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Combination Effects of African Leaf Ethanol Extract (*Vernonia amygdalina* Del.) with Red Onion Peel (*Allium cepa* L.) as Antidiabetes in Streptozotocin-induced Mice

Efek Kombinasi Ekstrak Etanol Daun Afrika (*Vernonia amygdalina* Del.) dan Kulit Bawang Merah (*Allium cepa* L.) sebagai Antidiabetes pada Mencit yang di Induksi Streptozotocin

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ABSTRACT

Type 2 diabetes arises when the body becomes resistant to insulin or when the pancreas fails to produce sufficient insulin. This study aimed to evaluate the antidiabetic effects of reducing blood glucose levels in white male mice through the administration of an ethanol extract of African leaves and onion peel, as well as to determine the duration of these effects. Streptozotocin was used to induce diabetes in the mice, and the experiment was conducted over a period of 21 days. The mice were divided into seven groups: Group 1 received a placebo (CMC Na 0.5%), Group 2 received glibenclamide (0.013 mg/20 g body weight), Group 3 received a single dose of African leaf extract (4.2 mg/20 g BW), Group 4 received a single dose of onion peel extract (4 mg/20 g BW), and Groups 5, 6, and 7 received combinations of African leaf and onion peel extracts at ratios of 1:1, 1:2, and 2:1, respectively. The results demonstrated that the combined administration of African leaf and onion peel extracts significantly reduced blood glucose levels, with decreases of 44.701%, 49.929%, and 51.996% in the 1:1, 1:2, and 2:1 group, respectively. The 1:1 combination was particularly effective, showing a reduction in blood glucose levels comparable to the positive control, which achieved a 45.957% decrease. The administration of the test preparations effectively reduced blood glucose levels over 21 days, with significant reductions observed on both the 14th and 21st days.

Keywords: African Leaf, Onion Peel, Combination, Streptozotocin, Antidiabetic

ABSTRAK

Diabetes tipe 2 terjadi ketika tubuh tidak merespons insulin dengan cara normal atau ketika pankreas tidak menghasilkan cukup insulin. Penelitian ini bertujuan untuk menentukan efek kombinasi ekstrak etanol daun afrika dan kulit bawang merah dalam penurunan kadar glukosa darah mencit putih jantan dan menetapkan lama pemberian yang efektif. Penelitian ini menggunakan model induksi streptozotosin dengan masa pengamatan selama 21 hari, menggunakan 7 kelompok hewan uji yaitu kelompok 1 sebagai kontrol negatif (CMC Na 0,5%), kelompok 2 sebagai kontrol positif (glibenklamid 0.013 mg/20 g BB), kelompok 3 ekstrak daun afrika tunggal 4.2 mg/20 g BB, dan kelompok 4 ekstrak kulit bawang merah tunggal 4 mg/20 g BB, kelompok 5, 6, dan 7 merupakan kelompok dosis kombinasi (ekstrak daun afrika : ekstrak kulit bawang merah) dengan perbandingan dosis 1:1, 1:2, dan 2:1. Hasil penelitian menunjukan bahwa pemberian kombinasi ekstrak etanol daun afrika dan kulit bawang merah mempunyai khasiat sebagai antidiabetes dengan persentase penurunan glukosa darah pada kelompok kombinasi (1:1), (1:2), dan (2:1) dengan cara berturut-turut sebanyak 44.701%, 49.929% dan 51.996%. Dosis kombinasi 1:1 merupakan dosis

terbaik sebagai antidiabetik karena mempunyai efek penurunan kadar glukosa darah yang paling mendekati dengan kontrol positif sebesar 45.957%. Lama durasi pemberian sediaan uji yang efektif menurunkan kadar gula darah pada mencit adalah 14 hari dengan potensi penurunan kadar glukosa darah signifikan di hari ke-14 dan hari ke-21.

Kata Kunci: Daun Afrika, Kulit Bawang Merah, Kombinasi, Streptozotosin, Antidiabetes.

