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Diabetic Wound Healing and Antimicrobial Activities of Gels *Melastoma malabathricum* L. and *Psidium guajava* L. in Sprague Dawley Rats

(Penyembuhan Luka Diabetes dan Aktivitas Antimikroba dari Gel Ekstrak *Melastoma malabathricum* L. dan *Psidium guajava* L. pada Tikus *Sprague Dawley*)

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Abstract: Diabetes mellitus (DM) is a degenerative disease characterized by abnormalities in carbohydrate, lipid, and protein metabolisms. This research was aimed at the gel's pharmacological activity, the extract's potential in treating diabetic wounds in male rats of *Melastoma malabathricum* L leaves (ML) and *Psidium guajava* L leaves (PL), as well as microbiological activity. Viscous extracts of ML and PL were prepared in gel dosage form with concentrations of 4% and 6%. Clindamycin was used as a positive control. This study was conducted using an experimental laboratory method, and the study population included white male rats. The pharmacological activity was tested in the form of a gel dosage formula, and the rats were made diabetic using alloxan. The potential of the extract was observed in healing diabetic wounds in male rats. ML and PL leaf extract gels affect wound healing in diabetic patients. This can be seen from the change in diameter. Wound swab examination revealed the presence of bacteria *Klebsiella pneumoniae* and *Staphylococcus aureus*. Secondary metabolites of flavonoids, tannins, steroids, and saponins help stimulate the regeneration of epithelial cells and tissues. The results for wound healing of 4% and 6% gel formulations were derived from maturation data on day 10.

Keywords: Alloxan, antimicrobial, *Melastoma malabathricum* L.*, Psidium guajava* L., wound healing

Abstrak: Diabetes melitus (DM), merupakan penyakit degeneratif yang ditandai dengan kelainan metabolisme karbohidrat, lipid, dan protein. Penelitian ini bertujuan untuk mengetahui aktivitas farmakologis gel dan potensi gel dalam mengobati luka diabetik pada tikus jantan dari ekstrak daun *Melastoma malabathricum* L. (ML) dan daun *Psidium guajava* L. (PL) serta aktivitas mikrobiologisnya. Ekstrak kental daun *Melastoma malabathricum* L dan *Psidium guajava* dibuat dalam bentuk sediaan gel dengan konsentrasi 4% dan 6%. Klindamisin digunakan sebagai kontrol positif. Penelitian ini dilakukan dengan metode eksperimen laboratorium, dan populasinya adalah tikus putih jantan. Aktivitas farmakologi diuji dalam bentuk formula sediaan gel, dan tikus dibuat menggunakan aloksan. Kemudian, potensi ekstrak tersebut terlihat dalam penyembuhan luka diabetes pada tikus jantan. Gel ekstrak daun *Melastoma malabathricum* L. dan *Psidium guajava* L. memiliki efek penyembuhan luka pada penderita diabetes. Hal ini terlihat dari perubahan diameternya. Hasil pemeriksaan swab luka menunjukkan adanya bakteri *Klebsiella pneumoniae* dan *Staphylococcus aureus*. Metabolit sekunder dari flavonoid, tanin, steroid, dan saponin, membantu merangsang sel dan jaringan epitel sehingga mereka beregenerasi. Hasil penyembuhan luka formulasi gel 4% dan 6% diperoleh dari data maturasi pada hari ke-10. Koloni pada hari ke-10 uji aktivitas mikroba lebih sedikit.

Kata kunci: Aloksan, antimikroba, *Melastoma malabathricum* L., penyembuhan luka, *Psidium guajava* L.

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INTRODUCTION

BLOOD glucose levels are elevated (hyperglycemia) as a result of insufficient insulin production, impaired insulin action, or both in diabetes mellitus (DM), a degenerative disease characterized by abnormalities in carbohydrate, lipid, and protein metabolism[1]. According to epidemiology, Indonesia will have 23.1 million DM patients with DM by 2030. In addition, the number of diabetics in Indonesia has risen over time. Diabetes Mellitus is a condition that can affect all of the body's organs and result in various symptoms, earning it the nickname "the silent killer" Visual impairment, heart illness, kidney disease, sexual impotence, wounds that are difficult to heal, and other disorders are among the conditions that will be brought on[2]. The development of sores that are challenging to cure, also known as diabetic ulcers, is a complaint in patients with DM [3].

