

Antibacterial activity and diabetic wound therapy of topical preparations of polyherbal combination extracts in vitro

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Article Info	ABSTRACT
<p>Keywords: Antibacterial, Diabetic Wounds, Polyherbal Combination Extract</p>	<p>Glucose metabolic disorders in cases of diabetes mellitus (DM) will affect other body metabolism, such as carbohydrate, protein, fat and water metabolism and cause cellular damage to several body tissues.. This research aims to determine the antibacterial activity test in vitro using aerobic bacteria (<i>S. aureus</i> and <i>P. aeruginosa</i>) based on the MIC value shown in the polyherbal formulation. Then a topical test was carried out on white mice which had previously been induced by STZ-NA with complications of diabetic ulcers characterized by wounds on the mice's backs. The samples used in this research were noni fruit simplicia, insulin leaves, turmeric, aloe vera and honey. Polyherbal plants that are still fresh and complete are determined at BBPPTOOT Tawangmangu, Kab. Karanganyar, Central Java. This research was carried out in-vitro and was a laboratory experiment. The polyherbal ethanol extract obtained was then tested for antibacterial activity against <i>S. aureus</i> and <i>P. aeruginosa</i> at several concentrations (20% and 50%). The test results showed that the concentration that had the most active MIC value was a concentration of 20% of the diameter of the best inhibitory zone, namely honey extract, noni fruit, insulin leaves, turmeric rhizomes, while at a concentration of 50% the diameter of the best inhibitory zone, namely aloe vera. From these results a new formulation series was created, expressed as F1. F1 was then replicated 3 times to get the best antibacterial activity results. From the replication results, the polyherbal formulation had inhibitory power due to the presence of a clear zone around the well. Diameter antibacterial zone of inhibition against <i>Staphylococcus aureus</i> has an inhibition zone diameter of 14.4 mm and <i>Pseudomonas auroginosa</i> has an inhibition zone diameter of 11.67 mm. From this research it was found that the polyherbal formulation had active antibacterial activity in inhibiting <i>Staphylococcus aureus</i> and <i>Pseudomonas auroginosa</i>. These results are continued to determine the healing activity of diabetic ulcers. The activity test used Wistar male white rats which were grouped into 10 groups with each group consisting of 5 rats. Rats were induced with STZ at 45 mg/kgBW and NA 110 mg/kgBW, until the rat's blood sugar level was > 250 mg/dL. Measurement of rat blood sugar levels using the GOD-PAP method. The mice were left for 3 weeks until nephropathy occurred, after which the mice's backs were injured using a biopsy punch with a diameter of ± 4 mm .</p>
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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia which is associated with abnormalities in carbohydrate, fat and protein metabolism caused by decreased insulin secretion or decreased insulin sensitivity, which causes chronic microvascular, macrovascular and neuropathy complications (Sukandar, et al., 2008) . This is because DM is a disease that has a high prevalence. In the world, it is estimated that 347 million people suffer from diabetes mellitus (WHO, 2013).

Glucose metabolic disorders in cases of DM will affect other body metabolism, such as carbohydrate, protein, fat and water metabolism and cause cellular damage to several body tissues. Other complications of DM are susceptibility to infection, pulmonary tuberculosis and infections of the legs which can then develop into gangrene. Gangrene is a condition characterized by the presence of dead tissue or necrosis (Waspadji, 2006). Gangrenous wounds are blackish red and have a foul smell due to blockages that occur in medium or large blood vessels in the legs.

Gangrenous ulcers are one of the chronic complications of DM that is most feared by every DM sufferer (Tjokroprawiro, 2007). DM sufferers are five times more likely to suffer from gangrene. Gangrene infection is usually caused by an organism from around the skin which is generally in the form of Gram-positive bacteria such as *Staphylococcus aureus* or *Streptococcus*. In chronic wounds, complex bacterial colonies can develop, including Gram-negative bacteria such as *Escherichia coli*, *Clostridium*. (Anandi, et al, 2004).

Many factors play a role in the length of the healing process for diabetic ulcers, including wound care, infection control, vascularization, age, nutrition, complications, history of smoking, medication, psychology (Yadi, 2000).

