

Spray gel film-forming system formulation of *Vernonia amygdalina* as diabetic wound healer

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Received: 26 August 2024/ Accepted: 25 April 2025

ABSTRACT: Poorly managed diabetes mellitus may elevate the risk of having a diabetic wound that may lead to non-traumatic lower limb amputation. Therefore, wound healing management in diabetic patients is essential to prevent such complications. African leaves (*Vernonia amygdalina*) has been widely used for wound treatment empirically for years including diabetic wounds. The current study focuses on developing an African leaves-based spray using a film-forming system for diabetic wound healing. The African leaves extract is formulated with polyvinyl alcohol (PVA) to create a viscous liquid and is then made into a spray gel-film form. The spray gel film formulation was further characterized and tested for its wound closure efficacy in animal testing. The characterization include watery form, green colour and a characteristic odour of African leaf extract. The spray gel film preparation of *Vernonia amygdalina* 1;2;4% extract have pH of 5.92 ± 0.0011 ; 5.96 ± 0.0011 and 5.42 ± 0.0011 . The viscosity value of the spray gel film 1;2 and 4% were 7.98 ± 0.0012 ; 8.43 ± 0.0019 and 5.70 ± 0.0013 cPs. Dry time of Spray Gel Film of *Vernonia amygdalina* 1;2;4% extract were 1.47 ± 0.0013 ; 1.50 ± 0.0010 ; and 1.56 ± 0.0010 minutes. The wound healing was evaluated using diabetic Wistar rats by measuring daily wound diameter. The result showed at a concentration 1%; 2%; 4% the percentage diameter of wound healing were 81.52 ± 0.06 ; 82.28 ± 0.05 ; and 100 ± 0.00 %. They were effective for wound healer in diabetic rats and it had antibacterial activities.

KEYWORDS: Diabetic wound; diameter; polyvinyl alcohol; spray gel film; *Vernonia amygdalina*; wound healing.

INTRODUCTION

Diabetes Mellitus (DM) is a degenerative disease with a growing number of patients each year in Indonesia. According to the World Health Organization (WHO), it is estimated that the number of diabetes patients in Indonesia will increase approximately two to five times, from 8.4 million to 21.4 million by 2030, and to 40.7 million by 2045, with individuals aged 40-60 years being the most affected [1],[2]. Unhealthy lifestyle habits such as high-carbohydrate food intake and lack of physical activity are the primary causes of DM. In addition, genetic factors can also increase the risk of developing DM. The main symptoms of DM include increased frequency of urination (polyuria), excessive hunger (polyphagia), and frequent thirst (polydipsia). Ultimately, long-term uncontrolled blood glucose levels in individuals with diabetes mellitus may increase the risk of cardiovascular complications and diabetic wounds [3].

The diabetic wound is a chronic symptom experienced by DM patients due to excessively high and uncontrolled blood sugar levels. Diabetic wounds involve impairments in peripheral and autonomic nerves, leading to a prolonged healing process and an increased risk of secondary infections that might worsen the existing wound. Inappropriate management of diabetic wounds, along with high and uncontrolled blood sugar levels might lead to the risk of amputation due to gangrene or tissue death, and potential mortality [4]. The prevalence of diabetic patients with diabetic wounds in Indonesia is approximately 15%, with an amputation rate of 30%. Based on Katz's Index, 51.7% of patients with severe diabetic wounds experience mortality within 6-7 years after initial diagnosis. The pathogenesis of diabetic wounds involves vascular and non-vascular factors, i.e. neuropathy, infection, and the immune system. Preventive measures to avoid further infection are key factors for treating diabetic wounds [5].

African Leaf (*Vernonia amygdalina*) is a tropical plant native to Africa and is widely known to have the potential to heal wounds. African leaves are characterized by dark green leaves with a bitter taste and are empirically utilized by the community for diabetes. The presence of alkaloids, saponins, tannins, and

How to cite this article: Novitasari PR, Nuari YR, Firdausi AA. Spray gel film-forming system formulation of *Vernonia amygdalina* as diabetic wound healer. JIFI. 2025; 23(1): 157-165.

flavonoids in these leaves may provide anti-inflammatory, antioxidant, and antibacterial effects [6]-[9]. The antibacterial activity in African leaves demonstrates significant potential for diabetic wound healing.

