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Research article

Evaluation of the Antioxidant and Antihyperglycemic Activity: A Comparative Study of Shallot (*Allium ascalonicum* L.) Peel and Bulb Ethanol Extracts

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Abstract

Diabetes Mellitus (DM), known as hyperglycemia, is a growing global health concern. Among medicinal plants explored for their potential in diabetes treatment, *Allium ascalonicum* L. (shallot) has gained significant attention. This study investigated the effectiveness of shallot peel and bulb extracts as antihyperglycemia activity in alloxan-induced diabetic rats. The total phenolic and flavonoid contents, and antioxidant activity of the extracts were also assessed. Shallot peel and bulb were extracted using ethanol-based maceration, followed by total phenolic content (TPC) and total flavonoid content (TFC) evaluation using the Folin–Ciocalteu and aluminium chloride methods, respectively. Antioxidant activity was determined by the DPPH radical scavenging assay. Diabetic rats were divided into four groups: negative control, positive control (metformin), and two treatment groups receiving 150 mg/kg of shallot peel or bulb extract for 10 days. Parameters such as fasting blood glucose, body weight, urine volume, food, and water intake were also monitored. The results showed significantly higher TPC in peel (347.6±1.7 mg GAE/g) than bulb (78.7±1.90 mg GAE/g), with $p = 0.001$ ($p < 0.05$). Peel also had higher TFC (56.60±1.63 mg QE/g vs. 30.4±0.81 mg QE/g in bulb), with $p = 0.001$ ($p < 0.05$) and superior DPPH scavenging capacity (IC_{50} of 124.814 µg/mL compared to 1,712 µg/mL in bulb extract). Both extracts significantly reduced fasting blood glucose levels, with the peel extract being the most effective in glycemic control, while the bulb extract showed greater improvement in diabetic symptoms. These findings suggest that ethanol extracts of shallot peels and bulbs are promising natural antioxidants with antihyperglycemic properties. Their phenolic and flavonoid richness supports their potential in diabetes management.

Keywords: *Allium ascalonicum*; shallot; peel; bulb; antihyperglycemic; antioxidant

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1. Introduction

Diabetes Mellitus (DM), commonly referred to as diabetes or hyperglycemia, is a chronic disease characterized by elevated blood glucose levels. The condition arises from metabolic disturbances in which the pancreas fails to produce sufficient insulin, a hormone necessary for effective blood sugar regulation, and/ or the body develops insulin resistance (American Diabetes Association, 2024). DM is associated with various comorbidities, including stroke, heart attack, visual impairment, renal failure, delayed wound healing, and amputation (Directorate of Prevention and Control of Non-Communicable Diseases, 2016). According to data from the International Diabetes Federation (International Diabetes Federation, 2021), Indonesia ranks seventh among the top ten countries globally, with 8.5 million reported cases of diabetes (Tamtaji et al., 2017).

The role of oxidative damage in the development of diabetes and its complications has been widely recognized (Asmat et al., 2016). Consequently, a solid recommendation exists to explore a novel antidiabetic compound possessing antioxidant characteristics (Hajleh et al., 2022). Traditional medicine has long utilized natural products for the treatment of diabetes mellitus. Plant extracts, particularly those with antioxidant properties, have demonstrated significant antidiabetic effects (Kooti et al., 2016). These extracts contain diverse antioxidant compounds, such as flavonoids, tannins, phenolic acids, and alkaloids. These enhance pancreatic cell efficiency and boost insulin levels by reducing glucose absorption through the intestinal wall (Ríos et al., 2015).

One medicinal plant believed to possess efficacy in treating diabetes is the shallot (*Allium ascalonicum* L.) (Galavi et al., 2021). Previous studies identified active compounds in *Allium sp*., such as polyphenols and flavonoids, including quercetin, which were found in greater abundance in shallots than in garlic (Tamtaji et al., 2017). The presence of an active compound in the peel of shallots, indicated by the longer shelf life of unpeeled shallots, was documented (Maryuni et al., 2022). Shallot peel exhibits higher levels of flavonoids compared to the bulb, with previous research demonstrating significantly elevated phenolic compounds and quercetin content in the peel that were at three to five times greater than those found in the bulb (Albishi et al., 2013). This suggests that shallot peel has potential applications as an antioxidant and antimicrobial agent in food, cosmetics, and the treatment of cancer and type 2 diabetes (Mobin et al., 2021; Chakraborty et al., 2022; Maryuni et al., 2022).