Research regarding the polyherbal combination of honey (*Mel depuratum*), aloe vera (*Aloe vera L*), noni (*Momocardica charantia*), turmeric rhizome (*Curcuma longa*), insulin leaves (*Smallanthus sonchifolia*), there is no supporting scientific evidence, because This research was developed to see the effect of the antibacterial activity of polyherbal combinations in vitro so that it becomes a scientific basis. The aim of this study was to determine the activity of a combination of topical polyherbal preparations as an antibacterial against *S. aureus*, *P. aeruginosa*, based on the inhibition zone/clear zone .

METHOD

Research design

The research carried out was experimental research. To determine the effects of ethanol extracts of noni fruit, black cumin, aloe vera, honey and turmeric rhizomes, this research carried out an animal model of diabetes in male Wistar rats. Research on male Wistar rats that were fattened with fat-fructose-rich feed, so they became hyperglycemic due to the emergence of insulin resistance. The research was carried out in 10 groups of experimental animals consisting of positive control group, negative control group, topical group, oral group, topical and oral groups.

Identify research variables

The first main variable in this study was the activity of topical preparations of polyherbal combination extracts in reducing blood sugar levels with diabetic complications. The second main variable in this study is antibacterial activity against aerobic bacteria (*S. aureus* and *P. aeruginosa*).

Classification of main variables

The main variables can be classified into various variables, namely independent variables, dependent variables and controlled variables. The independent variable in this research is a variable that was deliberately planned to examine its effect on the dependent variable. The independent variable in this study was the concentration of the polyherbal combination extract.

The dependent variable is the variable from the problem point which is the research criterion. The dependent variable in this study is the topical preparation of a combination of polyherbal extracts as an antibacterial against *S. aureus* and *P. aeruginosa* based on the inhibition zone/clear zone and the activity of reducing blood glucose levels in Wistar male rats. (*Ratus norvegicus*) which has been induced by DM with complications of diabetic ulcers and activities in healing diabetic ulcers.

Controlled variables are variables that influence the dependent variable other than the independent variable, namely research conditions, the laboratory used including tools, materials, temperature and pressure of the incubator, length of incubation time and the number of bacteria in each well and the physical condition of the test animals including body weight, feed, condition. pen.

Operational definition of main variables

First, a polyherbal combination, namely honey, aloe vera, noni fruit, insulin leaves, turmeric, all plants that are obtained fresh, not too old. Second, dry simplicia powder which is ground with a grinder and sieved with a 40 mesh sieve. Third, the test animals are the experimental animals used in research, the test animals used are male Wistar rats with a body weight of 150-200 g. Fourth, the test bacteria in this study used *S. aureus* and *P. aeruginosa* cultured at the Microbiology Laboratory of Setia Budi University, Solo. Fifth, Streptozotocin-Nicotinamide (STZ-NA) is given intraperitoneally which causes the pancreatic β cells of the islets of Langerhans to function to produce insulin, resulting in diabetes. Sixth, blood glucose levels are blood glucose levels taken from the eyes of Wistar rats.

RESULTS AND DISCUSSION

Macroscopic test results

Macroscopic results of honey

Macroscopic examination is carried out using organoleptic methods. Honey was carried out in the Phytochemistry laboratory at Setia Budi University, Surakarta. Organoleptics were carried out with the aim of matching the physical properties of honey used in the literature. Organoleptic examination includes examination of shape, smell, color and taste. Organoleptic results can be seen in table 1.

Table 1. Results of organoleptic examination of honey

Information	Organoleptic examination of honey
Form	Liquid
Smell	Typical
Color	Chocolate
Flavor	Sweet

Macroscopic results of aloe vera

Macroscopic examination is carried out using organoleptic methods. Aloe vera plants were carried out in the Phytochemistry laboratory at Setia Budi University, Surakarta. Organoleptics were carried out with the aim of matching the physical properties of the aloe vera plant to those used in the literature. Organoleptic examination includes examination of shape, smell, color and taste. Organoleptic results can be seen in table 2.

Table 2. Results of organoleptic examination of aloe vera plants

Information	Organoleptic examination of aloe vera plants
Form	Mucus or gel
Smell	Aromatic
Color	Clear
Flavor	Bitter

Macroscopic results of Insulin leaves

Macroscopic examination is carried out using organoleptic methods. Insulin leaves were carried out in the Phytochemistry Laboratory at Setia Budi University, Surakarta. Organoleptics were carried out with the aim of matching the physical properties of insulin leaves used in the literature. Organoleptic examination includes examination of shape, smell, color and taste. Organoleptic results can be seen in table 3.

