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Identification of bioactive from *Sansevieria Trifasciata Lauretii* extract as antidiabetic type 2 in obesity Wistar rats

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Abstract
Sansevieria Trifasciata Laurentii is a plant known for its antioxidant properties, including polyphenols, tannins, saponins, flavonoids, and steroids. This study aimed to investigate the antidiabetic effects of a methanol extract from the leaves of the snake plant in obese Wistar rats and to identify its bioactive constituents. A total of 24 obese Wistar rats were administered doses of 100 and 200 mg/kg of the methanol extract. The chemical composition of the extract was analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The results showed that the methanol extract significantly reduced the levels of free fatty acids, blood glucose, and triglycerides compared to the obese control group. Specifically, the 100 and 200 mg/kg doses decreased triglyceride levels by 78.2% and 67.8%, respectively, while blood glucose levels were reduced by 47.7% and 44.9%, respectively. Free fatty acid levels also decreased by 11.1% and 11.5% at the 100 and 200 mg/kg doses, respectively. LC-MS/MS analysis identified five compounds in the methanol extract with potential antidiabetic properties: anthranol, naringin, luteolin, quercetin, and sansevistatin 1. Based on these findings, it was concluded that the five bioactive compounds identified in the snake plant leaf extract showed promising antidiabetic activity.

Keywords: *Sansevieria Trifasciata*, Triglycerides, lipid, glucose, and antidiabetic

Introduction

Obesity is a disorder characterized by excessive accumulation of fat tissue in the body. Obesity is a dangerous condition where someone who is obese can trigger other diseases such as cancer, diabetes, and heart disease. Obesity is a predisposing factor to many diseases, one of which is an increase in blood sugar which can cause type 2 diabetes mellitus [1]. Human habits with inappropriate physical activity can cause an increase in cases of overweight and obesity which will cause diabetes mellitus [1]. Obesity is a factor in insulin resistance, so someone who is obese will experience the risk of developing diabetes mellitus. The main factor causing diabetes mellitus is inappropriate physical activity, this is revealed through research that has been carried out all respondents who have a genetic history of diabetes mellitus can be minimized by means of a healthy lifestyle [2].

Diabetes mellitus is a collection of metabolic disorders disorders defined by high blood sugar levels (hyperglycemia), which result from insufficient insulin production, poor insulin effectiveness, or a combination of both. When insulin is lacking, the transfer of fatty acids from the blood into fat cells is impaired, leading to an increase in blood triglyceride levels. In cases insulin resistance along with lipase-sensitive hormones in adipose tissue are activated, causing an enhanced breakdown of triglycerides (lipolysis) within the tissue. This leads to the overproduction of free fatty acids, which then enter the bloodstream. Some of these fatty acids are used as an energy source, while others are transported to the liver to be converted into triglycerides. The presence of free fatty acids decreases glucose absorption in adipose tissue and muscle, while also raising glucose production in the liver, contributing to insulin resistance and the development of diabetes mellitus [3].

Patients with diabetes mellitus can receive treatment through synthetic drugs and natural herbs. Synthetic drugs are drugs made from synthetic materials produced through chemical processes in laboratories and processed in a modern way and through prescriptions from doctors and medical circles to treat various diseases or increase the body's resistance to disease. In modern times, many people use synthetic drugs because of the effectiveness of fast treatment. However, long-term use of synthetic drugs also has side effects that can damage vital organs such as the heart, liver, and kidneys. Therefore, treatment with herbal medicine is expected to provide the same great benefits but have lower side effects than synthetic drugs. Traditional medicine has several advantages, including: lower side effects and relatively small if used properly and correctly [4].

