

**Anti-Hyperglycemic and Anti-Inflammatory Activities of *Moringa oleifera* Lam Leaves Extract**Jessica Novia^{1*}, Denny Juvi¹, Riskianto Riskianto¹, Marcelia Sugata², Dela Rosa¹¹Department of Pharmacy, Universitas Pelita Harapan, Jl. M.H. Thamrin Boulevard, Tangerang 15811, Indonesia²Department of Biology, Universitas Pelita Harapan, Jl. M.H. Thamrin Boulevard, Tangerang 15811, Indonesia

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ABSTRACT

Moringa oleifera has been known for its nutritional content and health benefit. As a medicinal plant, it has been used to control and treat diabetes with minimal or no side effects. Hence, this study investigated the anti-hyperglycemic and anti-inflammatory activity of *Moringa* leaf extract (MLE). Extracts were obtained by maceration with 1 L of 70% ethanol, ethyl acetate, and n-hexane. Anti-hyperglycemic activity of MLE was evaluated through alpha-amylase and alpha-glucosidase inhibition, while anti-inflammatory activity was analyzed by protein denaturation method. The results showed that ethyl acetate extract of *Moringa* leaves had the strongest inhibition against alpha-amylase ($IC_{50} = 0.243 \mu\text{g/mL}$) and alpha-glucosidase ($IC_{50} = 0.470 \mu\text{g/mL}$). Meanwhile, the greatest anti-inflammatory activity ($IC_{50} = 0.217 \mu\text{g/mL}$) was shown by n-hexane extract of *Moringa* leaves. In conclusion, *Moringa* leaves have anti-hyperglycemic and anti-inflammatory activities.

Keywords: *Moringa oleifera*, Anti-hyperglycemic, Anti-inflammatory, Alpha amylase, Alpha glucosidase.

Introduction

Foods play a critical role in physiological mechanisms by controlling metabolic processes. Inappropriate food consumption can lead to diseases in humans such as cardiovascular disease, metabolic syndrome, hyperlipidemia, thrombosis, hypertension, and diabetes.¹ There are two types of diabetes: type 1 and 2. In type 1 diabetes or insulin-dependent diabetes mellitus (IDDM), the body does not produce insulin, and therefore to maintain normal blood sugar levels, insulin injections are needed regularly. Meanwhile, in type 2 diabetes, the body does not generate enough insulin due to the damage of pancreatic cell function, the body does not respond to insulin appropriately (insulin resistance), or both.^{2,3} Type 1 diabetes accounts for 5-10% of diabetes cases, while type 2 diabetes accounts for about 90%-95% of all diagnosed patients. However, 9 out of 10 people with type 2 diabetes did not know they had a pre-diabetes condition.^{4,5} In 2013, Indonesia categorized type 2 diabetes as a non-communicable disease with a high mortality rate.⁶ In Indonesia, people with type 2 diabetes have reached 12.1 million in 2013 and estimated approximately 21.3 million people in 2030.⁷ There are various mechanisms of action of oral antidiabetic drugs. The sulfonylurea group stimulates insulin secretion in the pancreas; the biguanides group increases insulin sensitivity in liver and muscle tissue thereby increasing glucose absorption; the thiazolidinediones group increases insulin sensitivity in muscle, liver, and adipose tissue; the dipeptidyl peptidase-4 class (DPP-4) inhibitors reduce the post-meal increase in glucagon and stimulate insulin secretion; the glucosidase inhibitors prevent the breakdown of sucrose and complex carbohydrates in the small intestine.⁸

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However, some oral antidiabetic medications can cause serious side effects and is not recommended during pregnancy. Therefore, traditional medicine becomes an alternative diabetes treatment.

Medicinal plants have been reported for their ability to control and treat diabetes with minimal or no side effects. In addition, they are relatively cheaper than oral synthetic hypoglycemic agents.^{9,10} According to ethnobotanical reports, about 800 plants showed potential anti-hyperglycemic activity¹¹ and many of them have been recommended to treat diabetes.^{9,12} Plants for diabetes treatment generally contain various compounds such as carotenoids, flavonoids, terpenoids, alkaloids, and glycosides.^{13,14}