INTRODUCTION

Type 2 diabetes mellitus is a non-communicable metabolic disease associated with lifestyle. In 2045, the International Diabetes Federation (IDF) estimates that the number of people with diabetes in the world could reach 783.7 million. Type 2 diabetes mellitus accounts for 90-95% of global diabetes incidence, with the highest proportion in low- and middle-income countries (WHO, 2019).

The potential of African leaves as antidiabetics is supported by the presence of a number of active compounds, including flavonoids, alkaloids, tannins, saponins, and terpenoids (Putri, 2019). It is established that terpenoid compounds reduce blood glucose levels through their insulin-like activity, and they also inhibit gluconeogenesis and glycogenolysis processes. It has been demonstrated that saponins can mitigate hyperglycemia-related oxidative stress in patients with type 2 diabetes mellitus. Tannins have been demonstrated to inhibit the activity of alpha-amylase and alpha-glucosidase, which can subsequently reduce glucose transport to the intestinal epithelium. Additionally, flavonoids and alkaloids have been demonstrated to inhibit alpha-glucosidase activity (Asante et al., 2016). The administration of African leaf extract at a dose of 150 mg/kg BW has been demonstrated to be an effective method for reducing blood glucose levels in male white rats (Tuldjanah et al., 2020). Research results Ong et al. (2011), mentioned that the administration of African leaf extract to streptozotocin-induced diabetic rats decreased blood sugar by 32.1%.

Shallot skin is often wasted and becomes waste, according to research by Hidayat & Zahroh (2018) stated that shallot skin contains flavonoids that have an effect on lowering blood sugar levels. Based on research Lolok et al. (2019), showed that giving a combination of dayak onion skin extract and shallots at doses of 800 mg/kg BW and 200 mg/kg BW can reduce blood sugar levels in mice. Research results Harsono (2014) mentioned that the effect of giving shallot juice has an effect on reducing blood sugar levels in diabetic mice by 32%.

In light of the explanation provided above, it can be posited that the combination of African leaves and shallot skin has the potential to reduce blood sugar levels in a synergistic manner. It is anticipated that the administration of this combination will result in a synergistic effect and that further testing on male mice induced with streptozotocin will provide more effective data.

MATERIALS AND METHODS

1. Tools

Gastric sonde, blood sugar meter or glucometer (Easy Touch®), glucometer strips (Easy Touch®), digital scale, analytical balance (And®), chocolate bottle, aluminum foil, water bath, blender (Maspion®), oven (Memert®), thermometer, porcelain cup, filter paper, vacuum dry, glassware (Pyrex®), mortar, stamper, 40 mesh sieve, furnace (Ney®), crucibles, and test animal cages with complete feed supply.

2. Material

African leaves were obtained from Jonggol Village, Bogor, West Java and shallot skin was obtained from Brebes, Central Java. 28 male white mice, 96% ethanol (Merck®), distilled water, streptozotocin, glibenclamide 5 mg tablets (Indofarma), D-glucose (Merck®), Dragendorff reagent, Bouchardat reagent, Mayer's reagent, dilute HCl (dilute hydrochloric acid) (Merck®), 1% gelatin, $FeCl_3$ (ferric chloride) (Merck®), Na-CMC 0.5%, Mg (magnesium) powder (Brataco®), and concentrated HCl (concentrated hydrochloric acid) (Merck®).

3. Procedure

3.1 Plant Powder Preparation

African leaves 5 kg and shallot skin 5 kg that have been harvested are cleaned and wet sorted, then the leaves and skin are washed with running water until clean. Sample is dried by drying in the sun and the top of the sample is covered with a black cloth so that it is not exposed to direct sunlight. Sample that has been dried is sorted again which aims to clean up impurities that may be carried during drying. The dried sample is pulverized with a grinder and then sieved with a 40-mesh sieve, the obtained powder is then stored in a clean and tightly closed container.