People with diabetes mellitus (DM) may develop open sores on their skin surface. Diabetes ulcers that are not treated and treated can quickly become infected with germs, spread, and in more extreme cases, result in diabetic gangrene[4]. Disorders of the peripheral and autonomic nerves may contribute to diabetic injuries. Diabetes wounds with gangrene are composed of rotting or dead tissue because a sizable arterial embolism cuts off the blood supply to a specific body area. Protracted inflammatory processes, animal bites, labor accidents, burns, degenerative processes (arteriosclerosis), or metabolic diseases (diabetes mellitus) can all lead to this condition[5]. Fibroblasts, which create and maintain connective tissue, are the dominant cells that are crucial to healing. The inflammatory, proliferative, and remodeling phases are the three fundamental phases. The second phase's key component and the dominating cell are fibroblasts. When this mineral is administered, the fibrous component of the connective tissue proliferates and migrates more[6-7]. Antibacterials can prevent or eliminate microorganisms by interfering with the metabolism of dangerous organisms and wounds are synonymous with microbial infections. Because of their capacity to infect and cause disease, microorganisms can be harmful[8]. Maintaining a moist wound environment, preserving tissue fluid loss, and cell death are the foundations of the wound healing process[9].

Most people with weakened immune systems, especially the elderly, develop *Klebsiella pneumoniae* (KP) infections. Clinically, diseases of the upper respiratory tract, wounds, osteomyelitis, meningitis, pneumonia, cholecystitis, diarrhea, osteomyelitis, osteomyelitis, and septicemia can all be caused by *Klebsiella pneumonia*[10]. The community may experience morbidity and mortality due to *Staphylococcus aureus* (SA) infection. The bacterium can potentially lead to severe and sometimes fatal diseases, such as abscesses, endocarditis, osteomyelitis, pneumonia, and vasculitis[11,12]. Indonesians actively choose to use plants or herbs as their primary medical therapy[13]. The potential use of traditional plants as novel medicines is currently the subject of extensive research. In Indonesia, a tropical area, numerous therapeutic plants have been studied and processed to produce specific restorative raw components[14]. *Melastoma malabathricum* L. (ML) has pharmacological effects that include treating wounds, incisions, damage discharge, hemorrhoids, and dysentery[15] At the same time, *Psidium guajava* L. (PL) is used as a treatment for diarrhea, dysentery, sore throat relief, and menstrual cycle regulation[16] At a concentration of 75% (16 mm), *Melastoma malabathricum L* prevents the growth of *K. pneumonia*[17]. *Melastoma malabathricum* L was tested for its antibacterial action, and the results revealed that it was more effective against *S. aureus* than *E. coli*[18]. The antibacterial properties of *Psidium guajava* L. extract inhibit the growth of *Staphylococcus aureus*[19]. *Melastoma malabathricum* L. contains alkaloids, steroids, saponins, phenols, and tannins, among other phytochemicals[20]. *Psidium guajava* L., meanwhile, contains alkaloids, flavonoids, saponins, tannins, and terpenes[21].

Researchers are interested in combining these two herbs and analyzing their pharmacological mechanisms to investigate the effects of *Melastoma malabathricum* L and *Psidium guajava* L extracts on open wound healing in *Sprague Dawley* male white rats. This study aimed to examine the pharmacological activity of gel dosage forms using the inducing agent alloxan, and then to examine the potential of the extract in treating diabetic wounds in male rats using the condensed section of *Melastoma malabathricum* L leaves and *Psidium guajava* L leaves that will be made in a gel dosage form with a concentration of 4% and 6%, as well as information on the microbiological activity.