Table 3. Results of organoleptic examination of insulin leaves

Information	Organoleptic examination of insulin leaves
Form	Powder
Smell	Aromatic
Color	Green
Flavor	Bitter

Macroscopic results of noni fruit simplicia

Macroscopic examination is carried out using organoleptic methods. Noni fruit simplification was carried out in the Phytochemistry Laboratory of Setia Budi University, Surakarta. Organoleptics were carried out with the aim of matching the physical properties of noni fruit simplicia used in the literature. Organoleptic examination includes examination of shape, smell, color and taste. Organoleptic results can be seen in table 4.

Table 4. Results of organoleptic examination of noni fruit simplicia

Information	Organoleptic examination of noni fruit simplicia
Form	Powder
Smell	Aromatic
Color	Reddish brown
Flavor	Bitter

Macroscopic results of turmeric rhizomes

Macroscopic examination is carried out using organoleptic methods. Turmeric rhizomes were carried out in the Phytochemistry Laboratory at Setia Budi University, Surakarta. Organoleptics were carried out with the aim of matching the physical properties of turmeric rhizomes used in the literature. Organoleptic examination includes examination of shape, smell, color and taste. Organoleptic results can be seen in table 5.

Table 5. Results of organoleptic examination of turmeric rhizomes

Information	Organoleptic examination of turmeric rhizomes
Form	Powder
Smell	Aromatic
Color	Yellow
Flavor	Bitter

Phytochemical Test Results for Compound Content

Tests are carried out to determine the compound content contained in the powder and extract. The results of the compound content tests that have been carried out are seen in table 6 .

Table 6. Phytochemical Test

No	Compound class	Honey	Aloe vera	Noni powder	Insulin leaf powder	Turmeric rhizome powder
1	Saponins	+	+	+	+	+
2	Flavonoids	+	+	+	+	+
3	Tannin	-	+	+	+	+
4	Alkaloids	+	+	+	+	+
5	Curcumin	-	-	-	-	+

Explanation: (+) = gives positive results

(-) = gives negative results

Based on the results in the table above, noni powder, insulin leaf powder, aloe vera and honey contain positive saponins, flavonoids and alkaloids. In honey plants there is no tannin content, whereas in noni powder, insulin leaf powder, turmeric rhizome powder. Turmeric rhizome powder contains curcumin compounds which are not found in honey, aloe vera, noni powder, insulin leaf powder.

Results of Determination of Water Content of Noni Fruit Powder

Determination of the water content of noni fruit powder meets the requirements if the percentage is less than 10% (Ministry of Health, 2008). High water content can reduce the quality of the powder and can damage the quality of the powder because water is a medium for the growth of fungi and other microorganisms. The results of determining the water content of noni fruit powder can be seen in table 9.

Table 7. Results of determining the water content of noni fruit powder

Replication	Heavy (gram)	initial (ml)	Final (ml)	volume	Water (%)	content
I	20		0.8		4	
II	20		1.0		5	
III	20		1.0		5	
Average					4.67 ± 0.58	

The results of determining the water content of noni fruit powder obtained an average of 4.67%. These results meet the requirements for water content of simplicia powder, namely less than 10%. The results of calculating the water content of noni fruit powder can be seen in appendix 6.

Results of Determination of Water Content of Insulin Leaf Powder

Determination of the water content of insulin leaf powder meets the requirements if the percentage is less than 10% (Ministry of Health, 2008). High water content can reduce the quality of the powder and can damage the quality of the powder because water is a medium for the growth of fungi and other microorganisms. The results of determining the water content of insulin leaf powder can be seen in table 10.

Table 8. Results of determining the water content of insulin leaf powder

Replication	Initial (grams)	weight	Final volume (ml)	Water content (%)
I	20		0.8	3.5
II	20		0.8	4.5
III	20		1.0	5
Average				4.33 ± 0.77

The results of determining the water content of insulin leaf powder obtained an average of 4.33%. These results meet the requirements for water content of simplicia powder, namely less than 10%. The results of calculating the water content of insulin leaf powder can be seen in Appendix 6.