3.2 Extract Preparation

African leaf powder and shallot skin were each weighed 500 grams, and extracted using the maceration method with a solvent ratio of 1:10. The solvent used was ethanol 96% as much as 5000 mL. The solvent was divided into 3 parts, in the first maceration of 1500 mL, the second maceration of 1500 mL and the third maceration of 2000 mL. In the first stage of maceration, the powder was macerated using 1500 mL 96% ethanol for 24 hours, every 6 hours shaking for 15 minutes, then after 24 hours the filtrate was separated using filter paper. On the second day, the residue was re-macerated using 96% ethanol as much as 1500 mL using the same process as the first stage maceration. The residue was re-macerated using the remaining solvent as much as 2000 mL using the same process as before. The dregs were discarded, filtrate 1, filtrate 2, filtrate 3 were collected. The filtrate results were dried with a *vacuum dry* until it became a thick extract which was then carried out again quality testing including organoleptic, water content, ash content, and phytochemical analysis.

3.3 Characteristic Test

a. Determination of Moisture Content

Determination of moisture content using the gravimetric method. A 2 g sample was weighed in a crucible after being in the oven for 15 minutes. The sample was dried in a 105°C oven for 5 hours before being weighed again. If the weight change between two measurements exceeds 0.25%, the drying process is repeated until a constant weight is reached. The requirement for the moisture content is no more than 10% and the requirement for the moisture content of viscous extract is no more than 22.2%. (Departemen Kesehatan Republik Indonesia, 2013).

b. Determination of Ash Content

Determination of ash content is carried out by weighing 2 grams of sample, put into a platinum crust or silicate crust that has been incinerated and leveled, leveled. The sample was subjected to a gradual heating process in a 700°C furnace until the charcoal was completely consumed. Following this, the sample was cooled and then weighed. The Kurs were incinerated until the weight remained constant after which weighing was carried out. The ash content was calculated on air-dried material and repeated twice (duplo) by inserting Krus into the furnace simultaneously. Samples were then dried and weighed at 1-hour intervals until the difference between two consecutive weighings was no more than 0.25% or 0.0025 gram (Departemen Kesehatan Republik Indonesia, 1995). The requirements for the ash content of simplisia that meet the literature values should not be more than 2% to 14.1% and the requirements for the ash content of thick extracts are not more than 3.9% to 17.4% (Departemen Kesehatan Republik Indonesia, 2013).

c. Phytochemical Screening

Alkaloid, flavonoid, tannin, and saponin tests were carried out, as well as other phytochemical tests, in a qualitative way based on color and precipitation responses (Hanani, 2015).

3.4 Antidiabetic Effect Test

This study has met the research ethics rules of the Animal Ethics Committee of Pakuan University Bogor Based on Letter No. 019/KEPIIP-UNPAK/04-2022 dated April 16, 2022, by the Pharmacy Laboratory, Faculty of Mathematics and Natural Sciences, Pakuan University.

This study used 28 male white mice with a normal weight of 20 grams and an average age of 2-3 months. Mice were first weighed and calculated *Coefficient of Variance (CV)* to determine the homogeneity of the experimental animals to be used. If CV < 15%, it can be stated that the experimental animals used are still relatively homogeneous.

The experimental animals were divided into 7 treatment groups, each treatment consisting of 4 mice. The animals were acclimatized for 7 days so that the mice can adapt to the new environment and reduce the effects of stress on mice that can affect metabolism so that it can interfere with this study. Experimental animals are given food and drink *ad libitum* and maintained in cages or plastic tubs covered with sawdust which must be replaced and cleaned every 3 days so that the cage is maintained clean and healthy.

3.5 Streptozotocin Induction

Animals used in the study were first induced with streptozotocin. Animals were fed for 16 hours before streptozotocin administration and only given drinking water. Then the mice were induced with streptozotocin *intraperitoneally* at a dose of 0.8 mg/20 g BW of mice and measurements were taken on the 7th day after injection to determine the level of increase in blood sugar levels of mice, if the sugar level was still below 124 mg/dL then the injection was continued again.

Calculation example:

$$\%\ \text{Decrease in Blood Glucose Level} = \frac{H\ Early - H\ End}{H\ Early}\ x\ 100\%$$

$$\text{Negative Control} = \frac{199 - 203{,}25}{199}\ x\ 100\% = \text{-}2{,}010\%$$

3.6 Preparation of 0.5% CMC-Na

CMC-Na was weighed as much as 0.5 g and sprinkled over hot water 20 times CMC-Na to expand (± 15 minutes), then crushed to form *mucilago*. Put into a 100 mL volumetric flask, added *aquadest* ad mark, shaken until homogeneous.