MATERIALS AND METHODS

MATERIALS. The leaves of *Psidium guajava* L. and *Melastoma malabathricum* Linn., Ethanol 70%, $NaNO_3$ 5% (Merck, Germany), $AlCl_3$ 10% (Merck, Germany), NaOH 1N (Merck, Germany), Mayer's reagent, Bouchardat's reagent, Dragendroff's reagent, $FeCl_3$ 1% (Merck, Germany), HCl 2N (Merck, Germany),$(C_2H_5)_2O$ (Merck, Germany), $CHCl_3$

(Merck, Germany), $C_4H_6O_3$ (Merck, Germany), H_2SO_4 P (Merck, Germany), Aquadest, Ketamine (Bernofarm), Xylazine (NexGen Pharmaceuticals, U.S.A), Methylparaben (Ueno, Osaka Japan), Propylene glycol (USP, Singapore), Carboxymethyl cellulose (Alfa Kemika Indonesia), Nutrient broth (HiMedia), Folin-Cioucalteu reagent (Merk Millipore, Germany), Alloxan monohydrate (Sigma-Aldrich, U.S.A), *Klebsiella pneumoniae* and *Staphylococcus aureus*, Male *Sprague-Dawley* white rat, 4-5 months old, weighing 200-270 grams or more. White male *Sprague Dawley* rats with a weight between 250-270 grams that were in good health and engaged in regular movement activity served as test subjects. They were procured from BPPOM Jakarta.

Tools. Analytical balance (Wiggen Hauser), 1 mL syringe (Terumo), stopwatch (Olympic), vacuum rotary evaporator (Buchi b-740), rat cage, oral probe, and animal weighing, Gluco-Test (Dr Bio), spectrophotometer (Perkin Elmer Lambda 25) and Vernier calipers (Absolute Digimatic).

METHODS. Determination of Crops and Submission of Ethics Assessments. At the Indonesian Institute of Sciences (LIPI) Botanical Garden Plant Conservation Center in Bogor, West Java, 910/UN2. F3.09/PDP.02.00/2019, P. *guajava* L, and *Melastoma malabathricum* L were identified. Letter number B/2210/X/2019/KEPK requests ethical approval from the National Development University "Veteran" Jakarta's Health Research Ethics Committee, indicating that the research plan complies with the ethical standards for testing animals and is thus practical to implement.

Simplicia Preparation. *Melastoma malabathricum* Linn and *Psidium guajava* L produce up to 2 kg of dry leaves. The leaves of *Psidium guajava* L and *Melastoma malabathricum* L were then crushed to create a simplicial powder, which was then sieved through a mesh size of 40, weighed to determine its final weight, and stored in a tightly closed container. Next, dry sorting was performed to separate any dirt particles that could remain attached to the leaves. The resultant simplicial material was macerated by adding ten details of the solvent (70% ethanol) to a vessel containing one part of the dry simplicial powder. After the first six hours of covering and soaking, while sometimes stirring, it was allowed to sit for 18 h. The macerate must be separated by filtration or precipitation. The same type of solvent and amount were used at least twice during the extraction procedure. The macerates from *Psidium guajava* and *Melastoma malabathricum* were concentrated using a rotating vacuum evaporator to create a thick extract.

Phytochemical Screening. Flavonoids, saponins, alkaloids, tannins, and steroids/triterpenoids were among the substances measured in this test[22].

Phenolic Test in Total. *P. guajava* L and *Melastoma malabathricum* L (10 mg) were dissolved in 10 mL of distilled water. 0.5 mL of the sample was pipetted in addition to 0.3 mL of the Folin-Cioucalteu reagent, 2 mL of Na_2CO_3 (7%), and 5 mL of distilled water to create the final volume of the solution. A UV-VIS spectrophotometer was used to test the model's absorbance after the sample was vortexed and incubated for two hours[23].

DPPH Method for Testing Antioxidant Activity. Diphenylpicrylhydrazyl (DPPH) solution was prepared by weighing 1 mg of DPPH, placing it in a 10 mL vial, adding 6.26 mL of ethanol, and stirring until the DPPH was completely dissolved. Aluminum foil was used to completely cover the surface of the vial and protect it from light. The bottles were tightly closed. Weighing a sample of 0.01 grams and placing it in a 10 mL vial to create a stock solution. A 1000 ppm sample solution was obtained, and 5 mL of ethanol was added and mixed until dissolution. A vortex was used to help the model dissolve more quickly if challenging. Each test tube contained sample solutions at concentrations of 200 ppm (1 mL), 400 ppm (2 mL), 600 ppm (3 mL), and 800 ppm (4 mL). Each test tube contained 1 mL of DPPH solution, which was added to each test tube, and the line was then left for 30 min before being measured using a UV-Vis spectrometer with a wavelength of 517 nm[23].

