispa L.) stem ethanolic extract on the inhibition of Candida albicans biofilm formation. *Majalah Kedokteran Gigi*, *55*(1), 21–25. https://doi.org/10.20473/j.djmkg.v55.i1.p21-25

Kadja, G., & Ilmi, M. M. (2019). Indonesia natural mineral for heavy metal adsorption: a review. *Journal of Environmental Science and Sustainable Development*, *2*(2), 139-164. https://doi.org/10.7454/jessd.v2i2.1033

Kartini, K., Wati, N., Gustav, R., Wahyuni, R., Anggada, Y. F., Hidayani, R., Raharjo, A., Islamie, R., & Putra, S. E. D. (2021). Wound healing effects of Plantago major extract and its chemical compounds in hyperglycemic rats. *Food Bioscience*, *41*(June 2020), 100937. https://doi.org/10.1016/j.fbio.2021.100937

Li, D., & Wu, N. (2022). Mechanism and application of exosomes in the wound healing process in diabetes mellitus. *Diabetes Research and Clinical Practice*, *187*(January). https://doi.org/10.1016/j.diabres.2022.109882

Liu, Y., Li, C., Feng, Z., Han, B., Yu, D.-G., & Wang, K. (2022). Advances in the Preparation of Nanofiber Dressings by Electrospinning for Promoting Diabetic Wound Healing. *Biomolecules*, *12*(12), 1727. https://doi.org/10.3390/biom12121727

Mariani, H., Mahdi, N., & Umam, K. (2023). BIOSINTESIS NANOPARTIKEL PERAK (AgNPs) DENGAN EKSTRAK DAUN KIRINYUH (Chromolaena odorata) TERIMPREGNASI ZEOLIT DALAM MENGHAMBAT BAKTERI PENYEBAB JERAWAT. *Jurnal Biosilampari : Jurnal Biologi*, *5*(2), 187–198. https://doi.org/10.31540/biosilampari.v5i2.1833

Millah, U. (2021). *Identifikasi Bakteri Staphylococcus Sp. Pada Pasien Diabetes Mellitus Dengan Komplikasi Luka Gangren*. STIKes Ngudia Husada Madura.

Nasseri, S., & Sharifi, M. (2022). Therapeutic potential of antimicrobial peptides for wound healing. *International Journal of Peptide Research and Therapeutics*, *28*, 1–15. https://doi.org/10.1007/s10989-021-10350-5

Nurhayati, N. (2022). Identifikasi Senyawa Anti Mikroba Ekstrak Etanol Batang Brotowali (Tinospra Crispa (L.) Terhadap Staphylococcus Aureus, Bacillus Substillis, Dan Candida Albicans Dengan Metode Klt Bioautografi. *Jurnal Ayurveda Medistra*, *4*(1). https://doi.org/10.51690/medistra-jurnal123.v4i1.54

Philip, S., Tom, G., & Vasumathi, A. V. (2018). Evaluation of the anti-inflammatory activity of Tinospora cordifolia (Willd.) Miers chloroform extract – a preclinical study. *Journal of Pharmacy and Pharmacology*, *70*(8), 1113–1125. https://doi.org/10.1111/jphp.12932

Purwaningsih, N. S., Romlah, S. N., & Choirunnisa, A. (2020). Literature Review Uji Evaluasi Sediaan Krim. *Edu Masda Journal*, *4*(2), 108–120. https://doi.org/10.52118/edumasda.v4i2.102

Qi, Y., Qian, K., Chen, J., Yifeng, E., Shi, Y., Li, H., & Zhao, L. (2021). A thermoreversible antibacterial zeolite-based nanoparticles loaded hydrogel promotes diabetic wound healing via detrimental factor neutralization and ROS scavenging. *Journal of Nanobiotechnology*, *19*(1), 1–20. https://doi.org/10.1186/s12951-021-01151-5

Riswanto, W. (2022). Hubungan Patogen Pembentuk Biofilm Terhadap Perbaikaninfeksi Luka Pasca Antibiotik Definitif. *Doctoral Dissertation, Universitas Gadjah Mada*.

