Original Article

Therapeutic Efficacy of *Brotowali* Ointment for Diabetic Burn Streptozotocin-Nicotinamide-Induced Rats

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ABSTRACT

Introduction: Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia and is caused by factors such as genetics, obesity, improper diet and sleep patterns, stress, and lack of physical activity. One of the most common complications of DM is diabetic wound. The Dayak Ngaju people in Central Kalimantan use *brotowali* (*Tinospora crispa* L.) to treat skin diseases. The aim of this study is to pharmacologically assess the capacity of brotowali stem ointment in treating diabetic burn wound.

Materials & Methods: Diabetic rats were divided into six groups, each containing five rats, with wounds created on their dorsal regions. The first group received oral *brotowali* extract and no topical treatment. The second group received oral brotowali extract and topical Betadine ointment. The third group received oral *brotowali* extract and topical base ointment. The fourth group received oral *brotowali* extract and topical 20% *brotowali* ointment. The fifth group received oral brotowali extract and topical 35% *brotowali* ointment.

The sixth group received oral *brotowali* extract and topical 50% *brotowali* ointment. Diabetic burn wound healing was assessed based on wound scores, which were evaluated macroscopically by observing the color and the amount of scab detachment.

Results: The average wound scores at the end of the observation period indicated that all groups have a lower average wound score. Moreover the average wound scores at the end of the observation period indicated that Group K6 had a lowest average wound score ($p < 0.05$).

Conclusion: The 20% *brotowali* extract ointment has a healing capacity for diabetic burn wound comparable to Betadine ointment. Furthermore, the 35% and 50% *brotowali* ointments exhibit superior healing capabilities.

Keywords: Diabetic burn wound, Scabs, *Tinospora crispa* L, Wound healing

1. Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia [1] and is caused by factors such as genetics, obesity, improper diet and sleep patterns, stress, and lack of physical activity [2]. DM has long-term health impacts and, if not properly managed, can lead to chronic complications [3]. One of the most common complications of DM is diabetic gangrene [4]. Diabetic gangrene is a chronic wound resulting from excessive infection and inflammation in DM patients [4], often occurring on weight-bearing areas such as the lower extremities [3]. Infection arises due to delayed wound management caused by diabetic angiopathy and neuropathy [3,4], which can even extend to deeper tissues, including subcutaneous tissue, tendons, muscles, bones, and joints [3]. Many cases of diabetic gangrene can lead to amputation [3], but this risk can be minimized with appropriate and intensive wound care [4].

The evolving principle of wound care is moisturizing dressing, which involves maintaining moisture around the wound, often through the use of topical ointments. Ointments create a moist environment at the wound site, thereby enhancing epithelial tissue development and migration, which accelerates wound healing [5]. Concurrently, advancements in the healthcare field have led to numerous efforts to develop the most effective medications for wound management [5]. At the same time, the development of chemical-based drugs and traditional plant-based remedies has proven beneficial in wound healing [3]. The active compounds in plants have motivated researchers to develop plant-based medications, including ointments [3].

Brotowali (*Tinospora crispa* L.) is a plant from the Menispermaceae family native to Asia [6], including Indonesia [7]. Traditionally, *brotowali* has been used by various communities to treat a range of ailments [6]. The Dayak Ngaju people in Central Kalimantan use *brotowali* to treat skin diseases, rheumatism, and jaundice [6], while people in Bali use it for diabetic ulcers [8]. Scientific studies have shown that brotowali leaves can cleanse the digestive tract [9], while the stems can treat diabetes [1], and malaria [10]. Phytochemical studies reveal that *brotowali* leaves contain alkaloids, saponins, tannins, an d flavonoids [9]. Additionally, literature studies indicate that *brotowali* stems possess anti-inflammatory (11), and antioxidant effects [7].

