Jurnal Biodjati 9(1): 41-53, May 2024

e-ISSN : 2541-4208
p-ISSN : 2548-1606

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

Antidiabetic Potential of Ethanol Extract from *Moringa oleifera* Leaves in Streptozotocin-Nicotinamide-Induced Female *Mus musculus*

Vinsa Cantya Prakasita[*1], Nadya Aprina Theodora Pangaribuan[2], Enjelin Anjung Susilowati[3], Dwi Adityarini[4], Aniek Prasetyaningsih[5]

Received: November 09, 2023
Revise from: December 19, 2023
Accepted: March 10, 2024

DOI: 10.15575/biodjati.v9i1.30699

[1,2,3,4,5]Department of Biology, Faculty of Biotechnology, Universitas Kristen Duta Wacana, Jl. dr. Wahidin Sudirohusodo no. 5-25 Yogyakarta, Indonesia – 55224.

e-mail:

[1*]*vinsa.cantya.p@staff.ukdw.ac.id*
[2]*nadya.pangaribuan@students.ukdw.ac.id*
[3]*enjelin.susilowati@students.ukdw.ac.id,*
[4]*dwi.aditiyarini@staff.ukdw.ac.id*
[5]*aniek@staff.ukdw.ac.id*

**Corresponding author*

Abstract. *Type 2 diabetes mellitus (DM) is a chronic metabolic disorder characterized by elevated blood glucose levels due to insulin resistance. Type 2 diabetes is considerably more prevalent than other forms (85–90%). The risk of type 2 diabetes is higher in women (53.2%). There is an urgent requirement for better and more affordable treatment options considering DM therapy is expensive and may have adverse health effects. The study's objective is to examine how acute toxicity, blood glucose levels, and body weight are affected by Moringa leaf ethanol extract (MLEE). Maceration was employed to eliminate the leaves of the moringa plant. Phytochemical screening was completed to assess the total flavonoid content and screen for alkaloids, flavonoids, phenolics, saponins, and tannins. Acute toxicity testing was performed following OECD guideline 423. Clinical symptoms of acute toxicity were observed every 30 minutes for the first 24 hours post-treatment, followed by observations every 24 hours up to 14 days. The estimated LD50 range was determined. Streptozotocin/nicotinamide-induced female Mus musculus was administered to evaluate the antidiabetic potential of MLEE. Six groups of mice were utilized, which included a healthy control group (aquades not induced), a negative control group (induced aquades), a positive control group (induced glimepiride 0.8 mg/kg BW), and three treatment groups with varying dosages of MLEE (induced; 0, 100, and 150 mg/kg BW). A semi-auto chemical analyzer was employed on days 0 through 31 to determine blood glucose levels. An analytical digital balance was utilized to calculate the body weight. With a total flavonoid concentration of 20.75%, MLEE incorporated alkaloids, flavonoids, phenolics, saponins, and tannins. MLEE demonstrated a significant effect in lowering blood glucose levels at a dose of 100 mg/kg BW ($P<0.05$). A significant positive correlation has been identified between body weight and blood glucose levels ($P<0.05$).*

Keywords: *Antidiabetics, acute toxicity, blood glucose, body weight, moringa leaves, female mice*

Citation
Prakasita, V. C., Pangaribuan, N. A., Susilowati, E. A., Adityarini, D., & Prasetyaningsih, A. (2024). Antidiabetic Potential of Ethanol Extract from *Moringa oleifera* Leaves in Streptozotocin-Nicotinamide-Induced Female *Mus musculus*. *Jurnal Biodjati*, 9(1), 41-53.

Jurnal Biodjati 9(1): 41-53 May 2024

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease characterized by elevated blood glucose levels exceeding the normal range (Ministry of Health, 2020). Consistent high glycemic index food consumption can result in long-term hyperglycemia and consequences such as stroke, kidney failure, cataracts, and heart attacks (Almatsier, 2006). With a frequency of 11.3%, Indonesia ranked third among Southeast Asian countries with 463 million cases of diabetes worldwide, according to data from the International Diabetes Federation (IDF) (2019). By 2040, IDF projects that there will be 642 million DM cases globally (IDF, 2019). Type 2 diabetes is more prevalent (85–90%) in Indonesia. Insufficient physical exercise and bad lifestyle choices cause insulin resistance, which impairs the body's capacity to react to insulin (Ministry of Health, 2020). This results in type 2 diabetes. Type 2 DM can affect males and females, but females have a higher risk (53.2%).

The more intricate hormonal impacts on women, which could elevate blood sugar and body mass index, impact this (Bennett, 2000; Banner et al., 2009; Irawan, 2010). Most current treatments are synthetic medications such as insulin, biguanides, and sulfonylureas. Patients who require permanent or extended medical care incur high expenses. Health-related side effects must also be taken into consideration to prevent consequences such as kidney failure (Hardianto, 2020).

Indonesia has the second-largest biodiversity after Brazil with over 25,000–30,000 thousand plant species. About 1,200 are considered therapeutic plants, such as moringa, ginger, turmeric, and bitter melon (Zega et al., 2016). The herb Moringa oleifera is widely recognized for its medicinal properties. It is frequently used for therapeutic purposes and typically in tropical and subtropical locations (Aminah et al., 2015). Moringa's phytochemical components are primarily responsible for its health benefits. Alkaloids and flavonoids are the two main kinds of phytochemicals discovered in moringa. Pitriya et al. (2017) suggest that these substances have antidiabetic properties.

