# **Syzygium polyanthum** (Wight.) Walp Ethanol Extract Decreased Malondialdehyde Level in Type 2 Diabetic Patients

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#### **ABSTRACT**

**Background**: Beside conventional oral antidiabetic, many diabetic patients used plant as an alternative medicine. One of the plants is *Syzygium polyanthum* Wight. Walp. Previous study showed that ethanolic extract of *Syzygium polyanthum* (Wight.) Walp leaves (EESP) is safe to be consumed by healthy volunteer. **Aim**: The present study was conducted to investigate the effect of EESP on malondialdehyde (MDA) level in type 2 diabetes mellitus volunteers. **Materials and Methods**: EESP was obtained by maceration then formulated in capsules (weight of 350 mg). A total of 12 volunteers were randomly given EESP or Placebo/Pl (amylum) once daily for 14 days in conjunction with metformin 500 mg twice daily. Hemoglobin (Hb), liver and kidney function and MDA were measured at the beginning and at the end of the study. **Results**: After 14 days administration of EESP, Hb 12.52± 1.23 (g/dL), SGOT 24.16±13.57 (U/L), SGPT 27.50 ± 20.52 (U/L), ureum 23.12±13.27 (mg/dL), creatinine 0.71 ± 0.23 (mg/dL) while MDA 1041.63 ± 615.66 ng/mL in EESP treated group. The reduction of MDA level in EESP-treated group (24%) were higher than Pl-treated group (16%). **Conclusion**: *S. polyanthum* leaf extract is potential as antioxidant in type 2 diabetes mellitus patients

**Keywords:** Diabetes mellitus; Extract; Malondialdehyde; S. polyanthum

# **INTRODUCTION**

Diabetes mellitus (DM), commonly known as diabetes, is a group of metabolic disorders characterized by the presence of hyperglycemia in the absence of treatment. The heterogeneous aetiopathology includes defects in insulin secretion, insulin action, or both. The long-term specific complications of diabetes include retinopathy, nephropathy, and neuropathy. As a chronic illness, this disease requiring continuous medical care with multifactorial risk-reduction strategies beyond glycemic control.<sup>2</sup>

In the world and in all regions, DM is found in every population, including rural parts of low- and middle-income countries. WHO estimates there were 422 million adults with diabetes worldwide in 2014. The age-adjusted prevalence in adults rose from 4.7% in 1980 to 8.5% in 2014, with the greatest increase in low- and middle-income countries.<sup>1</sup>

There are considerable evidences that many of the biochemical pathways which generates reactive oxygen species (ROS) are activated by hyperglycemia which ultimately leads to oxidative stress.3,4 Increased rate of free radical production and/or impaired antioxidant mechanisms are responsible for the high of oxidative stress level. These condition are related to insulin resistance, impaired glucose tolerance and  $\beta$ -cell dysfunction. Malondialdehyde (MDA), an organic compound, which is produced by ROS of lipid peroxidation process can be used as a marker in the development and progression of diabetes and its complication.5 Therefore, the evaluation of MDA level can be used as a marker of oxidative stress which can predict the potency of antioxidant activity of an agent.

Medication using medicinal plants is believed has lower side effects and relatively low cost

than synthetic drugs.<sup>6-8</sup> In treatment perspective, the treatment of this disease need long term of antidiabetic agent consumption. Due to these issue, many diabetic patients used plants as the alternative medicine beside the conventional oral antidiabetic agent, one of which is *Syzygium polyanthum* Wight. Walp. <sup>9</sup> Its leaves has been reported to have biological activity such as antihyperlipidemic<sup>10,11</sup>, larvacid<sup>12</sup>, antidiabetic<sup>13-15</sup>, and antioxidant as well.<sup>16-18</sup> These beneficial activites related to the active constituents of this plant, i.e essential oil, eugenol, *methyl chavicol*, flavonoids, alkaloids, tannins, steroids, triterpenoids and squalene.<sup>9,19-20</sup>

The present study was conducted to evaluate the effect of extract ethanol of *Syzygium polyanthum* Wight. Walp leaf on malondialdehyde level in type 2 diabetic patients.