Traditionally, various plants have been used to treat diabetic wound, including soursop (*Annona reticulata*), *pegagan* (*Centella asiatica*) [7], guava (*Psidium guajava*), bitter melon (*Momordica charantia* L.), and combinations of *brotowali* (*Tinospora crispa* L.) with ginger (*Zingiber officinale*), black pepper (*Piper nigrum*), and calamus (*Acorus calamus*) [8]. Scientifically, plants known to treat diabetic wound include *bidara upas* (*Merremia mammosa* (Lour.)) [12]. The healing efficacy for diabetic wound is supported by flavonoid compounds that act as antibacterials [12], antioxidants [12], and anti-inflammatories by stimulating macrophage growth [12], modulating cytokines [13], and increasing lymphocyte count [12]. Additionally, in treating diabetic wound, phenolic compounds serve as antimicrobials, while alkaloids, saponins, tannins, and steroids play roles in stimulating cell proliferation, migration, and skin fibroblasts [13]. Thus, the aim of this study is to pharmacologically assess the capacity of *brotowali* stem ointment in treating diabetic burn wound in streptozotocin-nicotinamide induced rats.

2. Material and Methods

2.1. Materials

The study utilized *brotowali* stem, 96% ethanol, Vaseline album, adeps lanae, streptozotocin (Cayman, USA), nicotinamide (Nacalai, Kyoto), 30 male white rats (200-250 g), and betadine ointment (PT. Mahakam Beta Farma).

2.2. Extraction

The *brotowali* stems were obtained from Sanggau and Mempawah, West Kalimantan, Indonesia. The extraction process was performed using the maceration method as described by Panjaitan et al. [14] Fresh *brotowali* stems, weighing 13.5 kg, were cut, cleaned, and dried. Extraction was carried out with 96% ethanol for 24 hours and repeated three times. The resulting macerate was concentrated, yielding 172.52 grams of thick extract with a yield of 1.28%. The yield calculation using the following formula:

$$\%\ \text{Yield} = \frac{Weight\ of\ the\ thick\ extract\ (gram)}{Initial\ weight\ of\ the\ simplicia\ (gram)}\ x\ 100\%$$

2.3. Qualitative Phytochemical Analysis

Qualitative phytochemical testing of *brotowali* extract was conducted at the Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Tanjungpura University, Pontianak, Indonesia. The secondary metabolites tested qualitatively included alkaloids, flavonoids, saponins, terpenoids, steroids, and tannins. The results were reported in a certificate with the number 022/LABKIM/XI/2023.

2.4. Test Animals and Ethical Statement

A total of 30 male white rats (*Rattus norvegicus*), two months old and weighing 200-250 grams, were obtained from the Food and Nutrition Study Center, Gadjah Mada University, Yogyakarta, Indonesia. Prior to testing, the rats were acclimated for seven days and provided with standard food and water ad libitum. The use of test animals and the procedures for this study were approved by the Faculty of Health Sciences, Universitas Respati Yogyakarta, Indonesia, and received ethical clearance with the number 044.3/FIKES/PL/V/2023.

2.5. Formulation and Preparation of Ointment

The formulation of *brotowali* extract ointment follows the method described by Panjaitan et al. [14] The ointment was prepared in three concentrations: 20%, 35%, and 50%. The ointment base used consisted of 15g of adeps lanae and 85g of vaseline album. The preparation process adhered to the method outlined by Maru and Lahoti [15]. The ointment was made using a heated mortar and pestle, pre-warmed in an oven at 70°C. After removal from the oven, the mortar and pestle were used to mix the adeps lanae, vaseline album, and brotowali extract until the formulation was homogeneous.

a) *Brotowali* ointment (*Tinospora crispa* L.) 20% concentration
   Brotowali stem extract : 1g
   Ointment base : 4g
   Ointment formula : 5g

b) *Brotowali* ointment (*Tinospora crispa* L.) 35% concentration
   Brotowali stem extract : 1,75g
   Ointment base : 3,25g
   Ointment formula : 5g

c) *Brotowali* ointment (*Tinospora crispa* L.) 50% concentration
   Brotowali stem extract : 2,5g
   Ointment base : 2,5g
   Ointment formula : 5g

