Administration of ethanol extract of mustard greens (Brassica rapa L) leaves increased Superoxide Dismutase levels in Hyperglycemic rat

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Abstract. Mustard green leaves (Brassica rapa L) is one of the Indonesian medicinal plants that can be used as an antidiabetic drug. This study aims to prove that administration of ethylacetate fraction of ethanol extract of mustard greens (Brassica rapa L) leaves increased superoxide dismutase (SOD) level in hyperglycemic Wistar rats. Mustard green leaves powder (Brassica rapa L) was extracted by maceration using 96% ethanol to yield crude ethanol extract. 30 Wistar rats were divided into 5 groups consisting of 6 rats each group. Negative group, P0 (received food standart only), positive control, P1 (induced with streptozotocin and given glibenclamide drug); P2, P3, and P4 were treatment groups (induced with streptozotocin and given ethanol extract of mustard green at doses of 0.5, 2.0, and 5.0 mg/KgBw/day respectively). The dose of streptozotocin used to induce hyperglycemia in all rats was 125 mg/KgBw/day. The level of superoxide dismutase was examined before inducing streptozotocin and after the rats hyperglycemic. The ethanol extract was then fractionated into ethyl acetate fraction and then identified using GC-MS. The result showed that administration of ethanol extract at doses 5 mg/KgBW significantly increased SOD level (4.13 ± 1.18 ng/mL) of hyperglycemic rats as compared to negative control (2.75 ± 0.55ng/mL). Analysis GC-MS spectra of the ethylacetate fraction of ethanol extract of mustard green showed 6 major peaks assigned as vinyl propionste, buthyl formste, 2-methoxy4-vinylphrnol, 13-oxa-dispiro{5.0.5.1}trican-1-one, 1-methyl isoeugenol and 3-isopropoxy-5-methyl-phenol.

1. Introduction

Hyperglycemia is one of the basic diagnoses of diabetes mellitus(DM) Diabetes mellitus is highly prevalent disease in Indonesia. The lastest data in 2015 by Endocrinology Society stated that the number of diabetic patients in Indonesia reached 9.1 million people. Until this article was written Indonesia was the fifth for the highest number of diabetic patient in the world Since 2000. The number of patients with diabetes mellitus in Indonesia has increased. The World Health Organization predicted that by 2030 people with diabetes mellitus world reach 21.3 million people [1]. The latest epidemiology study had put type 2 DM as an epidemic in Indonesia.Nearly 80% of DM is caused bythe patient’s lifestyle. The life style in the word community, especially in Indonesia. In recent years have shown to be trans formed from onea traditional and nutritious one to a fastfood lifestyle that is low nutritious one to a fastfood lifestyle that is low in nutritional value ( junk food). The impact of these unhealthy life style is DM change is the emergence of various diseases one of which DM. which is a disease characterized by hyperglycemia that caused by abnormalities in insulin secretion or spesiesdisoxygen rder. This condition can increase the reactive oxygen species (ROS) and SOD (Superoxyde Dismutase) decrease compounds through a non enzymatic process. Hyperglycemic is a ondition with an decrease SOD (superoxy dismutase) and incorease ROS (reactive oxygen species) through the process of enzymatic reactions.

This reaction include oxidation and phosphorylation and ADPH oxidation reaction and through non enzymatic proceny abnors by forming of gluco oxidations and Glycation. Hyperglycemia is caused by abnormalities in insulin secretion or action of insulin disolder. The state of hyperglycemia is diabetes lead to increase formation of free radicals antioxidants and decrease a member of events that eventually occurs is called oxidative stress. This study aims to determine the effectiveness of mustard green leaves (Brassica rapa L) in fixing the rate of β-cell pancreas damage in hyperglycemia rat Wistar induced with
streptozotocin. Streptozotocin may exert diabetogenic effects by damage to beta cell membranes by increasing permeability. Streptozotocin sititosik action is mediated by free radicals. The streptozotocin toxic action in beta cells is initiated by free radicals formed by redox reactions. Streptozotocin and its reduction products, acids are grooved, forming a redox cycle with superoxide radical formation. This radical undergoes dismutase to hydrogen peroxide. The hydroxyl radical with high reactivity is formed by the Fenton reaction. The action of free radicals with high stimulation increases the concentration of cytosolic calcium which causes rapid destruction of beta cells. Avoiding the use of beta cells used therapy using the natural plants or as well known as herbal medicine has been known and used throughout the world since thousands of years ago. The community use herbal therapy for prevention efforts but often, it is also found for promotive and rehabilitative efforts. Herbal therapy is very popular because it is easily available and very economical. The community still assume that herbal therapy is relatively safer than synthetic drugs, but herbal therapy also has an advese effect if use appropriately. One of the natural plants that has properties as a drug and contains secondary metabolites is mustard green leaves.