Research conducted by Ambarwati et al. (2014) and Kamaliani et al. (2018) demonstrated that moringa leaf extract at a dose of 500 mg/kg body weight could lower blood glucose levels in male mice. To produce an antidiabetic effect, this dose is regarded as excessive. The phytochemical components in the extract can be influenced by the type of solvent and extraction technique, which in turn can change the biological activity that results. The maceration procedure with a 70% ethanol solvent is the most effective way to produce moringa leaf extract with the maximum flavonoid content, claim Laksmiani et al. (2020). It is widely recognized that flavonoids lower blood glucose levels. Despite type 2 diabetes cases being more prevalent in females, male experimental animals were frequently employed in previous studies on antidiabetic potential. Therefore, considering acute toxicity, blood glucose levels, and body weight, this study intends to investigate the antidiabetic potential of moringa leaf extract derived through maceration in female mice.

MATERIALS AND METHODS

Preparation of Moringa Leaf Ethanol Extract (MLEE)

Moringa leaf powder was obtained from the Laboratory of Herbal Materia Medica, Batu, Malang, East Java. The crude material was extracted by applying the re-maceration method. A total of 2000 g of moringa leaf powder was soaked in 70% ethanol (1:10 w/v) for 72 hours. The macerate was d-thickened using a rotary evaporator at 50°C until a dense extract was obtained. The yield was calculated by employing the formula (Fajarullah et al., 2014):

Yield (%) = (Weight of extract (g))/(Weight of sample (g)) x 100%

Phytochemical Screening

Flavonoids, alkaloids, saponins, phenols, and tannins were qualitatively examined in the ethanol extract of moringa leaves (Harborne, 1998). Pradana (2019) employed a significantly modified aluminum chloride colorimetric method to determine the total flavonoid concentration. The calibration employed quercetin, which was 25 mg dissolved in 96% ethanol and diluted to 6, 8, 10, and 12 ppm concentrations. Each concentration of the standard and sample solution was mixed with 1 mL of 2% aluminum chloride and 1 mL of 120 mM potassium acetate.

Using a UV-Vis spectrophotometer, the absorbance at 435 nm was measured after the combination was incubated for 60 minutes at room temperature, and it was compared to a blank that consisted solely of aluminum chloride. The total flavonoid content was calculated as mean ± SD (n = 3) and expressed as milligrams of quercetin equivalent (mg QE) per gram of extract.

Antidiabetic Potential of Moringa Leaf Ethanol Extract (MLEE) on Female Mice:

Test Animals

Female DDY (Deutschland Denken Yoken) strain mice aged 6-8 weeks with body weights of approximately 25-30 g were used in the study. The mice were acquired from UGM/LPPT. They were kept in cages with four randomly assigned mice per cage, 20–25°C temperature range, 45–55% humidity, and a 12-hour light/dark cycle (Yusuf & Al-Ghizar, 2022; Huet et al., 2013). Water and standard pellets were distributed openly, except for fasting periods. There was a one-week acclimatization phase. Animal care and handling were conducted under the globally recognized ethical principles for using laboratory animals, as established by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources Commission on Life Sciences, National Research Council (1985). The Ethics Committee for Health Research, Faculty of Medicine, Duta Wacana Christian University, Yogyakarta, authorized the research protocol under Ethical Clearance No. 1536/C.16/FK/2023.

Acute Toxicity Test

The acute toxicity test followed OECD guideline 423 (OECD Guideline for Testing of Chemicals, 2001). The mice were fasted overnight and provided only drinking water (Ragavan et al., 2006). The mice received 5, 50, 300, and 2000 mg/kg BW MLEE dosages, respectively, and were split into four groups (n = 3). Every 30 minutes for the first 24 hours following treatment (with special attention during the first 4 hours), then every 24 hours until 14 days, clinical symptoms of acute toxicity such as hypersalivation, skin irritation, changes in eyes and mucous membranes, respiratory disturbances, diarrhea, activity changes (lethargy), tremors, seizures, coma, and lethality were observed (Burger et al., 2005). Four hours after the extract was administered, food was served. The LD50 estimation range was measured. If mortality occurred during the test, mice were necropsied, and organ changes were observed.

Experimental Diabetes Induction

Blood glucose levels were assessed after the mice were starved for 12–14 hours. Diabetesogenic drugs, specifically streptozotocin and nicotinamide, were administered to induce diabetes. First, 120 mg/kg BW of nicotinamide and 50 mg/kg BW of streptozotocin induction were given intraperitoneally (Sari et al., 2017; Ali et al., 2015). The injections were performed in a citrate buffer solution at a pH of 4.5. According to Donovan and Brown (2006), the second induction was carried out on day 7 using streptozotocin (90 mg/kg BW) and nicotinamide (120 mg/kg BW). The mice's blood glucose levels were assessed 72 hours after induction using blood drawn from the retroorbital sinus. The research employed mice with blood glucose levels over 175 mg/dL.

Experimental Design

The blood glucose levels of the mice were assessed by grouping them into six groups of four

mice each. Group 1's mice were not stimulated as the healthy control group and received no medication. Group 2 was induced mice treated with aqua bides as a negative control. The induced mice in Group 3 were administered glimepiride at a dose of 0.0156 mg/30 g BW, serving as the positive control. Treatment groups 4, 5, and 6 received MLEE at dosages of 50, 100, and 150 mg/kg BW in that sequence. On days 0–7, 10, 17, 24, and 31, each group's blood glucose levels and body weights were determined (Nagappa et al., 2003). Aqua bides, glimepiride, and MLEE were administered orally at 0.5 mL per mouse per day for 21 days.

Blood Glucose Level Measurement

A microhematocrit capillary collected blood samples via the retroorbital sinus, which was then placed in microtubes. For ten minutes, the blood was centrifuged at 2500 rpm. Serum that had been separated was put into fresh microtubes. An auto-analyzer photometer with semi-chemistry was utilized to measure blood glucose levels. A 12 x 75 mm reaction tube was filled with 1 mL of glucose reagent and 20 µL of serum and gently mixed. The sample was then incubated at 37°C for 10 minutes, after which the glucose levels were determined using photometry.