Induction of Rats and Wound Formation. Employing the chemical substance alloxan, which weighed 10 g and was dissolved in saline solution, twenty-eight-week-old rats weighing between 250 and 270 g were administered a single intraperitoneal injection of 120 mg/kg BW to induce diabetes mellitus. After diabetic rat hair became white, the researchers observed that the rats' blood sugar levels increased. The rats were fully shaved on their backs. Punch biopsy was performed to create a wound with a diameter of 5 mm. The diabetic group of rats was anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (5 mg/kg) intramuscularly for pain treatment before the injury in the back area, and the mice were treated with an antiseptic by applying 70% ethanol. A complete thickness sheet of skin with a 5 mm diameter wound was peeled off under pressure after being demarcated in rectangles on the dorsal surface of the foot using a measuring tape. On day 1, after the injury was induced, there was a minor increase in damage.

The Blood Glucose Levels and Wound Diameters measurement. The glucose test was used to

Table 1. Formulations of *Melastoma malabathricum* L and *Psidium guajava* L gel.

Formulation	Amount			Description
	F1 (20%)	F2 (25%)	F3 (30%)	
Extracts of *Melastoma malabathricum* L. and *Psidium guajava* L.	4 % and 6%	4 % and 6%	4 % and 6%	It will be smeared on the wound for as much as 5 mL/3 days, one time wrapped with a transparent film.
CMC (Carboxymethyl cellulose)	6%	6%	6%	
Propylene glycol	8 %	8 %	8 %	
Methyl paraben	0.2 %	0.2 %	0.2 %	
Aquadest ad	ad 100 mL	ad 100 mL	ad 100 mL	

measure and determine blood glucose levels. Wound healing was assessed using a caliper to measure the closure after ten days.

Gel Preparation Formulation and Evaluation. The gel was created by mixing the developer, carboxymethyl cellulose, with methylparaben and developing it in hot water for 24 h. Once the gel was uniform, it was milled by the addition of propylene glycol to the mixture to create a transparent gel. The preparation mixture was blended with *Melastoma malabathricum* and *Psidium guajava* leaf extracts before being placed in a gel container. The formulas are shown in Table 1. Organoleptic, homogeneity, pH, and viscosity tests were used to evaluate gel preparations. The glucose test was used to measure and determine blood glucose levels. Wound healing was assessed by measuring incision closure using a caliper for ten days[24].

Antimicrobial Testing of Rat Wounds. The results of the microbiological study, specifically the type of bacteria and the number of bacterial colonies (CFU), were acquired both qualitatively and quantitatively. Wound swab examination revealed the presence of *Klebsiella pneumoniae* and *Staphylococcus aureus*, two different bacterial species. The rejuvenation was completed on medium agar. Active cells were obtained by growth in a liquid medium. A liquid medium (nutrient broth) is necessary for dynamic microbial growth. The press setup involves combining the nourishing broth with 50 mL of purified water, Erlenmeyer 0.4 grams each, and stirring for 30 min. The nutritional broth medium was chilled after mixing. After cooling, 1 mL of gel from each concentration and one dose of revived bacteria were added to each Erlenmeyer flask containing the nutritional broth media. The mixture was then shaken for 48 h to encourage microbial growth in the presence of turbidity in the press. Nourishing broth. When it clouds over, 1 mL of the microorganism was extracted and diluted between 10^{-1} and 10^{-10}. A colony counter was used to tally the colonies[25].

Data Analysis. The data obtained in this study were analyzed using analysis of variance with an experimental design and descriptive analysis. This study used a Randomized Block Design (RBD). Duncan's test was performed if the analysis results (ANOVA) are significantly different. Next, the wound-healing abilities of the treatment groups were analyzed.