This study focuses on the development of a gel spray formulation from African leaf extract (*Vernonia amygdalina*) for diabetic wounds by developing a thermosensitive hydrogel-based spray formulation utilizing a concentrated extract from African leaves, which exhibits excellent antibacterial activity for diabetic wound closure. A thermosensitive hydrogel-based spray gel formulation is chosen to facilitate the application process of medication to wound areas. The current study uses polyvinyl alcohol (PVA) as the thermosensitive gel, a hydrogel compound that is in liquid form at room temperature but solidifies at body temperature and immediately forms a thin-layer film on application areas [10]. An extract of African leaves is formulated with PVA to create a viscous liquid and is then made into a spray form. The spray gel formulation was further characterized and tested for its wound closure efficacy in animal testing.

The spray gel formulation of concentrated extract from the African leaf (*Vernonia amygdalina*) offers convenience and simplicity in the application to diabetic wounds as it only needs to be sprayed. When the spray liquid is applied to a diabetic wound, it immediately undergoes solidification (gelation) to form a gel and initiate a thin-layer film coverage, which can protect the sprayed wound area. The gel that solidifies and covers the diabetic wound is highly effective in the wound closure process thanks to the gel containing extracts from African leaves, which accelerate wound healing. Additionally, the gel provides physical protection to the wound, minimizing the risk of secondary infection from the surrounding environment. These factors highlight the effectiveness of diabetic wound closure offered by this spray formulation.

MATERIALS AND METHODS

Materials

African leaves (*Vernonia amygdalina*) was obtained from Nogotirto, Yogyakarta, Indonesia; animal test: male rats of Wistar strains with body weight of 150-200 gram obtained from Yogyakarta; Penicilin spray (*Jaya Supra Medika*→), alloxan monohydrate (*Sigma*→), filter paper, Ketamine HCl injection (*Dexa*→), Polivynil Alcohol (*Sigma*→), Magnesium powder, aquadest, glucose test (*Easytouch*→), ethanol 96% (*Sigma*→), gliserine (*Sigma*→), alcohol swab.

Preparation of extract african leaf (*Vernonia amygdalina*)

The African leaves (*Vernonia amygdalina*) was dried indoor for seven days, cut into small pieces and powdered using a blender. The extraction was carried out by maceration method with 96% ethanol to obtain a thick extract of *Vernonia amygdalina* in ratio (1:10) at temperature of 25 degrees Celsius in protected direct sunlight jar. Maceration was carried out by soaking simplicia powder in 96% ethanol solvent for 24 hours. The filtrate was concentrated by a rotary vacuum evaporator to obtain a good yield [15].

Phytochemical screening

Phytochemical screening of African leaf (*V. amygdalina*) extract employed standard procedures to reveal the presence of chemical constituents such as alkaloids, flavonoids, tannins, terpenes, and saponins [16].

Formulation of spray gel film-forming system of *Vernonia amygdalina* extract

The spray gel-film were made by dissolving the gel base: Polyvinyl Alcohol (PVA) with 20 ml of hot distilled water in a mortar. The glycerine and African leaves extract was added, then it was stirred homogenously. The formula used was listed in Table 1.

Table 1. Spray-film formulation.

Materials	Percentage (%)		
	Spray gel 1%	Spray gel 2%	Spray gel 4%
<i>Vernonia amygdalina</i> extract	1	2	4
PVA	2.5	2.5	2.5
Glycerine	1	1	1
Ad ethanol 70%	100	100	100

Evaluation of spray gel film-forming system formulation

The evaluation of spray gel-forming system was observed by organoleptic test (form, colour, odour); pH Test; viscosity; and dry time. Meanwhile the diameter size of the gel liquid after the spray is applied was producing uniform droplets with diameter sizes less than 10 μm [19].