Results of Determining Water Content of Turmeric Rhizome Powder

Determination of the water content of turmeric rhizome powder meets the requirements if the percentage is less than 10% (Ministry of Health, 2008). High water content can reduce the quality of the powder and can damage the quality of the powder

because water is a medium for the growth of fungi and other microorganisms. The results of determining the water content of turmeric rhizome powder can be seen in table 9.

Table 9. Results of determining the water content of turmeric rhizome powder

Replication	Initial weight (grams)	Final volume (ml)	Water content (%)
I	20	0.7	3.5
II	20	0.9	4.5
III	20	1.0	5
Average			4.33 ± 0.77

The results of determining the water content of turmeric rhizome powder obtained an average of 4.33%. These results meet the requirements for water content of simplicia powder, namely less than 10%. The results of calculating the water content of turmeric rhizome powder can be seen in Appendix 6.

Antibacterial Activity Test Results

Bacteria are divided into Gram positive and Gram negative groups, antibacterial testing against Gram positive *Staphylococcus aureus* using the well diffusion method to determine the diameter of the inhibition zone of the sample to be tested. The samples used were 5 polyherbal combination extracts consisting of honey, aloe vera, noni, insulin leaves. All polyherbal combination extracts are oriented first to obtain concentration results that have an inhibition zone/clear zone. The polyherbal combination extract was added approximately 100 mg into several wells in MHA (Muller Hinton Agar) media with a well diameter of ± 6 mm, then observed the diameter of the inhibition zone after incubation at 37 °C for 24 hours.

The antibacterial inhibition zone test was carried out to determine whether there was antibacterial activity in the polyherbal combination extract and the diameter of the antibacterial inhibition zone against *Staphylococcus aureus* and *Pseudomonas aeruginosa* . Results of the inhibition zone of antibacterial activity of polyherbal extracts against *Staphylococcus aureus* and *Pseudomonas a e r u ginosa* can be seen in tables 9 and 10.

Table 10. Results of antibacterial activity tests of polyherbal formulations on *Staphylococcus aureus* bacteria

Treatment	Zone of inhibition (mm) <i>S. aureus</i>
F1	14.4
Control +	18.2
Control -	10.6

Table 11. Results of antibacterial activity test of polyherbal formulations on *Pseudomonas a e r u ginosa* bacteria

Treatment	Inhibition zone (mm) <i>P. a e r u ginosa</i>
F1	11.67
Control +	28.6
Control -	7.9

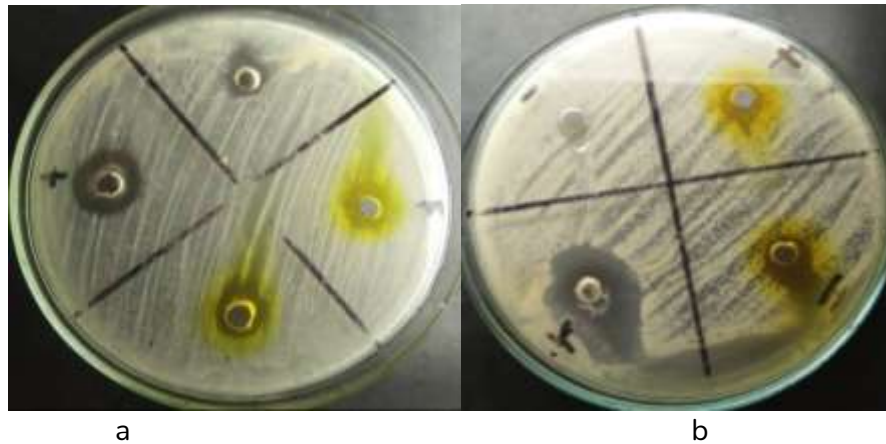


Figure 1. Antibacterial activity test of polyherbal extracts, a) *S. aureus* b) *P. aeruginosa*

Based on the table above, the replication results of the polyherbal combination extract have inhibitory power due to the presence of a clear zone around the well. Negative control (vaseline), positive control (nebacetin ointment) and F1 (polyherbal combination extract). From this *research*, it was found that the polyherbal combination extract had active antibacterial activity in inhibiting *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Results of Rat Blood Glucose Levels