This research aimed to investigate the differential effectiveness of shallot peel and bulb extracts (*Allium ascalonicum* L.) on alloxan-induced diabetic rats and determine the total phenolic and flavonoid contents and antioxidant activity. The results of this study are anticipated to contribute valuable information and enhance our understanding of the therapeutic potential of natural ingredients for treating diabetes.

2. Materials and Methods

2.1 Plant material

The shallots and their peels were purchased at the traditional market in Padang City, West Sumatra, Indonesia. A taxonomist in Andalas University Herbarium identified and authenticated the plants with voucher specimen number 089/K-ID/ANDA/II/2020).

2.2 Materials and reagents

All chemicals used in this study were analytical grade. Methanol p.a. (Emsure®), distilled ethanol, sodium carboxymethyl cellulose (NaCMC) (Sigma-Aldrich®), alloxan monohydrate (Sigma-Aldrich®), Folin Ciocalteu reagent (Supelco®), Sodium Hydroxide (NaOH) (Supelco®), aluminum chloride ($AlCl_3$) (Sigma-Aldrich®), sodium acetate (CH_3COONa) (Supelco®), quercetin (Sigma-Aldrich®), gallic acid (Sigma-Aldrich®), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) ((Sigma-Aldrich®) were used in this study. Metformin 500 mg was also utilized as the positive control.

2.3 Research instrument

UV-Vis spectrophotometer (Thermo Scientific Genesys®), rotary evaporator (IKA®), hot plate stirrer (Velp Scientifica®), oven (Memmert®), water bath (Memmert®), glucometer device and strip (Auto-check®) were used in this study.

2.4 Preparation of the extract

The outer peel and bulbs of shallots (*Allium ascalonicum* L.) were meticulously cleansed to remove any adhering impurities, followed by a consecutive three-day natural air-drying process. Once dried, they were finely ground into powder form. The powdered shallot peels and bulbs were then placed into separate glass bottles and immersed in 70% ethanol. The soaking process lasted 6 h, with intermittent stirring, followed by 18 h of standing undisturbed. This procedure was repeated thrice. Subsequently, the residue and filtrate were separated, with the filtrate collected and subjected to rotary evaporation to obtain a concentrated extract. The percentage of yield extract was determined according to the following formulation and was obtained at 10.66% and 41.9% for shallot peel and bulb ethanol extracts, respectively. The dosage used for testing the antidiabetic effectiveness of the peel and bulb extracts of shallots was 150 mg/kg body weight, formulated as a 1% (v/v) suspension, and administered orally to the experimental animals. The suspension was prepared using sodium carboxymethyl cellulose (Na-CMC) as the suspending agent with a concentration of 0.5%.

$$\text{Percentage of Yield Extract} = \frac{\text{Mass of Extract}}{\text{Mass of dry Simplicia}} \times 100\% \quad (1)$$

2.5 Extract characterization

Characterization of the extract involved conducting organoleptic assessments and physicochemical analysis, including measurements of water content, total ash content, and acid-insoluble ash content.

2.5.1 Organoleptic assessment

The organoleptic test was carried out to observe the shape and color of Extract according to Ministry of Health Indonesia (2017).

2.5.2 Physicochemical analysis

Physicochemical parameters encompassed determining water content, total ash content, and acid-insoluble ash content.

1) Determination of water content

The water content was determined using the gravimetric method. A one-gram sample was heated at 105ºC for 5 h and weighed. This process was repeated hourly until the weight difference between two consecutive measurements was less than 0.25% (Ministry of Health Indonesia, 2017).