2.6. Evaluation of The Healing Capacity of *Brotowali* Ointment for Diabetic Burn Wound (*Tinospora crispa* L.)

The rats were fasted for 8-12 hours, after which their initial blood glucose levels were measured. They were then induced with streptozotocin-nicotinamide (STZ-NA) at doses of 45 mg/kg and 110 mg/kg body weight, respectively, via intraperitoneal injection [16]. Nicotinamide induction was performed 15 minutes before streptozotocin induction. The STZ-NA induction was designated as day 0. After 72 hours (day 3), blood glucose levels were checked again; rats with glucose levels >200 mg/dl were considered diabetic [16]. Once diabetes was confirmed, wounds were created on day 4. The wound creation procedure followed Tumigolung et al. [17] First, a 3x3 cm area on the rats' backs was shaved and disinfected with 70% alcohol. A metal with a diameter of 2 cm was then heated for 3 minutes and applied to the rats' backs for 10 seconds. The wounds were left for 4 days (up to day 8), and observations of the wounds began on day 8 as an initial wound assessment.

Subsequent observations were conducted until day 22. On day 8, the diabetic burn wound rats were divided into six groups, each consisting of five rats. The first group (normal control) was given *brotowali* extract orally at a dose of 450 mg/kg without topical medication. The second group (positive control) received *brotowali* extract orally at a dose of 450 mg/kg and was treated with betadine ointment. The third group (negative control) was given *brotowali* extract orally at a dose of 450 mg/kg and treated with the ointment base. The fourth, fifth, and sixth groups received *brotowali* extract orally at a dose of 450 mg/kg and were treated with *brotowali* stem ethanol extract ointment at concentrations of 20%, 35%, and 50%, respectively. The oral extract dose followed Ashari et al [1]. Ointment application was performed twice daily at 08:00 AM and 02:00 PM, applied thinly and evenly to the wound surface [14]. Wound healing was assessed based on scores obtained from macroscopic observations over 15 days (from day 8 to day 22). A smaller score indicated better wound healing. On day 23, the rats' blood glucose levels were measured again to determine the final glucose levels.

2.7. Assessment of Diabetic Burn Wound Healing

Diabetic burn wound healing was evaluated using a macroscopic observation score adapted from the study by Priamsari and Yuniawati [18] (Table 1).

Table 1. Diabetic burn wound healing score in rats through macroscopic observation

Score	Wound description
1	White and clean, all crusts have fallen off
2	White with brownish, all crusts have fallen off
3	White with brownish, most crusts have fallen off
4	White with brownish, half of the crusts have fallen off
5	White with brownish, nearly half of the crusts have fallen off
6	White with brownish, a small amount of crusts have fallen off
7	White with brownish, crusts present and not fallen off
8	Dark brown, all crusts have fallen off
9	Dark brown, most crusts have fallen off
10	Dark brown, half of the crusts have fallen off
11	Dark brown, nearly half of the crusts have fallen off
12	Dark brown, a small amount of crusts have fallen off
13	Dark brown, crusts present and not fallen off
14	Brown, all crusts have fallen off
15	Brown, most crusts have fallen off
16	Brown, half of the crusts have fallen off
17	Brown, nearly half of the crusts have fallen off
18	Brown, a small amount of crusts have fallen off
19	Brown, crusts present and not fallen off
20	Reddish brown, all crusts have fallen off
21	Reddish brown, most crusts have fallen off
22	Reddish brown, half of the crusts have fallen off
23	Reddish brown, nearly half of the crusts have fallen off
24	Reddish brown, a small amount of crusts have fallen off
25	Reddish brown, crusts present and not fallen off
26	Reddish pale, crusts present
27	Reddish pale, no crusts present
28	Pale wound bed, no scab, no crusts present

2.8. Statistical Analysis

Wound scores during the observation period (days 8 to 22) were analyzed statistically using SPSS version 27. The analysis involved the *Kruskal-Wallis* test followed by the *Mann-Whitney* test.