RESULTS AND DISCUSSION

Phytochemical Results. The bioactive components of the extract were flavonoids, tannins, steroid saponins, total phenol (6.08%, quercetin 0.31 mg, and antioxidant activity >31.25 ppm in a mixed section of *Psidium guajava* L. *Melastoma malabathricum* L. Additional research has shown alkaloids, flavonoids, saponins, tannins, terpenoids[20], alkaloids, steroids, saponins, phenols, and tannins[21]. *Melastoma candidum* D. Don contains flavonoids, triterpenoids, steroids, saponins, tannins, and glycosides[26]. Polyphenols in *Psidium guajava* L can be used as antibacterial agents against *Vibrio*[27]. Phenolic compounds such as quercetin and kaempferol[28]. The glycoside compounds in *Melastoma malabathricum* L are thought to have wound healing properties[29]. The phenolic group and specific tannins from senggani leaves act as antimicrobials[30]. *Melastoma malabathricum* L. has selective antagonistic activity against PAF and is a potential candidate as a natural anti-inflammatory compound[31]. Hand sanitizer from *Psidium guajava* L. has the potential to inhibit the reproduction of *S. Aureus*[32]. Alkaloids and steroids was not, however, included in this investigation.

Evaluation of Gel Results. Based on the assessment results, the gel formulations from *Psidium guajava* L. and *Melastoma malabathricum* L at a concentration of 6% showed nearly identical evaluation results to those of clindamycin. This medicine is already commercially available. The evaluation findings include dense, transparent, and homogeneous, with a distinct scent, a viscosity value of 6.84 pa.S; a spreadability value of 3.5, and a pH value of 5.7. The data obtained complied with these standards. According to SNI Standard No. 06-2588, good gel preparation must have a uniform gel composition free of coarse granules. According to SNI 16 -4399-1996[33], fair viscosity value requirements (maintaining skin moisture) vary from 2000–50,000 mPs, and conditions for a suitable pH value are in the range of 4.5–6.5[34]. Except for the spreadability results, which are suspected to be due to

the excessive thickness of the preparation, the results demonstrate that the pH and viscosity values of the 6% extract preparation match the SNI standards. The table displays the effect of the four treatments in each group on the diameter of the rat wound.

Table 2 shows the mean results of the four treatments on wound diameter from days 0 to day 10th. The mean of ML gel and GL gel 4% was 2.00±0.70 mm, while the ML gel and GL gel 6% was 0.75±0.28 mm compared to Clindamycin gel, which was 1.87±0.25 mm. A significant increase was observed from day and to 10th day. The positive control and 4% and 6% gel preparation extracts showed the effectiveness of therapy on the onset of alloxan-induced wound healing in rats. Bonferroni test results on day 10 showed the effect of the treatments on the diameter of the rats in each group (Table 3). It shows the different results of the four treatments while there is a significant effect between the treatment in the negative control with 4% gel preparation, 6% gel, and positive control on the diameter of the rat wound.

Based on Figure 1, a reduction in the wound diameter was observed in each treatment group. Measurements were performed using calipers from day 0 to day 10th. Figure 1 shows that ML + GL gel treatment at concentrations of 4% and 6% can heal wounds in diabetes-induced rats for 10 days. Wound healing in diabetes is slightly different from that observed under normal conditions. Several physiological factors play a role in poor wound healing in individuals with diabetes. These factors include impaired blood flow and oxygenation due to increased blood glucose levels; decreased collagen and fibronectin synthesis; and decreased insulin levels, macrophage function, and growth hormone levels. The goal of wound healing in diabetes is to accelerate wound closure by stimulating growth factors to function normally[35].

Table 2. Effect of four treatments on rat wound diameter.

Treatment Group	Time	Wound Diameter (mm)	
		Mean ± SD	*p-value*
Control (-) CMC Na gel	Day 0	5.00 ± 0.00	-
Control (+) Clindamycin gel		5.00 ± 0.00	
ML+ PL 4% gel		5.00 ± 0.00	
ML+ PL 6% gel		5.00 ± 0.00	
Control (-) CMC Na gel	Day 2nd	4.87 ± 0.25	0.644
Control (+) Clindamycin gel		4.75 ± 0.28	
ML+ PL 4% gel		4.62 ± 0.25	
ML+ PL 6% gel		4.75 ± 0.28	
Control (-) CMC Na gel	Day 6th	4.37 ± 1.18	0.149
Control (+) Clindamycin gel		3.37 ± 0.47	
ML+ PL 4% gel		3.50 ± 0.70	
ML+ PL 6% gel		2.62 ± 1.31	
Control (-) CMC Na gel	Day 10th	4.00 ± 2.44	0.026
Control (+) Clindamycin gel		1.87 ± 0.25	
ML+ PL 4% gel		2.00 ± 0.70	
ML+ PL 6% gel		0.75 ± 0.28	

