

## Short Communication

# Antihyperglycemic and Antioxidant Activity of Nanoemulsion Extracts of *M. affine* D. Don Leaves in Alloxan-Induced Rat

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### ABSTRACT

This study determined the antihyperglycemic and antioxidant activity of nanoemulsion extracts of *M. affine* leaves in alloxan-induced rats. This research used 24 male Wistar rats around three months old which grouped as normal (untreated), negative control (treated with carboxymethyl cellulose sodium/Na-CMC<sub>s</sub>), positive control (treated with glibenclamide), and various concentration (30, 60, and 90%) of nanoemulsion extract of *M. affine* leaves groups. The extract of *M. affine* leaves had an antioxidant activity with IC<sub>50</sub> 5.30 ppm, categorized as a very strong antioxidant. Furthermore, the administration of this extract decreased glucose levels in antihyperglycemic rats. We concluded that *M. affine* leaves extract potential as antioxidants and be developed as an ingredient for diabetic drugs.

**Keywords:** diabetes, glucose levels, glibenclamide, *M. affine*, *Rattus norvegicus* strain wistar

Diabetes mellitus is a metabolic disorder that causes various complications. According to WHO, diabetic's incidences in Indonesia in 2000 were around 8.4 million and will be increased to 21.3 million in 2030 (Suharmiati, 2003). Currently, diabetes treatment is done by consuming synthetic drugs and maintaining a diet. Clinical management using synthetic drugs may cause irritation, resistance, and infection. Glibenclamide is an anti-hyperglycemic drug that is commonly used for the treatment of type 2 diabetes mellitus and has promising new medical indications. However, this drug is associated with high rates of serious hypoglycemic episodes as a result of its pharmacological activity (Hernández-Abad *et al.*, 2019). Glibenclamide spurs potassium channel activation (Assis *et al.*, 2018).

One of the natural approaches to overcome hyperglycemia is by consuming natural ingredients available in the environment. One of the plants which has antihyperglycemic potential namely

“senggangi” leaf from Aceh, Indonesia. Traditionally, Aceh people consume these leaves to reduce diabetes. *Melastoma malabathricum* leaf contains triterpene, alpha-amyrin, quercitrin, quercetin, and kaempferol-3-O-(2", 6" - di-Op-trans-coumaroyl) - β glucoside (Hasnah *et al.*, 2010). Flavonoids play a role in preventing diabetes and its complications (Jack, 2012). Some research has proven that plants containing flavonoids are able to fight diabetes mellitus (Brahmachari, 2011). The administration of ethanolic extract of *Melastoma malabathricum* leaf by 400 mg/kg body weight can reduce glucose levels in diabetic mice (Sahara *et al.*, 2019), ethanolic extract of *M. malabathricum* leaves has significant antidiabetic and antihyperlipidemic activity in diabetic rats (Karappasamy *et al.*, 2014). However, this research uses *M. affine*, which belongs to the same genus as *M. malabathricum*, leaves extraction method carried out by nanoemulsion technique, one of the advantages of nanoemulsion technique is that it is easily absorbed by the small intestine wall thereby increasing the bioavailability of a compound. The rate of absorption of nano herbal in the human body can almost reach 100% (Poulain & Nakache, 1998). At present, there has never been reported the effect of

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nanoemulsion-based of *M. affine* leaves extract in diabetic conditions. The aim of this study was to determine the antihyperglycemic and antioxidant activity of nanoemulsion extracts of *M. affine* leaves in alloxan-induced rats.

The research was carried out at the Laboratory of Animal, Universitas Syiah Kuala. The preparation of *M. affine* leaves nanoemulsion extract was carried out at the Laboratory of Biology and Chemistry Faculty of Teacher Training and Education, Universitas Syiah Kuala, and Laboratory of Pharmacology, Faculty of Veterinary Medicine, Universitas Syiah Kuala. Antioxidant activity test was carried out at the Laboratory of Chemistry, Faculty of Teacher Training Education, Universitas Syiah Kuala.

This research used 24 male Wistar rats three months old with an average of body weight 180-200 gram. The rats were divided into 6 groups: N (normal group, untreated), NC (negative control, treated with carboxymethyl cellulose sodium/Na-CMC), PC (positive control, treated with glibenclamide), and nanoemulsion extract of *M. affine* leaves groups: P1 (30 % of nanoemulsion extract of *M. affine* leaves), P2 (60 % of nanoemulsion extract of *M. affine* leaves), and P3 (90 % of nanoemulsion extract of *M. affine* leaves). The extract treatment was given orally once a day for 21 days.