# **MATERIALS AND METHODS**

#### Time and place

The study was conducted from April to November 2019 at Pharmacy Faculty and Medical Faculty of Universitas Sumatera Utara, Primary Health Care Glugur Darat, Medan and Integrated Laboratory of Medical Faculty, Universitas Sumatera Utara. A protocol has been approved by Health Research of Ethical Committee (No.616/TGL/KEPKFKUSU-RSUP HAM/2019).

#### **Extract preparation**

The leaves of *Syzygium polyanthum* Wight. Walp were collected from Medan region, North Sumatera, Indonesia. The selected leaves were washed in tap running water, then were dried in drying cabinet. The dried leaves, then were mashed into powder form. The powder was macerated using 70% ethanol, to obtain the Ethanol Extract of *S. polyanthum* Leaves



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(EESP). Plant identification was confirmed by Herbarium Medanense (MEDA), Universitas Sumatera Utara, Medan, Indonesia.

# Determination of total phenolics

Total phenolic content was determined with the Folin–Ciocalteu reagent. A calibration curve was obtained by using gallic acid as standard. An amount of 5 mg gallic acid was dissolved in 100 ml methanol as a standard solution. Then it was diluted to 62.5; 125; 500  $\mu g/ml$ . 10 mg sample was diluted in 10 ml methanol on the test tube. As mush as 0.1 ml of both standards and samples solutions were taken and mixed with 0.5 ml of Folin–Ciocalteau and 7.9 ml of distilled water, vortexed for  $\pm$  1 minute, and added 1.5 mL of Na2 CO3 20%, then incubated for 90 minutes. The absorbance of all standards and samples were measured at 400 nm to 800 nm using Shimadzu 1800 UV-Vis spectrophotometer and the results expressed as milligrams of gallic acid equivalents (GAE) per g of extract.

#### Determination of total flavonoids

Total flavonoid was analyzed using aluminium chloride colourimetric method.  $^{21}$  Quercetin was used to make the calibration curve. 10 mg of quercetin was dissolved in 100 ml ethanol 96% and diluted to 6, 10, 14.5, 19, and 23.5 µg/mL. 25 mg sample was diluted in 25 ml ethanol 96%. 2 ml of each concentration of standard solutions, as well as 1 ml of each sample solution, were mixed with 3 mL ethanol 96%, 0.2 mL of aluminium chloride, 0.2 mL potassium acetate 1 M and 5.6 mL of distilled water. The mixture was incubated at room temperature for 30 minutes. The absorbance was measured at 440 nm using Shimadzu 1800 UV-Vis spectrophotometer and the results expressed as weight of Quercetin Equivalent (QE) per gram of extract.

#### Capsule preparation

The EESP mixed with maydis starch to obtain a homogeneous mass. The mass was dried in oven at 55°C and then filled into 0-sized capsule shells using a semi-automatic capsule filling device. The capsule contained 350mg ethanol extract of *S. polyanthum* leaves.

# Volunteer recruitment

The volunteers were outpatients at Glugur Darat Primary Health Care, Medan, North Sumatera, Indonesia. Each volunteer was explained the study protocol and signed informed consent before acceptance. To participate in the study, the volunteer must fulfill the inclusion criteria, i.e diabetes mellitus type 2, consumed metformin during the study, no gastritis history, not in terminal illness, liver function (Serum Glutamic-Oxaloacetic Transferase (SGOT) and Serum Glutamic-Pyruvic Transferase (SGPT)) and kidney function (Ureum (Ur) and Creatinin (Cr)) not more than 2 times normal range. Any adverse effect or subjective symptoms that could not be tolerated by the subject was excluded from the study.

# Design

The minimum sample size required in this study was calculated using the G power software. A total of 12 volunteers was randomly given capsule contained EESP or placebo (Pl) (amylum) once daily along with metformin 500 mg twice daily. Complete blood tests, liver (SGOT and SGPT) and kidney (Ur and Cr) function were carried out at the beginning and after 14 days of taking the capsules. During the administration any complaints experienced were recorded.