Data Analysis

Data were presented as mean ± standard deviation. Bar and line diagrams were created using Ms. Excel 365 software. Blood glucose data were analyzed using Kruskal-Wallis's and Dunn's post hoc test using SPSS software version 20.0.

RESULTS AND DISCUSSION

Moringa Leaves Ethanol Extract (MLEE)

The Moringa leaf crude material used in this study was obtained from the Laboratory of Herbal Materia Medica, Batu, East Java. The plant utilized is a member of the Moringa oleifera Lamk species, according to the results of the Herbal Materia Medica Laboratory, Batu, No. 067/193/102.20/2023. Using 70% ethanol as the solvent, the maceration method was the extraction technique utilized for the crude material made from Moringa leaves.

This technique was employed to break down and soften plant cell walls to promote the release of soluble phytochemical substances (Handa et al., 2008). Maceration was selected because it is a straightforward process that does not involve heat, which could harm plant chemicals. It was selected since ethanol is a polar solvent and can dissolve polar substances, including flavonoids, alkaloids, saponins, and tannins. Ethanol can penetrate cell walls, enabling the diffusion of cells and facilitating the quicker extraction of bioactive compounds (Prayitno & Rahim, 2020). Furthermore, ethanol is safe, easily obtainable, and highly efficient in extraction (Chen et al., 2020). According to Lee et al. (2017), ethanol has qualities that make it acceptable for consumption and food grade, making it safer to use than methanol or acetone.

A concentrated extract weighing 504 g was recovered from the 2000 g crude material, yielding 25.2%. Vongsak et al. (2013) reported a yield of 40.5%, higher than this. In contrast, Fatmawati and Aji (2019) reported a yield of 13.66%, while our yield was greater. Factors like the length of the extraction process can be responsible for variations in yield numbers. Larger yields are produced since the solvent and sample are in contact for a longer period during longer extraction durations (Handayani et al., 2016). Furthermore, the solvent to sample volume ratio is another factor affecting yield differences; a greater solvent volume leads to higher yields (Aziz, 2009). Vongsak et al. (2013) used a sample-to-solvent ratio of 1:40 (w/v), while Fatmawati and Aji (2019) used a ratio of 1:5 (w/v), resulting in a smaller yield.

Phytochemical Compounds in Moringa Leaves Ethanol Extract (MLEE)

Plants that contain phytochemical compounds can benefit humans in several methods and have pharmacological effects on the body. Phytochemical screening is a technique that identifies groups of phytochemical compounds by examining color or shape changes brought on by interactions with particular solutions. The ethanol extract of Moringa leaves underwent phytochemical screening, yielding positive results for tannins, alkaloids, phenolics, flavonoids, and saponins (Table 1).

These results are consistent with those of Putra et al. (2016) and Prasetyana et al. (2022). It should be highlighted, nonetheless, that environmental and geographic factors might cause variations in the secondary metabolite composition even within the same plant species (Katuuk et al., 2018; Agustina et al., 2016; Nurfitriani, 2016). Plant secondary metabolites provide the body with biological advantages and functions. The five secondary metabolites are alkaloids, flavonoids, phenolics, tannins, and saponins. They are antioxidants that counteract free radicals in the body, preventing oxidative damage and reducing the aging process of cells (Saputra et al., 2020). Through a variety of processes,

Table 1. Results of Phytochemical Compound Screening in *Moringa oleifera* Leaves.

Compound Group		Result	Remark
Alkaloid	Mayer	Positive	Presence of precipitate
	Dragendroff	Positive	Presence of precipitate
	Wagner	Positive	Presence of precipitate
Phenolic		Positive	Purple-blue coloration
Flavonoid		Positive	Orange to reddish coloration
Saponin		Positive	Presence of froth or foam
Tannin		Positive	Dark blue-black coloration

Total Flavonoid Content in Ethanol Extract of Moringa Leaves

Determining total flavonoid content aims to quantify the number of flavonoids present in a sample. It is widely recognized that flavonoids are present in almost every component of a plant. Because flavonoids may lower blood glucose levels in mice, this study concentrated on them.

The total flavonoid content analysis demonstrated that the MLEE had 20.75 mg/g of flavonoids. This figure is greater than the findings of the same extraction method employed by Pradana (2019) and Susanty et al. (2019), who reported a total flavonoid content of 7.9 mg/g and 8.04 mg/g, respectively. Variations in the location of the moringa leaf sample collection could cause discrepancies in the results. Nutrients from photosynthesis are required for the synthesis of flavonoids. Considering photosynthesis depends on light, places with strong light levels are necessary. This element affects the number of flavonoids and how they function in plants (Sari, 2015).

Acute Toxicity Test

The acute toxicity study revealed that the graded doses of the ethanol extract did not exhibit observable signs of toxicity up to a dose of 2000 mg/kg. This result is consistent with the study of Moodley (2017). There

were no significant changes in behavior, motor activity, lethargy, paralysis, respiration, restlessness, tremors, seizures, or coma. Furthermore, no deaths were observed over the two weeks. This indicates that the lethal dose 50 (LD50) of MLEE is greater than 2,000 mg/kg body weight of mice, and MLEE is non-toxic (Loomis & Hayes, 1996).