Moringa oleifera Lam is a tropical plant that grows easily in Indonesia. Previous studies have reported that *Moringa* leaves showed pharmacological activities such as antidiabetic,¹³ antiatherosclerosis,¹⁵ anticancer,¹⁶ antibacterial,¹⁷ antioxidant,¹⁸ anti-inflammatory,¹⁹ immunomodulatory,²⁰ and hepatoprotective.²¹ *Moringa* leaves contain secondary metabolites such as alkaloids, phenols, flavonoids, tannins,¹⁸ carotenoids, anthraquinones, anthocyanins, proanthocyanidins,²² saponins, steroids, triterpenoids, coumarins, and quinines.^{23,24} The flavonoid compounds in *Moringa* leaves can regulate pleiotropic mechanisms to relieve the effects of diabetes, stimulate glucose absorption in peripheral tissues, and control the activity of various enzymes involved in the carbohydrate metabolism process. During inflammation, *Moringa* leaves can downregulate the expression of iNOS, interferon-gamma, and CRP.²⁵ This study evaluated the antihyperglycemic and anti-inflammatory activity of *Moringa* leaves extract *in vitro*.

Materials and Methods

Materials

All solvents (ethanol 70%, ethyl acetate, n-hexane) and sodium chloride were procured from Merck, Germany. Phosphate Buffered Saline (PBS) was purchased from Oxoid, England. Alpha-amylase, α -glucosidase and other materials were of analytical grade: starch, DNS Reagent, acarbose, maltose, and egg white.

Sample collection

Moringa oleifera leaves were collected in April 2019 from Pangandaran Regency, West Java, Indonesia. Sample was authenticated by Pusat Riset Biosistemika dan Evolusi (BRIN, Indonesia) with voucher number B-855/V/DI.05.07/3/2022.

Extraction

Moringa oleifera leaves were dried and powdered, then the extraction was done by macerating the powdered leaves (200 g) in 1 L solvent (ethanol 70%, ethyl acetate, or n-hexane) for 72 h at room temperature. Maceration was done separately with each solvent. Stirring and solvent replacement were done every 24 h, the extract was filtered, then the residue was re-extracted with each solvent until a clear extract was obtained. The filtrate was collected and evaporated (Heidolph®, Germany) at 50°C, followed by drying in an oven (Memmert®, Germany) at 50°C to obtain a dry extract (MLE).

α -Amylase inhibition

A starch solution (0.5%) was prepared by dissolving 0.125 g of potato starch into 25 mL of a solution containing 20 mM phosphate buffered saline (PBS) and 6.7 mM NaCl (pH 6.9) in boiling water for 15 min. The reagent was prepared by mixing a solution of sodium potassium tartrate and 96 mM 3,5-dinitrosalicylic acid (DNS reagent) in a volume ratio of 1:1. One mL of starch solution was mixed with one mL of various concentration of MLE (1, 2, 4, 6, 8, and 10 g/mL). Then, one mL of α -amylase (1 U/mL in 20 mM PBS and 6.7 mM NaCl pH 6.9) was added to each mixture and incubated at 25°C for 3 min. The reaction was terminated by adding one mL of DNS reagent, followed by heating in boiling water for 15 min. After cooling, distilled water was added to the initial volume of 4 mL. A standard was prepared using the same procedure replacing the extract with acarbose (50-1000 g/mL). The absorbance (Abs_s) was measured at a wavelength of 540 nm using a spectrophotometer (DLAB®, USA). Double-distilled water was used as control (Abs_c).²⁶ The percentage of α -amylase inhibition was calculated by the following formula:

$$\% \text{ inhibition} = \frac{(Abs_c - Abs_s)}{Abs_c} \times 100\%$$

The extract concentration resulting in 50% inhibition of enzyme activity (IC_{50}) was determined graphically.

α -Glucosidase inhibition

The inhibition of α -glucosidase was determined by incubating the mixture of α -glucosidase (1 U/mL, 100 μ L), PBS (pH 7.0, 100 μ L), and various extract concentrations (25-1600 μ g/mL, 100 μ L) at 37°C for 60 min in maltose solution. The reaction was stopped by heating the solution in boiling water for 2 min. After cooling, Benedict's reagent (2 mL) was added to each mixture. A standard was prepared using the same procedure replacing the extract with acarbose (0.1 - 3.2 μ g/mL). The absorbance (Abs_s) was measured at a wavelength of 540 nm using a spectrophotometer (DLAB®, USA). Double-distilled water was used as control (Abs_c). The concentration of glucose resulting from the breakdown of maltose by α -glucosidase was estimated.²⁷ The percentage of inhibition was then calculated by the following formula:

$$\% \text{ inhibition} = \frac{(Abs_c - Abs_s)}{Abs_c} \times 100\%$$

The extract concentration resulting in 50% inhibition of enzyme activity (IC_{50}) was determined graphically.