The mice's blood glucose levels were measured three times. The 1st measurement is before the drug treatment is given to the test animals. Before measuring blood glucose levels, the mice were first induced with STZ-NA and then left without treatment for 5 days. The second measurement was carried out in the first week after administering the test preparation. The 3rd measurement was carried out in the second week after administering the test preparation. The 1st to 3rd blood glucose levels increased in each group. The occurrence of DM in the treatment group after being induced by STZ-NA was characterized by hyperglycemia, namely blood glucose levels above 200 mg/dL. Streptozotocin (STZ) or 2-deoxy-2-[3-(methyl-3-nitrosoureido)-D-glucopyranose] obtained from streptomyces achromogenes can be used to induce type 1 and type 2 DM in test animals. The mechanism is influenced by the formation of NO and the generation of free radicals, NO and reactive oxygen are the main causes of damage to pancreatic β cells by activating poly ADP-ribosylation which then results in a reduction in cellular NAD⁺, then a decrease in the amount of ATP, ultimately inhibiting insulin secretion and synthesis (Szkudelski, 2001). Rat blood glucose data can be seen in table 14.

Table 12. Data on mouse blood glucose levels

Treatment group	Rat Glucose Levels (mg/dL)		
	number 1	2nd	the 3rd
Normal	81±2.36	81±2.42	82±2.55
Control - (CMC)	213±10.15	214±10.89	215±10.27
Control - (Vaseline)	215±3.26	210±13.89	212±29.24
Control - (CMC & Vaseline)	216±6.52	215±15.12	214±30.03
Control + (Glibenclamide)	210±3.64	184±1.83	150±2.45
Control + (Nebacetin)	211±8.29	212±8.93	213±8.44
Control + (Glibenclamide & Nebacetin)	216±6.89	184±1.89	149 ± 1.73
Oral (noni + insulin leaves)	204±7.39	188±1.90	160±3.13*
Polyherbal topical	217±10.24	218±11.07	216±9.56*
combination (oral + topical)	216±10.24	185±11.07	161±9.56*