#### Data analysis

Data were analyzed using IBM SPSS Statistics 22 software. Results were presented as mean  $\pm$  standard deviation. Statistical significance was assessed with Mann-Whitney U or Independent T-Test; Wilcoxon Signed Ranks Test or Dependent T-Test.

#### **RESULTS**

#### Total phenolics and total flavonoids

Table 1 showed that EESP of this study contained phenol 161.64 mg/g in GAE and flavonoid 45.75 mg/g in QE.

# Volunteer observation

During the observation, voluntary vital signs as recorded in normal range (data were not shown). Feeling sleepy, itchy, increased appetite, polyuria and numb were submitted by the Pl-group.

Table 1: Total phenolics and total flavonoids.

	Total phenolics (mg/g) in GAE	Total flavonoids (mg/g) in QE
EESP	161.64	45.75

(GAE: Gallic Acid Equivalent; QE: Quercetin Equivalent)

Table 2: Hb, liver and kidney function of the volunteers.

Group	Hb ( <i>Male</i> : 14.00-17.00) ( <i>Female</i> :12.00-16.00) g/dl Mean±SD		<b>p</b> <sup>b</sup>
	Day 0	Day 14	
EESP	12.60 ±1.27	12.52 ±1.23	0.52
Pl	$12.88 \pm 1.57$	$12.76 \pm 1.36$	0.68
$\mathcal{P}^{a}$	0.93	0.48	

Group	SGOT (5-35) U/L Mean±SD		<b>p</b> <sup>b</sup>
	Day 0	Day 14	
EESP	$20.83 \pm 9.51$	24.16 ± 13.57	0.27
Pl	$16.00 \pm 6.32$	$19.83 \pm 13.45$	0.22
$\mathcal{P}^{a}$	0.39	0.31	

Group	SGPT (5-35) U/L Mean±SD		<b>p</b> ⁵
	Day 0	Day 14	
EESP	$25.66 \pm 18.25$	$27.50 \pm 20.52$	0.34
Pl	$17.83 \pm 8.61$	$20.66 \pm 13.61$	0.27
$p^a$	0.69	0.93	

Group	Ureum (<50) mg/dl) Mean±SD		<b>p</b> ⁴
	Day 0	Day 14	
EESP	21.57 ±7.04	23.12 ± 13.27	0.91
Pl	$26.33 \pm 5.87$	$21.62 \pm 6.34$	0.12
$p^c$	0.67	0.31	

Group	Creatinin (0.6-1.3) mg/dl Mean±SD		<b>p</b> <sup>b</sup>
	Day 0	Day 14	
EESP	$0.65 \pm 0.10$	$0.71 \pm 0.23$	0.17
Pl	$1.01 \pm 0.40$	$1.03 \pm 0.40$	0.83
$\mathcal{P}^{a}$	0.09	0.24	

a:Mann-Whitney U Test; b:Wilcoxon Signed Ranks Test; c: Independent T-Test; d: Dependent T-Test

Table 3: Effect of EESP on MDA level.

Group	MDA (ng/ml) Mean±SD		<b>p</b> <sup>b</sup>
	Day 0	Day 15	
EESP	1381.55 ± 977.79	$1041.63 \pm 615.66$	0.22
Pl	$1418.38 \pm 503.24$	$1177.74 \pm 589.39$	0.34
$\mathcal{P}^a$		0.05	

a:Mann-Whitney U Test; b:Wilcoxon Signed Ranks Test

# Effect of EESP on hemoglobin, liver and kidney function

As shown in Table 2, the level of Hb, SGOT, SGPT, ureum and creatinin were in normal level. Statically, no significant different between day 0 and day 14 of the treatment.

#### Effect of EESP on MDA level

Table 3 showed the MDA level before and after 14 days intervention. The results showed that MDA reduction in the EESP-treated group was higher than Pl group. In EESP-, MDA decreased to 1041.63  $\pm$  615.66 ng/ml from 1381.55  $\pm$  977.79 ng/ml (24%), while in Pl-treated group from 1418.38  $\pm$  503.24 ng/ml to 1177.74  $\pm$  589.39 ng/dl (16%). However, statistically, there was no significant different between both of intervention (p>0.05).