3. Results

3.1. Phytochemical Content of *Brotowali* Extract (*Tinospora crispa* L.)

Qualitative phytochemical testing was conducted to identify secondary metabolites in brotowali stem extract that contribute to the healing of diabetic burn wound. Based on the results of the qualitative phytochemical test, *brotowali* extract was reported to contain secondary metabolites including flavonoids, saponins, steroids, and tannins.

3.2. The Capacity for Healing Diabetic Burn Wound of *Brotowali* Ointment (*Tinospora crispa* L.)

Diabetic burn wound was observed macroscopically from day 8 to day 22. The macroscopic description of the wound condition was then matched with the descriptions in the wound score table. A smaller wound score indicates better healing capacity. After assigning scores to each wound, a more specific analysis of the healing capacity was performed by analyzing the obtained wound scores. The results of the wound score data analysis are presented in Table 2.

Table 2. Results of the *Mann-Whittney* post-hoc test for the average diabetic burn wound scores in rats over 15 days, from day 8 to day 22.

Day	K1	K2	K3	K4	K5	K6
8	28,0^{a} ± 0,00	28,0^{a} ± 0,00	28,0^{a} ± 0,00	28,0^{a} ± 0,00	28,0^{a} ± 0,00	28,0^{a} ± 0,00
9	26,0^{a} ± 0,00	25,8^{a} ± 0,45	26,0^{a} ± 0,00	26,0^{a} ± 0,00	26,0^{a} ± 0,00	26,0^{a} ± 0,00
10	26,0^{a} ± 0,00	25,8^{a} ± 0,45	26,0^{a} ± 0,00	26,0^{a} ± 0,00	25,8^{a} ± 0,45	25,8^{a} ± 0,45
11	26,0^{a} ± 0,00	25,8^{a} ± 0,45	26,0^{a} ± 0,00	26,0^{a} ± 0,00	25,8^{a} ± 0,45	25,8^{a} ± 0,45
12	22,6^{a} ± 3,29	22,6^{a} ± 5,37	25,2^{a} ± 0,45	25,8^{a} ± 0,45	24,2^{a} ± 2,95	23,8^{a} ± 2,77
13	21,4^{a} ± 3,29	22,6^{a} ± 5,37	21,6^{a} ± 2,19	19,4^{a} ± 0,89	18,4^{a} ± 0,55	20,0^{a} ± 3,39
14	20,2^{b} ± 2,68	20,2^{b} ± 5,02	21,6^{b} ± 2,19	19,0^{ab} ± 1,22	18,4^{a} ± 0,55	16,2^{a} ± 3,42
15	20,2^{b} ± 2,68	18,8^{ab} ± 3,90	21,6^{b} ± 2,19	18,6^{ab} ± 0,55	17,8^{ab} ± 1,30	15,4^{a} ± 3,78
16	19,0^{bc} ± 0,00	17,8^{b} ± 2,68	21,6^{c} ± 2,19	15,2^{ab} ± 3,03	16,0^{ab} ± 2,55	14,4^{a} ± 3,21
17	19,0^{bc} ± 0,00	17,8^{b} ± 2,68	21,2^{c} ± 2,49	14,8^{ab} ± 3,49	14,8^{ab} ± 1,92	14,2^{a} ± 3,03
18	19,0^{bc} ± 0,00	17,0^{bc} ± 4,47	19,0^{c} ± 0,00	14,4^{ab} ± 3,36	12,6^{a} ± 4,16	11,8^{a} ± 1,10
19	17,8^{bc} ± 2,68	14,0^{ab} ± 4,36	19,0^{c} ± 0,00	13,0^{ab} ± 5,00	12,4^{ab} ± 4,04	11,0^{a} ± 1,41
20	17,8^{c} ± 2,68	11,4^{abc} ± 4,39	17,4^{bc} ± 2,19	12,6^{bc} ± 5,08	12,0^{b} ± 4,53	6,8^{a} ± 3,49
21	16,2^{bc} ± 3,03	11,0^{ab} ± 4,53	16,2^{c} ± 2,68	12,2^{abc} ± 4,60	10,6^{ab} ± 4,39	6,6^{a} ± 3,29
22	15,6^{d} ± 3,13	9,2^{bc} ± 2,28	13,6^{d} ± 1,14	9,6^{bc} ± 1,52	7,8^{ab} ± 2,68	4,6^{a} ± 3,58