Three kilograms of *M. affine* leaves were weighed, washed thoroughly, and air-dried for  $\pm$  3 days. After dry, these leaves were blended until smooth. Maceration was done with leaves powder and a 96 % ethanol ratio of 1:10 for 2 days to withdraw all the compounds contained in *M. affine* leaves. The preparation nanoemulsion of *M. affine* leaves extract includes the oil phase and water phase. The oil phase is a mixture of 180 mL of *M. affine* leaves extract and the water phase consists of 180 gram of maltodextrin, 18 mL tween 80, and 102 mL of phosphate buffer solution. Homogenization of the oil phase in the water phase was done using centrifugation at 15,000 rpm for 15 min (Safrida *et al.*, 2020)

The Antioxidant activity test was done using the DPPH (2,2 -difenil -1-pikrilhidrazil) method. The test was carried with 0.5 mL sample solution at various concentrations (2, 4, 6, 8, and 10 ppm) to create a standard curve. 3.5 mL of DPPH was added to each solution. Homogenization was processed with vortex and incubated at 37 °C in a dark room. The absorbance was measured at the wavelength of 517 nm using a spectrophotometer (X Series UV VIS 100 DA-X) (Musri *et al.*, 2017).

The use of experimental animals in this study is in accordance with the code of ethics with ethical clearance (Ref: 5/KEPH/I/2020) from the Faculty

of Veterinary Medicine, Universitas Syiah Kuala. Rats were kept in animal cages. Cages were cleaned before used by spraying formalin 10 % as a disinfectant. Cages were placed at room temperature and get indirect light. Animal cages were wire cages. Food and drink were administrated to rat in containers, cages were cleaned every day. The rats were acclimatized for 1 week and food was provided ad libitum. The initial blood glucose level was measured after being fasted for 8-12 h. Furthermore, rats were injected by intraperitoneal with alloxan 120 mg kg<sup>-1</sup> body weight. Blood glucose was measured after alloxan induction. One week after alloxan injection, fasting blood glucose was measured to verify the incidence of hyperglycemic. Rats are declared hyperglycemic if glucose concentration above 200 mg dL<sup>-1</sup>, the rats were treated with the extract of *M. affine* orally.

Rat's blood glucose examination was done using Gluco Dr strips. Blood was drawn from the tip of the rat's tail with a little massage and then pierced with a sterile needle  $\pm$  as deep as  $\leq$  0.5 cm. Blood was taken from the tail of the rats and then dropped on a blood glucose strip. The blood glucose level was indicated on the numbers read on the blood sugar strips. Examination of blood sugar levels was done on day 0, 7, 14, and 21 after alloxan injection.

A compound has a very strong antioxidant if the IC<sub>50</sub> is less than 50 ppm, strong (50-100 ppm), moderate (100-150 ppm), and weak (151-200 ppm). The smaller the IC<sub>50</sub> value the higher the antioxidant activity (Musri *et al.*, 2017). The results showed that nanoemulsion extract of *M. affine* leaves has strong antioxidant activity with IC<sub>50</sub> 5.30 ppm while the IC<sub>50</sub> value of Vitamin C was 6.35 ppm (Table 1). This means that the antioxidant activity of *M. affine* extract is categorized as a very strong antioxidant. It was reported that methanolic extract of *Melastoma malabathricum* leaves has high antioxidants properties (Suhaimy *et al.*, 2017). Methanolic extract of *Melastoma malabathricum* leaves was considered as a compound which has a high antioxidant value (Mamat *et al.*, 2013). *M. affine* is related to *M. malabathricum*, which belongs to the family Melastomataceae and genus Melastoma. Ethanolic extract of green grass jelly leaves was reported to have high antioxidant activity with IC<sub>50</sub> 6.3 ppm (Mahadi *et al.*, 2018).

