

# Effect of mother-in-law's tongue leaves (*Sansevieria trifasciata*) extract's solvent polarity on anti-diabetic activity through in vitro $\alpha$ -glucosidase enzyme inhibition test

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**Abstract.** Diabetes Mellitus (DM) is a metabolic disease caused by elevated blood glucose levels above normal values (hyperglycemia). Natural ingredients that contain hypoglycemic substances, can become an alternative treatment of DM, i.e. mother-in-law's tongue leaves (*Sansevieria trifasciata*), which is known to contain flavonoids, glycosides, alkaloid, and steroids that have anti-diabetic activity. The goal of this research is to obtain mother-in-law's tongue leaves crude extract which has the highest anti-diabetic activity as an  $\alpha$ -glucosidase inhibitor. The result showed that from all solvents used, which is aquadest, 70% ethanol, and 70% ethyl acetate, extracted with 70% ethanol has the highest yield value, which is 12.69%. The percentage of inhibition  $\alpha$ -glucosidase was 90.72% at the inhibitor concentration of 4708.71. This shows that the extract with 70% ethanol has the required bioactive compound content to act as the  $\alpha$ -glucosidase enzyme inhibitor. Based on the identification of compounds found in the sample mother-in-law's tongue leaves extract with 70% ethanol using Gas Chromatography and Mass Spectrometry (GC-MS), compounds that have anti-diabetic activity is steroids, alkaloids, terpenoids, tocopherol, and flavonoid.

## 1 Introduction

People have experienced degenerative metabolic diseases, one of many diseases is diabetes mellitus (DM). DM is a metabolic disease caused by elevated blood glucose levels above normal values (hyperglycemia). The disease is caused by impaired glucose metabolism due to insulin deficiency both in absolute and relative. DM is the most common metabolic syndrome worldwide with an incidence rate of 1-8%. According to WHO [1], Indonesia ranks 4<sup>th</sup> largest in the world.

Oral application of hypoglycemic substances from synthetic materials can cause excessive side effects. One of the natural ingredients that can lower blood glucose levels is the leaves of mother-in-law's tongue (*Sansevieria trifasciata*). The decoction of the mother-in-law's tongue leaves is commonly used by people from Sabah, Malaysia for the treatment of DM disease [2]. The medical efficacy of the leaves of the mother-in-law's tongue is related, among others, to the treatment of DM, earache, pharyngitis, skin itching, and urinary diseases as an analgesic and antipyretic [3][4][5][6].

The hypoglycemic effect of the plant bioactive component can restore pancreatic  $\beta$  cell function, thereby increasing insulin secretion, inhibiting glucose absorption in the intestine and inhibiting the action of the  $\alpha$ -glucosidase enzyme. The leaves of the mother-in-law's tongue extracted with ethanol or aquadest solvent contain various phytochemical compounds commonly grouped in the form of alkaloids, flavonoids, saponins, glycosides,

terpenoids, tannins, proteins, and carbohydrates [6]. Several studies that have proven the biological effects of the ethanol extract of the leaves of the mother-in-law's tongue are the analgesic and antipyretic effects [6], antibacterial [7] and antioxidants [8]. In addition, the leaves of mother-in-law's tongue also have an anti-diabetic effect with in vivo test [9]. The soluble active compounds in ethyl acetate are active compounds which can be well extracted. One of the compounds contained in the ethyl acetate fraction is flavanol [10].

From the description above it is known that differences in the nature of polarity of the solvent can affect the number of extractive substances dissolved in the sample extract tested [11]. Polar solvents commonly used for flavonoid extraction are methanol, acetone, ethanol, aquadest, ethyl acetate and isopropanol [12]. Therefore, the solvents to be used in this study were aquadest, 70% ethanol, and 70% ethyl acetate to see how the polarity ability of each type of solvent was tested. In this research, the method to be performed is by testing the activity of the  $\alpha$ -glucosidase enzyme that plays a role in the process of glucose *in vitro* metabolism on the leaves of the mother-in-law's tongue which has been extracted by sonication extraction method. The results of the proposed research are expected to be used as one of the basic in the manufacture of DM drugs with the basic ingredients of mother-in-law's tongue leaves extract.

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## 2 Materials and Methods

### 2.1 Materials

#### 2.1.1 Substances

The dried leaves of *Sansevieria trifasciata* were obtained from Bogor, Indonesia. Aquadest, ethanol, and ethyl acetate were obtained from bioprocess engineering laboratory. The  $\alpha$ -glucosidase enzymes (from *Saccharomyces cerevisiae* type I) and 4-nitrophenyl  $\alpha$ -D-glucopyranoside substrate were purchased from Sigma-Aldrich (Singapore). The acarbose as a comparison solution sample positive control.

