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Research Article
Wound Healing and Blood Sugar Effect of *Psidium guajava* L. Leaves and *Melastoma malabathricum* L. Leaves on Rats with Diabetic Foot Ulcer

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Abstract

Background and Objective: Approximately 15% of people with DM will experience ulcers during their illness and 3-4% will have severe infections. This study aimed to examine the wound healing and blood sugar effect of *Psidium guajava* L. and *Melastoma malabathricum* L. (senduduk) leaves using diabetic foot ulcer rats. **Materials and Methods:** Diabetes were induced in the rat by single intraperitoneal injection of Alloxan monohydrate (120 mg kg^{-1}) and was injured by excision. The 30 rats were divided into following group: untreated control (group 1), group treated with metformin (group 2), group treated with senduduk leaves extract (group 3), group treated with guava leaves extract (group 4) and group treated with mixed extract of senduduk leaves and guava leaves (group 5). Blood sugar levels and wound area were measured before and after the intervention. **Results:** *In vitro*, the bioactive components of the mixed extract found were Flavonoid, Tannin, Saponin Steroid, total phenol 6.08%, quercetin 0.31 m g^{-1} and high antioxidant activity >31.25 ppm in the mixed extract. *In vivo* analysis of rat group with mixed extract of senduduk leaves and guava leaves significantly ($p<0.05$) was able to decrease blood glucose level and reduce wound area in rat's foot and was better than other groups. **Conclusion:** Senduduk leaf extract and guava leaf extract significantly ($p<0.05$) reduce blood glucose levels and reduce the area of injury in rat feet.

Key words: *Psidium guajava* L., *Melastoma malabathricum* L., wound area, blood sugar, diabetic foot ulcer

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes Mellitus is a degenerative disease that becomes a global threat. The data of the International Diabetes Foundation (IDF) in 2013 indicated that diabetes mellitus affects 382 million people worldwide. While the number of diabetics in Indonesia is about 8.6 million people and the 7th ranked in the world[1]. The majority (60-80%) of foot ulcers will heal, while 10-15% of them will remain active and 5-24% of them will finally lead to limb amputation within a period of 6-18 months after the first evaluation. Neuropathic wounds are more likely to heal for 20 weeks, while neuroischemic ulcers take longer and will more often lead to limb amputation[2].

Melastoma malabathricum (*M. malabathricum*) is an upright shrub as high as 0.5-4 m that has many branches and can grow in places that get enough sunlight such as on a mountain slope, bush, a field that is not too arid at altitudes up to 1,650 above sea level. The leaves are single, stemmed, located cross-faced and ovoid with a pointed tip and the surface is short-haired which is rare and stiff so palpable rough and has three curved leaf bones[3]. *Melastoma malabathricum* has a different name depending on the location where the plant was found and the community or tribe who use it traditionally for therapeutic purposes[4]. Generally, a different part of the *M. malabathricum* are used in folk medicine to the treatment of dysentery, diarrhea, hemorrhoids, leucorrhoea, wounds and cut mainly in India, Malay and Indonesia[5].

Same as *M. malabathricum* and *Psidium guajava* (guava) trees can be found in almost all tropical regions because they thrive in various types of soil, multiply easily and produce fruit quickly. The leaves and bark of guava trees are often used as traditional medicines such as diarrhea, dysentery, omitting and sore throat and to regulate the menstrual cycle. Guava leaves are rich in β flavonoids, especially quercetin which has an anti-bacterial and anti-diarrheal effect and can relax the small intestine. Five constituents including one new pentacyclic triterpenoid: Guajanoic acid and four compounds known as beta-sitosterol, uvaol, oleanolic acid and ursolic acid, were recently isolated from guava leaves[6]. Supplementing a balanced diet with guava leaf extracts may provide health-promoting effects[7].

Diabetic wounds are different from wounds under normal conditions. High blood sugar is a medium that is good for bacterial growth and if it occurs in diabetes ulcer patients will result in infection. This wound should be treated immediately to avoid damage leading to amputation. The wound management usually performed for diabetic foot ulcer (DFU) is debridement maintaining wound moisture, controlling inflammation and infection and increasing epithelial margins. One of the most common efforts is to control inflammation and infection by using topical and antibacterial anti-inflammation[8].

Many parts of the senduduk leaves and guava leaves have been studied regarding their chemical components of bioactive compounds and their use for human health. However, research combining senduduk leaves and guava leaves extracts for wound healing due to diabetes had not been proven *In vivo*. Therefore, the present study aims to find out wound healing and blood glucose effect of local guava leaves and senduduk leaves extract.

