

Testing the Effectiveness of Kepok Banana (*Musa paradisiaca* L) Skin Extract on Reducing Blood Sugar Levels in Male Mice (*Mus musculus*)

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ABSTRACT

One natural ingredient that has the potential to be used as traditional medicine is the peel of the Kepok banana (*Musa paradisiaca* L). This study aims to determine the effect of administering Kepok banana peel extract with different solvents on reducing blood sugar levels in male mice and to determine the secondary metabolite content of Kepok banana peel. This research uses extraction methods, effectiveness testing, fractionation and identification. The effectiveness test was carried out using male mice. Data analysis was carried out by ANOVA using SPSS V 20 for Windows 10 and the Least Significant Difference (BNT) test. Fractionation was carried out using TLC and KKC. Identification of secondary metabolite compounds was carried out using phytochemical tests and Gas Chromatography Mass Spectrometer (GC-MS). The results of the study showed that the extract that had the greatest effect on lowering blood sugar levels was the ethyl acetate extract of Kepok banana peel which was predicted to contain flavonoid secondary metabolite compounds in the form of flavonoids 5, 7, 3', 5'-tetrahydroxy-3-4-flavon- diol (m/z 306 g/mol).

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1. INTRODUCTION

Diabetes Mellitus DM is a deadly disease that is suffered by quite a lot of people. This disease is characterized by high blood sugar levels due to lack of secretion of the insulin hormone or disruption of the performance of the insulin hormone (AKBAR, 2022). The International Diabetes Federation IDF estimates that 8.3 of adults or around 382 million people suffer from diabetes and the number of sufferers of this disease will increase beyond 592 million in less than 25 years IDF, 201311. Apart from adults around 79,100 children aged 15 It is also estimated that people suffer from diabetes every year and the number will continue to increase every year throughout the world IDF, 2013 42. Although it is not classified as an infectious disease, diabetes mellitus can cause several complaints such as cataracts, heart disease, kidney disease, sexual impotence, wounds that are difficult to heal and rot or gangrene, lung infections, blood vessel disorders, stroke and so on (MARBUN, 2021).

Not infrequently, people with severe diabetes mellitus have to undergo limb amputation due to rotting wounds (Hazni et al., 2021). So proper treatment is very necessary to overcome Diabetes Mellitus. The expression for every disease has a cure, the meaning can be general, so it includes deadly diseases. Because if the drug is given in the wrong way, for example with an excessive

dose for the stage of the disease in its use or the amount or quantity is more than it should be, it can actually trigger the emergence of other diseases (Dwicahya, 2023). However, if the dose is not enough, it cannot be treated.

So far, the treatment used to overcome diabetes only consists of consuming chemical drugs and a heavy diet (Fandinata & Ernawati, 2020). Treatment with chemical drugs requires a lot of money because the price is relatively expensive, besides long-term use it can also cause side effects such as digestive disorders, GI disorders, itching, pruritus, nausea and anemia. (Berniyanti, 2020). So the development of herbal medicines is very necessary to avoid the side effects of chemical medicines (Suliasih & Mun'im, 2022). One of the plants that has medicinal properties is the banana plant, where banana peels are usually used for various types of medicine for health and beauty (Hasanah & Purwanti, 2023).

Banana plants consist of several types. However, the morphology of banana plants is not different. Banana plants are plants with fibrous roots without a tap root. Banana plant roots are usually 75-150 cm long depending on the variety (Lubis, 2021). The stem of the banana plant itself is a true stem or tuber and is usually known as a hump (LENI, 2023). The true stem of the banana plant is hard and has a growing point (bud eye) which will produce banana leaves and flowers. Apart from the true stem, the banana plant also has a false stem (Danang, 2022). This false stem consists of long leaf midribs that wrap and cover each other to form a strong stem. The false stem of a banana plant is usually 3-8 m long depending on the variety (Sirruhu & Sulaiman, 2020). Banana plants also have oval-shaped flowers with pointed ends. Newly emerged banana flowers are also known as banana blossoms (Ryan & Pigai, 2020). The type of banana whose peel is most widely used is the kapok banana plant because kapok bananas contain many ingredients such as carbohydrates, calcium, phosphorus, vitamins A, B and C. Several secondary metabolite compounds that can be used as medicine for tonsillitis and anemia can also be found in this banana (Angelina et al., 2021).