3.7 Preparation of Glibenclamide Suspension

This study used glibenclamide as a positive control, the dose of glibenclamide used was 0.013 mg/20 g BW of mice. Glibenclamide was suspended into CMC Na 0.5% little by little while stirring until homogeneous, put into a 100 mL volumetric flask, and added CMC Na 0.5% until the limit mark, shaken until homogeneous.

3.8 Giving Treatment

Following the elevation of blood sugar levels in the mice, the thick extracts of African leaves and shallot skin were administered to several treatment groups, each comprising 4 mice. The mice were divided into the following groups:

1. Group I: The negative control was given 0.5% CMC Na.
2. Group II: The positive control was given glibenclamide 0.013 mg/20 g BW of mice.
3. Group III: Dose 1 was given a single dose of African leaf extract 4.2 mg/20 g BW of mice.
4. Group IV: Dose 2 was given a single dose of shallot skin extract 4 mg/20 g BW of mice.
5. Group V: Dose 3 was given a combination of African leaf extract and shallot skin (1:1) with a dose of African leaf 4.2 mg/20 g BW of mice and a dose of shallot skin 4 mg /20 g BW of mice.
6. Group VI: Dose 4 was given a combination of African leaf extract and shallot skin (1:2) with a dose of African leaf 4.2 mg/20 g BW of mice and a dose of shallot skin 8 mg/20 g BW of mice.
7. Group VII: Dose 5 was given a combination of African leaf extract and shallot skin (2:1) with a dose of African leaf 8.4 mg/20 g BW of mice and a dose of shallot skin 4 mg /20 g BW of mice.

The dose used is based on research by Tuldjanah et al. (2020) and Lolok et al. (2019).

3.9 Blood Sugar Level Measurement

Antidiabetic testing is carried out on mice by measuring blood sugar levels using the Easy Touch® GCU tool, blood is taken from the tail of the mice and then dripped on a strip which is then installed on the Easy Touch® GCU tool to see blood glucose levels. Observation of blood glucose levels was carried out after acclimatization, on the day before induction was normal blood sugar levels. On day 0, blood glucose levels were measured after induction, on days 7, 14, and 21 after induction were checked during treatment or until glucose levels had returned to normal levels. Blood glucose levels are expressed in mg/dL.

4. Data Analysis

The results of this research data were analyzed using an experimental design *post-test only control group* random sampling technique. Observation data were analyzed using one-way ANOVA analysis of variance method using SPSS. To get a conclusion, the data obtained were analyzed using the method of Randomized Group Design (RAK) factorial pattern.

RESULTS AND DISCUSSION

1. Results of Simplisia Powder and Extract Preparation

The results of the production of African leaf powder simplisia indicate that the yield of African leaves is 77.82%, while the yield of shallot skin is 75.42%. The results of the extraction of African leaves and shallot skin using the maceration method with 96% ethanol as the solvent demonstrate the production of a thick extract of African leaves (98.9 grams) with a yield of 19.78%, and a thick extract of shallot skin (62.3 grams) with a yield of 12.46%.

2. Moisture Content Determination Result

The water content of the powder and African leaf extract was found to be 5.53% and 4.84%, respectively. Similarly, the water content of the powder and shallot skin extract was determined to be 6.55% and 5.33%, respectively. These results are in accordance with the provisions set forth by the Departemen Kesehatan Republik Indonesia (2013) regarding the requirements for water content in powders and simplisia extracts. The requirements for water content in African leaf powder are less than 10%, and extracts are not more than 22.2%. Similarly, the requirements for water content in shallot skin powder are less than 10%, and extracts are not more than 22.2%.