- The number following the ± symbol indicates the standard deviation (SD)
- Alphabetical notations$^{a, b, c, d}$ on the same row indicate significant differences based on the Mann-Whitney test ($p<0.05$)
- KI: normal control (no treatment), K2: positive control (Betadine ointment), K3: negative control (ointment base), K4: 20% *brotowali* ointment, K5: 35% *brotowali* ointment, K6: 50% *brotowali* ointment**.**

In this study, the initial blood glucose measurement and diabetes induction in the test animals were conducted on day 0. Subsequently, blood glucose levels were rechecked on day 3, and it was found that the glucose levels in all test animals were >200 mg/dl. According to Panjaitan et al. [14] animals are considered diabetic if their blood glucose levels exceed 200 mg/dl. After confirming diabetes in the test animals, wound creation was performed on day 4, with the initial condition of the wound area showing redness, slight dampness, and some burned surface areas. From day 5 to day 7, the wounds were left untreated both orally and topically. On day 8, before treatment was administered, the condition of the wounds was first observed. Macroscopic description and wound healing of diabetic burn wound on days 8: normal control (no treatment), K2: positive control (betadine oinment), K3: negative control (ointment base), K4: 20% *brotowali* ointment, K5: 35% *brotowali* ointment, K6: 50% *brotowali* ointment are the condition of the wounds on each individual test animal showed similar changes ($p>0.05$), with the skin appearing white but without any scabs (score 28). Initially, the wounds displayed redness and areas of burned surface. The differences in wound condition on day eight compared to previous days indicate natural healing efforts. Macroscopic description and wound healing of diabetic burn wound on days 9 : normal control (no treatment), K2: positive control (betadine oinment), K3: negative control (ointment base), K4: 20% *brotowali* ointment, K5: 35% *brotowali* ointment, K6: 50% *brotowali* ointment are observations revealed changes in wound condition across all treatment groups, characterized by reddish-white scabs on the wound surface (score 26), except in the K2 group where the scabs were reddish-brown (score 25.8). Statistically, the K2 group had a lower average score, but there was no significant difference compared to the other groups (p>0.05). Macroscopic description and wound healing of diabetic burn wound on days 22: normal control (no treatment), K2: positive control (betadine oinment), K3: negative control (ointment base), K4: 20% *brotowali* ointment, K5: 35% *brotowali* ointment, K6: 50% *brotowali* ointment are by the end of the observation period (day twenty-two), the K5 and K6 groups displayed wound conditions with a whitish-brown crust, with K6 showing half of the crust detached (score 4.6) and K5 with no crust detached (score 7.8). The K2 (score 9.2) and K4 (score 9.6) groups showed dark brown crusts with almost all of the crusts detached. The K3 group had a dark brown crust with no detachment (score 13.6), while the K1 group had a brown crust with nearly all of the crusts detached (score 15.6). The average wound scores presented in Table II indicate that the K6 group demonstrated the best healing capacity with the lowest average wound score, although statistically there was no significant difference from the K5 group (p>0.05), but significant differences were observed compared to the K1, K2, K3, and K4 groups (p<0.05). The order of groups from the best to worst healing based on average wound scores is K6, K5, K2, K4, K3, and K1, with scores evaluated based on crust color and amount of crust detached.