Salsabila, L. S., Shafira, I., Azhar, M. A., Ryanti, G. A. S., Aurelia, R., & Sitorus, M. A. H. (2023). Formulasi Dan Evaluasi Balsam Aroma Terapi Menggunakan Minyak Lemon (Oleum Citri. L) Dengan Cera Alba Sebagai Stabilizing Agent. *Indonesian Journal of Health Science*, *3*(2), 155–160. https://doi.org/10.54957/ijhs.v3i2.448

Sharma, P., Dwivedee, B. P., Bisht, D., Dash, A. K., & Kumar, D. (2019). The chemical constituents and diverse pharmacological importance of

Tinospora cordifolia. *Heliyon*, *5*(9). https://doi.org/10.1016/j.heliyon.2019.e02437

Singh, A. K., Preethi, B. O., Singh, H., Gangwar, A., Niyogi, D., & Devi, K. S. (2016). Comparative evaluation of the wound healing potential of Tinospora cordifolia and its combination with local Insulin therapy in diabetic rabbits. *Journal of Pharmacognosy and Phytochemistry*, *6*(4), 1812–1817.

Singh, B., Nathawat, S., & Sharma, R. A. (2021). Ethnopharmacological and phytochemical attributes of Indian Tinospora species: A comprehensive review. *Arabian Journal of Chemistry*, *14*(10), 103381. https://doi.org/10.1016/j.arabjc.2021.103381

Soesilowati, S. (2021). Implementasi Visual Manajemen Terhadap Kegiatan Operasional dan Keamanan Laboratorium Biokimia. *Jurnal Pengelolaan Laboratorium Pendidikan*, *3*(1), 8–13. https://doi.org/10.14710/jplp.3.1.8-13

Subandi, E., & Sanjaya, K. A. (2020). Efektifitas Modern Dressing Terhadap Proses Penyembuhan Luka Diabetes Melitus Tipe 2. *Jurnal Kesehatan*, *10*(1), 1273–1284. https://doi.org/10.38165/jk.v10i1.7

Sun, H., Saeedi, P., Karuranga, S., Pinkepank, M., Ogurtsova, K., Duncan, B. B., Stein, C., Basit, A., Chan, J. C. N., Claude Mbanya, J., Pavkov, M. E., Ramachandaran, A., Wild, S. H., James, S., Herman, W. H., Zhang, P., Bommer, C., Kuo, S., Boyko, E. J., & Magliano, D. J. (2023). Erratum to "IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045" [Diabetes Res. Clin. Pract. 183 (2022) 109119] (Diabetes Research and Clinical Practice (2022) 183, (S0168822721004782), (1. *Diabetes Research and Clinical Practice*, *204*(October), 110945. https://doi.org/10.1016/j.diabres.2023.110945

Susanti, L., Wahidah, L. K., & Viogenta, P. (2018). Optimasi Zeolit Alam Lampung (ZAL) Sebagai Penstabil Sediaan Antibakteri Esktrak Buah Mengkudu (Morinda citrifolia L.). *Jurnal Farmasi Lampung*, *7*(2). https://doi.org/10.37090/jfl.v7i2.54

Vijendren, S., Jothi, S., Rajandran, K., & Muruganandham, V. (2017). Assessment of in-vitro wound healing activity of the Tinospora crispa Extracts. *Assessment*. https://doi.org/10.47893/IJPPT.2017.1016

Yang, D. J., Moh, S. H., Son, D. H., You, S., Kinyua, A. W., Ko, C. M., Song, M., Yeo, J., Choi, Y. H., & Kim, K. W. (2016). Gallic Acid Promotes Wound Healing in Normal and Hyperglucidic Conditions. *Molecules*, *21*(7), 1–15. https://doi.org/10.3390/molecules2107089