ML gel = *Melastoma malabathricum* Linn Leaves; PL gel = *Psidium guajava* Leaves gel; N=16

Table 3. Bonferroni test results on day 10 - effect between treatments on rat wound diameter.

(I) Group Treatment	(J) Group Treatment	Mean Difference (I-J)	*p-value*
A1= Negative Control	A2= Positive Control	2.1250	0.228
	A3= 4% gel	2.0000	0.292
	A4= 6% gel	3.2500*	0.023
A2= Postive Control	A1= Negative Control	-2.1250	0.228
	A3= 4% gel	-0.1250	1.000
	A4= 6% gel	1.1250	1.000
A3= 4% gel	A1= Negative Control	-2.0000	0.292
	A2= Positive Control	0.1250	1.000
	A4= 6% gel	1.2500	1.000
A4= 6% gel	A1= Negative Control	-3.2500*	0.023
	A2= Positive Control	-1.1250	1.000
	A3= 4% gel	-1.2500	1.000

* states significant differences

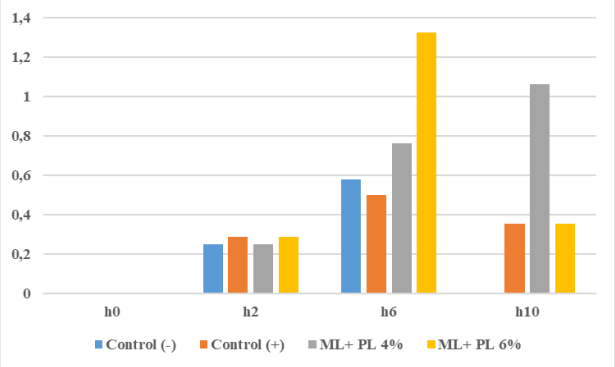

Figure 1. The difference in wound diameter in each treatment on day 10th.

Wound Healing and Pharmacological Mechanisms Wound healing is a complex process. Which involves 4 phases: the coagulation or hemostatic phase, the inflammatory phase, the proliferation phase, and the remodeling phase. The hemostatic phase suppresses the initial bleeding when the wound occurs, which is seen 10-30 minutes after the wound occurs. In this phase, the wound still appears red, bleeding occurs, and there is no wound closure. Furthermore, the inflammatory phase is characterized by a reddish reaction, warm sensation, and pain. This phase lasts 1-6 days after the wound occurs and functions to clean the wound, marked by the number of neutrophils and macrophages in the wound, which helps the phagocytosis of bacteria and foreign bodies[36]. The proliferation phase is the phase of new tissue formation in re-epithelization, neovascularization, and collagen formation on days 5-21 after injury[37]. The mechanism by which flavonoids prevent inflammation in burns involves several different mechanisms, including inhibition of capillary permeability, inhibition of serotonin and histamine release to the site of inflammation, inhibition of arachidonic acid metabolism by inhibition of cyclooxygenase, and inhibition of the secretion of inflammatory mediators such as lysosomal enzymes. It reduces the growth of neutrophils, endothelial cells, and inflammatory processes[38]. The oil palm leaves contain flavonoids, which are potent antioxidants with anti-inflammatory properties. These flavonoids may be linked to faster wound healing because they stimulate fibroblasts, endothelial cells, and macrophages in response to skin damage. Fibroblast proliferation is associated with the recovery of structure and function in wound tissue[39]. The α-glucosidase enzyme is competitively inhibited by flavonoid substances like quercetin, which lowers postprandial blood glucose levels[40]. Phenolic substances reduce inflammation by scavenging free radicals, which damage tissue, promote arachidonic biosynthesis to produce prostaglandins, which are inflammatory mediators, and block cyclooxygenase enzymes[41]. Tannins have anti-inflammatory properties due to their antioxidant activity, which prevents neutrophils, monocytes, and macrophages from producing oxidants (O_2). Hypochlorous acid (HOCl) and OH production will be inhibited by reducing the synthesis of O_2 oxidants, which will also lower H_2O_2 creation. Inhibits immediately reactive oxidants like hypochlorous acid[42]) and hydroxy radicals (OH). Many enzymes, including α-amylase and α-glucosidase enzymes, which are crucial in the pharmacological treatment of diabetes mellitus, can be inhibited by tannin and saponin substances[43].