There are more than 500 plants that have been proven to have the ability to reduce blood sugar levels because the compounds contained in these plants are believed to have antidiabetic properties, such as flavonoids, curcuma, chromium, tannins, isoflavones [5]. One of the plants containing flavonoids, saponins, terpenoids and phenols is *Sansevieria Trifasciata*. *Sansevieria Trifasciata* plant is a plant that is abundantly available in Indonesia. This plant has antioxidant activity. Based on the study carried out by Monisa (2016) [6] this *Sansevieria Trifasciata* leaf has the activity to decrease blood glucose levels in Wistar rats because the ethanol extract of *Sansevieria Trifasciata* leaves contains flavonoid compounds, saponins, and tannins [6]. The extract from *Sansevieria Trifasciata* leaves contains flavonoids, gallic acid, and vitamin C. Flavonoids are recognized for their antioxidant characteristics, which help protect pancreatic cells from damage caused by free radicals.

The high corelation between body weight and the incidence of increased blood sugar levels, triglyceride levels, and free fatty acids requires research to determine whether the content of methanol extract of *Sansevieria Trifasciata* leaves can prevent increased blood sugar levels, triglyceride levels, and free fatty acids. In this study, this plant was used because this plant is easy to find, contains compounds as antioxidants and is cultivated in tropical areas such as in Indonesia. To test the *Sansevieria Trifasciata* leaf extract, it will be carried out on Wistar rats that are made in a naturally obese state by being given a high-fat diet within a certain time while still calculating the obesity index using the Lee index. By giving *Sansevieria Trifasciata* extract, it is expected to reduce the levels of free fatty acids, blood glucose, and blood triglycerides in obese Wistar rats.

Material and Methods

Materials

The materials to be used in this study include *Sansevieria Trifasciata* leaves, 90% technical methanol (pa), distilled water, female Wistar rats, high-fat feed, standard feed, Wagner reagent, $FeCl_3$, Lieberman-Burchard reagent, $C_2H_2O_4.H_2O$ (pa), chloroform (pa), NaOH, PP indicator, HCl, and Mayer reagent.

Equipment

The equipment used in this study are UV-VIS Spectro, LC-MS / MS (Shimadzu), spatula, blender, glass jar, glassware, funnel, drip plate, serum tube, rotary vacuum evaporator Buchi R 215 and vacuum Buchi B169, analytical balance Shimadzu Type ATY224, gauze, aluminum foil, drip pipette, micro pipette, water bath, sonde tool, glucometer, burette, clamp, and stative.

Methods

Sansevieria Trifasciata leaf extraction

The dried *Sansevieria Trifasciata* leaves were then blended. A total of 1 kg of *Sansevieria Trifasciata* leaf powder was soaked using methanol solvent for 24 hours. After 24 hours, filtering was carried out. The filtrate from the maceration is collected and the pulp of the *Sansevieria Trifasciata* is macerated again, for 24 hours. The maceration process was carried out three times until it was believed that the active compounds in the *Sansevieria Trifasciata* leaves had been fully extracted. A total of 1 kg of *Sansevieria Trifasciata* leaf powder was subjected to methanol extraction for 24 hours. Following this, the mixture was filtered, and the resulting filtrate was obtained was collected and evaporated using a rotary vacuum evaporator to yield a concentrated extract of *Sansevieria Trifasciata*.

Phytochemical Test

1. Alkaloid Test

The alkaloid test involves dissolving the sample in dilute sulfuric acid. This solution is then subjected to two separate tests using Dragendorff's and Mayer's reagents. A positive result is indicated by the appearance of a red-orange precipitate with Dragendorff's reagent and a yellowish-white precipitate with Mayer's reagent.

2. Terpenoid and Steroid Test

Several samples were dissolved in 2 ml of chloroform in a dry test tube, followed by the addition of 10 drops of acetic anhydride and 3 drops of concentrated sulfuric acid. A positive result was marked by the appearance of a red solution initially, which then shifted to blue and green.

3. Saponin Test

The presence of saponins can be detected through the foam test in hot water. A stable foam that remains for 5 minutes and does not disappear after adding a drop of 2 N HCl indicates the presence of saponins.

4. Fenol Test

After extracting the sample with 70% methanol, 1 ml of the extract was combined with two drops of 5% ferric chloride solution. A positive reaction, indicating the presence of phenolic compounds, was observed when a green or blue-green color appeared.