Determination of phase transition and film formation of spray gel film-forming system

Based on previous study, the solution phase changes to gel at a temperature of around 31°C [17] and the film formation in 2 to 10 minute [18].

Antibacterial test of spray gel-film preparation test

Bacterial rejuvenation

Streptococcus aureus (SA) bacteria were grown in streak plates on Nutrient Agar (NA) media, incubated at $35 \pm 1^\circ\text{C}$ for 24 hours. After incubation, 1 dose of bacteria was added to physiological NaCl. Then matched it with 0.5 McFarland (equivalent to $1-2 \times 10^8$ CFU/mL for SA) [20].

Antibacterial test

Cotton swabs were dipped into the prepared inoculum, then streaked into Mueller Hinton Agar (MHA) media. Empty well were soaked with spray gel-film in various extract concentration (1;2;4 %), negative control and positive control, attached to the MHA medium, then incubated at $35 \pm 1^\circ\text{C}$ for 24 hours [21].

Experimental animal preparation

Male rats of 3 month Wistar strains (150-200 gram) were acclimatized in individual cage for one week prior to the induction of diabetes. The male rats were fed with growers feed (by Top feeds Indonesia Limited) and water *ad libitum*. The cage was illuminated with 12 hours light/12 hours dark cycle in laboratory condition (temperature $22 \pm 2^\circ\text{C}$, humidity 60-70%). The characteristic of the rats were white, red eyes, elongated head and tail that exceed the body's length [22].

Diabetes induction

Male Wistar rats were fasted overnight. Their blood was taken for initial blood glucose measurement. The rats were induced 150 mg/kgBB of body weight. Alloxan was dissolved in NaCl 0.9%. Blood glucose examination was performed on 3rd day after induction of Alloxan [23].

Measurement of blood glucose

Blood glucose examination was performed on third day post-diabetic induction at Faculty of Pharmacy Universitas Ahmad Dahlan (UAD), Yogyakarta Laboratory. Blood glucose levels of male Wistar rats were checked by glucose test (*Easy Touch*) before, after induction of alloxan, and 14th day during the topical treatment to ensure that rats actually in a diabetic condition. After being fasted overnight, 1 mL mouse blood was taken from the orbital plexus using a capillary pipe and was tested by glucose test (*Easy Touch*) [24].

Wounding of rats

Ketamine (10 mg/mL pf 0.4 mL i.p) were used to anesthetize of rats to create wound. The rat's hair was shaved on the right and left sides of its back. The wound was made with a 1-2 cm diameter using punch biopsy [25].

Experimental design

Wounded rats were grouped into 6 groups with 5 rats in each group. They were normal, negative, positive, and spray gel-film in various concentration (1,2,4% VA extracts) groups. The positive group was given by Penicillin spray (*Jaya Supra Medika*→) and negative group was given by base spray without extract. Spray gel-film treatments were applied to every rat twice daily in the morning and afternoon. Spray distances between wound and the tools were ± 1 cm (± 1 mL) [26].

Observation and data collection

Wound diameter of the rats ((normal, negative, positive, and treatment: spray gel-film in various concentration (1,2,4% VA extracts) groups)) were measured using vernier calliper on four sides of the wound

diameter average from the development of wound healing process on the rat's back every three days by comparing the healing process of diabetic wound. Thus, the percentage of wound closure was obtained [27]. Observations started from day 1st until day 16th after the wounds were made. Measurements were carried out in various direction using the Morton method. Percentage of Diabetic Wound Healing Area using the percentage conversion formula, the wound diameter measurement was then converted into the percentage of healing (%). The measurement of wound diameter is then carried out by calculating the percentage of wound healing using the percentage conversion equation 1 [28].

$$Px = \frac{d1-dx}{d1} \times 100\% \quad (1)$$

Information :

Px : Percentage of wound healing on day x (in %)

d1 : First-day wound diameter (cm)

dx : wound diameter x day (cm)

Data analysis

Data analysis was carried out by measuring the diameter of the healing wound. Data processing used IBM SPSS Version 26.0 with the Shapiro Wilk method to test normality, the Levene test was used to test homogeneity. To determine the respective differences in wound healing activity in test animals using Kruskal-wallis and Mann-Whitney [29].