3. Results of Ash Content Determination

In this study, the ash content of the powder and extract of African leaves was found to be 5.04% and 5.73%, respectively. Similarly, the ash content of the powder and extract of shallot skin was determined to be 5.12% and 6.85%, respectively. These results are consistent with the standards set forth by the Department of Health of the Republic of Indonesia

(2013) regarding ash content in powders and simplisia extracts. The permissible range for ash content in African leaf powder is 2-14.1%, and for extracts, it is 3.9-17.4%. Similarly, the permissible range for ash content in shallot skin powder is 2-14.1%, and for extracts, it is 3.9-17.4%.

4. Phytochemical Test Results

The results of phytochemical screening of simplisia and extracts of African leaf and shallot skin were found to contain several active compounds including flavonoids, alkaloids, saponins, and tannins. The screening test results can be seen in **Table 1**.

The results of this phytochemical test show that 96% ethanol solvent is a universal solvent because it can dissolve almost all organic compounds present in the simplisia powder and extracts of African leaf and shallot skin. The results of the phytochemical test obtained are in accordance with the results of the phytochemical test in the study of Tambusai (2018). Tambusai (2018)The phytochemical test results obtained are in accordance with the results of the phytochemical test in Tambusai's research (2018), namely powder and ethanol extracts of African leaves containing flavonoids, saponins, alkaloids, and tannins. Setiani et al. (2020) that shallot skin extracted using the MAE (Microwave-Assisted Extraction) method positively contains flavonoids, saponins, tannins, and alkaloid compounds.

Table 1. Phytochemical Screening Results of African Leaves and Shallot Skins

Compound Identification	Reagents	Parameters	African leaf		Skin Red Onion	
			Powder	Extract	Powder	Extract
Flavonoid	96% Ethanol + Mg and Zn powder	Yellow Color	+	+	+	+
Alkaloid	Dragendorff	Red to Orange Sediment	+	+	+	+
	Mayer	White precipitate	+	+	+	+
	Bouchardat	Brown precipitate	+	+	+	+
Saponin	Hot aquadet + HCL 2 N	Foam Forms	+	+	+	+
Tanin	Nacl + Gelatin	White precipitate	+	+	+	+
	Gelatin	White precipitate	+	+	+	+
	$Fecl_3$	Blue-black	+	+	+	+

Description:
+ (Positive) = Contains Chemical Compound
– (Negative) = Does Not Contain Chemical Compounds

5. Antidiabetic Effect Test Results

The mice used were previously fed for ± 16 hours, mice were measured fasting blood sugar levels to determine initial blood sugar levels, after measuring fasting blood sugar levels, mice were induced with streptozotocin *intraperitoneally* to raise blood glucose levels in test animals (hyperglycemia), then measured blood sugar levels of mice on the 7th day after induction until the next day until showing an increase in blood sugar levels, mice are considered diabetic if blood sugar levels ≥ 124 mg/dL and are said to be normal if the blood sugar levels of mice range from 71-124 mg/dL. (Safitri et al., 2022). The results of measuring blood sugar levels before and after treatment induction can be seen in **Table 2**.

Based on **Table 2**, the administration of a streptozotocin dose of 0.8 mg/20 g BW has been observed to elicit an increase in blood sugar levels. On the 0th day following the administration of streptozotocin, the blood glucose level was found to be ≥124 mg/dL. On the 7th day, the results demonstrated a notable increase in blood sugar levels in the test animals. The objective of testing on the 7th day was to confirm the effect of streptozotocin induction on elevated blood sugar levels. These findings suggest that streptozotocin has a significant potential for elevating blood sugar levels in test animals. Streptozotocin selectively damages pancreatic beta cells, with low doses only affecting a subset of these cells, thereby maintaining a stable state of hyperglycemia (Husna et al., 2019).