MATERIALS AND METHODS

This study was an experimental study with a Randomized Block Design. This study was carried out for 30 days and used *in vitro* analysis. Local guava leaves and senduduk leaves are obtained from the Bengkulu city area. Male rat (Sprague Dawley) strains are 8 weeks old, weighing 250-270 g. The tools used are TLC (Thin Layer Chromatography), spectrophotometry, IC-DPPH, FTIR (Fourier Transform Infra Red), HPLC, glucoDR biosensor, extraction equipment, Alloxan Monohydrate, ketamine, xylazine, Metformin and Quercetin.

This study has received ethics approval from Mohammad Hoesin Central General Hospital and Faculty of Medicine Sriwijaya University, number 104/kepkrsmhfkunsri/2017.

Extract procedure: Guava leaves and senduduk leaves were prepared by drying it up by using the oven at ±50°C so that the water content was 14%. Both types of leaves were cut into pieces and then blended, the second material of the leaves was macerated with 70% ethanol solvent. Followed by centrifuging at 10000 rpm, 4°C.

Phytochemical screening: Analysis of nutraceutical of guava leaves and senduduk leaves extract used proximate test with AOAC method[9]. Component analysis of phytochemical compounds with TLC (Thin Layer Chromatography made in Japan, Merck Hitachi, 2004) and HPLC (Japan, Merck Hitachi, 2005) for the components of β-carotene, triterpenoids, glycans and alkaloids. Analysis of antioxidants with 1,1-diphenyl-2-pikrilhidrazil (DPPH-IC50). The FTIR (Fourier Transform Infra Red) was measured by Bruker Tensor 37 tools (German, 2004).

Grouping of animals: Thirty rats were grouped into 5 groups in a hyperglycemic condition, so the rats were induced by using an injection of Alloxan Monohydrate 10 g which was

dissolved in saline solution with a single dose of 120 mg kg^{-1} BW intraperitoneally. Alloxan functioned to damage the pancreas, so that blood sugar level increased. *Psidium guajava* leaves, *M. malabathricum* leaves and mixtures given orally via sonde (oral) were given 0.5 mL at a dose of 300 mg kg^{-1} b.wt., once a day. Six rats represented each treatment group for each method of diabetic foot wound induction. In group 1 was given a negative control (aquadest), group 2 was given a positive control (metformin), group 3 was given senduduk leaves extracts, group 4 was given guava leaves extract and group 5 was given a mixed extract of senduduk leaves and guava leaves.

Excision wound ulcer: The rats of the diabetic group (day 0) were anesthetized with a combination of ketamine (100 mg kg^{-1}) and xylazine (5 mg kg^{-1}) intramuscularly. It was done to eliminate the pain while performing the excision method. A rectangle tool was marked on the dorsal surface of the foot and then the skin layer in the full thickness (standard area 4×10 mm) was exfoliated and modified[10]. One day after wound induction (day 1), the wound became larger.

Blood glucose level: Blood samples were taken from the base of a rat tail after 1 night fasting and blood glucose level were measured with a tool of gluco DR Biosensor made in Korea type AGM 2100.

Statistical analysis: Result obtained has been expressed as Mean±SEM. Data were analyzed using one-way analysis of variance (ANOVA) with a significant level of $p<0.05$.

RESULTS

Phytochemical analysis (*In vitro*)

Qualitative identification of 3 sample extracts: The results of the qualitative phytochemical analysis in the samples of senduduk leaves extract (sample 1), guava leaves extract (sample 2) and the mixed extracts of senduduk leaves extract and guava leaves extract (sample 3) showed that all the extracts had the same group of secondary metabolic such as flavonoids, tannins and steroids, except the saponin content found in sample 1 and 3 (Table 1).

Quantitative analysis and antioxidant activity: In the antioxidant activity, the results of the 3 leaf extracts of senduduk leaves, guava leaves and mixed extract were very strong, especially in the mixed extract which has an antioxidant activity of <31.25 ppm compared with senduduk leaves. Quantitatively, senduduk leaves extract had a total phenol of 4.23%, quercetin 0.35 mg g^{-1}. Besides, guava leaf extracts had a total phenol of 6.19%, quercetin 0.36 mg g^{-1} and mixed extracts of senduduk leaves and guava leaves extract quantitatively had total phenol of 6.08%, quercetin 0.31 mg g^{-1} (Table 2).