Nanoemulsion extract of *M. affine* leaves have very strong antioxidant activity. This extract has the potential to reduce glucose concentration in diabetic rats. The results showed that glucose levels decreased after the administration of nanoemulsion extract of *M. affine* leaves. The average blood glucose level of each treatment including N (normal group, untreated), NC (negative control, given

**Table 1.** Antioxidant Activity of Nanoemulsion Extract of *M. affine* Leaves.

No.	Control (EtOH, Absorbance)	Concentration (ppm)	Absorbance		% Inhibition		IC <sub>50</sub>	
			Vitamin C	<i>M. affine</i> leaves	Vitamin C	<i>M. affine</i> leaves	Vitamin C	<i>M. affine</i> leaves
1		2	0.071	0.078	42.74	37.10	6.35ppm	5.30 ppm
2		4	0.064	0.074	48.39	40.32		
3	0.124	6	0.063	0.051	49.19	58.87		
4		8	0.058	0.047	53.23	62.10		
5		10	0.057	0.043	54.03	65.32		

carboxymethyl cellulose sodium/Na-CMC), PC (positive control, given glibenclamide), P1 (given 30 % nanoemulsion extracts of *M. affine* leaf), P2 (60 % nanoemulsion extracts of *M. affine* leaf), and P3 (90 % nanoemulsion extracts of *M. affine* leaf) can be seen in Table 2.

The results showed that the initial blood glucose level of rats was in the range of 75-98 mg/dL. According to Wolfensohn and Lloyd (2013), the range of normal blood glucose levels in rats is 50-135 mg/dL. The results showed that the administration of alloxan caused increases in blood glucose in rats (Table 2). This is in line with a previous study by Safrida and Sabri (2019) that alloxan can induce diabetes in rats.

At the first week of observations, the amount of blood sugar in PC (positive control, treated with glibenclamide), and treated nanoemulsion extract of *M. affine* leaves groups; P1 (30 % of nanoemulsion extract of *M. affine* leaves) and P2 (60 % of nanoemulsion extract of *M. affine* leaves) were decreased compared to diabetic rats, but had not reached normal blood sugar levels. The rat blood

sugar levels in the P3 (90 % nanoemulsion extracts of *M. affine* leaves) treatment was almost close to normal rat blood sugar levels.

The administration nanoemulsion extract of *M. affine* leaves for 21 days was able to reduce the blood glucose level of rats to normal. This is presumably because *M. affine* leaves contain several bioactive compounds that have potency as antidiabetic. Mohd *et al.*, (2012) reported that *M. malabathricum* extract contains bioactive compounds namely ursolic acid, 2-hydroxyursolic acid, asiatic acid, glycerol-1,2-dilinolenyl-3-O-β-D galactopyranoside, glycerol 1,2-dilinolenyl-3-O- (4,6-di-O-isopropylidene) -β-D-galactopyranoside, methyl-2,5,6-trihydroxynaphthalene carbonate, flavonol glycoside derivatives, 2,5,6-trihydroxynaphthoic carbonic acid, kaempferol-3-O- ( 2 ', 6'-di-O-p-trans-coumaroyl) -β-glucoside, and quercitrin.

Quercetin is a type of flavonoid, a subclass of flavonols. It has a hypoglycemic ability to inhibit the enzyme alpha-amylase in the hydrolysis of carbohydrates. Quercetin is able to inhibit glucose

**Table 2.** Average of Blood Glucose Concentration of Rats in Various Treatments.

Treatment	Initial blood glucose (mg/dL)	Blood glucose after alloxan injection (mg/dL)	Blood glucose levels (mg/dL)		
			Day 7	Day 14	Day 21
N	75,00 ± 5,887 <sup>a</sup>	75,00 ± 5,887 <sup>a</sup>	96,25 ± 20,56 <sup>a</sup>	86,50 ± 1,91 <sup>a</sup>	76,00 ± 10 <sup>a</sup>
PC	85,25 ± 5,315 <sup>a</sup>	263,25 ± 39,508 <sup>b</sup>	173,75 ± 14,31 <sup>b</sup>	82,50 ± 3,87 <sup>a</sup>	87,75 ± 4,11 <sup>a</sup>
NC	88,25 ± 7,135 <sup>a</sup>	239,75 ± 41, 732 <sup>b</sup>	216,25 ± 46,77 <sup>c</sup>	247,50 ± 77,73 <sup>b</sup>	194,5 ± 5,32 <sup>b</sup>
P1	74,00 ± 2,708 <sup>a</sup>	316,50 ± 35,519 <sup>b</sup>	157,00 ± 17,26 <sup>b</sup>	104,75 ± 6,50 <sup>ab</sup>	87,00 ± 4,24 <sup>a</sup>
P2	98,25 ± 8,995 <sup>a</sup>	307,00 ± 73,787 <sup>b</sup>	158,50 ± 11,95 <sup>b</sup>	98,25 ± 8,57 <sup>a</sup>	88,50 ± 8,18 <sup>a</sup>
P3	98,25 ± 6,130 <sup>a</sup>	259,50 ± 61,733 <sup>b</sup>	137,50 ± 39,49 <sup>ab</sup>	91,25 ± 8,53 <sup>a</sup>	82,5 ± 10,34 <sup>a</sup>