Apart from the Kepok banana fruit (*Musa paradisiaca* L), the peel of the Kepok banana fruit also contains biochemical components in the form of cellulose, hemicellulose, chlorophyll pigments and pectin substances containing galacturonic acid, arabinose, galactose (Azzakiah Isna Salsabila, 2022). The biochemical components contained in Kepok banana peel are known to be used to absorb heavy metals (Syakri, 2019). Kepok banana skin also contains secondary metabolite compounds such as flavonoids 5, 6, 7, 4'-tetrahydroxy-3-4-flavan-diol (MARISEP, 2023). Other research conducted by Saputri & Augustina, 2020, shows that Kepok banana peel also contains several other metabolites such as terpenoids and tannins.

When using fruit peels on bananas, it is necessary to extract and separate the substances from the fruit peels using extraction. Extraction is a process of separating compounds contained in a sample from a mixture using an appropriate solvent until there is a balance in the concentration of compounds and solvents in plant cells. Extraction is carried out to isolate bioactive compounds from plants whose existence is known or unknown and is also used to identify types of secondary metabolites from an organism (Pratiwi et al., 2021). Extraction is a separation method that involves moving a substance from one layer to another. In the organic solvent extraction method that is generally chosen is a solvent with a boiling point that is much lower than the boiling point of the compound to be extracted, usually the solvent chosen is a solvent that is cheap and contains non-toxic compounds (Soekamto et al., 2023). Meanwhile, the separation of substances is called Fractionation, which is the process of separating the components in the extract into fractions. The fractionation process can be carried out using several methods, such as Thin Layer Chromatography (TLC), column chromatography, HPLC and Gas Chromatography (GC) (Mierza et al., 2022; SELFIANA, 2019).

Diabetes can usually be overcome by extracting banana peels (Syamsiyah, 2022). Diabetes mellitus is a chronic disease that occurs due to hormonal disorders which can cause various chronic diseases such as cataracts, kidney, nerve and blood vessel disorders (Setiawan, 2021). Meanwhile, according to the Big Indonesian Dictionary (KBBI), diabetes mellitus is a disorder of carbohydrate metabolism because the pancreatic glands are unable to secrete enough insulin. Diabetes mellitus is usually characterized by weight loss, always thirsty and hungry and frequent urge to urinate. The pancreas is an endocrine gland that produces the peptide hormones insulin, glucagon and somatostatin and an exocrine gland that produces digestive enzymes. Peptide hormones are secreted from cells located in the islets of Langerhans (B-cells that produce insulin, A-cells that produce glucagon and D-cells that produce somatostatin). These hormones play an

important role in regulating the body's metabolic activities and help maintain blood glucose homeostasis (Syukri, 2022).