RESULTS

Plant determination

Determination of African leaves (*Vernonia amygdalina*) conducted in laboratory of Biology Study Centre of Science and Technology Application Universitas Ahmad Dahlan, Yogyakarta with the certificate number of 135/Lab.Bio/B/III/2024 showed that the plant used was the species *V. amygdalina* Delille.

Phytochemical screening

The African leaves (*V. amygdalina*) or VA extract contained alkaloids, flavonoids, tannins, terpenes, and saponins. This result was in line with the results of previous test [30].

Formulation and evaluation of spray gel film-forming system of *Vernonia amygdalina* extract

The spray gel film forming system have watery form, green colour and a characteristic odour of African leaf extract (Figure 1). The spray gel film preparation of spray gel film-forming system of *V. amygdalina* 1;2;4% extract have pH of 5.92 ± 0.0011 ; 5.96 ± 0.0011 and 5.42 ± 0.0011 . There were safe for the skin (pH between 4.5 - 6.5) [14]. The viscosity of the spray gel film was prepared by rheosys viscosimeter. The viscosity value of the spray gel film 1;2 and 4% were 7.98 ± 0.0012 ; 8.43 ± 0.0019 and 5.70 ± 0.0013 cPs. This water-like spray gel film were applicable for skin. Dry time of of spray gel film-forming system of *V. amygdalina* 1;2;4% extract were 1.47 ± 0.0013 ; 1.50 ± 0.0010 ; and 1.56 ± 0.0010 minutes.

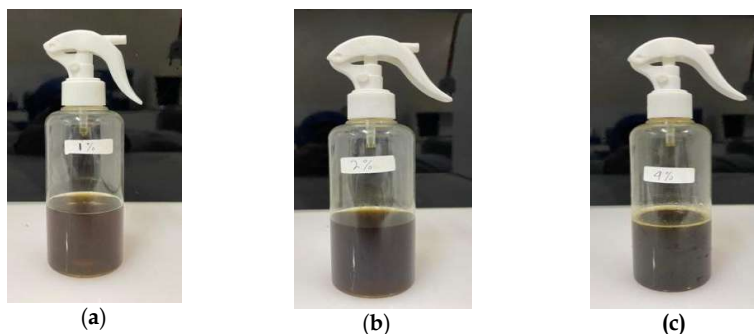


Figure 1. Spray gel film-forming system as: (a) Spray gel-film 1%; (b) Spray gel-film 2%; (c) Spray gel-film 4%.

Determination of phase transition and film formation of spray gel film-forming system

The phase transition and film formation of the spray gel film-forming system have been investigated in earlier studies. It was reported that the solution undergoes a phase change to a gel at approximately 30-31°C, and the film forms within a period of 5 to 10 minutes. So they were in accordance with previous research [18], [19].

Antibacterial test of spray gel-film preparation test

Antibacterial activity test of spray gel-film of VA extract conducted by using well plate diffusion method. The inhibition zone of spray gel film shown in Table 2 and Figure 2.

Table 2. Antibacterial activity of spray gel-film preparation test of African leaves (*Vernonia amygdalina*) extract.

Group	Inhibition zone (average \pm SD (mm))
Positive control	16.66 \pm 0.12
Negative control	5.67 \pm 0.01
Spray gel-film 1%	7.12 \pm 0.15
Spray gel-film 2%	7.70 \pm 0.1
Spray gel-film 4%	7.91 \pm 0.02

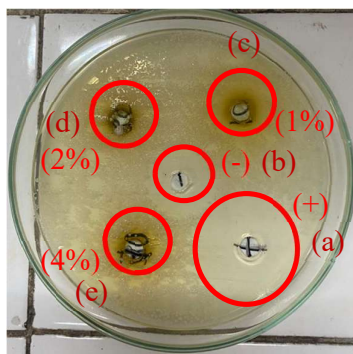


Figure 2. Antibacterial Activity Test as: (a) Positive Control (Penicillin spray 1%); (b) Negative control (spray gel film without extracts); (c) Spray gel-film 1%; (d) Spray gel-film 2%; (e) Spray gel-film 4%.