5. Flavonoid Test

The sample was combined with 0.1 mg of magnesium powder, 0.4 ml of amyl alcohol (a 1:1 mixture of 37% hydrochloric acid and 95% ethanol), and 4 ml of alcohol, then shaken. A positive result was observed when a red, yellow, or orange color developed in the amyl alcohol layer [7].

LC-MS/MS Analysis

The methanol extract of *Sansevieria Trifasciata* leaves was prepared using the SPE technique. First, the Sep-Pak C18 column (1 cc, 100 mg) was conditioned with 1 ml of an acetonitrile-water mixture (80:20), followed by the addition of 0.5 ml of a protein precipitation solution. The column was then eluted with 0.5 ml of the acetonitrile-water mixture, and 0.25 ml of 200 mM ammonium formate in a 50:50 acetonitrile-methanol mixture was added. The final eluate was combined with 0.2 ml of a 25:75 acetonitrile-buffer (25 mM ammonium formate, pH 4.5) solution. The resulting mixture was filtered through a 0.45 µm cellulose acetate filter membrane, degassed, and injected into the LC-MS for analysis. The data were processed using LC-MS/MS, and the chromatograms were analyzed with Masslynx V.4 software.

Test animal treatments

Concentrated *Sansevieria Trifasciata* leaf methanol extract was weighed and applied *in vivo* to 18 Wistar rats according to the following post-test treatment groups:

K_1: Normal Group
K_2: High Fat Group
K_3: LM+100 group
K_4: LM+200 group

The body weight of mice was measured weekly for 4 weeks, mice were declared obese if the Lee obesity index value > 0.3 or showed an increase in body weight of more than 50% compared to control mice with an induction period of 4 weeks, after 4 weeks the mice were fasted for approximately 10 hours to take blood samples. Blood from the rat was collected from the orbital eye using a 3mL syringe. The sample was left at room temperature for 30 minutes before being centrifuged at 1000 rpm for 10 minutes. The serum was then collected and stored in a sealed container. This serum was subsequently used to measure levels of triglycerides, glucose, and free fatty acids.

Determination of Triglyceride and Blood Glucose Levels

Measurement of triglyceride levels in serum was carried out using a UV-Vis spectrophotometer. A total of three test tubes were prepared for standard, blank, and sample containers. In test tube 1 (blank), 10 µL of distilled water was added; in test tube 2 (standard), 10 µL of cholesterol standard was added; and in test tube 3 (sample), 10 µL of blood serum was added. Each test tube received 1000 μL of triglyceride reagent. The mixtures were incubated at 37°C for 10 minutes, followed by analysis using a UV-Vis spectrophotometer at 546 nm. Triglyceride levels below 150 mg/dL are deemed normal, whereas levels exceeding 150 mg/dL indicate hypertriglyceridemia in the rat [8].

Determination of Free Fatty Acid Content

The measurement of free fatty acid levels in rats is performed through acid-base titration using the Folch method. The Folch method is a technique for extracting and purifying lipids, commonly employed to assess lipid levels in the blood. The measurement of free fatty acid levels was carried out by means of 10 mL of neutral solution of methanol and chlorophom (1:2) v/v plus 200 μL (0.2 mL) of blood serum and 2-3 drops of phenolphthalein indicator then titrated with 0.01 N NaOH until a color change to pink occurs. The volume of NaOH used was recorded and the free fatty acid content was calculated in mmol/L.

Results and Discussion

The results of the phytochemical analysis of the methanol extract of *Sansevieria Trifasciata* leaves revealed positive for alkaloids, flavonoids, phenols, saponins, and steroids in the following table 1.