Table 1: Identify the qualitative 3 types of extracts

Samples	Identify the sample condition	Bioactive compound (Phytochemical)	Results
Extract 1	Solids	Flavonoids	Positive negative
Senduduk (*Melastoma malabathricum* L.) leaves		Alkaloids tannins saponins quinone steroids triterpenoids	Positive
			Positive
			Negative
			Positive
			Negative
Extract 2	Solids	Flavonoids	Positive negative
Guava leaves (*Psidium guajava* L.)		Alkaloids tannins saponins quinone steroids triterpenoids	Positive
			Negative
			Negative
			Positive
			Negative
Extract 3	Solids	Flavonoids	Positive negative
Mixed of senduduk leaves and guava leaves		Alkaloids tannins saponins quinone steroids triterpenoids	Positive
			Positive
			Negative
			Positive
			Negative

Table 2: Quantitative analysis and antioxidant activities

Samples	Identity the sample condition	Parameters	Units	Results
1st sample senduduk leaves	Solids	Total phenol antioxidant IC_{50} DPPH quercetin	% (b/b), ppm, mg/g	4.23 37.13 0.35
2nd sample guava leaves	Solids	Total phenol antioxidant IC_{50} DPPH quercetin	% (b/b), ppm, mg/g	6.19<31.25 0.36
3rd sample mixed senduduk leaves and guava leaves	Solids	Total phenol antioxidant IC_{50} DPPH quercetin	% (b/b), ppm, mg/g	6.08 <31.25 0.31

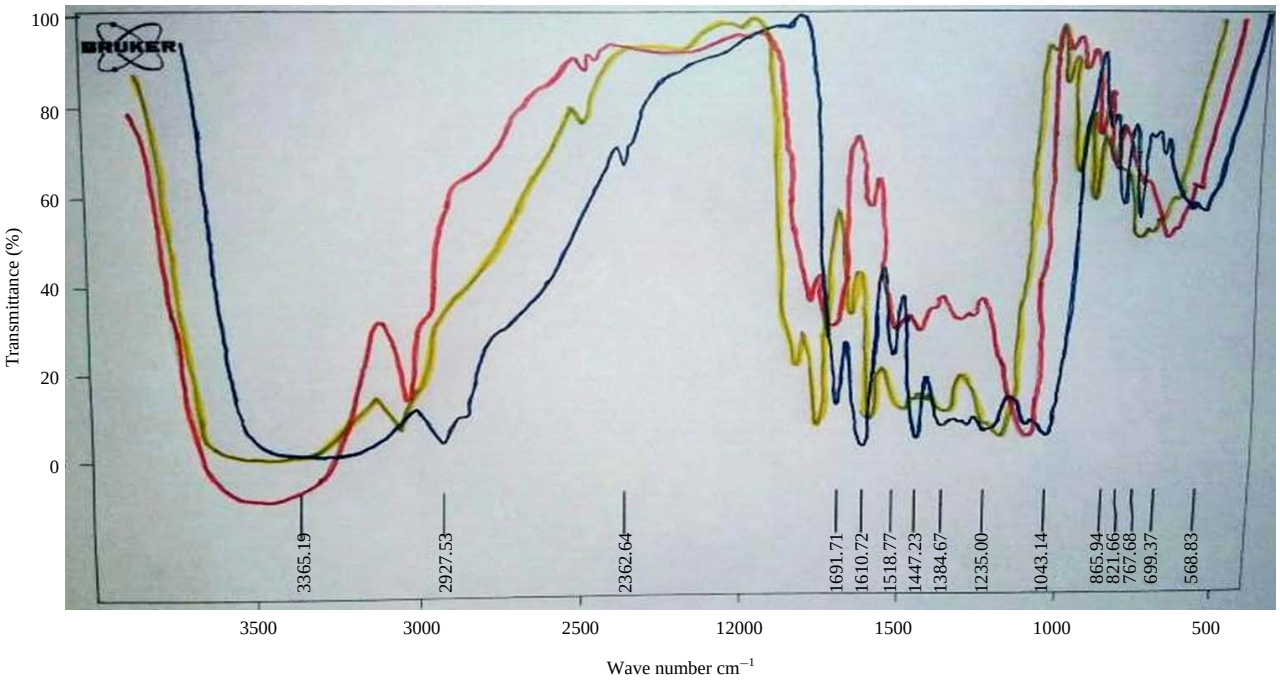

Fig. 1: Spektra FTIR and phytochemical component group 3 leaves extract
Blue line: Guava leaves extract, Yellow line: Mixed extract of guava leaves and senduduk leaves, Redline: Senduduk leaves

Antioxidant activity with 1,1-difenil-2-pikrilhidrazil (DPPH)-IC_{50} method: The three samples of senduduk leaves extracts, guava leaves and mixed extract of senduduk leaves+guava leaves had an IC_{50} value <50 ppm, with this result, it means that the potent antioxidant activity potency is expected *in vivo* to have systemic effect, as anti-inflammation, wound healing and phenol content, quercetin which will play a role in lowering blood sugar levels.