In vitro anti-inflammatory activity

Anti-inflammatory activity of the MLE was carried out by protein denaturation method. Diclofenac sodium was used as standard. The reaction mixture consisted of 2 mL egg albumin, 2.8 mL of phosphate buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations of MLE (100-500 μ g/mL) or sodium diclofenac (100 and 200 μ g/mL). The mixtures were incubated at 27°C for 15 min and then heated at 70°C for 10 min. After cooling, the absorbance of the mixtures (Abs_s)

was measured at a wavelength of 660 nm using a spectrophotometer (DLAB®, USA). Double-distilled water was used as control (Abs_c).²⁸ Percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition} = \frac{(Abs_c - Abs_s)}{Abs_c} \times 100\%$$

The extract concentration resulting in 50% inhibition of protein denaturation (IC_{50}) was determined graphically.

Statistical analysis

Data are presented as the mean \pm standard deviation (n = 3). Statistical analysis was done using Minitab 15 (Minitab Inc., USA). A p-value < 0.05 was considered as statistically significant.

Results and Discussion

Moringa leaves were extracted with various solvents, namely n-hexane, ethyl acetate, and ethanol 70%. The different polarities in solvents allow the extraction of several active compounds in *Moringa* leaves. Anti-hyperglycemic activity of MLE was evaluated *in vitro* based on α -amylase and α -glucosidase inhibition assay. Acarbose was used as a positive control because it can inhibit the breakdown of sucrose, maltose, dextrin, and starch by α -glucosidase and α -amylase. The inhibition of carbohydrate metabolism leads to lower blood sugar levels, hence acarbose is commonly used in the treatment of diabetes, especially type 2 diabetes with a failed response to insulin properties. Acarbose is an oligosaccharide compound obtained from the fermentation of *Actinoplanes uthensis*.²⁹

As seen in Figure 1 and Table 1, MLEs inhibited the activity of α -amylase with IC_{50} values as follow: n-hexane extract (0.268 μ g/mL), ethyl acetate extract (0.243 μ g/mL), ethanol extract (0.744 μ g/mL). The ethyl acetate extract showed the highest inhibition of α -amylase activity among other extracts, even higher than acarbose as the positive control ($IC_{50} = 0.299$ μ g/mL). Based on Figure 2 and Table 2, MLEs demonstrated the ability to inhibit α -glucosidase activity with IC_{50} values as follows: n-hexane extract (0.516 μ g/mL), ethyl acetate extract (0.470 μ g/mL), ethanol extract (0.824 μ g/mL). Unlike the α -amylase inhibition assay, the inhibition of α -glucosidase activity by acarbose, positive control was greater than the extracts ($IC_{50} = 0.352$ μ g/mL), with ethyl acetate as the highest inhibitor among the extracts. The results from α -amylase and α -glucosidase inhibition assay indicated that ethyl acetate extract had the best antihyperglycemic activity.

A previous study reported that *Moringa* leaves lower high blood glucose level caused by a high-fat diet (HFD) and that induced by streptozotocin (STZ), a harmful substance to mammalian pancreatic cells.³⁰ The antihyperglycemic effect of *Moringa* leaves extract comes from the ability of the extract to prevent pancreatic damage caused by Reactive Oxygen Species (ROS). The presence of antioxidant activity in *Moringa* leaves also reduces hyperglycemia by increasing glucose consumption in skeletal muscle, stimulating insulin secretion, inhibiting α -amylase and α -glucosidase.³¹ In addition, *Moringa* leaves could inhibit ATP-sensitive potassium channels in pancreatic β -cell, thus causing depolarization of the cell membrane and the opening of voltage-dependent calcium channels (VDCC). When VDCC is open, there will be an increase of intracellular calcium in beta cells which then triggers insulin secretion.³²

Flavonoids are known to have hydroxyl groups that play a role in preventing lipid peroxidation and oxidation reactions by free radicals.³³ Tannins are a central glucose molecule present in the hydroxyl group with one or more galloyl residues. In the presence of copper ions, tannins act as antioxidants that can form hydroxyl radicals.³⁴ Flavonoid and tannin can negate the development of insulin resistance or insulin dysfunction in diabetes by maintaining beta-cell function and reducing oxidative stress-induced tissue damage.³⁵ The anti-inflammatory activity of *Moringa* leaves extract was evaluated *in vitro*. All extracts showed anti-inflammatory activity by inhibiting protein denaturation (Figure 3).