# **DISCUSSION**

Diabetes is strongly co-associated with oxidative stress induction. The use of antioxidants in both the treatment and prevention of diabetes was scrutinized in several studies. Recent studies reported contrasting findings regarding the benefits of antioxidant therapeutics in the management of diabetes.<sup>23</sup>

*Syzygium polyanthum* (Wight) Walp. or bay leaf commonly used as food flavoring in Indonesia. For herbal purpose, safety data in preclinical study reported that ethanol extract of *S. polyanthum* 1 g/kg for 90 days not toxic on the hematology, creatinine and SGPT.<sup>24</sup> In this study, safety report on liver (SGOT and SGPT level), renal function (Ur and Cr), and hemoglobin level showed that those parameters were in normal range. There were no significant changes on those profiles during observation.

The phytochemicals are responsible for the therapeutic activities of the plants including phenols and flavonoids. These compounds have been reported play a role to the antioxidant activity of plants. The present study showed that EESP contained phenolics (161.64 mgGAE/g extract) and flavonoids (45.57 mgQE/g extract). This results support the previous preclinical studies that reported the antioxidant properties of this plant.  $^{16,17,28,29}$ 

Evidences suggest that hyperglycemia plays a role in generation of reactive oxygen species (ROS). These ROS ultimately increase oxidative stress in a variety of tissues. 15 One indicator of oxidative stress is malondialdehyde (MDA).30 MDA is a product that results from the degradation of polyunsaturated fatty acids in cell membranes. Reported levels of this product are higher found in diabetic patients.<sup>31</sup> This condition may trigger an inflammatory process associated with blood vessel abnormalities in type 2 DM. The agents which can delay or prevent oxidative stress are called as antioxidants.<sup>32</sup> Imbalance between oxidants and antioxidants can cause oxidative stress in pancreatic beta cells, subsequently involved in the pathophysiology of DM and its complications. $^{33,34}$  MDA level was measured in the present study to evaluate the potency of S. polyanthum extract as an antioxidant agent in diabetic patients. In this study, administration of capsule containing EESP decreased MDA level greater than placebo for 14 days observation. High serum levels of MDA correlates with hyperglycemia because of self-oxidation of glucose and could generate free radicals.<sup>5</sup> S. polyanthum leaves could inhibit a α-glucosidase enzyme which yields an effect similar to acarbose. The flavonoid compounds of the extract could help regenerate the dysfunctional pancreatic  $\beta\text{-cell}$  which could improve glucose control by optimizing insulin production.<sup>35</sup> Its antioxidant activities mainly due to its flavonoids and phenols content that has strong antioxidant properties. 11 However, the limitation of this study needs to be further investigated with wider range of volunteer and longer time of use. Along with this study, the treatment of 350mg of EESP was effective to lower fasting blood glucose level in patients with type 2 diabetes mellitus within 14 days observation. Fasting blood glucose level of EESP decreased from 186.25±58.57 to 169.79±47.31 (8.85%; BGL difference 16.5mg/dl).36

#### CONCLUSION

The reduction of MDA level after *S. polyanthum* ethanol extract leaf at dose of 350 mg for 14 days administration showed its potency as antioxidant in type 2 diabetes mellitus patients.

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# **CONFLICTS OF INTEREST**

The authors declare no conflicts of interest.

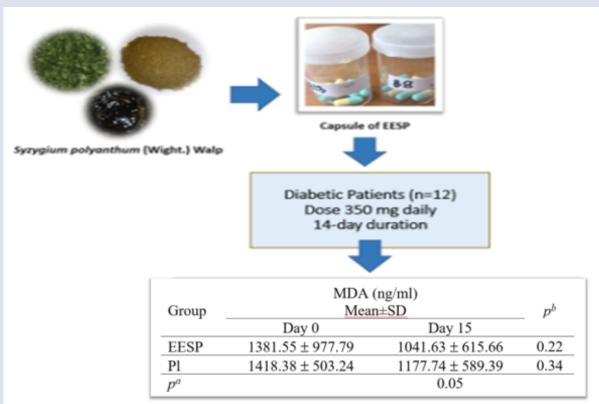
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# GRAPHICAL ABSTRACT



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