Potential Anti-diabetic Effects of Moringa Leaves Ethanol Extract (MLEE) in Female Mice

Type 2 diabetes (DM2) is a condition characterized by insulin resistance despite normal insulin production (Garcia et al., 2020). The diabetogenic drugs streptozotocin (STZ) and nicotinamide (NA) are used to cause type 2 diabetes in mice. Streptomyces achromogens is the bacteria that produces STZ, an antibiotic. An analog of N-acetylglucosamine, this inducer damages DNA in β pancreatic cells by generating nitric oxide (NO) molecules during cellular metabolism. It is transported into the cells by the glucose transporter (GLUT-2) (Tesch & Allen, 2007; Rakieten et al., 1963). To replicate the conditions of type 2 diabetes, NA is required to safeguard β pancreatic cells, resulting in insulin resistance equivalent to that of type 2 diabetes patients (Ruskar, 2010; Knip, 2000).

Malole & Pramono (1989) show that mice's normal blood glucose ranges from 62.8 to 175 mg/dL. Blood glucose levels were not elevated above normal levels seventy-two hours after the initial induction, meaning a second induction using a higher dose of STZ—90 mg/kg body weight—was required. This is because, in comparison to male mice, female mice have greater resistance to STZ (Saadane et al., 2020). In female mice, estrogen enhances insulin production in response to STZ and protects β pancreatic cells from oxidative stress and death (Tiano et al., 2012; Liu et al., 2010). Three days after the initial induction, on day 10, blood glucose levels were already higher than 175 mg/dL in every group. After the induction on day 10, treatment was continued for all groups. Treatment was administered once daily for 21 days. Compared to blood glucose levels on day 10, all groups experienced a decrease on day 17 and returned to the normal range (Table 2).

Day 31 of the 21-day treatment period revealed a nonsignificant drop in blood glucose levels in groups 4, 5, and 6 (MLEE 50, 100, and 150 mg/mL) when compared to the positive control ($P>0.05$; post hoc Dunn's). This indicates that glimepiride, a commercial medication employed as a positive control, and MLEE have comparable potential. Group 5 (MLEE 100 mg/mL) had the least blood glucose reduction, at 91.2±2.6 mg/dL. In contrast, Yasaroh et al.'s study from 2021 discovered that the greatest reduction in blood glucose levels was observed at a dose of 400 mg/kg body weight of MLEE administered for 21 days (78.4±5.5 mg/dL). This indicates that MLEE administered at lower dosages can lower blood glucose levels in female mice.

The ability of MLEE to reduce glucose levels is attributed to the presence of secondary metabolite compounds, one of which is flavonoids. Flavonoids are members of the phenolic group and are frequently identified as secondary metabolites in plants. Flavonoids have a fundamental heterocyclic ring containing oxygen and one aromatic B ring, making up their C6-C3-C6 molecular structure (Buraerah, 2010; Hernani et al., 2007). Flavonoids play a biological role in stabilizing free radicals by acting as antioxidants. Persistent hyperglycemia can cause free radicals called reactive oxygen species (ROS) and cause β cells in the pancreas to undergo apoptosis. When ROS accumulates in the mitochondria due to electron leakage, oxygen attaches itself to free electrons that are liberated (Annisa et al., 2014; Yan, 2014).

Antioxidants contribute hydrogen atoms that neutralize the oxidation of flavonoids and free radical compounds, stabilizing them (Winarsi, 2007; Panjuantiningrum, 2010; Widharna et al., 2010; Widharna et al., 2015).

Quercetin is one of the flavonoid chemicals. The intestinal mucosa absorbs less glucose due to quercetin's inhibition of GLUT-2. This process is consistent with the activity of tannins, which lower blood glucose levels by preventing glucose absorption by creating a barrier in the intestines (Rotblatt, 2002; Song et al., 2002; Tandi et al., 2018). Furthermore, by blocking PDE, flavonoids can raise cAMP, activating protein kinase A and improving insulin production (Harapan et al., 2010). By reactivating insulin signals and releasing insulin from the pancreatic β cells, saponins and tannins can reduce blood glucose levels. Additionally, these substances suppress the expression of glucose 6-phosphate and glycogen phosphorylase mRNA, impede the action of α-glucosidase, and elevate the expression of GLUT-4 (Kumari & Jain, 2012; Andrie et al., 2014; El Barky et al., 2017). Alkaloids can neutralize toxins in the body and have the ability to regenerate pancreatic β cells (Meiyanti et al., 2006; Kardono et al., 2003). A synergistic effect of various phytochemical compounds in Moringa leaves contributes to stable blood glucose levels (Simarmata et al., 2012).

Relationship between Blood Glucose Level and Body Weight of Female Mice

Each group had weekly weight rise or decrease variations based on the weight measurement data (Table 3). In all groups, the average weight began to decline on day 17. Stress may have affected the mice's appetite and energy intake, leading to weight loss (Francois et al., 2022). After 21 days of therapy, weight growth was observed in both the MLEE treatment group at 100 mg/kg BW and the positive control group. This weight gain is thought to be related to moringa leaves' capacity to restore pancreatic β cells, which progressively increases insulin output (Robertson et al., 1992). Moringa leaves also contain many other nutrients, including protein, carbs, vitamins, and minerals. The vitamin content encompasses vitamins A and C, which are abundant in β-carotene (Fuglie & Lowell, 2005). These contents contribute to the weight gain of mice given MLEE.

Blood glucose levels and body weight exhibit a correlated relationship ($P<0.05$; Spearman's rho). The correlation that results has a moderately positive coefficient value of 0.256. This suggests that improved weight gain (increased-normal) is correlated with higher-quality blood glucose levels (normal). This relatively significant connection implies that other factors, such as the mice's stress levels, may be responsible for weight increase or loss variations.