transport by intestinal GLUT2 and GLUT5 which have a function in the absorption of glucose in the small intestine, so that quercetin is able to reduce blood glucose. Some evidence showed that flavonoids from vegetables and medicinal plants have a beneficial effect on diabetes by increasing glycemic control, lipid profile, and antioxidant status. Mechanisms of antihyperglycemic effects include reduction of carbohydrate absorption from the small intestine, inhibition of tissue gluconeogenesis, elevation absorption of tissue glucose, stimulation of insulin secretion from beta cells, and protection of the islets of Langerhans against degeneration (Ghorbani, 2017). Antioxidants can repair cells by capturing free radicals thus protecting the islets of Langerhans against damage (Musri *et al.*, 2017)

The amount of rat blood glucose reached normal blood glucose levels after 14 and 21 days of treatment in all concentrations of nanoemulsion extract of *M. affine* leaves. Previous research by Karmilah (2018) showed that the ethanolic extract of *M. affine* leaves with the dose of 360 mg/g body weight could reduce the amount of blood sugar to normal with a duration of 28 days. According to Justina (2014), nanoemulsion is a technology that can change drug particles into a nano size. One of the advantages of nanoemulsion is that it is easily absorbed by the intestinal wall.

Nanoemulsion extract of *M. affine* leaves have high antioxidant activity and potentially reduce glucose levels in diabetic rats. Nanoemulsion extract of *M. affine* leaves has potency as a source of antioxidants and antihyperglycemic agents.

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## REFERENCES

- Assis KS, Araújo IGA, de Azevedo FLAA, Maciel PMP, Machado Calzerra NT, da Silva TAF, Assis VL, de Vasconcelos AP, Santos CAG, Meireles BRLA, Cordeiro AMTM, Araújo DAM, Medeiros IA, 2018, Potassium Channel Activation Is Involved in the Cardiovascular Effects Induced by Freeze Dried *Syzygiumjambolanum* (Lam.) DC Fruit Juice. *Biomed Res* 1:1-12.
- Brahmachari, G., 2011, Bio- Flavonoids with Promising Antidiabetic Potentials: A Critical Survey, *Research Signpost*, 187-212.
- Ghorbani A., 2017, Mechanisms of Antidiabetic Effects of Flavonoid Rutin. *Biomed Pharmacother*. 96:305-312.
- Hasnah M S, Deny S, Farediah A, Hiromitsu T, Mriko K, 2010, Amides, Triterpene and Flavonoids from the Leaves of *Melastoma Malabathricum* L. *J Nat Med*. 64(4):492-5.
- Hernández-Abad VJ, Sánchez-González EG, Espinosa-Contreras C, Marroquín-Segura R, Mora-Guevara JLA, Flores-Cabrera Y, 2019, Controlled release of glibenclamide from monolithic silica subdermal implants produced by the sol-gel process and its use for hyperglycaemia treatment in a murine model. *Mater SciEng C Mater Biol Appl*. 1; 94:1009-1019.
- Jack, 2012, Syntetis Of Antidiabetic Flavonoids and Their Derivative. *Medical Research*.
- Justina, N., 2014, Produksi Nanoemulsi Ekstrak Temulawak dengan Metode Homogenisasi [Production of Temulawak Extract Nanoemulsion by Homogenization Method], Thesis, Bogor: Institut Pertanian Bogor.
- Karappasamy B, Antony N, Veerabahu RM, 2014, Antidiabetic and Antihyperlipidaemic Activity of Ethanol Extract of *Melastoma Malabathricum* Linn. Leaf in Alloxan Induced Diabetic Rats. *Asian Pac J Trop Biomed*. 4(1): S442–S448.
- Karmilah, 2018, Efek Antidiabetik Ekstrak Daun Senggani (*Melastoma polyanthum* BI.) pada Mencit (*Mus musculus*) Jantan yang Diinduksi Streptozotocin [Effects of Antidiabetic Extracts of Senggani (*Melastoma polyanthum* BI.) On Male Mice (*Mus musculus*) Induced Streptozotocin]. *Jurnal Mandala Pharmacon Indonesia*, 4(1), 28-32.
- Mahadi, R, Mustafid Rasyiid, Krisnanda Surya Dharma, Lindia Anggraini, Rahma Nurdiyanti, Tri Rini Nuringtyas, 2018, Immunomodulatory and Antioxidant Activity of Green Grass Jelly Leaf Extract (*Cyclea barbata* Miers.) *In Vitro. J. Trop. Biodiv. Biotech.*, 3 (2), 73—79
- Mamat, Siti Syariah, Mohamad Fauzi Fahmi Kamarolzaman, Farhana Yahya, Nur Diyana Mahmood, Muhammad Syahmi Shahril, Krystal Feredoline Jakius, Norhafizah Mohtarrudin, Siew Mooi Ching, Deny Susanti, Muhammad Taher, and Zainul Amiruddin Zakaria., 2013, Methanol extract of *Melastoma malabathricum* leaves exerted antioxidant and liver protective activity in rats. *BMC Complement Altern Med*. 13: 326.