Fourier transform infra red (FTIR) analysis: The spectra of extracts phytochemical components analysis from samples 1, 2 and 3 by using FTIR can be seen in Fig. 1.

Characterization of the electrical conductivity of complex compounds used a conduction meter aimed to determine the type of complex mixtures whether they were molecular or ionic. The complex compound which was measured by its electrical conductivity then was compared with the electrical conductivity of the solvent used, DMSO (Dimethyl Sulfoxide).

This study showed that the three samples had the gallic acid and methyl error compounds based on the absorption area 3373.81-3396.05 and 1692.26-1633.19 cm^{-1}, it was similar to previous research[11].

***In vivo* analysis of influence 5 treatment of blood glucose level and wide foot wound on diabetes rats:** In all treatment groups before the blood sugar level intervention was relatively similar, this indicated that no sample had high sugar content due to the derivative. After 5 days post-alloxan induced, it increased blood sugar levels in all treatment groups. Furthermore, the effect of intervention on blood glucose level in 5 treatment groups were negative control (aquadest) 311.83 mg dL^{-1}, positive control (metformin) 175.16 mg dL^{-1}, guava leaves extract 297 mg dL^{-1}, senduduk leaves extract 199.8 mg dL^{-1} and mixed extracts of 195.75 mg dL^{-1}. The five treatment groups of rats that were given a significantly mixed extract (<0.000) proved to be able to lower blood sugar.

Effect of 5 treatments on blood sugar level: In 5 treatment groups of rats on blood sugar level after given alloxan, the blood sugar level increased 300 mg dL^{-1} on average. It was different from blood sugar level after post-intervention that decreased 310-175 mg dL^{-1} on average. Two extract groups had a relatively similar decrease, i.e., senduduk leaves extract and mixed extracts at 198.8 and 195 mg dL^{-1}. Out of 3 samples, extract and positive control which was given metformin were significantly (<0.05) able to lower blood sugar glucose level.

This study showed that the blood sugar level after 5 days of alloxan induction increased by an average of 300 mg dL^{-1}. Blood sugar level that given positive control by giving metformin antihyperglycemic drugs can significantly lower blood sugar level with an average of 310-175 mg dL^{-1}, but it needed to be alerted and controlled regularly because at a

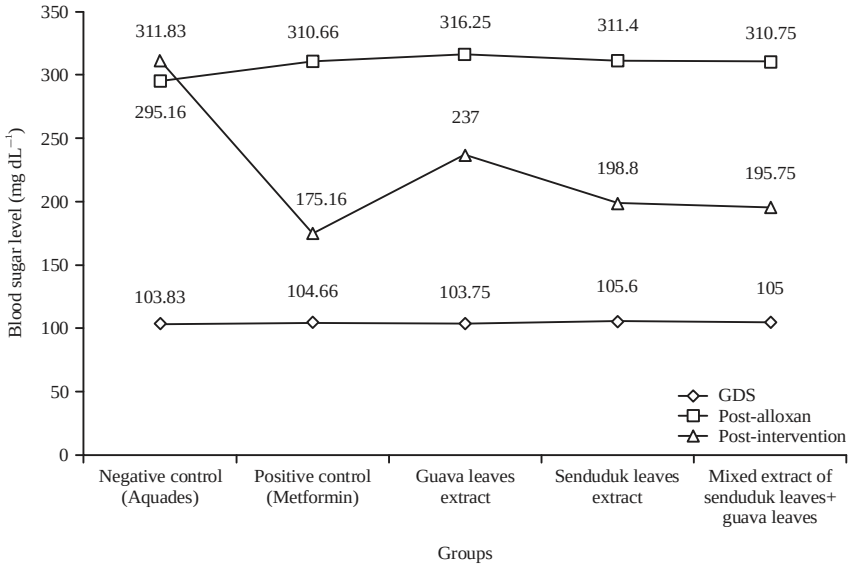

Fig. 2: Graphics of the difference between blood sugar level during, post-alloxan and post-intervention