Table 2. Results of Blood Glucose Level Measurements in Female Mice Over 31 Days

Group	Mean blood glucose level ± SD (mg/dL)						
	Day 0	Day 3	Day 7	Day 10	Day 17	Day 24	Day 31
Healthy control[a]	43±26.5	94.6±5.9	140.8±7.1	105.5±10.5	100.6±9.3	96.6±10.5	102.5±3.8
Negative control[b]	45.6±19.9	115.3±13.2	132.8±17.9	175.3±10.4	144.6±7.8	132.9±5.5	132.3±3.5
Positive control[a,b]	33.4±12.6	120.6±25.4	123.3±15.1	181.2±14.1	112.7±6.5	104±12	99.7±3.8
MLEE 50 mg/kg BW[b]	58.3±29	110.4±12.4	140±16.6	175.9±13.7	133.2±14	131.5±14	110±17.9
MLEE 100 mg/kg BW[b]	51.1±3.1	165.6±18.6	137.3±10.8	177.8±5.2	121.2±12.7	108.9±11.5	91.2±2.6
MLEE 150 mg/kg BW[b]	88.3±46.7	68.9±13.3	136.8±4.1	187±4.5	131.6±28.9	129.4±36.6	105.1±14.9

Remark: [a-b]represents [a] notation difference indicating a significant difference ($p<0.05$), MLEE: *Moringa oleifera* Ethanol Extract

Table 3. Results of average body weight measurements in female mice over 31 days

Group	Average Body Weight ± SD (g)				
	Day 3	Day 10	Day 17	Day 24	Day 31
Healthy Control	32.5±1.7	32.8±2.1	33.3±2.6	31±2	30.5±2.1
Negative Control	31.3±2.1	31.3±1.7	28.8±1	29±0.8	29.5±1.3
Positive Control	30.8±1.3	31.8±2.2	31±2.3	30.8±2.1	32.5±1.7
MLEE 50 mg/kg BW	31±2.8	32.5±2.5	29.3±1.9	28.8±2.4	30±2.2
MLEE 100 mg/kg BB	24.5±0.6	33.3±1.5	32.3±2.1	31.5±1.3	32.3±1
MLEE 150 mg/kg BW	33±3.2	33.8±3	29.3±2.2	31.3±1.7	30.3±3.2

CONCLUSION

The acute toxicity study demonstrated that the ethanol extract of moringa leaves (MLEE) at doses ranging from 5 to 2000 mg/kg BW showed no acute toxicity symptoms. In female mice, MLEE administered at 100 mg/kg BW for 21 days was essential in the notable reduction in blood glucose levels (within the normal range). There was a favorable correlation between this drop in blood glucose levels and body weight. As a secure and practical substitute for traditional diabetes management methods, the MLEE reveals tremendous potential

AUTHOR CONTRIBUTION

VCP: Designed the research, supervised all processes, and prepared and edited the manuscript. NATP, EAS, DA, AP: Participated in laboratory work, data abstraction and analysis, and the preparation and editing of the manuscript. All authors read and approved the final manuscript.

ACKNOWLEDGMENTS

We express our gratitude to the Research and Community Service Institute (LPPM) of Duta Wacana Christian University for the financial support provided for this research grant under agreement letter No. 115/D.01/LPPM/2023

CONFLICT OF INTEREST

The authors declare that they have no competing interests

REFERENCES

Agustina, S. R., dan Aggrippina, W. (2016). Skrining Fitokimia Tanaman Obat Di kabupaten Bima. *Jurnal Cakra Kimia*. 4(1): 71-75.

Ali, G., Subhan, F., Abbas, M., Zeb, J., Shahid, M., & Sewell, R. D. (2015). A Streptozotocin-Induced Diabetic Neuropathic Pain Model For Static Or Dynamic Mechanical Allodynia And Vulvodynia: Validation Using Topical And Systemic Gabapentin. *Naunyn-schmiedeberg's Archives of Pharmacology*, 388, 1129-1140.

Almatsier, S. (2006). Prinsip Dasar Ilmu Gizi, edisi ke-6. Jakarta: Gramedia Pustaka utama.

Ambarwati, A., Sarjadi, S., Johan, A., & Djamiatun, K. (2014). Efek *Moringa oleifera* terhadap Gula Darah dan Kolagen Matrik Ekstraseluler Sel Î² Pankreas Diabetes Eksperimental. *Jurnal Kedokteran Brawijaya*, 28(2), 74-78.

Aminah, S., Ramdhan, T., & Yanis, M. (2015). Kandungan Nutrisi dan Sifat Fungsional Tanaman Kelor (*Moringa oleifera*). *Buletin pertanian perkotaan*, 5(2), 35-44.

Andrie, M., Taurina, W., & Ayunda, R. (2014). Activities test of "Jamu Gendong Kunyit Asam"(*Curcuma domestica* Val.; *Tamarindus indica* L.) as an antidiabetic in streptozotocin-induced rats. *Majalah Obat Tradisional*, 19(2), 95-102.

Annisa, F., Viryawan, C., & Santoso, F. (2014). Hipoksia Berpeluang Mencegah Kerusakan Sel β Pankreas pada Pasien Diabetes Melitus Tipe 2: Tinjauan Biologi Molekular. *Cermin Dunia Kedokteran*, 41(3), 398975.

Aziz, T., KN, R. C., & Fresca, A. (2009). Pengaruh Pelarut Heksana dan Etanol, Volume Pelarut, dan Waktu Ekstraksi Terhadap Hasil Ekstraksi Minyak Kopi. *Jurnal Teknik Kimia*, 16(1).

Banner, A., Zirie, M., Janahi, I. M., Al-Hamaq, A. O., Musallam, M., & Wareham, N. J. (2009). Prevalence Of Diagnosed and Undiagnosed Diabetes Mellitus and Its Risk Factors In A Population-Based Study Of Qatar. *Diabetes research and clinical practice*, 84(1), 99-106.

Bennett, P. (2000). Epidemiology of Type 2 Diabetes Mellitus. Diabetes Mellitus: A Fundamental and Clinical Text. 2nd ed. Philadelphia: Lippincott Williams & Wilkins, 544-70.