2) Determination of total ash content

A one-gram sample was placed in a silicate crucible, weighed, and evenly distributed within the crucible. The sample was then gradually heated to 800±25°C until complete carbon combustion occurred. After cooling in a desiccator, the crucible was reweighed. This procedure was repeated until a stable and consistent weight was obtained (Ministry of Health Indonesia, 2017).

3) Determination of acid-insoluble ash content

The ash obtained from the ash content test was subjected to further processing. It was boiled with 25 mL of 10% HCl for 5 min, then filtered using non-ash filter paper. Afterwards, it was washed with 5 mL of hot water, transferred to a crucible, and dried on a hot plate. Subsequently, the sample was ignited by gradually increasing the heat to 800±25°C, cooled in a desiccator, and finally weighed (Ministry of Health Indonesia, 2017).

2.6 Determination of total phenolic content (TPC)

2.6.1 Preparation of standard gallic acid for calibration curve

The determination of total phenolic content (TPC) in the shallot peel and bulb extracts (*Allium ascalonicum* L.) was conducted using the Folin–Ciocalteu colorimetric method, with slight modification according to the Indonesian Herbal Pharmacopeia (Ministry of Health Indonesia, 2017). A standard solution of gallic acid was prepared by dissolving 10 mg of gallic acid in 25 mL of ethanol to achieve a concentration of 400 µg/mL. Various concentrations of gallic acid solutions in ethanol (25, 30, 45, 50, and 60 µg/mL) were prepared from this standard solution. For each concentration, 1 mL was mixed with 5 mL of 7.5% Folin–Ciocalteu reagent and left to stand for 8 min. Afterwards, 4 mL of 1% NaOH was added, resulting in a blue-colored mixture, which was incubated for 1 h at room temperature. The absorbance was then measured at 730 nm against a blank sample. All experiments were conducted in triplicate, and the average absorbance values for the different concentrations of gallic acid were used to create a calibration curve.

2.6.2 Preparation of samples for total phenolic content in shallot peel and bulb extracts (*Allium ascalonicum* L.)

Each extract from the shallot peel and bulb was prepared by diluting 0.2 g of the extract in 100 mL of ethanol. The same procedure described for gallic acid was applied to these shallot peel and bulb extracts. The total phenolic content of the shallot peel and bulb extracts was then determined by plotting their absorbance values on the gallic acid standard solution calibration curve. The resulting data were expressed as milligrams per gram of dry extract (mg GAE/g) in terms of gallic acid equivalents.

2.7 Determination of total flavonoid content

2.7.1 Preparation of standard quercetin for calibration curve

The flavonoid content of the extracts was determined using the aluminum chloride method (Ministry of Health Indonesia, 2017). A stock solution of quercetin with a concentration of 400 µg/mL was prepared by dissolving 10 mg of quercetin in 25 mL of ethanol. This standard solution was subsequently serially diluted to generate 3, 12, 25, 50, and 75 µg/mL concentrations. For each of these concentrations, 0.5 mL of quercetin was mixed with 1.5 mL of ethanol, 0.1 mL of a 10% aluminum chloride solution, and 0.1 mL of 1 M sodium acetate. To reach a final volume of 4 mL, 2.8 mL of distilled water was added. The resulting mixtures were then incubated for 30 min at room temperature, followed by the absorbance measurement at 415 nm against a blank. All experiments were conducted in triplicate, and the average absorbance values for the different concentrations of quercetin were used to create a calibration curve.

2.7.2 Preparation of samples for total flavonoid content in shallot peel and bulb extracts (*Allium ascalonicum* L.)

The same procedure used for quercetin was applied to the extract. Specifically, 0.5 mL of the extract solution (0.2 g in 100 mL) was combined with 1.5 mL of ethanol, 0.1 mL of a 10% aluminum chloride solution, 0.1 mL of 1 M sodium acetate, and 2.8 mL of water. These mixtures were then incubated for 30 min at room temperature, after which the absorbance at 415 nm was measured against a blank. The resulting data were expressed as milligrams of quercetin equivalents per gram (mg QE/g) of dry extract, determined using a linear equation derived from the calibration curve.