Table 3: Effect of treatment groups on blood glucose level and rat foot extension area post-induced alloxan and post-intervention

Treatment groups	Phase	Blood sugar level	Areas of wound
Negative control (Aquadest)	Post-Alloxan	295.16±19.75	40.00±0
Positive control (Metformin)		310.66±6.28	40.00±0
Guava leaves extract senduduk leaves extract mixed extract		316.25±9.94	40.00±0
		311.40±6.42	40.00±0
		310.75±6.39	40.00±0
p-value		0.071	NS
Negative control (aquadest)	Post-intervention	311.83±35.54	71.83±10.36
Positive control (Metformin)		175.16±12.57	29.83±6.27
Guava leaves extract senduduk leaves extract mixed extract		297.00±49.93	31.33±16.44
		199.80±8.10	29.6±4.66
		195.75±4.5	23.5±3.69
p-value		0.000	Significance

NS: No significance

particular time it was possible that hypoglycemic occurs. In the 3rd of group extract, the blood sugar decrease was relatively the same in the group of senduduk leaves extract and the mixed extract of senduduk leaves and guava leaves of 198.8 and 195 mg dL^{-1} (Fig. 2).

The result showed that the positive control group had given metformin and 3 sample extracts were able to decrease blood glucose level at the end of the intervention and proved significantly ($p<0.05$) (Table 3).

Effect of 5 treatments on the areas of wound of diabetic rats: In all treatment groups, incision of the wound after 5 days of alloxan was made 4×10 mm wound. After being given metformin, the areas of the injury in the diabetic rat's feet decreased with an average of 23.83-29.83 mm^2. The diabetic rat's treatment group that with the lowest reduction rate of wound area was the group given the mixed extract of senduduk leaves and guava leaves. Significantly (<0.000), the mixed extract of senduduk leaves and guava leaves had a significant effect of reducing wound area (Fig. 3).

The results of this research showed that the positive control group had given metformin the areas of diabetic rat's feet decreased from 40-29.6 mm^2 and the reduced level of wound areas was the lowest in the group given the mixed extract of guava leaves and senduduk leaves orally and topical treatment rubbed into the feet.

DISCUSSION

Qualitatively and quantitatively, the three of senduduk leaves extract, guava leaves extract and mixed extracts of senduduk and guava leaves had a potential as nutraceutical which is beneficial for health.

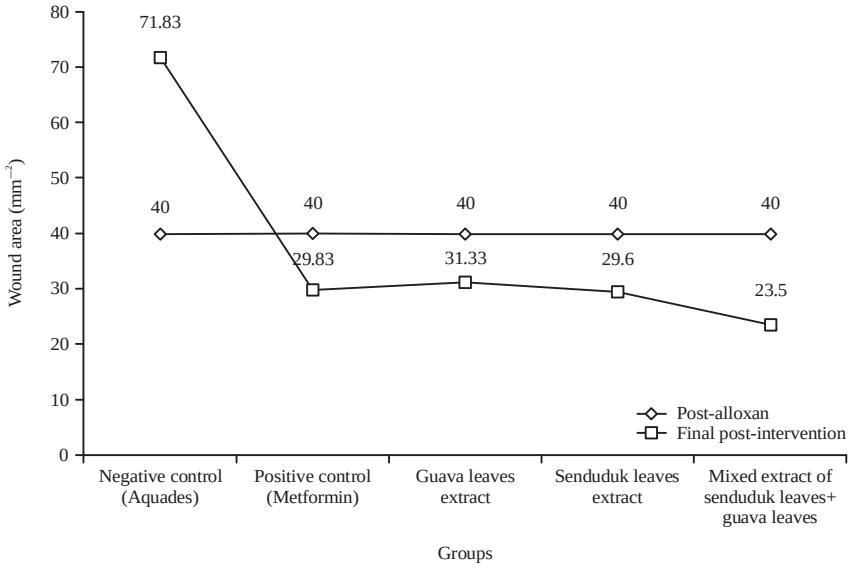

Fig. 3: Graphics of the difference between wound areas of rats' feet post-alloxan and post intervention

The mixture extract of senduduk and guava leaves have a high antioxidant activity. It means that the potential of capturing free radicals by DPPH-IC_{50} method depends on the value of ppm (part per million), the lower value of ppm indicated that the stronger the ability of antioxidant activity in food material[12].