Buraerah, H. (2010). Analisis Faktor Risiko Diabetes Melitus Tipe 2 di Puskesmas Tanrutedong, Sidenreg Rappan. *Jurnal Ilmiah Nasional*, 35(4), 228-237.

Burger C, Fischer DR, Cordenunzzi DA, Batschauer APD, Filho VC, Soares ARD. (2005). Acute & Sub-Acute Toxicity of The Hydroalcoholic Extract From Wedelia Paludosa (*Acmela brasiliensis*) (*Asteraceae*) in mice. *J Pharm Sci*. 2005;8(2):370–373.

Chen, H., Xiao, H., & Pang, J. (2020). Parameter Optimization and Potential Bioactivity Evaluation of A Betulin Extract From White Birch Bark. *Plants*, 9(3), 392.

Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal, Resources Commission on Life Sciences, National Research Council. Guide for the Care and use of Laboratory Animals. U.S. Department of Health and Human Services, Public Health Service National Institutes of Health (NIH), NIH Publication No. 86–23, Revised 1985.

Donovan, J., & Brown, P. (2006). Blood collection. *Current Protocols in Immunology*, 73(1), 1-7.

El Barky, A. R., Hussein, S. A., Alm-Eldeen, A. E., Hafez, Y. A., & Mohamed, T. M. (2017). Saponins and Their Potential Role In Diabetes Mel-

litus. *Diabetes Manag*, 7(1), 148-58.

Fajarullah, A., Irawan, H., & Pratomo, A. (2014). Ekstraksi Senyawa Metabolit Sekunder Lamun Thalassodendron Ciliatum Pada Pelarut Berbeda. *Repository UMRAH*, 1(1), 1-15.

Fatmawati, A., & Aji, N. P. (2019). Penetapan Kadar Flavonoid Total Ekstrak Etanol Daun Kelor (*Moringa oleifera* Lam) Dengan Metode Kromatografi Lapis Tipis Densitometri. Published online 2019: 1-7.

Francois, M., Canal Delgado, I., Shargorodsky, N., Leu, C. S., & Zeltser, L. (2022). Assessing The Effects of Stress on Feeding Behaviors in Laboratory Mice. *Elife*, 11, e70271.

Fuglie, Lowell J., ed. (2005). The Miracle Tree: Moringa oleifera: Natural Nutrition for The Tropics. Training Manual. 2001. Church World Service, Dakar, Senegal.

Garcia-Galicia, U., Benito-Vicente, A., Jebari, S., Larrea-Sebal, A., Siddiqi, H., Uribe, K. B., ... & Martín, C. (2020). Pathophysiology of type 2 diabetes mellitus. *International journal of molecular sciences*, 21(17), 6275.

Handa, S. S., Khanuja, S. P. S., Longo, G., & Rakesh, D. D. (2008). Extraction Technologies For Medicinal And Aromatic Plants, no. 66. Italy: United Nations Industrial Development Organization and the International Centre for Science and High Technology. Trieste, 21-25.

Handayani, H., Sriherfyna, F. H., & Yunianta. (2016). Ekstraksi Antioksidan Daun Sirsak Metode Ultrasonic Bath (Kajian Rasio Bahan: Pelarut dan Lama Ekstraksi). *Jurnal Pangan Dan Agroindustri*, 4(1), pp. 262–272.

Harapan, J. K., Hayati, Z., & Muhammad, I. (2010). Peran Puasa Dalam Remodelling Sel Enteroendokrin Untuk Mencegah Diabetes Melitus Tipe 2. *JIMKI*, 1(1), 36-40.

Harborne, J.B. (1998). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd Edition, Chapman and Hall Ltd, London, 21-72.

Hardianto, D. (2020). Telaah Komprehensif Diabetes Melitus: Klasifikasi, Gejala, Diagnosis, Pencegahan dan Pengobatan. *Jurnal Bioteknologi dan Biosains Indonesia (JBBI)*, 7(2), 304-317.

Hernani, Marwati, T., dan Winarti, C. (2007). Pemilihan Pelarut pada Pemurnian Ekstrak Lengkuas (*Alpinia galanga*) Secara Ekstraksi. *Jurnal Pascapanen*. 4(1):1–8.

Huet O, Ramsey D, Miljavec S, Jenney A, Aubron C, Aprico A, Sterfanovic N, Balkau B, Head GA, de Haan JB, Chin-Dusting JPF. (2013). Ensuring Animal Welfare While Meeting Scientific Aims Using A Murine Pneumonia Model of Septic Shock. Shock 39(6): 488-494.

International Diabetes Federation (2019). IDF Diabetes Atlas Ninth Edition 2019. IDF.

Irawan, D. (2010). Prevalensi dan Faktor Risiko Kejadian Diabetes Melitus Tipe 2 di Daerah Urban Indonesia (Analisa Data Sekunder Riskesdas 2007). *Thesis*. Jakarta: Sekolah Pascasarjana, Universitas Indonesia.

Kamaliani, B. R., Setiasih, N. L. E., & Winaya, I. B. O. (2018). Gambaran Histopatologi Ginjal Tikus Wistar Diabetes Melitus Eksperimental yang Diberikan Ekstrak Etanol Daun Kelor. *Buletin Veteriner Udayana*, 11(1), 71-77.

Kardono, L. B. S. (2003). Chemical constituents of Phaleria Macrocarpa (Scheff.) Boerl. In Phaleria Macrocarpa seminar, RND center for pharmacy and traditional medicines. Indonesian Ministry of Health, Jakarta, Indonesia.