Microbiology Activity. Table 4 shows that providing *Melastoma malabathricum* L and *Psidium guajava* L. leaves extract gel preparations at a concentration of 4% and 6% can significantly affect the type and number of bacterial colonies on the 10th day against KP-CFU (*Colony Forming Unit*) and SA-CFU. Compared to the positive control, there was a significant difference between the gel extract preparations against KP-CFU bacteria, but not against SA-CFU on day 10th. The effects of the four test treatments on the type and number of bacterial colonies in each group are shown in Table 4.

Melastoma malabathricum L. leaves potentiate extracts to inhibit or killing pathogens[44]. *Psidium guajava* L. leaf extract inhibited *B. cereus* and *S. aureus*[45]. The results of the Bonferroni test for the four treatments (the number of bacteria in each group) are presented in Table 5. There was a statistically significant difference between the control group and the treatment group containing *Melastoma malabathricum* L. extract and *Psidium guajava* L leaf extract. Based on short-term cytotoxicity analysis, *Psidium guajava* L. extract reduced the cell population in vitro and accelerated wound healing[29]. The 5% and 7% concentrations of *Psidium guajava* L. leaf ethyl acetate fraction gel effectively accelerated scab formation and wound healing[46]. The activity of *Melastoma malabathricum* L. and *Psidium guajava* L. leaves extract gel in healing wounds in alloxan-induced rats, specifically. The higher the concentration of the extract gel used, the greater the effect of reducing wound diameter. The phytochemical composition of the two plant materials has been found to potentially provide antimicrobial and antidiabetic effects, as well as promote the regeneration of epithelial cells and tissues. The secondary metabolites present in the extracts of the test preparations have different mechanisms for assisting the healing process.

Alginate can accelerate wound healing in rats with diabetes mellitus, with healing taking at least ten days and no longer than 22 days for complete wound closure (100%), decreased local inflammatory response,

Table 4. The effect of four treatments on the type and number of bacterial colonies.

Types of Bacteria	Time	Treatment	Mean ± SD - n_{bc}	*P-Value*
KP-CFU		Negative Control	1100.00 ± 0.000	
	Day 2nd	Positive Control	500.00 ± 0.000	
		4% (ML+PL) gel	300.00 ± 0.000	
		6% (ML+PL) gel	275.00 ± 28.868	
KP-CFU		Negative Control	500.00 ± 0.000	
	Day 10th	Positive Control	0.00 ±0.000	
		4% (ML+PL) gel	225.00 ± 28.868	
		6% (ML+PL) gel	125.00 ± 28.868	0.000
SA-CFU		Negative Control	1200.00 ± 0.000	
	Day 2nd	Positive Control	1075.00 ± 28.868	
		4% (ML+PL) gel	1100.00 ± 0.000	
		6% (ML+PL) gel	1100.00 ± 0.000	
SA-CFU		Negative Control	500.00 ± 0.000	
	Day 10th	Positive Control	0.00 ± 0.000	
		4% (ML+PL) gel	0.00 ± 0.000	
		6% (ML+PL) gel	0.00 ± 0.000	

KP-CFU: *Klebsiella pneumonia – Colony Forming Unit*; SA-CFU: *Staphylococcus aureus* – Coloni Forming Unit; N=8.

Table 5. Bonferroni test results on four treatments on the number of bacteria (colonies).