2.8 Antioxidant activity

The antioxidant activity of the extracts was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method to measure radical scavenging activity (RSA) (Phuyal et al., 2020). The solution (0.2 mL) —at concentrations ranging from 6.25 to 50 µg/mL for gallic acid and 125 to 8000 µg/mL for the extract solution—was dissolved in methanol and mixed with 3.8 mL of a 50 µM DPPH solution. These mixtures were then placed in a dark environment for 30 min, after which their absorbance was measured at a wavelength of 517 nm against a blank containing an equal amount of DPPH and methanol.

The percentage of DPPH scavenging (RSA %) was calculated using the following equation 2:

$$\%\ scavenging\ of\ DPPH\ =\ [(A_{control}\ -\ A_{sample})/A_{control}]\ \times\ 100 \quad (2)$$

The IC_{50} value of the samples was determined by plotting the sample concentration on the x-axis and the percentage of DPPH scavenging (RSA %) on the y-axis. A linear regression equation of the form (y = a + bx) was employed to fit the data. The IC_{50} value was then calculated using equation 3:

$$IC_{50}\ =\ (50\ -\ a)\ /\ b \quad (3)$$

2.9 Antihyperglycemic activity

2.9.1 Experimental animal

Twenty male rats, aged 2-3 months and weighing on average 200-230 g, were sourced from the 'Magek' Animal Health Center in Agam regency, West Sumatra, Indonesia. These rats were housed in a controlled environment with access to food and water *ad libitum*. All animal procedures followed institutional protocols and the guidelines for animal care established by The Ethics Committee of The Faculty of Medicine, Universitas Andalas, under approval number 183/UN.16.2/KEP-FK/2020.

2.9.2 Diabetic induction using alloxan monohydrates

The rats underwent a 12-h overnight fast while having unrestricted access to water. To induce diabetes mellitus (DM) in these fasted rats, a single intraperitoneal injection of alloxan monohydrate was administered at 125 mg/kg of body weight (Ibrahim et al., 2023). In the first three days following the injection, the animals were provided with free access to a 10% glucose solution to counteract drug-induced hypoglycemia (Dhandapani et al., 2002). The confirmation of diabetes was performed by assessing the blood glucose levels of these rats seven days after the administration of Alloxan. Blood samples were collected from the tip of their tails using an Auto-check® digital glucometer. Rats with fasting blood glucose levels equal to or exceeding 140 mg/dL were classified as diabetic and included in the experimental group (Prince & Menon, 2001).

2.9.3 Animal grouping and experimental design

The rats were categorized into four experimental groups. The negative control group received an injection of alloxan and had access to water. The positive control group received an injection of alloxan and was administered metformin at a dose of 45 mg/kg of body weight. The treatment groups received an injection of alloxan and were given ethanol extract of shallot peel or bulb at a dose of 150 mg/kg of body weight. The doses used for the shallot bulb and peel were based on a previous study, which reported that 100-200 mg/kg BW of Persian shallot was effective in reducing HbA1c levels and controlling blood glucose in a dose-dependent manner (Mehdi et al., 2013). On the first day following the induction of diabetes, fasting blood glucose levels were reassessed before the commencement of treatments. The test formulations were administered to each group daily at the same time for ten days. Fasting blood glucose levels were monitored during the treatment period on days 1, 3, 5, and 10. Other parameters, such as body weight, urine volume, water intake, and food consumption, were also measured.

2.10 Data analysis

The results were presented as mean±standard error of the mean (SEM). Statistical analysis was performed using an independent T-test for evaluating total phenolic content (TPC) and total flavonoid content (TFC), and two-way analysis of variance (ANOVA) was used for evaluating the percentage decrease of fasting blood glucose and diabetic symptoms, followed by Duncan's multiple range test. Significance was determined at $p < 0.05$.