Katuuk, R.H.H., Sesilia A.W., Pemmy T. (2018). Pengaruh Perbandingan Ketinggian Tempat Terhadap Kandun-

gan Metabolit Sekunder Pada Gulma Babadota (*Ageratum conyzoides* L.). *Jurnal Agroteknologi*. 2(1): 1-6

Ministry of health. (2020). Profil Kesehatan Indonesia Tahun 2020. Jakarta: Kementerian Kesehatan RI.

Knip, M., Douek, I. F., Moore, W. P. T., Gillmor, H. A., McLean, A. E. M., Bingley, P. J., ... & ENDIT Group. (2000). Safety of High-Dose Nicotinamide: A Review. *Diabetologia*, 43, 1337-1345.

Kumari, M., & Jain, S. (2012). Tannins: An Antinutrient With Positive Effect To Manage Diabetes. *Research Journal of Recent Sciences ISSN*, 2277, 2502.

Laksmiani, N. P. L., Widiantara, I. W. A., Adnyani, K. D., & Pawarrangan, A. B. S. (2020). Optimasi Metode Ekstraksi Kuersetin Dari Daun Kelor (*Moringa oleifera* L.). *J Kim*. Published online.

Lee, W. K., Lim, Y. Y., Leow, A. T. C., Namasivayam, P., Abdullah, J. O., & Ho, C. L. (2017). Biosynthesis of Agar in Red Seaweeds: A Review. Carbohydrate polymers, 164, 23-30.

Liu, S., & Mauvais-Jarvis, F. (2010). Minireview: Estrogenic Protection of B-Cell Failure In Metabolic Diseases. *Endocrinology*, 151(3), 859-864.

Loomis, T. A., & Hayes, A. W. (1996). Toxicologic testing methods. Loomis's Essentials of Toxicology. Academic Press, Inc., San Diego, CA, 205-248.

Malini DM, Madihah, DA Khoirunnisa, I Sasmita, N Ratningsih, K Alipin & W Hermawan. (2019). Ekstrak Etanol Kulit Buah Jengkol Menurunkan Kadar Glukosa dan Meningkatkan Hormon Insulin Tikus Diabetes yang Diinduksi Streptozotocin. *Veteriner*, 20(36): 65–73.

Malole, M.B.M., Pramono. C.S.U., (1989), Penggunaan Hewan-Hewan Percobaan di Laboratorium. Bogor: PAU Pangan dan Gizi, IPB.

Meiyanti, Dewoto, H. R., & Suyatna, F. D. (2006). Efek Hipoglikemik Daging Buah Mahkota Dewa (*Phaleria macrocarpa* (Scheff) Boerl) Terhadap Kadar Gula Darah Pada Manusia Sehat Setelah Pembebanan Glukosa. *Universa Medicina*, 25(3).

Moodley, I. (2017). Acute toxicity of Moringa oleifera leaf powder in rats. *J. Med. Plants Stud*, 5(5), 180-5.

Nagappa, A. N., Thakurdesai, P. A., Rao, N. V., & Singh, J. (2003). Antidiabetic Activity of *Terminalia catappa* Linn fruits. *Journal of ethnopharmacology*, 88(1), 45-50.

Nurfitriani, E., Mulyani, Y., & Agung, M. U. K. (2016). Hubungan Kualitas Air dengan Profil Metabolit Sekunder Ekstrak Daging Holohuria atra di Perairan Teluk Lampung dan Perairan Garut. *Akuatika Indonesia*, 2(2), 146-154.

OECD Guideline for testing of Chemicals, 2001. Guideline 423: acute Oral Toxicity – Acute Toxic Class Method 2001.

Panjuantiningrum F. (2010). Pengaruh Pemberian Buah Naga Merah (H.Polyrhizus) Terhadap Kadar Glukosa Darah Tikus Putih yang Diinduksi Aloksan. Surakarta : Fakultas Kedokteran Universitas Sebelas Maret.

Pitriya, I. A., Rahman, N., & Sabang, S. M. (2017). Efek Ekstrak Buah Kelor (*Moringa oleifera*) Terhadap Penurunan Kadar Gula Darah Mencit (Mus musculus). *Jurnal Akademika Kimia*, 6(1).

Pradana, D. L. C., & Wulandari, A. A. (2019). Uji Total Flavonoid Dari Ekstrak Air Aun Kelor (*Moringa oleifera*) Dan Secang (*Caesalpinia sappan* L.). *Jurnal Insan Farmasi Indonesia*, 2(2), 271-277.

Prasetyana, V. A. (2022). Uji Aktivitas Antiinflamasi Kombinasi Daun Kelor (*Moringa oleifera* L.) dan Natrium Diklofenak pada Tikus Putih (*Rattus norvegicus*) yang Diinduksi Karagenan.

Universitas Kristen Duta Wacana.

Prayitno, S. A., & Rahim, A. R. (2020). Comparison of Extracts (Ethanol And Aquos Solvents) Muntingia Calabura Leaves on Total Phenol, Flavonid, and Antioxidant (Ic50) Properties. *Kontribusia: Research Dissemination for Community Development*, 3(2), 319-325.

Putra, I. W. D. P., Dharmayudha, A. A. G. O., & Sudimartini, L. M. (2016). Identifikasi Senyawa Kimia Ekstrak Etanol Daun Kelor (*Moringa oleifera* L) di Bali. *Indonesia Medicus Veterinus*, 5(5), 464-473.

Ragavan, B., & Krishnakumari, S. (2006). Antidiabetic effect of T. arjuna Bark Extract in Alloxan Induced Diabetic Rats. *Indian Journal of Clinical Biochemistry*, 21, 123-128.

Rakieten, N., Rakieten, M. L., & Nadkarni, M. V. (1963). Studies on The Diabetogenic Action of Streptozotocin. *Cancer Chemotherapy Reports Part* 1,29, 91.