Dependent Variable	(I) Treatment	(J) Treatment	Mean Difference (I-J)	*p-value*
KP-CFU Day 2nd	Negative Control	Positive Control	600.000*	0.000
		4% (ML+ PL) gel	800.000*	0.000
		6% (ML+ PL) gel	825.000*	0.000
	Positive Control	Negative Control	-600.000*	0.000
		4% (ML+ PL) gel	200.000*	0.000
		6% (ML+ PL) gel	225.000*	0.000
	4% (ML + PL) gel	Positive Control	-800.000*	0.000
		Negative Control	-200.000*	0.000
		6% (ML + PL) gel	25.000	0.184
	6% (ML + PL) gel	Positive Control	-825.000*	0.000
		Negative Control	-225.000*	0.000
		4% (ML + PL) gel	-25.000	0.184
CFU-KP Day 10th	Negative Control	Positive Control	500.000*	0.000
		4% (ML+ PL) gel	275.000*	0.000
		6% (ML+ PL) gel	375.000*	0.000
	Positive Control	Negative Control	-500.000*	0.000
		4% (ML+ PL) gel	-225.000*	0.000
		6% (ML+ PL) gel	-125.000*	0.000
	4% (ML + PL) gel	Positive Control	-275.000*	0.000
		Negative Control	225.000*	0.000
		Gel 6% (ML + PL)	100.000	0.000
	6% (ML + PL) gel	Positive Control	-375.000*	0.000
		Negative Control	125.000*	0.000
		Gel 4% (ML + PL)	-100.000	0.000
SP-CFU Day 2nd	Negative Control	Positive Control	125.000*	0.000
		Gel 4% (ML+ PL)	100.000*	0.000
		Gel 6% (ML+ PL)	100.000*	0.000
	Positive Control	Negative Control	-125.000*	0.000
		4% (ML+ PL) gel	-25.000*	0.184
		6% (ML+ PL) gel	-25.000*	0.184
	4% (ML + PL) gel	Positive Control	-100.000*	0.000
		Negative Control	25.000*	0.184
		6% (ML + PL) gel	0.000	1.000
	6% (ML + PL) gel	Positive Control	-100.000*	0.000
		Negative Control	25.000*	0.184
		4% (ML + PL) gel	0.000	1.000

KP-CFU: *Klebsiella pneumonia* - Colony Forming Unit; SA-CFU: *Staphylococcus aureus* – Colony Forming Unit; N=8 *: states the significance different.

reduced exudate to keep the wound area moist during the healing process, decreased neutrophils and macrophages, increased fibrocytes and fibroblasts, accelerated formation of granulation tissue, and increased angiogenesis on VEGF examination[47]. The most prevalent bacterial pathogen, *Staphylococcus aureus*, is a gram-positive bacterium that causes foot ulcer. Gram-negative bacteria are exceedingly diverse, as are their negative effects. Knowledge of the microbiology of diabetic foot ulcer infections is essential for monitoring antimicrobial resistance and provides an overview of anti-infective targets because bacterial colonization and proliferation in diabetic foot ulcer wounds are believed to significantly inhibit wound healing[48]. Quercetin has demonstrated outstanding antioxidant, anti-inflammatory, wound healing, and antimicrobial effects in recent preclinical tests[49]. Quercetin activity in the 4FPBA-Q complex is suitable for treating diabetic foot ulcers because of its decreased primary irritation index (PDII), increased antibacterial activity, and wound healing[50]. In therapy utilizing gel formulations or clindamycin, Table 5 shows that the CFU value of the bacteria used was reduced to less on day ten compared to day two. This result suggests a reduction in the number of CFU and a wound-healing effect in alloxan-induced rat wounds. This combination is anticipated to accelerate recovery from diabetes and lower the associated mortality risk. Infections caused by bacteria can worsen diabetes and can defeat microorganisms that have developed antibiotic resistance.

CONCLUSION

Based on the results of the extraction process using 70% ethanol solvent, the phytochemical composition of the two plants included secondary metabolites of flavonoids, saponins, tannins, and steroids. The gel dose concentration of 6% was determined by gel evaluation at concentrations of 4% and 6%, and satisfied all requirements except for the spreadability observation. The results for wound healing of 4% and 6% gel formulations were derived from maturation data on day 10. Fewer colonies were on the 10th day of the microbial activity test.

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