4. Discussion

Wound healing is a complex and dynamic process [19,20]. This process consists of three stages: inflammation, proliferation, and maturation [18-20]. The inflammatory phase typically occurs from the onset of the wound until the third day post-injury [14,18,20]. The proliferation phase usually begins around the third or fourth day [14,20] and continues through the second week after the injury [20]. The maturation phase, the longest phase of wound healing, typically starts in the second week after the injury [18] and can last for months to years [14,18,20].

Each stage of wound healing exhibits distinct macroscopic characteristics [18,19,20]. The first stage, the inflammatory phase, involves the inflammatory response where immune cells combat bacteria and remove dead tissue [20]. Additionally, this phase generates growth factors that stimulate the formation of new tissue in the wound [20], promotes coagulation, recruits repair cells, releases cytokines [21], and fills the empty lumen with blood due to changes in capillary conditions [19]. Macroscopically, the inflammatory phase is characterized by a reddish wound surface [18-20] and a wet appearance [19].

The second stage is proliferation, characterized by the formation of epithelial cells, blood vessels, and new tissue growth [20,21]. This phase is marked by the formation of a scab [13,14,18,19]. The proliferation phase ends with the shedding of the scab, during which epithelial cells migrate from the edges of the wound towards its center [14,18]. The detachment of the scab indicates that the underlying tissue has dried, signaling the transition to the maturation phase [20,21]. The third stage, and the final one, is maturation. During this phase, epithelialization occurs [18,21] along with gradual tissue recovery through increased collagen deposition [14]. Epithelialization is observed when the scab falls off, ideally without leaving a scar [18], and the epithelial tissue appears pale white [21]. If a scar remains, it indicates that the scab has not fully matured, leading to a delay in the maturation phase as the wound may dry out and form a new scab [19].

In this study, the inflammatory phase of wound healing lasted five days, beginning from the onset of the wound (day four) and ending on day eight. This phase was characterized by wounds showing redness and a moist appearance on the day of wounding (day four). This observation is consistent with Priamsari and Yuniawati [18]; Rahman and Kamri [19], who state that the inflammatory phase is marked by redness and moisture in the wound. By the end of the inflammatory phase (day eight), the wounds appeared whitish, yet there was no visible scab formation. According to Panjaitan et al. [14]; Rahman and Kamri [19], scab formation indicates that the wound healing has transitioned to the proliferative phase. Thus, the absence of a scab suggests that the healing process remains within the inflammatory phase.

On day nine, the wound healing process in all treatment groups entered the proliferative phase, as indicated by the formation of scabs. By the end of the observation period (day twenty-two), the wounds in all treatment groups remained in the proliferative phase and had not yet transitioned to the maturation phase, as evidenced by the presence of residual scabs and the lack of a pale appearance. This suggests that the scabs formed were not fully matured, resulting in a delay in advancing to the maturation phase due to the scabs re-forming as the wounds dried. This is consistent with Priamsari et al. [18] who note that the maturation phase begins when scabs are shed without leaving residual marks.

Based on the wound observations, the 50% brotowali ointment demonstrated a superior healing effect compared to the 35% and 20% concentrations. This suggests that higher concentrations of the extract result in a greater amount of secondary metabolites, which are hypothesized to enhance wound healing capacity. Additionally, Betadine ointment also effectively promotes wound healing due to its content of povidone-iodine, which addresses bacterial infections and aids in stimulating the formation of new blood vessels at the wound site [22].