Table 1: Alpha-amylase inhibition by Moringa leaves extracts (MLEs)

Sample	Concentration	% Inhibition	Linear Regression Equation	IC ₅₀ (µg/mL)
Acarbose	0.10%	38.69 ± 0.10	y = 49.43x + 35.21	0.299
	0.50%	62.56 ± 0.05	R ² = 0.9896	
	1.00%	83.47 ± 0.07		
N-Hexane	0.10%	42.42 ± 0.01	y = 41.135x + 38,948	0.268
	0.50%	60.67 ± 0.04	R ² = .09971	
	1.00%	79.57 ± 0.03		
Ethyl acetate	0.10%	44.74 ± 0.08	y = 35.443x + 41.354	0.243
	0.50%	59.36 ± 0.07	R ² = 0.9998	
	1.00%	76.67 ± 0.05		
Ethanol 70%	0.10%	12.40 ± 0.11	y = 56.611x + 7.8372	0.744
	0.50%	38.12 ± 0.12	R ² = 0.9955	
	1.00%	63.57 ± 0.05		
Negative control		0.00		-

Table 2: Alpha-glucosidase inhibition by Moringa leaves extracts (MLEs)

Sample	Concentration	% Inhibition	Linear Regression Equation	IC ₅₀ (µg/mL)
Acarbose	0.10%	33.06 ± 0.10	y = 60.749x + 28.614 R ² = 0.9914	0.352
	0.50%	61.92 ± 0.08		
	1.00%	88.06 ± 0.13		
N-Hexane	0.10%	25.19 ± 0.06	y = 55.311x + 21.457 R ² = 0.9875	0.516
	0.50%	52.35 ± 0.07		
	1.00%	75.33 ± 0.06		
Ethyl Acetate	0.10%	30.88 ± 0.02	y = 47.002x + 27.869 R ² = 0.9847	0.470
	0.50%	54.41 ± 0.13		
	1.00%	73.52 ± 0.11		
Ethanol 70%	0.10%	8.53 ± 0.06	y = 56.321x + 3.552 R ² = 0.9984	0.824
	0.50%	32.89 ± 0.10		
	1.00%	59.35 ± 0.10		
Negative control		0.00		-

Table 3: Inhibition of protein denaturation by Moringa leaves extracts (MLEs)

Sample	Concentration	% Inhibition	Linear Regression Equation	IC ₅₀ (µg/mL)
Diclofenac Sodium	0.10%	48.32 ± 0.06	y = 43.091x + 45.251 R ² = 0.9902	0.110
	0.50%	69.03 ± 0.03		
	1.00%	87.35 ± 0.04		
N-Hexane	0.10%	46.88 ± 0.16	y = 24.126x + 44.749 R ² = 0.9984	0.217
	0.50%	57.32 ± 0.03		
	1.00%	68.65 ± 0.07		
Ethyl Acetate	0.10%	45.71 ± 0.15	y = 23.352x + 43.992 R ² = 0.9917	0.257
	0.50%	56.78 ± 0.04		
	1.00%	66.85 ± 0.04		
Ethanol 70%	0.10%	22.46 ± 0.08	y = 29.169x + 19.983 R ² = 0.9973	1.029
	0.50%	35.36 ± 0.02		
	1.00%	48.80 ± 0.04		
Negative control		0.00		-

The IC₅₀ values of n-hexane, ethyl acetate, and ethanol extract were 0.217 µg/mL, 0.257 µg/mL, and 1.029 µg/mL, respectively (Table 3). The results show that the n-hexane extract exhibited the highest anti-inflammatory activity. However, its activity was still lower than diclofenac sodium as positive control (IC₅₀ = 0.110 µg/mL). Moringa leaves extract contain phenolic compounds that can modulate lipid peroxidation and prevent free radicals through antioxidant and anti-inflammatory activities.^{36,37,38} A previous study reported that one of the constituents of Moringa plants, namely isothiocyanates, plays a role in the NFκB pathway. Under oxidative conditions in cells and tissues, NFκB will be activated and cause the release of pro-inflammatory cytokines. The released NFκB will also accumulate in the nucleus and trigger the transcription of genes that play a role in the inflammatory response.^{39,40}

Conclusion

Ethyl acetate extract of Moringa leaves had the best inhibition against α-amylase and α-glucosidase, while n-hexane extract of Moringa leaves has the highest anti-inflammatory activity. Further studies are needed to isolate specific compounds in Moringa leaves that are responsible for the inhibitory activity of α-amylase, α-glucosidase enzymes, and anti-inflammatory activity.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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