*) $p < 0.05$ is significantly different from the negative control group

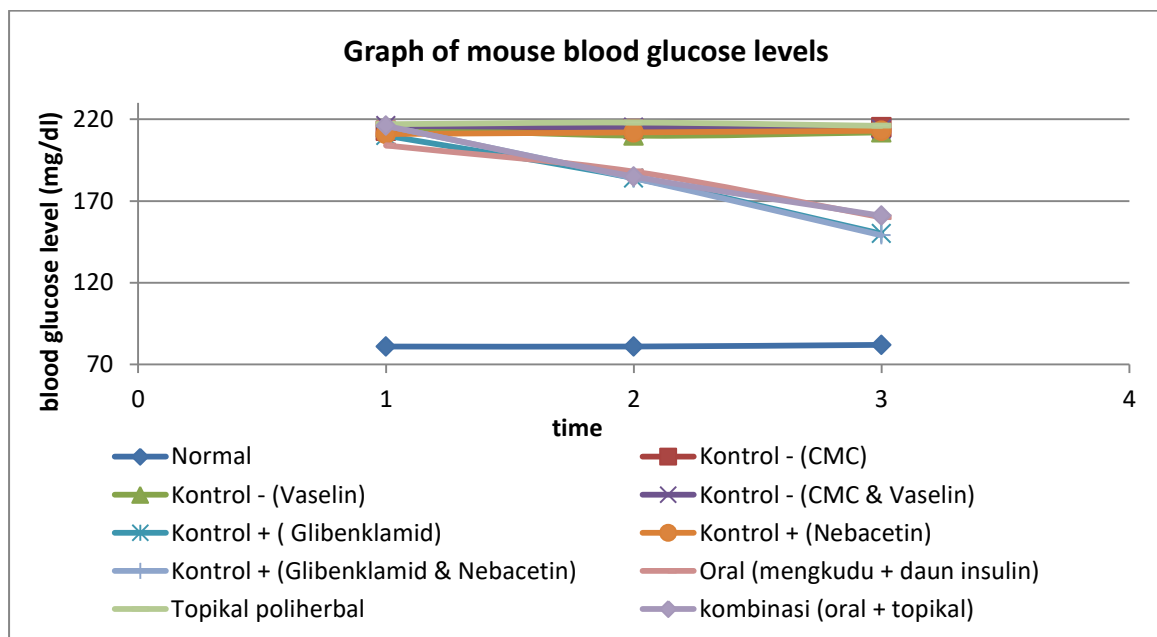


Figure 2. Graph of rat blood glucose levels

Based on the data above, it shows a high increase in blood glucose levels in the treatment group after being induced by STZ-NA in the 1st measurement. This shows that giving STZ-NA induction can increase blood glucose levels in mice, indicating that the induction was successful in making mice become DM with levels glucose above 200 mg/dL. On the 3rd day, some groups showed a decrease in glucose levels. The decrease in glucose levels was obtained from the difference between the initial DM glucose level and the glucose level on the day of measurement. There was a decrease in glucose levels in the positive control group due to the effect of glibenclamide and in the polyherbal group, glibenclamide had a hypoglycemic effect through selective inhibition of ATP-sensitive K^{+} ion

channels (K_{ATP}) in pancreatic β cells. The opening of K_{ATP} channels is regulated by the intracellular concentration of ATP. If there is an increase in ATP the channel will close, and if the ATP level decreases the channel will open. The activity of K_{ATP} channels in pancreatic β cells is regulated by blood glucose levels. If blood glucose increases, glucose will be transported into the pancreatic β cells and undergo metabolism. Glucose metabolism results in an increase in ATP levels which causes the closure of K_{ATP} channels. Closing this channel depolarizes which will open intracellular Ca^{2+} channels which in turn trigger insulin secretion (Ikawati, 2008). The effect of glibenclamide results in a decrease in glucose levels (Baroroh *et al.*, 2011 ; Pasaribu *et al.*, 2012; Rabbani *et al.*, 2010).

The polyherbal topical treatment group and the positive group with the negative group (nebacetin) showed blood glucose levels that increased but were not significant, this could be because topical administration was not effective in increasing insulin secretion in pancreatic β cells. The results of the statistical analysis test of rat blood glucose levels in the 1st measurement showed that the data was not normal because the significance value was (0.00) ($p < 0.005$), then the Kruskal-Wallis test was carried out, there was a significance value of (0.045) ($p > 0.05$) , in the 2nd measurement the data shows that it is not normal because the significance value is (0.00) ($p < 0.05$) so it cannot be analyzed because it is non-parametric, in the 3rd measurement the data shows normal by fulfilling the requirements of the parametric test with a significance value of 0 0.06 > 0.05 , then a *one way* ANOVA test was carried out and the results were significantly different from each control group and treatment group.

Results of Healing Skin Wounds in DM Rats

Wound healing can be observed macroscopically and microscopically. Macroscopic wound healing can be seen from the diameter of the wound. Measuring the diameter of the wound is the most important part of observing wound healing, measuring the wound is also a benchmark in the healing process. The diameter of wound healing in the positive and negative control groups as well as the treatment group with ethanol extract of insulin leaves can be observed by comparing the wound diameter of the normal group (without treatment). The diameter of skin wounds in DM mice can be seen in table 13.

Table 13. Diameter of ulcer wounds in DM rats

treatment group	Wound Diameter (mm)		
	Day 0	Day 7	Day 14
Normal	4.00	1.63 ± 0.50	1.02 ± 0.11
Control - (CMC)	4.00	4.11 ± 0.04	5.61 ± 0.63
Control - (Vaseline)	4.00	4.14 ± 0.07	5.63 ± 0.89
Control - (CMC & Vaseline)	4.00	4.08 ± 0.03	5.26 ± 0.60
Control + (Glibenclamide)	4.00	3.74 ± 0.11	1.48 ± 0.06
Control + (Nebacetin)	4.00	3.94 ± 0.08	1.21 ± 0.10
Control + (Glibenklami & Nebacetin)	4.00	3.56 ± 0.06	1.27 ± 0.20
Oral (noni & insulin leaves)	4.00	3.87 ± 0.07	1.35 ± 0.70
Polyherbal topical	4.00	3.9 ± 0.13	1.67 ± 0.09
Combination (noni & insulin leaves)	4.00	3.67 ± 0.025	1.07 ± 0.035