3. Results and Discussion

3.1 Organoleptic assessment and phytochemical analysis

The results of organoleptic and phytochemical analysis of shallot peel and bulb ethanol extracts (*Allium ascalonicum* L.) can be observed in Table 1. These data serve as a benchmark for assessing the quality of raw materials and extracts, ensuring they meet the standards expected for good simplicia. Determining water content in the samples was a critical step in maintaining the quality of the extracts and ensuring their suitability for further testing and applications. Maintaining low water levels in extracts minimizes the potential for mold growth and contamination. According to the literature, it is advised that the water content should not exceed 10% (Ministry of Health Indonesia, 2017).

Table 1. The results of the organoleptic test and physicochemical analysis from shallot peel and bulb ethanol extracts (*Allium ascalonicum* L.)

Parameters	Peel Ethanol Extract	Bulb Ethanol Extract
Organoleptic profiles		
shape	thick	thick
color	brown	brown
smell	distinctive	distinctive
Physicochemical analysis		
Water content (%)	16.13 %	21.64 %
Total ash content (%)	3.98 %	4.76 %
Acid-insoluble ash content (%)	0.54 %	0.73 %

The total ash content was analyzed to assess the combined mineral content present in simplicia or extracts throughout the manufacturing process. A higher total ash content signifies the presence of minerals in the sample. Additionally, the acid-insoluble ash content measurement indicates the quantity of minerals that remain unaffected by acid treatment. A higher acid-insoluble ash content suggests the presence of silicate components, which may be linked to soil, sand, silver, lead, or mercury within the sample (Ministry of Health Indonesia, 2017).

3.2 Total phenolic content (TPC)

The total phenolic content (TPC) in the shallot peel and bulb extracts was assessed using the Folin–Ciocalteu method, with gallic acid as the standard. The TPC of the extracts was determined by applying the calibration curve equation ($y = 0.0135x + 0.0279$; $R^2 = 0.9916$).

The study showed significantly higher TPC in the peel (347.6±1.7 mg GAE/g) than in the bulb (78.7±1.90 mg GAE/g), with $p = 0.001$ ($p < 0.05$), as shown in Figure 1.

This outcome aligns with the findings in a previous study (Albishi et al., 2013), in which the content of phenolics extracted from onion (*Allium cepa* L.) peels was approximately six times higher (62.65±0.60 mg GAE/g) than that of their flesh counterparts (17.33±0.98 mg GAE/g). Another study also reported a similar result, showing a higher TPC in shallot (*Allium ascalonicum* L.) peel compared to the bulb (94±0.9 mg GAE/g vs 0.016±0.16 mg GAE/g) (Mobin et al., 2021). Phenolic compounds are known for their health-promoting effects, encompassing antidiabetic and anti-cancer properties, enzyme inhibition, and cardiovascular benefits (Zeb, 2020; Chadorshabi et al., 2022). Phenolic compounds function as reducing agents, donating hydrogen atoms, and can scavenge and neutralize free radicals (Wojdyło et al., 2007). This means they can protect tissues against free radical damage caused by oxidation. Hence, they also play a crucial role in enhancing the antioxidant potential of foods, making them a valuable natural source of antioxidants (Balasundram et al., 2006).

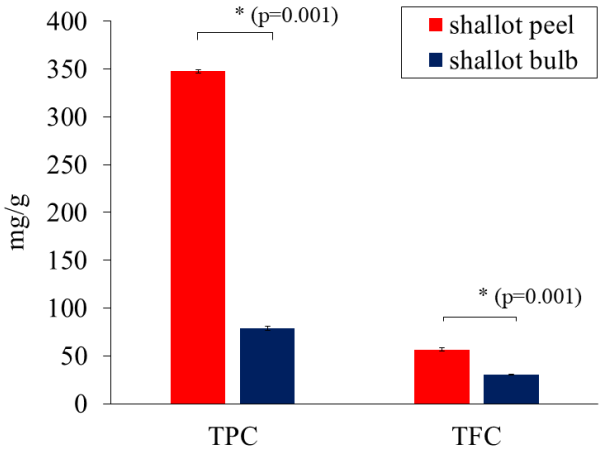

Figure 1. Total phenolic content (TPC) and total flavonoid content (TFC) of shallot peel and bulb (*Allium ascalonicum* L.) ethanol extracts