#### 2.1.2 Instruments

Ultrasonic cleaner bath (sonicator), ELISA microplate reader (410 nm), Gas Chromatography and Mass Spectrometry (GC-MS) Agilent Technologies 6890N Network GC system for gas chromatography.

### 2.2 Methodology

#### 2.2.1 Preparation of Mother-in-Law's Tongue Leaves Powder

Sorting, washing, and draining the sample of the mother-in-law's tongue leaves. Cut the leaf sample and weigh the wet weight of 1000 g. Next, dry the leaves by using the oven at 60°C for 8 h/day. Smooth the leaves with a blender and sift the leaves with a sieve analyzer to obtain leaf diameter  $\pm 0.15$ - $0.18$  mm.

#### 2.2.2 Extraction of Mother-in-Law's Tongue Leaves

Mother-in-law's tongue leaves powder was dissolved in variation type of solvents, i.e. aquadest, 70% ethanol, and 70% ethyl acetate by 1:10 ratio of mass and solvent (m/v). Then, extraction using sonicator for 60 minutes at a frequency of 53 kHz and 27°C, continued by solvent evaporating using a vacuum rotary evaporator.

#### 2.2.3 In vitro $\alpha$ -Glucosidase Enzyme Inhibition Test

The enzyme inhibition activities for  $\alpha$ -glucosidase were evaluated according to the method previously reported by Sancheti *et al.*<sup>[13]</sup> with minor modifications. The reaction mixture consisted 50  $\mu$ L of 0.1 M phosphate buffer (pH 7.0), 25  $\mu$ L of 0.5 mM 4-nitrophenyl  $\alpha$ -D-glucopyranoside (dissolved in 0.1 M phosphate buffer, pH 7.0), 10  $\mu$ L of test sample (main concentration for each extract: 1000  $\mu$ g mL<sup>-1</sup>) and 25  $\mu$ L of  $\alpha$ -glucosidase solution (a stock solution of 1 mg mL<sup>-1</sup> in 0.1 M phosphate buffer, pH 7.0 was diluted 0.04 Units mL<sup>-1</sup> with the same buffer, pH 7.0 just before assay). This reaction mixture was then incubated at 37°C for 30 min. Then, the reaction was terminated by the addition of 100  $\mu$ L of 0.2 M sodium carbonate solution.

The enzymatic hydrolysis of a substrate was monitored by the amount of p-nitrophenol released in the reaction mixture at 410 nm using ELISA microplate reader. All experiments were carried out in triplicates.

#### 2.2.4 Quantitative test using Gas Chromatography-Mass Spectrometry (GC-MS)

The sample was taken 6  $\mu$ L and insert the sample by injecting into the GC-MS inlet. Conducting quantitative analysis of component and separation of a compound on GC by capillary column with diameter 0.25 mm and length 60 m with initial temperature 40°C (temperature rise in 15°C/min until temperature reaches 290°C and the end time of 10 min). The identification of compounds is done by matching the mass spectral data with the database present in the WILEY 9<sup>th</sup> library.

## 3 Result and Discussion

### 3.1 Yield percentage value of Mother-in-Law's Tongue extract

Extraction by sonication produces yield from all of the solvent used. Extract with 70% ethanol had the highest yield value, 12.69%, followed by extract aquadest and extract 70% ethyl acetate with yield 10.37% and 6.65%. Based on the result, the highest percentage of extract yield was obtained from mother-in-law's tongue leaves extract with 70% ethanol. This showed that the leaves of mother-in-law's tongue have the same characteristics of polarity compounds as ethanol solvents.