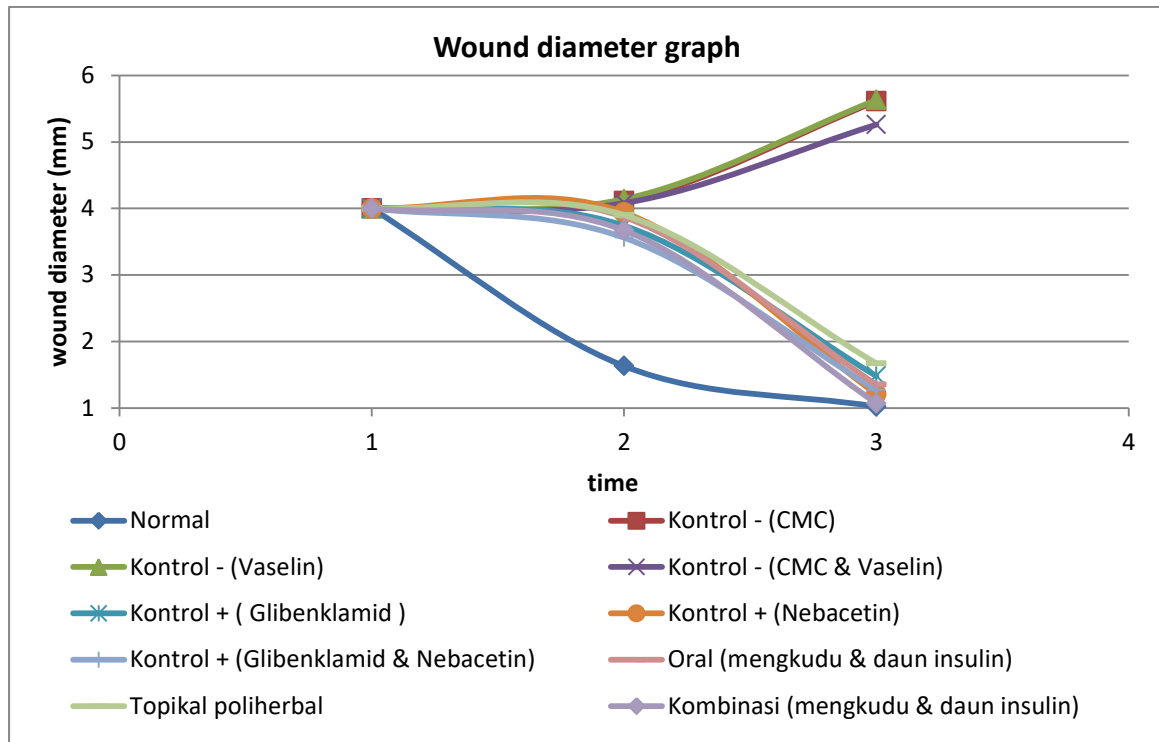


Figure 3. Wound diameter graph

Based on the table above, macroscopically, the level of healing in the group of mice induced by STZ-NA and treated using positive control in the form of nebacetin, glibenclamide and nebacetin+glibenclamide cream on the 14th day of treatment showed good healing with the wound closed as in the normal group. The same thing was shown in the treatment group, namely oral and topical healing. The negative group is the group that was induced by STZ-NA without any treatment. The negative control group did not show any healing compared to the other groups. Wound healing in diabetes sufferers takes a long time. This occurs due to various complications due to diabetes which interfere with physiological processes and the wound healing process. Administration of a polyherbal combination dose of 150 mg/kg BW orally and topically for 14 days was more effective in accelerating the healing of diabetic wounds in DM rats compared to the positive group. Apart from topical treatment, oral treatment can speed up wound healing because wound tissue also regenerates damaged cells from within the body. Based on the results of statistical analysis tests on days 7 and 14, it showed that there were significant differences between each group ($p < 0.05$).

CONCLUSION

The concentration of the polyherbal combination that has the best antibacterial activity against *S aureus* and *P aeruginosa* is a concentration of 20% with an inhibitory zone diameter of 19.33 mm and 23.33 mm. Administration of a polyherbal combination with an

oral dose of 150 mg/kg BW and a topical dose of 20% concentration has activity in healing diabetic ulcers.

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