3.3 Total flavonoid content (TFC)

The extracts' total flavonoid content (TFC) was calculated from the regression equation of the calibration curve ($y = 0.0095x + 0.2247$; $R^2 = 0.99$). The TFC values followed a similar trend to the TPC values, with the peel showing significantly higher TFC (56.60±1.63 mg QE/g) compared to the bulb (30.4±0.81 mg QE/g), with $p = 0.001$ ($p < 0.05$), as illustrated in Figure 1. The findings were relevant to previous studies that reported that the waste fractions of the onions and shallots showed greater quercetin content and higher antioxidant capacity than the edible counterparts (Crnivec et al., 2021). Additionally, another species of shallot (*Allium cepa* L.) was shown to contain significantly higher levels of flavonoids than the edible portion, by around 2-10 g/kg (Suh et al., 1999). A similar result was also reported that the flavonoid content in *Allium cepa* L. peel was also higher than those in its flesh and indicated that the outer layers of onions were rich in flavonoids compared to the whole onion bulb or edible parts (Albishi et al., 2013).

In comparing our present study to previous research, Mobin et al. (2021) reported a higher total flavonoid content (68±0.6 mg QE/g) in the aqueous methanolic (20:80) crude extract of the outer peel of *Allium ascalonicum* L. This finding highlights the potential variability in flavonoid content among different solvent and shallot samples. Another previous study identified 14 compounds of flavonoid in shallot peel ethanol extract, such as kaempferol-3-lucuronide, robinetin, pedalitin, quercetin-4′-glucoside, quercetin-7-O-[β-D-glucopyranosyl (1→6)-β-D-glucopyranoside], quercetin-3-O-β-D-glucopyranoside (Maryuni et al., 2022). Furthermore, Bonaccorsi et al. (2008) discovered that shallot bulbs (*Allium ascalonicum* L.) also displayed the most abundant flavonoid composition among various onion varieties. Notably, the levels of quercetin 3,4'-diglucoside and quercetin-4'-glucoside in red shallot bulbs were nearly double those found in the other onions studied (Kwak et al., 2017). This observation accentuates the significant differences in flavonoid content even within the same species.

3.4 Antioxidant activity

Antioxidants are vital in preventing and managing chronic diseases, including diabetes mellitus, by inhibiting or slowing down reactions between biomolecules and free radicals (Losada-Barreiro et al., 2022). Natural antioxidants found in plants are crucial in mitigating the harmful effects of oxidative stress. They predominantly come from plants, including various edible vegetables, fruits, spices, and herbs. These plants are rich in vitamins, phenolic compounds, and trace elements (Flieger et al., 2021).

Polyphenolic compounds are commonly present in various plants, including shallots, and are known for their diverse biological effects, notably their antioxidant activity (Wojdyło et al., 2007). This study assessed the antioxidant activity of ethanol extracts from shallot peel and bulb (*Allium ascalonicum* L.) using the DPPH free radical scavenging assay. Their reducing power was determined based on the concentration required to achieve 50% inhibition (IC_{50}) of DPPH free radicals (Phuyal et al., 2020). Among the various methods used to assess antioxidant potential, the DPPH assay is particularly convenient. This assay measures the presence of antioxidant compounds with hydrogen-donating groups, such as flavonoids and phenols. These compounds reduce the methanolic DPPH solution, forming non-radical compounds (Mensor et al., 2001; Aberoumand & Deokule, 2008). The findings, which include the average percentage of DPPH free-radical scavenging activity at various extract concentrations and a standard solution, are presented in Table 2.

The radical scavenging activity of the various extracts increased in a concentration-dependent manner. Notably, the shallot peel, which exhibited higher total phenolic content (TPC) and total flavonoid content (TFC), demonstrated a potent DPPH scavenging capacity, with an IC_{50} value indicating the greater antioxidant capacity of the shallot peel (124.814 µg/mL) compared to the bulb (1,712 µg/mL) (Table 2). This finding is consistent with another study that reported the waste fraction of red shallot ethanol extract to have a higher antioxidant capacity (11.47 ± 0.04 mg TE/g) compared to its edible part (0.09 ± 0.001 mg TE/g) (Črnivec et al., 2021). Additionally, it aligns with a study indicating that the antioxidant capacity as free phenolic content in red onion peel (0.1520 ± 0.004 mmol TE/g) was higher compared to the flesh (0.027 ± 0.001 mmol TE/g) (Albishi et al., 2013).