The results obtained indicate that in the positive control treatment group and three variations of combination doses, namely 1:1, 1:2, and 2:1, the blood sugar levels of the test subjects on day 14 fell within the normal range of 71-124 mg/dL. Consequently, the administration of the test preparations was terminated to prevent the occurrence of hypoglycemia in the test animals. On the 21st day, the blood sugar levels of the test animals were re-evaluated, revealing a decline in the positive control group and the test solution group. It is postulated that this phenomenon is attributable to the mechanism of action of glibenclamide, ethanol extracts

of African leaves and shallot skin in the short term, namely, the stimulation of insulin secretion by pancreatic beta cells, while in the long term, the primary effect is the augmentation of insulin's action on peripheral tissues and the reduction of glucose output from the liver (Toby et al., 2020). The most effective reduction in blood sugar levels was observed on day 14, demonstrating a consistent and sustained impact on blood sugar levels. This aligns with the objective of antidiabetic drug therapy in patients with diabetes, which aims to achieve a stable and predictable decline in blood sugar levels. In the negative control group, there was no observed decrease in blood sugar levels. In this case, the negative control was a comparison control administered CMC-Na, which is an inert substance that does not have a therapeutic effect on the disease under investigation.

Table 2. Measurement of Blood Sugar Levels Before Induction and Administration of Extracts

Treatment Group	Before Induction (Normal)	After Induction (Day 0)	Treatment		
			Day 7	Day 14	Day 21
Negative Control (CMC-Na 0.5%)	$85.25^{bc} \pm 5.68$	$199^{i} \pm 23.71$	$203.5^{i} \pm 22.37$	$207^{i} \pm 21.65$	$208.75^{i} \pm 20.98$
Positive Control (Glibenclamide)	$78.75^{ab} \pm 12.12$	$209.25^{i} \pm 17.38$	$148.5^{fg} \pm 7.32$	$108.5^{de} \pm 9.29$	$82.25^{abc} \pm 17.63$
Single African Leaf (TDA)	$104.75^{cde} \pm 11.24$	$205.5^{i} \pm 6.85$	$165^{h} \pm 11.74$	$136.25^{f} \pm 11.24$	$121.25^{ef} \pm 0.96$
Single Red Onion Peel (TKB)	$102.5^{cde} \pm 13.96$	$204.5^{i} \pm 4.20$	$177.5^{h} \pm 10.08$	$143^{fg} \pm 2.16$	$123.25^{ef} \pm 0.96$
Combination TDA: TKB (1:1)	$88^{bc} \pm 8.88$	$237.5^{j} \pm 13.40$	$173^{h} \pm 4.24$	$123.75^{ef} \pm 11.74$	$97.25^{bcd} \pm 8.06$
TDA combination : TKB (1:2)	$78.5^{ab} \pm 11.33$	$237^{j} \pm 2.58$	$158.25^{gh} \pm 5.56$	$112^{de} \pm 6.68$	$85.75^{bc} \pm 10.24$
TDA combination : TKB (2:1)	$73.25^{a} \pm 11.32$	$214.75^{i} \pm 2.58$	$138.75^{f} \pm 2.94$	$97^{bcd} \pm 3.74$	$77.25^{ab} \pm 10.28$

The positive control utilized in this study was glibenclamide. Glibenclamide was selected as the positive control due to its efficacy in reducing blood glucose levels, a characteristic of oral antidiabetic drugs. The mechanism of action of this drug in reducing blood sugar levels is through the stimulation of insulin secretion from pancreatic beta cells. This occurs through the interaction of ATP-sensitive K channels in the pancreatic beta cell membrane, which can cause membrane depolarization and the opening of calcium channels, allowing Ca^{2+} ions to enter the pancreatic beta cells. This stimulates the insulin-filled granules, causing insulin secretion (Solikhah et al., 2020).

The compounds present in African leaf extract and shallot skin have been observed to possess a number of mechanisms associated with the reduction of blood glucose levels, thereby exhibiting antidiabetic properties. The results of the aforementioned study indicated that compounds such as flavonoids function by inhibiting phosphodiesterase, thereby increasing cAMP in pancreatic beta cells and stimulating insulin secretion. Additionally, they inhibit GLUT 2 (glucose transporter) intestinal mucosa, which ultimately reduces glucose absorption from the intestine, consequently leading to a decrease in blood glucose levels (Puspati et al., 2013; Song et al., 2002). Alkaloid compounds exert their effects by increasing glucose transport in the blood, inhibiting glucose absorption in the intestine, stimulating glycogen synthesis, and inhibiting glucose synthesis (Prameswari & Widjanarko, 2014). Tannins function by increasing glycogenesis and acting as astringents or chelators that can cause wrinkling of the epithelial membrane of the small intestine, thereby reducing the absorption of food juice. This results in the inhibition of glucose intake and a relatively low rate of glucose increase (Tuldjanah et al., 2020). Saponins exert their effect by inhibiting the α-glucosidase enzyme, which is responsible for the conversion of carbohydrates into glucose, thereby reducing blood glucose levels (Fiana & Oktaria, 2016).