One of the medicinal plants that can anti be used as an antidiabetic drug is mustard green leaves (*Brassica rapa* L) is specries from the Brasscaceae family which play a major role in vegetable and consumption throughout the world. Mustard greens leaves have been cultivated for centuries throughout Europe which eventually spread to central and eastern Asia. Plant parts such as roots, leaves, and seeds have been used in traditional medicine, generally for the treatment of several diseases such as diabetes.

Based on [2]. Mustard greens contain alkaloid, terpenes, tannins, saponins, and glycosides. Alkaloid have been shown to have the ability to regenerate damaged pancreatic β-cells. Terpen serves as an antidiabetic because terpenes are the main component of essensial oil while saponins functions to increase to glucose homeostasis by increasing insulin sensitivity. Corrugated mustard green leaves contain biologically active compounds such as flavonoid including isorhamnetin, kaempferol and quercertin glycosides. Phenylpropanoid derivatives, indole alkaloids, and glucoside sterol that polyphenols have beneficial effects. Especially in diabetes. The use of mustard greens as an antidiabetic drug is still rare. Generally, People use mustard green to make a home cooked dish.

There has been no scientifc study of the effects of mustard greens ethanol extract to decrease superoxide dismutase levels is antioxidants are compound that play an important role in maintaining and improving the quality of body health. Antioxidants are needed by the body to inhibit oxidation reactions by binding to free radicals that are widely found in nature so that cell damage does not occur. Antioxidants consist of two types, namely enzymatic, and non enzymatic, namely enzymatic antioxidants produced in the body include superoxide dismutase (SOD), glutation peroxidase (GPx), and enzyme catalase (CAT). Non enzymatic antioxidants can be micronutrient in the form of vitamins, where both types of antioxidants can be obtained from food or suplements [3] Oxidative stress is a state of imbalance between oxidants and antioxidants. One that can cause an increase in oxidative stress is a pattern of life [4] Lifestyle that can cause an increase in oxidative stress one of which is consumption high glucose. Diet high glucose considered hyperglycemia. Hyperglycemia is a condition with an increase of blood glucose fasting levels (about 109 mg/dL) and 2 hour blood sugare post prandial (above 158 mg/dL).

### 2. Methodology

#### 2.1. Plant material

The leaves of mustard green (*Brassica rapa* L) from Sumber Village Sanankulon District Blitar Regency.

#### 2.2. Chemical

Analytic grade of ethanol, N-hexan, Ethyl acetate, FeCl3 were purchased from local supplier and analytic biokimia kit Superoxide Dismutase etc from BPOM (Badan Pemeriksaan Obat dan Makanan) Bali.
2.3. Animal
30 male Wistar rats (200-250 g) housed at 25 ± 2°C and with received standard food and water libitum were used in these experiment that were performed during after a rats were randomly assigned into 5 groups consisting of 6 rats eachive. Which will P0 control positip, P1 control negatip group (induced with streptozotocin with treatmen glibenclamide); P2 induced with streptozotocin treatment 0,5 mg/kgbw/day, P3 induced with streptozotocin treatment 2,0 mg/kgbw/day and P4 induced streptozotocin treatment 5,0 mg/kgbw/day. All control treatment groups have streptozotocin induced at a dose of 125 mg/kg bw to obtain hyperglycemia. Before inducing diabetes, all Wistar rats were fasted for 6-18 hours (drinking waster was still given sufficiently) and examined for superoxyde dismutase test in hiperglycemic Wistar rats (Table 3).one week adaptation process.the sample were grouped. Based on Federer’s formula (1977)[5].

2.4. Procedures

2.4.1. Extract preparation. Extraction of mustard greens: a total of 1000gram of mustard green powder (Brassica rapa L) were extracted by maceration process using 96% ethanol ethanol as solvent until all the mustard green was immersed in the solvent.Soaking was done for ±48 hours repeatedly until a clear filtrate is obtained.The clear filtrate was then run with a thin layer chromatography plate to confirm complete extraction.The ethanol extract was filtered and seperated from the solvent using a rotary vacuum. Evaporator until a thick extract was produced which from now on will be called as ethanol extract of thick mustard green (Brassica rapa L). The thick ethanol extract was fractionated using water, n-hexane, and ethyyl acetate. The fractionated products were thhe evaporated, and dosage from was made for the initial test of the most effective dose to oxygen dismutase levels in hyperglycemic rats.

2.4.2. Analysis of Phytochemical.
Phytochemical screening performed on ethyl acetate fraction includes alkaloids. Flavonoids, terpenoids. Steroid.saponins.adn phenolics. Phytochemical screening result show that ethyl acetate fraction doesnot steroid compound, but contains alkaloids, flavonoids, terpenoids, saponins,and phenolic compounds (Table 1).