Table 1: Phytochemical test results

No.	Phytochemical test	Reagents	Results	Description
1	Alkaloids	Wagner's reagent	Brown precipitate forms	+
2	Flavonoids	Mg and HCl powder	Color change to orange	+
3	Phenol	$FeCl_3$	Color change to blackish green	+
4	Saponins	HCl 1%	Froth does not disappear after being tested with HCl	+
5	Steroids	Lieberman buchard	Bluish-green discoloration occurs	+

From the results of phytochemical screening of methanol extracts of *Sansevieria Trifasciata* leaves, it shows that it positively contains alkaloids, flavonoids, phenols, saponins and steroids. This is also shown in research conducted by Putra and Bogoriani (2022) [8] which states that methanol extracts of *Sansevieria Trifasciata* leaves positively contain alkaloid compounds, flavonoids, phenols, saponins, and steroids [8].

The results of measuring the body weight of rats for 4 weeks can be seen in the following diagram

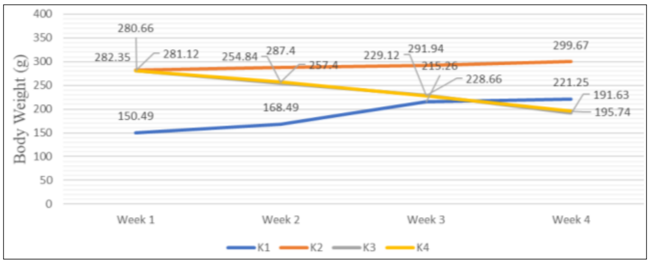


Fig 1: Body weight of mice every week

From the results of measuring the body weight of rats every week in Figure 1. indicates a rise in body weight in the group given high fat feed (K_2) compared to the normal group (K_1). In the treatment group given *Sansevieria Trifasciata* at a dose of 100 mg/kg (K_3) and 200 mg/kg (K_4) showed that there was a decrease. The average levels of triglycerides, blood glucose, and free fatty acids after 4 weeks of treatment are shown in Table 2 below.

Table 2: The average of triglycerides, blood glucose, free fatty acids

Groups	K_1	K_2	K_3	K_4
Triglycerides	$135.00 \pm 3.35^{b,c,d}$	$209.66 \pm 2.16^{a,c,d}$	$45.50 \pm 3.44^{a,b,d}$	$67.33 \pm 7.84^{a,b,c}$
Glucose	$80.66 \pm 2.65^{b,c,d}$	$232.66 \pm 9.13^{a,c,d}$	$121.50 \pm 26.69^{a,b}$	$128.16 \pm 12.28^{a,b}$
Free Fatty Acids	$7.75 \pm 0.57^{b,c,d}$	$10.58 \pm 0.69^{a,c,d}$	$9.35 \pm 0.35^{a,b}$	$9.27 \pm 1.14^{a,b}$

Note: **K1:** Normal group a: signifies a substantial difference from K1 (p<0.05)
K2: High fat group b: signifies a substantial difference from K2 (p<0.05)
K3: LM+100 group c: Signifies a substantial difference from K3 (p<0.05)
K4: LM+200 group d: signifies a substantial difference from K4 (p<0.05)

From the results of the data in Table 2. shows that the high-fat diet treatment group (K_2) had an increase in triglyceride levels, blood glucose, and free fatty acids and mouse body weight data in Figure 1. tends to increase compared to the normal treatment group (K_1), therefore it can be interpreted that mice in the K_3 and K_4 treatment groups are already included in the obesity category. The data in Table.2 shows the mean ± SD (n = 6) followed by superscripts on the same line indicating substantial distinction with $p < 0.05$. The triglyceride levels of group K_2 showed a substantial distinction with group K_3 and group K_4 because p<0.05 (p=0.001), which means that the addition of *Sansevieria Trifasciata* leaf extract has the effectiveness in reducing triglyceride levels. Blood glucose levels of group K_2 showed a substantial distinction with group K_3 and group K_4 because p<0.05 (p=0.001) but in group K_3 and group K_4 did not have a substantial distinction because p>0.05 (p = 0.463) which means that LM+100 and LM+200 have the same effectiveness in reducing blood glucose levels. Free fatty acid levels in group K_2 showed a substantial distinction between group K_3 and group K_4 because p<0.05 (p=0.001) but in group K_3 and group K_4 did not have a substantial distinction because p>0.05 (p=0.858) which means that LM+100 and LM+200 have almost the same effectiveness in reducing free fatty acid levels in obese Wistar rats.