- Mohd, Joffry S, Yob NJ, Rofiee MS, Meor Mohd Affandi MMR, Othman F, Abdah MA, Mohd Desa MN, Suhaili Z, Zakaria ZA., 2012, *Melastoma malabathricum* (L.) Smith: A review of its ethnomedicinal, chemical and pharmacological uses. *Evid Based Complement Alternat.* 13:258434.
- Musri Musman, Safrida Safrida, Viqqi Kurnianda, Erlidawati Erlidawati., 2017, Evaluation of Antihyperglycemia Property from *Syzygium moleana* (Magnoliopsida: Myrtaceae) Pericarp, *Research Journal of Medicinal Plants*, 11 (3), 100-106, 1819-3455.
- Poulain N and Nakache E., 1998, Nanoparticles from vesicles polymerization. II. Evaluation of their encapsulation capacity *J Polym Sci A: Polym Chem.* 36: 3035–3043.
- Safrida S, and Sabri M., 2019, Effect of *Muntingia calabura* L. Stem Bark Extracts on Uric Acid Concentration and Renal Histopathology in Diabetic Rats. *Medicina Journal*, 55(10), 2-8.
- Safrida S, K Khairil, W Artika and, R Rinaldi., 2020, *Pandanus amaryllifolius* Roxb. leaf extract prepared by nanoemulsion technique as a natural mouthwash. *Journal of Physics: Conference Series.* 1460 (1-7).
- Sahara M, Maxwel Simanjntak, Yoridha Aulia, Yustina Zai, Masdalena., 2019, Uji Aktivitas Anti Diabetes Ekstrak Etanol Daun Senggani (*Melastoma Malabathrium* L) pada Mencit Jantan yang Diinduksi Aloksan [Anti-Diabetic Activity Test of Ethanol Leaf *Melastoma Malabathrium* L in Aloksan-induced Male Mice]. *Seminar Nasional Teknologi Komputer & Sains (SAINTEKS)* 1: 174 – 176.
- Suhaimy NWI, Ahmad Khusairi Noor Azmi, Norhafizah Mohtarrudin, Maizatul Hasyima Omar, Siti Farah Md. Tohid, Manraj Singh Cheema, Lay KekTeh, Mohd. Zaki Salleh, Zainul Amiruddin Zakaria., 2017, Semipurified Ethyl Acetate Partition of Methanolic Extract of *Melastoma malabathricum* Leaves Exerts Gastroprotective Activity Partly via Its Antioxidant-Antisecretory-Anti-Inflammatory Action and Synergistic Action of Several Flavonoid-Based Compounds. *Oxid Med Cell Longev.* 1: 6542631
- Suharmati., 2003, Pengujian Bioaktivitas Anti Diabetes Melitus Tumbuhan Obat [Testing the Anti-Diabetes Mellitus Bioactivity of Medicines]. Surabaya: Badan Riset Pengembangan dan Kesehatan. Pusat Riset dan Pengembangan Pelayanan dan Teknologi Kesehatan. Departemen Kesehatan RI.
- Wolfensohn, S., dan Lloyd, M., 2013, *Handbook of Laboratory Animal Management And Welfare, 4th ed.*, Wiley Blackwell, WestSussex.