Robertson, R.P., Kahn, C.R., Kahn, B., D. Moller, & Abrahamson, M. (1992). "Seminars in medicine of the Beth Israel Hospital, Boston: pancreatic and islet transplantation for diabetes—cures or curiosities?" *New England Journal of Medicine*, 327 (26): 1861–1868.

Rotblatt M, Zimet I. (2002). Evidence-Based Herbal Medicine. London: Haney & Belfus, INC.

Ruskar, A. G. N. (2010). Kajian Efektivitas Streptozotosin Dalam Induksi Tikus Putih Diabetik (Doctoral dissertation, Universitas Airlangga).

Saadane, A., Lessieur, E. M., Du, Y., Liu, H., & Kern, T. S. (2020). Successful Induction of Diabetes in Mice Demonstrates No Gender Difference in Development of Early Diabetic Retinopathy. *PLoS One*, 15(9), e0238727.

Saputra, A., Arfi, F., & Yulian, M. (2020). Literature Review: Analisis Fitokimia dan Manfaat Ekstrak Daun Kelor (*Moringa oleifera*). *AMINA*, 2(3), 114-119.

Sari, A. K. (2015). Penetapan Kadar Polifenol Total, Flavonoid Total, Dan Uji Aktivitas Antioksidan Ekstrak Etanol Daun Sirsak (*Annona muricata*) Dari Jember Pada Ketinggian Tanah Yang Berbeda.

Sari, S. A., & Budiasih, K. S. (2017). Pengaruh Pemberian Senyawa Cr (No3)· 9H2O Terhadap Kadar Glukosa Darah Tikus Wistar Jantan yang Diinduksi Dengan Streptozotocin-nicotinamide. *Jurnal Elemen Kimia*, 6(2), 21-28.

Simarmata, Y. B. C., Saragih, A., & Bahri, S. (2012). Efek Hipourikemia Ekstrak Daun Sidaguri (*Sida rhombifolia* L) Pada Mencit Jantan. *Journal of Pharmaceutics and Pharmacology*, 1(1).

Song, J., Kwon, O., Chen, S., Daruwala, R., Eck, P., Park, J. B., & Levine, M. (2002). Flavonoid Inhibition of Sodium-Dependent Vitamin C Transporter 1 (SVCT1) and Glucose Transporter Isoform 2 (GLUT2), Intestinal Transporters For Vitamin C and Glucose. *Journal of Biological Chemistry*, 277(18), 15252-15260.

Susanty, S., Yudistirani, S. A., & Islam, M. B. (2019). Metode Ekstraksi Untuk Perolehan Kandungan Flavonoid Tertinggi Dari Ekstrak Daun Kelor (*Moringa oleifera* Lam). *Jurnal Konversi*, 8(2), 6.

Tandi, J., Rahmawati, R., Isminarti, R., & Lapangoyu, J. (2018). Efek Ekstrak Biji Labu Kuning Terhadap Glukosa, Kolesteroldan Gambaran Histopatologi Pankreas Tikus Hiperkolesterolemia-Diabetes. *In Talenta Conference Series: Tropical Medicine.* 1(3):144-151.

Tesch, G. H., & Allen, T. J. (2007). Rodent models of streptozotocin-induced diabetic nephropathy (Methods in Renal Research). *Nephrology*, 12(3), 261-266.

Tiano, J. P., & Mauvais-Jarvis, F. (2012).

Importance of Oestrogen Receptors To Preserve Functional β-Cell Mass in Diabetes. *Nature Reviews Endocrinology*, 8(6), 342-351.

Vongsak, B., Sithisarn, P., Mangmool, S., Thongpraditchote, S., Wongkrajang, Y., & Gritsanapan, W. (2013). Maximizing Total Phenolics, Total Flavonoids Contents and Antioxidant Activity of *Moringa oleifera* Leaf Extract by The Appropriate Extraction Method. I*ndustrial crops and products*, 44, 566-571.

Widharna, R. M., Ferawati, Hendriati, L., Surjadhana, A., Jonosewo, A., dan Widjajakusuma, E. C. (2010). Antidiabetic Properties of Andrographis Paniculata and Eugenia Polyantha Wight Leaves in Wistar Rats by Oral Glucose Tolerance Test. *The Journal of Indonesian Medicinal Plants*. 3 (2): 88-93.

Widharna, R. M., Ferawati, Wahyu, D. T., Lucia, H., Iwan, S. H., dan Elisabeth, C. W. (2015). Antidiabetic Effect of The Aqueous Extract Mixture of Andrographis Paniculata and *Syzygium polyanthum* Leaf. *European Journal of Medicinal Plants*. 6 (2): 82-91.

Winarsi H. Antioksidan Alami dan Radikal Bebas. (2007). Yogyakarta: Kanisius. P.77-81.

Yan, L. J. (2014). Pathogenesis of Chronic Hyperglycemia: From Reductive Stress To Oxidative Stress. Journal of diabetes research, 2014.

Yasaroh, S., Christijanti, W., Lisdiana, L., & Iswari, R. S. (2021). Efek Ekstrak Daun Kelor (*Moringa oleifera*) Terhadap Kadar Glukosa Darah Tikus Diabetes Induksi Aloksan. In Seminar Nasional Biologi UNNES (Vol. 9, pp. 224-229).

Yusuf, M. Y., & Al-Gizar, M. R. (2022). Teknik Manajemen dan Pengelolaan Hewan Percobaan (Memahami Perawatan Dan Kesejahteraan Hewan Percobaan).

Zega, V. L., Wowor, P. M., & Mambo, C. (2016). Uji Beberapa Dosis Ekstrak Buah Mengkudu (*Morinda citrifolia* l.) Terhadap Kadar Glukosa Darah pada Tikus Wistar (*Rattus norvegicus*) yang Diinduksi Aloksan. *Jurnal e-Biomedik*, 4(2).