Examination of blood glucose levels in rats

All rats have obtained an ethical approval from the Research Ethics Committee of Universitas Ahmad Dahlan (KEP UAD) (No.012404075). The increase in blood glucose levels is shown in Table 3.

Table 3. Blood glucose levels in rats.

Groups	Blood glucose level (mg/dL)		
	Before alloxan induction	After alloxan induction	Day 14 th
Positive control	98.33 \pm 17.62	310.75 \pm 73.14	319.75 \pm 73.14
Negative control	141.67 \pm 21.94	395.33 \pm 134.60	398.33 \pm 134.60
Normal control	148.67 \pm 62.07	134.50 \pm 20.27	134.80 \pm 20.27
Spray gel-film 1%	104.33 \pm 8.51	492.67 \pm 32.53	495.67 \pm 32.53
Spray gel-film 2%	134.67 \pm 13.58	359.20 \pm 54.280	427.50 \pm 81.32
Spray gel-film 4%	137.33 \pm 13.05	603.33 \pm 5.77	570.50 \pm 13.44

Wound healing activity spray gel film-forming system of african leaves (*Vernonia amygdalina*)

All rats had to be in diabetic conditions before treatment, except for normal control group. Male Wistar rats anaesthetised with Ketamine to reduce pain due to injury, because it could cause a muscle relaxation [16]. Wound diameter measurements were carried out every three days until the wound diameters were close to zero. The dry process of the wound is characterized by the appearance of a scab on the skin as a marker that wound healing is in progress and narrowing diameter of wounds in each groups. The statistical test results

showed a significant difference ($P < \alpha 0.05$) between the treatment groups in the observation of wound healing days (Table 4).

Table 4. Wound diameter result in rats.

Groups	Wound diameter in several days (cm)					
	1	4	7	10	13	16
Positive control	2.46±0.00	1.92±0.001	1.33±0.00	1.08±0.00	0.75±0.01	0.25±0.01
Negative control	2.15±0.001	1.62±0.001	1.28±0.00	0.98±0.18	0.86±0.02	0.63±0.02
Normal control	1.56±0.00	1.39±0.00	1.11±0.04	0.96±0.01	0.48±0.03	0.40±0.03
Spray gel-film 1%	2.17±0.00	1.65±0.00	1.15±0.01	0.66±0.01	0.60±0.19	0.40±0.02
Spray gel-film 2%	1.98±0.00	1.19±0.00	1.11±0.12	0.55±0.02	0.45±0.03	0.35±0.01
Spray gel-film 4%	2.18±0.00	1.21±0.01	0.76±0.02	0.54±0.01	0.40±0.02	0.00±00.00

Furthermore, the wound diameter data (Table 4) were used to calculate the percentage of wound diameter in rats in each groups. The percentage of wound diameter in rats described in Table 5 and the wound appearances in Figure 3.

Table 5. Percentage of wound diameter in rats.

Groups	Percentage of wound diameter in rats (%)					
	1	4	7	10	13	16
Positive Control	0±0.00	22.03±0.00	45.90±0.00	55.93±0.02	69.49±0.02	89.83±0.02
Negative Control	0±0.00	24.70±0.00	40.23±0.02	54.59±0.01	59.83±0.03	70.89±0.00
Normal Control	0±0.00	10.93±0.11	28.80±0.00	38.40±0.12	69.60±0.02	74.40±0.12
Spray gel-film 1%	0±0.00	23.79±0.02	47.11±0.01	69.40±0.06	72.29±0.03	81.52±0.06
Spray gel-film 2%	0±0.00	40.00±0.01	43.80±0.15	72.15±0.01	77.29±0.02	82.28±0.05
Spray gel-film 4%	0±0.00	44.60±0.03	65.06±0.00	75.29±0.02	81.61±0.01	100±0.00