In this study, the oral and topical preparations of *brotowali* stem extract synergistically heal diabetic burn wound. So far, previous research results have shown that plants that can heal diabetic wound through oral administration include *bidara upas* (*Merremia mammosa* (Lour.)) [12], black pepper (*Piper nigrum*), peanuts (*Arachis hypogaea*) [13], and *insulin* (*Smallanthus sonchifolius*) [12], but their topic al use has not been reported. Plants reported to heal diabetic wound topically in the form of ointments include *binahong* leaves (*Anredera scandes* (L.) Moq.) [23], lime peel (*Citrus aurantifolia*) [24], and *mahkota dewa* (*Phaleria macrocarpa*) [25]. *Binahong* leaves ointment heals diabetic gangrene supported by the content of flavonoids, saponins, triterpenoids, and tannins by accelerating proliferation [23]. Lime peel ointment can heal diabetic gangrene with an ability comparable to the positive control, supported by the content of flavonoids, alkaloids, and saponins [24]. Furthermore, *mahkota dewa* ointment helps improve the healing process of diabetic gangrene through the reduction of oxidative stress, supported by the content of flavonoids, saponins, tannins, and phenolics [25].

Further qualitative phytochemical analysis revealed that the ethanol extract of *brotowali* stems contains secondary metabolites such as flavonoids, saponins, tannins, and steroids. These secondary metabolites contribute to the wound healing capacity of *brotowali* ointment. Panjaitan et al. [14] state that the most common mechanisms by which secondary metabolites aid wound healing include their roles as antioxidants, anti-inflammatory agents, and antibacterial agents. Specifically, in the context of diabetic wound, flavonoids act as antioxidants by binding to excess free radicals produced at the wound site and as antibacterial agents by inhibiting bacterial invasion during the early phases of wound healing [12]. Moreover, flavonoids can enhance fibroblast proliferation, accelerate re-epithelialization, and improve wound contraction rates [3,15]. Additionally, research by Nurwahita et al. [3] suggests that saponins can shorten the epithelialization period and prevent cellular damage. Saponins also stimulate vascular endothelial growth factor (VEGF) and increase the number of fibroblasts and macrophages migrating to the wound area [13].

Panjaitan et al. [14] explain that tannins aid in wound healing by acting as astringents, which help stop bleeding and promote faster drying of the wound, as well as enhance the formation of capillaries and fibroblasts. Additionally, in the context of diabetic wound healing, tannins provide anti-inflammatory effects [13]. Furthermore, steroids contribute to wound healing by preventing excessive growth of granulation tissue [22].

The limitation of this research is that the wound model created was limited to burn wounds only. Furthermore, this research is not only related to topical preparations, but also involves oral consumption of preparations and this research is only limited to the use of experimental animals and has not been conducted on human research. Based on Cachet et al. [25], patients who consume *brotowali* stems in the form of liquid extract or tablets regularly and continuously can cause hepatitis. However, it was also reported by Cachet et al. [25] that hepatitis patients caused by consuming brotowali stems can recover after several weeks without special medical treatment. In this regard, controlled clinical trials are needed to determine the safety of using *brotowali* properly.

Conclusion

We conclude, the 20% *brotowali* extract ointment has a healing capacity for diabetic burn wound comparable to Betadine ointment. Furthermore, the 35% and 50% *brotowali* ointments exhibit superior healing capabilities

Acknowledgment

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Authors' Contribution

Study concept and design: R.G.P.P and F.
Acquisition of data: R.G.P.P, F, T, M.W.A, and A.S.
Analysis and interpretation of data: R.G.P.P, F, T, M.W.A, and A.S.
Drafing of the manuscript: R.G.P.P and F.
Critical revision of the manuscript for important intellectual content: R.G.P.P, F, T, M.W.A, and A.S.
Statistical analysis: R.G.P.P and F.
Administrative, technical, and material support: R.G.P.P, F, T, M.W.A, and A.S.

Ethics

The use of test animals and the procedures for this study were approved by the Faculty of Health Sciences, Universitas Respati and received ethical clearance with the number 044.3/FIKES/PL/V/2023.

Conflict of Interest

The authors declare No. conflict of interest.

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Data Availability

The data that support the findings of this study are available on request from the corresponding author and Fitriyani.

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