Obesity is a condition that results from an imbalance in the body's energy, specifically a positive energy balance that is eventually stored as fat in body tissues [9]. Several theories suggest that obesity plays a crucial role in the development of insulin resistance. The greater the amount of fat tissue in the body, the more resistant the body becomes to insulin, particularly when excess fat is concentrated in the central or abdominal area (central obesity). Fat can interfere with insulin function, preventing glucose from being transported into cells, leading to its accumulation in the blood vessels and causing elevated blood glucose levels. In addition, obesity is a major contributor to the development of type 2 diabetes mellitus, with approximately 80-90% of individuals diagnosed with the condition being obese.

Diabetes mellitus is a long-term condition marked by consistently elevated blood sugar levels. This arises when the body either cannot generate an adequate amount of insulin or is unable to effectively utilize the insulin it produces [10]. Diabetic is a metabolic disorder where the body has problems producing insulin or using it effectively, resulting in elevated blood sugar levels. Diabetics are unable to produce the hormone insulin in sufficient quantities or the body cannot use it effectively, resulting in excess sugar in the blood. This chronic excess sugar in the blood (hyperglycemia) becomes toxic to the body. Factors that influence the levels of triglycerides, glucose, and FFA in each treatment group are energy intake and the composition of the feed given. An increase in energy or fat from food will cause an increase in lipogenesis activity and Free Fatty Acid (FFA) or fatty acids are also formed more and more. The FFA formed will be mobilized from fat tissue to the liver and bind to glycerol to form triacylglycerol. In adipose tissue, triglycerides are degraded into fatty acids and glycerol. The glycerol produced will undergo gluconeogenesis to be transformed into glucose [11]. The levels of triglycerides, blood glucose, and free fatty acids in Wistar rats during the 4 weeks of treatment can be seen in Figure 2 below:

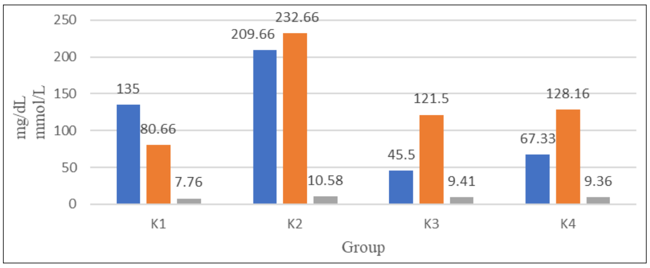


Fig 2: Triglyceride, Blood Glucose, and Free Fatty Acid Levels

The data presented in Figure 2 indicates that the levels of triglycerides, glucose, and free fatty acids in the treatment groups K_3 and K_4 are lower compared to the high-fat group (K_2), while the average levels of triglycerides, glucose, and free fatty acids in the normal group (K_1) remain within the normal range or below the threshold. Normal glucose levels in rats range from 50-145 mg/dL, and normal triglyceride levels are less than 150 mg/dL. This suggests that the administration of methanol extract of *Sansevieria Trifasciata* leaves at doses of 100 mg/kg and 200 mg/kg effectively reduced triglyceride levels by 78.2% (K_3) and 67.8% (K_4), respectively. Reduction of triglyceride levels in K_3 is greater than K_4 because the content of secondary metabolites in excessive amounts will reduce their effectiveness. The decrease in blood glucose levels was 47.7% K_3 and 44.9% K_4, the decrease in glucose levels of K_3 and K_4 did not have a significant difference and the decrease in K_3 was greater than K_4. The K_4 treatment group showed a higher state of oxidative stress than the K_3 treatment group because the consumption of higher than usual doses of antioxidants can reduce the effect of antioxidants themselves. The decrease in free fatty acid levels was 11.1% K_3 and 11.5% K_4 in obese Wistar rats. This demonstrates the effect of administering the methanol extract of the leaves is able *Sansevieria Trifasciata* to reduce the free fatty acid levels of obese rats.