2.4.3. Analysis of Gaschromatography MassSpektofotometry.
Gas Chromatography-Mass Spectrophotometry identification obtained chromatogram with 6 peaks in the fraction. The chromatogram result can be seen in (Figure 1). And the identification of alleged compounds containeds based on GC-MS spectrometer database can be seen in Table 2.

2.7 Analysis of Superoxyde Dismutase
Superoxyde Dismutase (SOD) levels were determined biochemically using an ELISA kit. The reaction in this kit consisted of available Plasma Biotinylated Ab and Horseradish Peroxidase (HRP) Plasma added 50µL Biotinylated Detection Ab for each tube. Let stand for 45 minutes at room temperature 37°C Aspiration and wash 3 times and then add 100µL HRP conjugate with the same tube. Incubate for 30 minutes at room temperature 37°C Aspiration and wash again 5 times add 90µL reagent substrate let stand for 15 minutes at room temperature 37°C Add stop solution. Color changes were measured by spectrophotometry at a wavelength of 450 nm ± 2 nm. SOD levels in the standard curve sample are units of ng /mL. Analysis of oxygen dismutase level was obtained from venous blood of Wistar rats using kit Elisa in mg/dL after rats fasted for 10 -12 hours units [6]

3. Result and discussion

3.1. The effect ethyl acetate fraction
The result of the chemical product Thined from result of the chemical products obtained from the green mustard were 8.69% i.e from the 1000 gram powder. 86.93 gram of thick tracetethanol extract were produced. The reaction products were calculated to determine the amount of extract produced by the mustard green powder. The thick ethanol extract was fractionated using water, n-hexane, and ethyl acetate. The fractionated result were then evaporated and dosage from made to determine the most effective dose to superoxide Dismutase levels in hyperglycemia. Wistar rats (already stated in methods section. Hence unnecessary. Consider to remove this statement) The result of the preliminary test showed that the ethyl fraction decreased a higher than other fractions. Phytochemical screening performed on ethyl acetate fraction includes alkaloids, Flavonoids, terpenoids, Steroid, saponins, and phenolic phenolics. Phytochemical screening result show that ethyl acetate fraction does not steroid compound, but contains alkaloids, flavonoids, terpenoids, saponins, and phenolic compounds (Table 1).

### Table 1. Phytochemical screening of Mustard Green Ethanol Extract

| Tests  | Reagents          | Outcome |
|--------|-------------------|---------|
| Phenolic | FeCl3             | +       |
| Alkaloid | Wagner           | +       |
| Terpenoid | Lieberman Burchard | +     |
| Steroid | Lieberman Burchard | -     |
| Saponin | Hot aquadest + HCL | +      |

Mass spectrometer identification obtained chromatogram with 6 peaks in the fraction. The chromatogram results can be seen in figure 1. And the identification of alleged compounds based on GC-MS spectrofotometry database can be soon in Table 2. The mass spectrum of each peak is the identified by comparing, the mass spectrum is the database so that these compound contained in the fraction can be predicted. Estimates of compounds based on database can be seen in Table 2.

### Table 2. Identification of six possible compound

| Peaks | Tr  | % Area | M*  |
|-------|-----|--------|-----|
| 7     | 4.59| 2.85   | 73  |
| 3     | 13.60| 2.29   | 150 |
| 4     | 17.90| 1.71   | 194 |
| 5     | 19.68| 19.68  | 178 |
| 6     | 20.14| 20.14  | 166 |

namely phenol compoundes. Phenol compounds are widely used as antioxidants. Phenol works as an antioxidant which inhibits the formation of free radicals and protects cells from oxidation. Phenol has a cardioprotective effect. Which inhibits a very powerful antioxidant 2-methoxy-4-vinylphenol compound is a phenolic group that has antioxidant, antimicrobial and antiinflammatory
3.2. Analysis of Superoxide Dismutase

Superoxide Dismutase (SOD) levels were determined biochemically using an ELISA kit and levels SOD data were tested for normality using the shapiro-Wilk test and homogenety using the leven´s test. Noting that SOD level data in all groups of Wistar rats were normally distributed (p>0.05) and data variance was homogeneous (p>0.05) to determine the difference in mean SOD levels between groups carried out a significance analysis using the one way Anova test. Resume pre and postest presented in Table 3.