The three extracts samples of senduduk leaves, guava leaves and mixed extract of senduduk leaves and guava leaves had an IC_{50} value <50 ppm, with this result, it means that the potent antioxidant activity potency is expected *in vivo* to have systemic effect, as anti-inflammation, wound healing and phenol content, quercetin which will play a role in lowering blood sugar levels. It can be concluded that *in vitro* the three extract materials have the potential of bioactive substance such as Flavonoids, Alkaloids, Tannin, Saponin, Quinone, Steroids and Triterpenoids and in quantity it has a component phenol and high antioxidant activity. The steroid content serves as an anti-inflammatory, saponin serves as an antiseptic that has a benefit to kill and prevent the growth of micro-organisms and tannins are useful as astringents that can cover the skin pores, harden the skin and stop light bleeding[13].

Phenol compounds have a strong association with antioxidants because they can contribute hydrogen to free radicals. The antioxidant activity of the extract which is suppressed by a polar organic solvent such as methanol is greater than the extract obtained from by non-polar organic solvents such as hexane and chloroform shows that polar compounds such as phenolics are responsible for antioxidant activity[14,15].

Guava leaf extracts showed potential antioxidant activity. The higher the concentration used the higher the free radical-scavenging effect. The parameter, antiradical efficiency (AE) has been found to be an adequate parameter for selecting antioxidants than the widely[7] used EC_{50}. Nurdiana's research on the content of flavonoids and tannins in Melastoma m leaves showed that the quantitative determination of the total flavonoid content of extracts expressed as a percentage of catechins per 10 mL of water extract showed a content value of 10.8 w/v. Whereas, for total tannin levels expressed as the equivalent of mg tannic acid and per 10 mL of the sample showed a content value of 6.2 w/v. The results show Malabathricum leaf water extract contains less tannin than flavonoids. This may be because the solubility of the tannin in a solution of water is less than that of flavonoids[16].

Blood sugar level that given positive control by giving metformin anti-hyperglycemic drugs can significantly lower blood sugar level with an average of 310-175 mg dL^{-1}, but it needed to be alerted and controlled regularly because at a particular time it was possible that hypoglycemic occurs. In the third of group extract, the blood sugar decrease was relatively the same in the group of senduduk leaves extract and the mixed extract of senduduk leaves and guava leaves of 198.8 and 195 mg dL^{-1}.

The result showed that the positive control group had given metformin and 3 sample extracts were able to decrease blood glucose level at the end of the intervention and proved significantly ($p<0.05$). Furthermore, another research

conducted intervention by giving 4-ethyloxychalcone on alloxan-induced rats concluded that the very significant decrease was significant ($p<0.001$) when compared to the diabetic control group during the experiment. The reduction in blood glucose caused by chalcone was also proportional to the standard of glibenclamide drug. The action mechanism of this compound is currently under further research[17].

Significant blood glucose lowering effects of the *P. guajava* leaves extract were observed by Oh W.K. *et al.*[18], after intraperitoneal injection of the extract at a dose of 10 mg kg^{-1} in both 1 and 3 month old Lepr (db)/Lepr (db) mice.

According to Nurdiana and Marziana[16], rats which treated by *M. malabathricum* leaves extract showed the finest scar with little inflammation and no microbe infection compared to other treatments. The aqueous extract of *M. malabathricum* leaves showed the highest concentration of flavonoids and also the presence of tannins had improve wound healing activities for the excised wound.

Statistically, each group significantly reduced the areas of wound to rat's feet, but reduced signs of inflammation such as swelling, redness and shrinkage of the injury surface were better in the intervention group given the extract guava leaves and senduduk leaves orally and on topical wound treatment with the principle of "Moisture wound healing dressing"[19]. It was proved that the mixed extract group of guava leaves and senduduk leaves was more potential and had minimum side effects.

CONCLUSION

Based on the results of research which had been conducted on male rats (Sprague Dawley) induced with alloxan extracts intervened with mixed extract of senduduk leaves and guava leaves orally and topical treatment on injury were significantly ($p<0.05$) able to made lower blood glucose level and reduced the areas of wound to rat's feet.

SIGNIFICANCE STATEMENT

This study discovered the mixed extract group of guava leaves and senduduk leaves was more potential and had minimum side effects that can be beneficial for healing and preventing diabetic foot ulcer from amputation, although this research used rats for the animal model in our experiment.

This study will help the researchers to uncover the critical areas of wound healing using mixed extract guava leave and senduduk that many researchers were not able to explore. Thus a new theory on foot diabetic ulcer with innovation may have arrived.

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