The decrease in triglyceride, blood glucose, and free fatty acid levels is due to the content of secondary metabolites that are anti hyperglycemia and antioxidants. Other studies have been conducted by Kumala, 2021 [12] the antioxidant content of methanol extracts of *Sansevieria Trifasciata* leaves such as flavonoids and saponins can act as antidiabetics, antihyperglycemia, and can inhibit excessive free radicals in the body [12]. How antioxidant compounds work as antihyperglycemia by inhibiting lipid peroxidation that increases due to high-fat feeding, by inhibiting oxidative stress that can damage tissues in the body. Flavonoid compounds have antioxidant properties so that they can protect pancreatic cell damage from free radicals.

The results of the analysis of methanol extracts of *Sanseviera Trifasciata* leaves using LC-MS / MS have 5 peaks of compounds that can reduce triglyceride levels, blood glucose, and free fatty acids, namely anthranol, narigin, luteolin, quercetin and sanseviestatin 1 which are presented in table 3 below.

Table 3: Compound analysis data of methanol extract of *Sansevieria Trifasciata*

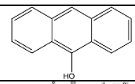
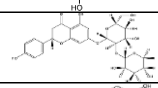
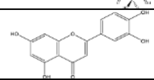
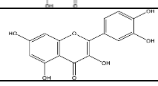
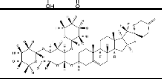
Retention time	M+ ion (m/z)	Chemical formula	Suspected Compound	Chemical Structure
5,051	194.0732	$C_{14}H_{10}O$	anthranol	
33,453	580.1792	$C_{27}H_{32}O_{14}$	naringin	
10,256	286.0477	$C_{15}H_{10}O_6$	luteolin	
11,427	302.0427	$C_{15}H_{10}O_7$	quercetin	
49,972	866.4664	$C_{45}H_{70}O_{16}$	Sansevistatin 1	

Table.3 shows the identification results of bioactive compounds of *Sansevieria Trifasciata* leaves. Luteolin, naringin, and quercetin are flavonoids found in various plants. Flavonoid compounds like quercetin and naringin contain phenolic hydroxyl groups that can form hydrogen interactions with amino acid residues at the active site of α-glucosidase. This interaction prevents the enzyme from binding to carbohydrates, thereby reducing glucose production. In research carried out by Alam *et al*., (2023) the antidiabetic potential of luteolin compounds is very high, as evidenced by the results of research with the treatment of test animals given luteolin compounds were able to reduce daran glucose levels and significant body weight [13]. Anthranol is an anthraquinone glycoside derivative compound that is widely found in the aloe plant group as aloin. This aloin compound has antiglycation properties by reducing advanced glycation end products (AGEs) by 50%. Excess glycation and AGE accumulation in involved tissues will cause diabetic complications [14]. Anthranol is thought to stimulate AMP-activated protein kinase (AMPK), which plays a role in increasing fatty acid oxidation and regulation of glucose metabolism, helping to lower triglyceride and blood sugar levels. Sanseviestatin is a compound of the saponin group, this compound can reduce triglyceride synthesis through regulating enzymes involved in lipid biosynthesis, such as hydroxymethylglutaryl-CoA reductase (HMGCR).

These bioactive compounds can interact directly with triglyceride molecules, free fatty acids, or glucose through non-covalent bonds. Hydrophobic groups on bioactive compounds can interact with hydrocarbon chains on fatty acids, thereby reducing the availability of fatty acids for triglyceride synthesis.

Conclusion

The study results imply that the methanol extract of *Sansevieria Trifasciata* leaves at a dose of 100 mg/kg has the potential to lower triglyceride levels, blood glucose, and free fatty acids in obese Wistar rats. This effect is attributed to the secondary metabolite compounds found in the methanol extract, including sansevistatin 1, naringin, luteolin, quercetin, and anthranol.

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