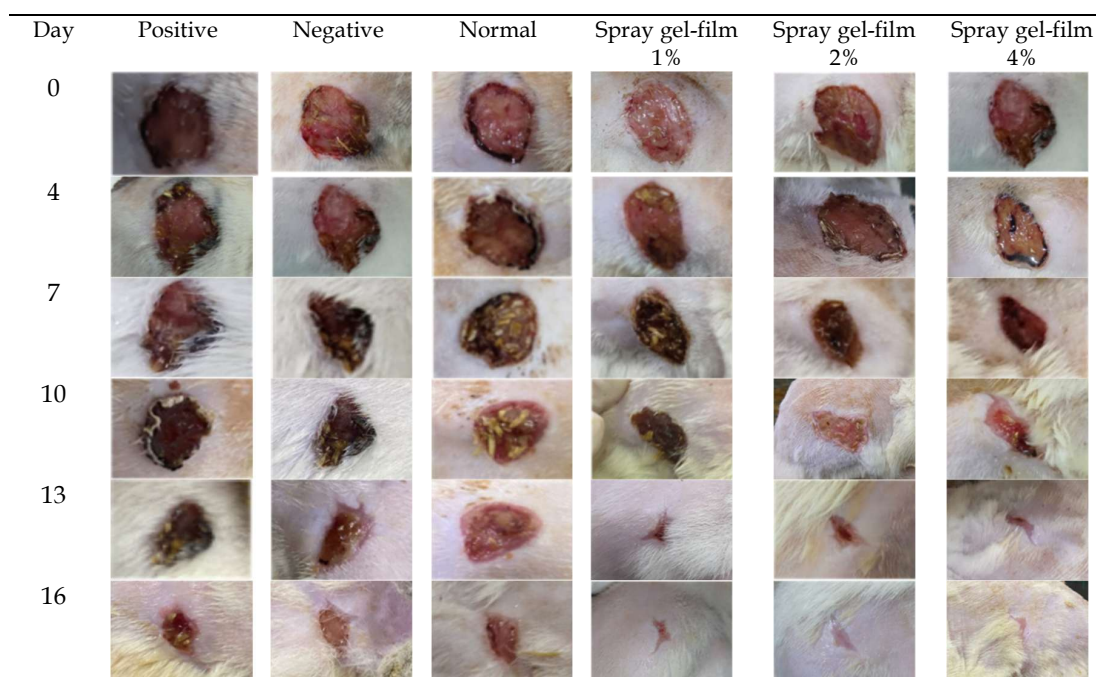


Figure 3. Figure of the wound in rats.

DISCUSSION

The African leaves (*Vernonia amygdalina*) used in this formulation were previously determined, and the extract has undergone a phytochemical screening showing it contained alkaloids, flavonoids, tannins, terpenes, and saponins, which are in line with the previous study [30]. The spray gel film-forming system is watery, green in colour, and has the characteristic odour of African leaf extract. The evaluations demonstrated that the spray gel film has a pH within the required range, with a low viscosity that indicates the spray was aqueous before application. Also, the dry time was below 2 minutes, and the phase transition of sol-to-gel was observed between 5-10 minutes at 30-31°C. These results were in agreement with previous studies [18],[19], and demonstrating that this spray was conveniently applicable.

The concentrations of Spray Gel-Film-Forming System were set at 1%, 2%, and 4% to evaluate whether the increase in antibacterial activity occurs with increasing concentrations. The antibacterial extract had been studied in the previous study [32]. In addition, the positive control used was penicillin spray 1%, which generally can be used to treat *Staphylococcus aureus* infection, and the negative control was spray gel film without extracts. The results of the antibacterial activity test showed inhibition of the *Staphylococcus aureus* in positive, negative, and spray gel film (1;2;4%) groups, as shown in Figure 2. These results were in line with the purpose of this research, which is to prevent the infection of diabetic wounds, which is mostly caused by bacteria. Furthermore, the antibacterial activity of the spray gel film is important to minimize the risk of secondary infection from the surrounding environment of the wound. Furthermore, the blood glucose levels of male Wistar rats were checked by glucose test (Easy Touch) before, after alloxan induction, and on the 14th day throughout the topical treatment to guarantee that rats were really in diabetic conditions. Rats were confirmed to have diabetes if their blood glucose levels were ≥ 200 mg/dL. This research used alloxan as a diabetogenic induction, as it causes diabetes via fractional destruction of the beta (β) cells from pancreatic islets and significant reduction of the quality and quantity of insulin released by these cells [33].