### 3.2 In vitro $\alpha$ -Glucosidase Enzyme Inhibition Test

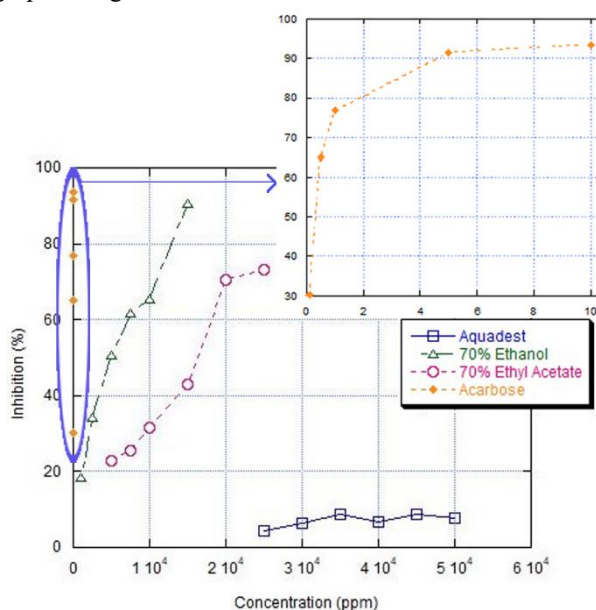
Inhibition of the  $\alpha$ -glucosidase enzyme was performed to determine the presence or absence of anti-diabetic activity in the leaves of mother-in-law's tongue extracts. Enzyme  $\alpha$ -glucosidase is an enzyme that plays a role in the formation of glucose in the human small intestine through carbohydrate hydrolysis. Inhibition of the action of this enzyme can be done to prevent the drastic increase of glucose levels in the body of type II DM through delaying the process of breaking carbohydrates so that it can delay the absorption of glucose by the intestine into the blood. Based on research conducted by Yulianti *et al.*<sup>[14]</sup>, a preliminary test has been conducted at a concentration of 125 ppm in order to find the optimum condition for the inhibition test. This mother-in-law's tongue leaves inhibition test using concentration at 125 ppm results shown in Table 1 below.

**Table 1.** % Inhibition at 125 ppm

Sample	Concentration (ppm)	Inhibition (%)
Aquades Extract	125	5.427
70% Ethanol Extract	125	5.561
70% Ethyl Acetate Extract	125	4.121

Based on the data showed in Table 1, the percentage value of inhibition showed that  $\alpha$ -glucosidase enzyme in the sample extract at concentration 125 ppm was already active because it showed the result. However, the control positive (acarbose) has a higher percentage value of inhibition at a concentration of 10 ppm with 93.52%. This indicates that the sample extract should be increased the concentration range to obtain concentration value when the percentage of inhibition reaches 50% (IC<sub>50</sub>). The purpose of using IC<sub>50</sub> parameter is to know the activity of sample extract at how many concentration when the percentage value of inhibition reaches 50%.

Since the trial data didn't show inhibition percentage more than 50%, then the sample concentration should be increased. The increased concentration showed on the graph in Fig. 1.



**Fig. 1.** Comparison of All Sample

The graph in Fig 1 shows the relationship between the percentage of inhibition and the concentration of the sample extract. The orange line in Fig 1 shows that acarbose as a positive control has a higher activity than the extract. This is due to a patented diabetes-specific acarbose that contains 100 pounds of pure acarbose, while the leaves of the mother-in-law's tongue extract contain a mixture of various bioactive and inactive compounds as anti-diabetics.

The correlation between the concentration range of each sample shows that the sample extract will reach its active point of inhibition percentage when the concentration is increased. This is due to the sample extract using different solvents, which have different polarity index values and dielectric constants. 70% ethanol solvent has a good polarity profile and having a fairly active concentration range when compared with 70% ethyl acetate and aquadest. This indicates that the bioactive compounds in the extract with 70% ethanol are more bounded due to its better polarity properties than the 70% ethyl acetate and aquadest.

The percentage inhibition of acarbose was 93.52% with a concentration of 10 ppm, while the percentage

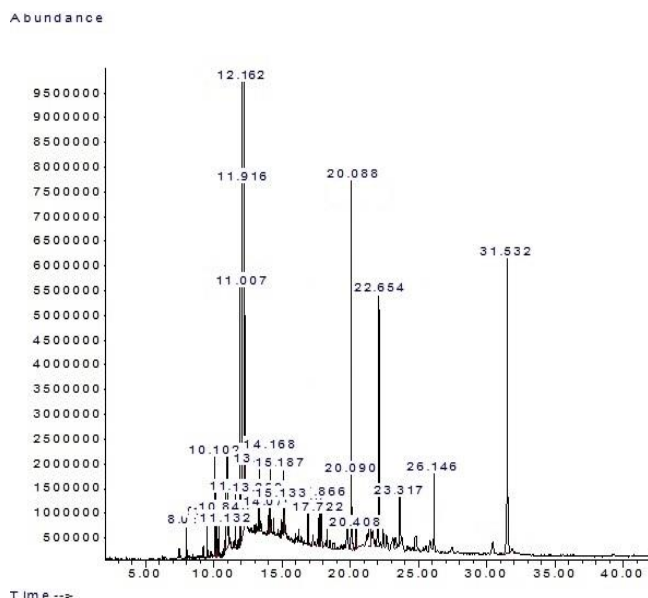
value of 70% ethanol extract inhibition was 90.72% at 15000 ppm concentration. The test results on Table 2 also showed that inhibitory activity of the  $\alpha$ -glucosidase enzyme by acarbose drug, extract of 70% ethanol, and extract of 70% ethyl acetate has an IC<sub>50</sub> value of 0.25 ppm, 4708.71 ppm, and 15161.72 ppm.