Table 3. Mean Differences in SOD levels between Groups after Being iven Ethanol Extract of Mustard Green leaves

| Groups    | Observation SOD (Oxygen dismutase) levels ng/mL |
|-----------|-------------------------------------------------|
| ?pretest ± SD | ttest ± SD           |
| P0        | 2.75 ± 0.55                                   |
| P1        | 2.91 ± 2.30                                   |
| P2        | 2.22 ± 0.65                                   |
| P3        | 2.27 ± 1.91                                   |
| P4        | 3.99 ± 1.19                                   |
| P5        | 2.83 ± 0.55                                   |
| P6        | 2.96 ± 2.30                                   |
| P7        | 2.60 ± 0.64                                   |
| P8        | 2.70 ± 1.90                                   |
| P9        | 4.13 ± 1.18                                   |

Table 3 The result of this study also showed that the higher the does of mustard green (Brassica rapa L) leaves ethanol extract the lower the SOD level. This decrease in SOD levels is possible because the phenol contained in mustard leaves not only act as antioxidants; but also act as procolsins are certain conditions [6]. The imbalance betwen free radicals and antioxidants in the body result in oxidative stress. Oxidative stress cause superlipid peroxidation thereby increasing malondialdehyde (MDA) levels and Increasing superoxide dismutase (SOD) activity [7].

4. Discussion

Mustard green (Brassica rapa L) leaves to make a home cooked dish and plants have been tradiational used for treatment of antihyperglycemia. Phytochemical study revealed that ethanol extract of mustard greens (Brassica rapa L) a numerous class of secondary metabolits including flavonoid, phenolic, terpenoid, tanin and saponin. The mechanism of action of the mustard green ethanol extract in
increasing superoxyintgen dismutase (SOD) level is due to the compounds contained in the extract. Some compounds are potentially active as antihyperglycemic and antioxidants, namely the phenol compounds. Phenol compounds are widellusiony used as antioxidants [10]. Phenol works as an antioxidant which inhibits the formation of free radical and protects cell from oxidantion. Phenol has a cardioprotective effect. Which is a very powerful antioxidants 2-methoxy-4-vinyl phenol. Methyl isoeugenol 1 and 3-isopropoxy-5-methylphenol compound are thought to be the active compound that contribute to antioxidant and antihyperglycemic activity in the mustard green ethyl acetate fraction. According[11] the 2-Methoxy-4-vinyl phenol compound is a phenolic group that has antioxidant. Antimicrobial and antinflammatory activities. The mean SOD level are presented in Table 3. While SOD level profile from various dose of mustard green ethyl acetate fraction are present in figure1. Table3 shows that the highest increase in SOD levels of hiperglycemia Wistar rats occured in the administration of 5.0 mg/kg BW extract. Increased SOD levels are effected by the antioxidant endogenous compounds in the fractions that can supply electrons to the radical compounds. Increases SOD is the negative effects of oxidatives stress.

5. Conclusion
In general, this study proved that the administration of ethanol extract of mustard green (Brassica rapa L) ethanol extract containing phenolic, flavonoid, Alkaloid, Saponin and antioxidant activity against DPPH with IC50 = 4.33 mg/mL can inhibition oxidative stress due to exposure hyperglycemic reaction at a dose of 5.0 mg/kg BW/day can significantly increase superoxide dismutase (SOD). Administration of phenol extract of mustard green (Brassica rapa L) ethyl acetate fraction at a dose of 5.0 mg/kg BW can significantly increased superoxygen dismutase (SOD) levels in hiperglycemic Wistar rats (p<0.05). Phenol group compounds contained in ethyl acetate fraction of mustard extract (Brassica rapa L) have function as a powerful antioxidant to increase SOD hyperglycemic Wistar rats

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References
[1] Fard, M.H.Naseh G, Lotfi, N.,Hosseini,M.2015 .Effect of aqueous of Brassica rapa L in alloxan induced diabetic rats. Avicenna Journal of Phytochemical 5 (2) 148-156
[2] Hamid; A.A; Aiyelaagbe, O.O; Usman.,L.A.; Ameen, O.M., and Lawal, A.2010. Antioxidant it’s Medical and Pharmacological Applications African Journal of Pure and Applied Chemistry Vol 4 (8):142-151 cited 2016 Peb.08 Availablef.org/Ajrom http://www.academiajournals.org/AJPAC
[3] Mahmudah, FK.2011. Uji Aktivitas anti diabetes dengan Metode Penghambatan Enzim a-Glukosidase dan skrining Fitokimia Pada beberapa tanaman Indonesia Depok.,Skripsi F.MIPA Universitas Indonesia
[4] Paine; M.A.,Owollah, Rudar, E.H;Harman, T.J; blumberg, J.and Goldman,M.B.2013. Chapter 4. Oxidative Sress Oogenesis zisolliculogenesis, In: Argawal, A., Azis,N; Kizk, D. Editors. Studies on Woman’s Health; Oxidative Stress in Applied Basic Research and Clinical Practice.New York: Spinger Science Business Media P.75-76.ha
[5] Prochazkova,D.; Bousava, L.and Wilhelmove,N; 2011. fito Antioxidant and prooxidant properties of flavonoids Fitoterapia. Vol 82 (4). Pp 513-23.