2. METHOD

This research was carried out at the Organic Chemistry Laboratory, Faculty of Science and Technology, UIN Alauddin Makassar. Mice blood sugar measurements were carried out at the Pharmacy Laboratory, Faculty of Health Sciences, UIN Alauddin Makassar, while GC-MS was carried out at the Makassar POLRI Forensic Laboratory. The equipment used is a gas chromatography-mass spectrometer (GC-MS), rotary evaporator, liquid vacuum column chromatography (KCCV), glucometer (Nesco Multi Check), distillation circuit, UV lamp 254-366 nm, 4 digit analytical balance, analytical balance 2 digits, bath, chamber, blender, animal scales, mortar and mortar, oven, fraction container, jar, porcelain drops, scissors, cannula, 1 ml syringe, spatula, tweezers and glass tools. The materials used are distilled water (H₂O), concentrated sulfuric acid (H₂SO₄), iron (III) chloride (FeCl₃) 5%, ethyl acetate (C₄H₈O₂), glucose (C₆H₁₂O₆), kepok banana peel (*Musa paradisiaca* L), filter paper Whatman no.42, n-hexane (C₆H₁₄), sodium hydroxide (NaOH) 10%, sodium carboxymethylen cellulose (Na CMC) 1%, methanol (CH₃OH), metformin, male mice (*Mus musculus*), liebermanburcharad reagent, mayer reagent, Wagner's reagent, TLC plate, G60 silica (230-400 mesh) no. catalog 7330 and 7733, tween 80 and vaseline. The skin of the Kepok banana (*Musa paradisiaca* L) is cleaned, then cut into pieces and dried in the sun at room temperature until dry. The dried fruit skin is then blended to form powder. Dry kepok banana peel powder (*Musa paradisiaca* L) was weighed in the amount of 1500 grams, then the powder was divided into three parts of 500 grams each. Then each was macerated for 3x24 hours using three different solvents, namely n-hexane (C₆H₁₄), ethyl acetate (C₄H₈O₂) and methanol (CH₃OH). The extract is then filtered and evaporated using a rotary evaporator until it forms a thick extract or until the volume becomes ¼ of the initial volume. During the extraction process on banana peels, several tests are also carried out to determine whether the extraction results are good for making herbal medicine, such as testing the effectiveness of kepok banana peel extract (*Musa paradisiaca* L) on reducing blood sugar levels of male mice (*Mus musculus*) such as making a solution and testing the extraction and carrying out calculations using SPSS as well as the effectiveness of the fraction of ethyl acetate extract of kepok banana peel (*Musa paradisiaca* L) on reducing blood sugar levels of male mice (*Mus musculus*).

3. RESULTS AND DISCUSSION

3.1 Research results

3.1.1 Extraction of kepok banana peel (*Musa paradisiaca* L)

The following are the results of extraction from banana peel using several types of solvents which can produce a good type of extraction on banana peel.

Table 1. Results of extraction of Kepok banana peel

No.	Solvent	Initial weight of kapok banana peel sample	Extract weight
1	n-Hexane	500 grams	64, grams
2	Ethyl acetate	500 grams	32, 7160 grams
3	Methanol	500 grams	55, 3994 grams

3.1.2 Effectiveness of kepok banana peel extract (*Musa paradisiaca* L) on reducing blood sugar levels in male mice (*Mus musculus*)

Table 2. Results of mice blood glucose measurements.

Sample	Fasting Blood	Initial Blood	Initial Blood Glucose Levels (mg/dL)/minute					
	Glucose Levels (mg/L)	Glucose Levels (mg/L)						
NaCMC 1%	54	92	92	139	163	160	139	139
Metformin	71	285	285	332	332	159	130	97
N-Hexane Extract	80	182	273	292	185	173	139	135
Ethyl Acetate Extract	101	206	259	157	139	120	119	119
Methanol Extract	75	139	139	120	119	119	107	107

Based on the significant probability value (Sig) anova, it can be seen that there are real differences between the data groups being compared or it can be said that the three test extracts

have significant differences in the effectiveness of reducing blood sugar levels in male mice (*Mus musculus*). To compare the effectiveness of the three extracts (n-hexane, ethyl acetate and methanol), a least significant difference test (BNT) was carried out. Based on the value of the smallest real difference (BNT) of n-hexane, ethyl acetate and methanol extract from Kepok banana peel, it can be seen that ethyl acetate extract has greater effectiveness in reducing blood sugar levels in male mice (*Mus musculus*) than n-hexane extract and methanol. To compare the increase and decrease in average blood sugar levels in mice before, after glucose induction and after treatment between negative control (1% Na-CMC), positive control (Metformin) and n-hexane, ethyl acetate and banana peel methanol extracts. given up.

3.1.3 Phytochemical test results of kapok banana peel extract (*Musa Paradisiaca L*)

The kepok banana peel extract (*Musa paradisiaca L*) which was most effective in reducing blood sugar levels in an effectiveness test using mice was ethyl acetate extract. The ethyl acetate extract was then tested for phytochemistry to determine the components of secondary metabolite compounds contained in the extract.