**Table 2.** IC<sub>50</sub> Value of All Sample

Sample	IC <sub>50</sub> (ppm)
Aquades Extract	>50000
70% Ethanol Extract	4708.71
70% Ethyl Acetate Extract	15161.72
Acarbose	0.25

IC<sub>50</sub> value of aquadest extract can't be calculated, because of the inhibition activity is less than 50% although the concentration has been extended to 25000-50000 ppm range. The data showed that among the aquadest extract, 70% ethanol, and 70% ethyl acetate, 70% ethanol extract had the best anti-diabetic activity because it had the highest inhibition percentage value, 90.72% and the lowest IC<sub>50</sub> was 4708.71 ppm. However, when compared with the positive control (acarbose), 70% ethanol extract is still classified as weak (inactive) in inhibiting anti-diabetic activity. It also indicates that the sample concentration is influenced by the solvency polarity index used in each extract.

### 3.3 Quantitative test using Gas Chromatography and Mass Spectrometry (GC-MS)



**Fig. 2.** GC-MS chromatogram of crude extract from Mother-in-Law's Tongue leaves

Based on the identification of compounds found in the sample mother-in-law's tongue leaves extract with 70% ethanol using GC-MS, compounds that have anti-diabetic activity is steroids, alkaloids, terpenoids, tocopherol, and

flavonoid. These compounds are found in the retention time of 11.007, 11.916, 12.162, 20.088, 22.654, and 31.532 min respectively (see Fig. 2).

Matthew *et al.*<sup>[15]</sup> explain that natural compounds with anti-diabetic activity in descending frequency of occurrence include, complex carbon hydrates, alkaloids, glycopeptides, terpenoids, peptides, amines, steroids, flavonoids, lipids, coumarins, sulfur compounds and inorganic ions. The results of the GC-MS test showed in Table 3 below.

**Table 3.** The bioactive compound from GC-MS test

No	Compounds Name	Concentration (%)	Bioactivity
1	Linoleic Acid	20	Antioxidants <sup>[16]</sup>
2	Campesterol	17,46	Antidiabetic <sup>[17]</sup>
3	Palmitic Acid	17,05	Anticancer <sup>[18]</sup>
4	Pyridine	5,51	Antidiabetic <sup>[19]</sup>
5	Phytol	1,87	Antidiabetic <sup>[20]</sup>
6	Cycloeucalenol	1,13	Antidiabetic <sup>[21]</sup>
7	Tocopherol	1,03	Antioxidants <sup>[22]</sup> , Antidiabetic <sup>[23]</sup>
8	Pyranosoflavones	0,54	Antidiabetic <sup>[24]</sup>
9	Quinolone	0,35	Antibacterials <sup>[25]</sup>
10	Other	35	-

Based on Table 3 above, we can conclude that bioactive compounds such as a steroid (campesterol), an alkaloid (pyridine), terpenoid (phytol and cycloeucalenol), tocopherol (vitamin E), and flavonoid (pyrano-isoflavones) have potential antidiabetic compounds. These bioactive compounds consistent with previous researches using mother-in-law's tongue leaves extracted using 70% ethanol such as campesterol by Vats *et al.*<sup>[17]</sup>, pyridine by Wang *et al.*<sup>[19]</sup>, phytol by Elmazar *et al.*<sup>[20]</sup>, cycloeucalenol by Ragasa *et al.*<sup>[21]</sup>, tocopherol by Monfalouti *et al.*<sup>[23]</sup>, and pyranosoflavones by Nigel *et al.*<sup>[24]</sup>. It shows that, although 70% ethanol in-vitro extract has a weak IC<sub>50</sub> value that is 4708.71 ppm compared to positive control (acarbose) that has the IC<sub>50</sub> value of 0.25 ppm, the extract still has the same bioactive compounds described by previous researchers.

## 4 Conclusion

Based on the results of the research, it can be concluded that the leaves of mother-in-law's tongue extract with 70% ethanol had the highest yield value, that is 12.69% and the percentage of inhibition  $\alpha$ -glucosidase was 90.72% at the inhibitor concentration of 4708.71. This suggests that the extract with 70% ethanol has the required bioactive compound content as the  $\alpha$ -glucosidase enzyme inhibitor. Based on identification using the GC-MS, compounds that have anti-diabetic activity is steroids, alkaloids, terpenoids, tocopherol, and flavonoid.

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