The results of the ANOVA statistical tests of blood sugar levels on days 0, 7, 14, and 21 revealed significant differences ($P < 0.05$). Subsequently, Duncan's further test was employed to ascertain the discrepancy between each treatment. The results of the analysis demonstrated that there were statistically significant differences between the treatment doses, with a notable decrease in blood sugar levels. The data on blood glucose levels (in

mg/dL) were calculated as a percentage of the reduction in blood sugar levels. This was done to ascertain the percentage of the potential generated from the combination of African leaf extract and shallot skin in reducing blood sugar levels. The data were then statistically analyzed using ANOVA, and Duncan's further test was used to identify the real differences between treatments. The results of the measurement of the percentage of blood sugar level reduction are presented in **Table 3**.

Table 3. Percentage of Blood Sugar Level Reduction

Treatment Group	Percentage decrease (%)			
	Day 7	Day 14	Day 21	Average
Negative Control (CMC-Na 0.5%)	-2.010	-4.020	-4.899	-3.643[a]
Positive Control (Glibenclamide)	29.032	48.148	60.693	45.957[c]
Single African Leaf (TDA)	19.708	33.698	40.997	31.467[b]
Single Red Onion Peel (TKB)	13.202	30.073	39.731	27.668[b]
TDA combination: TKB (1:1)	27.157	47.894	59.053	44.701[c]
TDA combination: TKB (1:2)	33.227	52.746	63.818	49.929[c]
TDA combination: TKB (2:1)	37.136	54.831	64.022	51.996[c]

As evidenced in **Table 3**, the single dose of African leaf ethanol extract, shallot skin ethanol extract, and combination doses (1:1, 1:2, and 2:1) have been observed to reduce blood sugar levels. It can be inferred that the greater the percentage reduction in blood sugar levels, the more pronounced the antidiabetic effect. The results of the percentage decrease in blood sugar levels in a row from the highest to the lowest are as follows: the combination group (2:1), combination (1:2), positive control, combination (1:1), single dose of African leaf ethanol extract, and single dose of shallot skin ethanol extract. The negative control yielded a percentage result of -3.643%. The percentage results for the aforementioned groups were 51.996%, 49.929%, 45.957%, 44.701%, 31.467%, and 27.668%, respectively. The combined dose (1:1) was identified as the most effective dose in this study, exhibiting a percentage reduction in blood sugar levels that was most similar to that of the positive control, glibenclamide. It can be posited that the administration of a combination dose (1:1) is more efficacious, as a smaller dose already produces an effect that is comparable to that of the positive control. At a combination dose of 1:2 and 2:1, the results of lowering blood sugar levels are higher. This is due to the fact that the greater the dose of the combination used, the greater the potential for reducing blood sugar levels. This is supported by the findings of Liem et al., (2015), which indicate that in the use of a combination dose, the higher the dose used, the greater the effect of lowering blood glucose levels produced. Widiana & Marianti (2022) posited that glibenclamide and flavonoids operate via a similar mechanism within the body, namely by stimulating insulin production and increasing insulin secretion from pancreatic beta cells. This assertion aligns with the hypothesis that the mechanism of action of glibenclamide with flavonoids present in numerous plants has the same potential for reducing blood glucose levels.

CONCLUSION

The administration of a combination of ethanol extracts of African leaves and shallot skin has been observed to exert an antidiabetic effect on male white mice induced by streptozotocin. The combination dose of 1:1 has been identified as the dose that produces the most pronounced reduction in blood glucose levels, closely approximating the positive control of glibenclamide. The administration period for test preparations that are effective and stable in reducing blood sugar in mice is 14 days.

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