Table 3. Phytochemical test results of ethyl acetate extract of Kepok banana peel (*Musa paradisiaca L*.)

Compound Classes	Test results	Description (Color Change)
Flavonoids		Brick red
•Concentrated H ₂ SO ₄	+	A yellow precipitate is formed
•NaOH 10%	+	Forms a yellowish green color
•FeCl ₃	+	
Alkaloids		An orange color forms
•Wagner	+	No brown color forms
•Dragondorff	+	No yellow or white precipitate is formed
•Mayer	+	
Terpenes and Steroids		
•Lieberman Burhard	-	No red-purple or blue-green color is formed
Tannin		
•FeCl ₃	-	No white precipitate was formed

The kepok banana peel extract (*Musa paradisiaca L*) which was most effective in reducing blood sugar levels in an effectiveness test using mice was ethyl acetate extract. The extract was then fractionated using vacuum liquid column chromatography (KKCV). This fractionation process produces 12 fractions, then each fraction is reanalyzed for its constituent components using thin layer chromatography (TLC). The following is an image of the results of fraction analysis using thin layer chromatography (TLC). The dry powder of Kepok banana peel was weighed as much as 1500 grams, then divided into three parts of 500 grams each. Each Kepok banana peel powder is then extracted. Extraction of Kepok banana peel samples was carried out using three different solvents, namely n-hexane, ethyl acetate and methanol. The aim of using three types of solvents is to maximize the withdrawal of sample compounds, both polar, semi-polar and non-polar. The extraction process was carried out for 24 hours (3 times) and a brownish red colored extract was obtained. Then each extract was evaporated using a rotary evaporator until a thick, brownish red extract was obtained of 55.399 grams for the n-hexane extract, 64.881 grams for the ethyl acetate extract and 32.716 grams for the methanol extract.

The Anova test (Analysis of Variance) is a test used to compare the means of more than two samples. Based on the ANOVA test that was carried out, a significant probability value of 0.043 was obtained. A group of data is said to have significant differences or is not homogeneous if the significant probability value (Sig) obtained is equal to or less than 0.050. On the other hand, if the Sig value of the groups being compared is greater than 0.050, it means that there is no real difference between the data groups or the data is said to be. Based on the values obtained, it can be said that giving ethyl acetate, n-hexane and methanol extract to mice made a significant difference in reducing blood sugar levels. It can be seen from the significant value obtained, $0.043 < 0.05$. Based on the results of the BNT test carried out, it shows that the average difference between fraction D - fraction B is 76.124. Meanwhile, the extract from fraction D – fraction A was 78.948 and the average difference between fraction B – fraction A was 2.824. Based on the mean difference between these fractions, it is known that the fractions that have the potential to have an effect on reducing blood sugar levels are fraction B and fraction A because the mean difference between the two fractions is closest to the B value, namely 7.701. From the two fractions, we can find out which fraction has the highest effectiveness by looking at the average value of each

fraction. Fraction A has an average value of 94.166 while fraction B has an average value of 172.5. Thus, fraction B was continued for GC-MS characteristics.

4 CONCLUSION

Based on effectiveness tests on each kepok banana peel extract (*Musa paradisiaca* L) in reducing blood sugar levels in male mice (*Mus musculus*) with different solvents, it is known that ethyl acetate extract has greater effectiveness than n-hexane extract and methanol. 2. The secondary metabolite compounds in kepok banana peel extract (*Musa paradisiaca* L) which play a role in reducing blood sugar levels in male mice (*Mus musculus*), are predicted to be flavonoid compounds in the form of flavonoid compounds 5, 7, 3', 5'-tetrahydroxy- 3-4 flavon-diol.

THANK-YOU NOTE

Research suggestions are that it is best to isolate bioactive compounds from the peel of the Kepok banana (*Musa paradisiaca* L) which can reduce blood sugar levels and also carry out antimicrobial tests from the peel of the Kepok banana (*Musa paradisiaca* L) which is known to contain antimicrobial compounds in the form of stigmaterol.

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