The data from the wound diameter study were analysed using parametric statistical tests; SPSS Version 26.0 indicated a significant effect between treatments and days on the results of the diameter of the wound, with the value of Sig. 0.000, which is smaller than the value of the significance level (α) of 0.05. Based on the results from the Kruskal-Wallis and Mann-Whitney tests to determine whether there was a significant difference or not for the parameter of decreasing wound diameter, subsequently, spray gel-film 1%, 2%, and 4% showed significantly different results from the negative control. The percentage of spray gel-film 1%, 2%, and 4% was higher than that of the negative control groups. This indicates that applying the spray gel-film with 1%, 2%, and 4% of VA extracts had effective healing in diabetic rats. In addition, spray gel-film 1%, 2%, 4%, and normal control provided significantly different results from the normal control ($P < \alpha 0.05$), even though the rats were not in a diabetic condition in the normal control groups. The percentage of spray gel-film 1%, 2%, and 4% was higher than that of the normal control group. It shows that the healing rate could match this normal control since the body was homeostatically capable of restoring itself from damaged tissue components by forming a new functional structure similar to the previous condition [35]. Moreover, spray gel-film of 4% was significantly different from the positive control ($\alpha < 0.05$). The percentage healing of this group was higher than that of the positive control group, but the percentage healing of spray gel film of 1% and 2% was smaller than that of the positive control group. It represents that applying spray gel film 4% was more effective than applying spray gel film of 1% and 2%, respectively.

Based on the observation of appearance in wound healing (Figure 3), it could be seen that there were differences between each group in the decrease in the diameter of the wound. The characteristic wound healing by the appearance; there were scabs on the skin, and epithelial cells began to form. Based on Figure 6, the 16th day of wound healing showed wound closure in all treated groups, with the 4% VA extract spray formula exhibiting the fullest wound closure. These results are in line with previous studies that report bioactive compounds in VA extract with strong antioxidant activity, such as flavonoids and polyphenols, which were able to reduce oxidative stress, a factor that impairs wound healing in diabetic ulcers [36]. In addition, the previous study [36] showed that VA exerts anti-inflammatory activity to accelerate wound closure in diabetic Wistar rats. Additionally, studies also suggest that VA upregulates vascular endothelial growth factor (VEGF) and promotes angiogenesis [37] as well as enhances collagen synthesis by stimulating fibroblast proliferation [11]. Combined with its potential blood glucose-lowering effects [13] and the antimicrobial activity, as presented in this study, suggests that the VA extract creates a favourable metabolic

environment for accelerated wound closure in diabetic rats. Therefore, the spray gel film-forming system of VA extract has the potential to be developed as a wound healer for diabetic patients.

CONCLUSION

The spray gel film-forming system of African leaves (*Vernonia amygdalina*) at a concentration 4% were the most effective for wound healing in diabetic rats and it had antibacterial activities and good evaluation formulation tests. This formula was applied twice a day and needs 16 days to recover after injury. It was effective for wound healer in diabetic rats and it had antibacterial activities.

Acknowledgements: The author would not only like to thank Riset Nasional Muhammadiyah Batch VII, 2024, with the number of contract 0258.572/I.3/D/2024 for providing funding, but also thank the Faculty of Pharmacy Universitas Ahmad Dahlan which has helped facilitate the laboratory materials needed during the research process

Funding: Riset Nasional Muhammadiyah Batch VII, year 2024.

Conflict of interest statement: The authors declared no conflict of interest in the manuscript.

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