Table 2. Percentage of DPPH Inhibition and IC_{50} values of shallot peel and bulb (*Allium ascalonicum* L.) extract and gallic acid at different concentrations

Sample	Concentration (µg/mL)	% Inhibition	Linear Regression	IC_{50} (µg/mL)
Galic Acid	6.25	19.53	y = 1.5331x + 10.671; R^2 =0.9988	25.68
	12.5	31.21		
	25	48.20		
	50	87.47		
Shallot peel	125	50.32	y = 0.0808x + 39.915; R^2 =0.9994	124.814
	250	59.66		
	375	70.22		
	500	80.47		
Shallot bulb	2,000	49.77	y = 0.0052x + 1.095; R^2 =0.9823	1,712
	4,000	63.93		
	6,000	73.07		
	8,000	81.28		

There is a significant correlation between plant phenolic content and antioxidant activities (Yu et al., 2021). The remarkable antioxidant activity observed in shallots can be attributed to their rich content of flavonoids and polyphenol compounds, particularly quercetin, kaempferol, myricetin, and catechin (Maryuni et al., 2022). Among these, quercetin monoglucoside and quercetin diglucoside are the two predominant components, comprising approximately 80% of the total flavonoids found in onions. Interestingly, the levels of quercetin glucosides are notably higher in onions than in other vegetables (Proteggente et al., 2002; Bonaccorsi et al., 2008). Similarly, Price & Rhodes (1997) reported that quercetin 3,4'-O-glucoside and quercetin monoglucoside (quercetin 4'-O-glucoside) were the primary flavonols present in the edible parts of onions, with a higher concentration found in the peel. These findings are consistent with the results obtained in our study, reinforcing the notion that quercetin and related compounds are critical contributors to the antioxidant properties of shallots.

3.5 Antihyperglycemic activity

Hyperglycemia is a primary symptom of diabetes mellitus and a risk factor for cardiovascular diseases (Pistrosch et al., 2011). However, many bioactive molecules derived from plants, like polyphenols, have proved their efficacy in the treatment of metabolic disorders and have high nutraceutical values, including antihyperglycemic activity, antihypertensive and cardioprotective and cytotoxic properties (Tijjani et al., 2020).

The administration of shallot peel extract and shallot bulb extract (both at a dosage of 150 mg/kg) and the positive control resulted in a significant reduction in fasting blood glucose levels in diabetic rats compared to the negative control group. On the initial and third days of observation, the positive control and shallot bulb groups showed considerable decreases in fasting blood glucose levels. In contrast, the shallot peel group had the least impact. However, by the 5^{th} and 10^{th} days, the reduction in fasting blood glucose levels was most pronounced in the shallot peel extract group, surpassing the effects observed in shallot bulb groups and performing comparably to the positive control group. This made the shallot peel extract group the most effective in reducing blood glucose levels over time

(Table 3 and Figure 2). Herbal extracts or natural substances typically need time to exert their effects on the body. On the first days of treatment, the body begins to absorb the active compounds, resulting in gradual pharmacological activity. The dosage may also not be sufficient to produce a significant effect initially. However, the cumulative dose had a more noticeable impact (Kar et al., 2003; Rodino & Butu, 2019).

Table 3. Effect of treatment in percentage decrease of fasting blood glucose

Treatment given	Dose (mg/kg)	Percentage Decrease of Fasting Blood Glucose (%)±SEM				Mean±SEM
		Day 1	Day 3	Day 5	Day 10	
Negative control	-	-35±15.5	-51±15.5	-61±15.5	5±15.5	-36±7.8^{a}
Positive control	45	18±15.5	22±15.5	34±15.5	66±15.5	35±7.8^{b}
Shallot peel	150	5±15.5	2±15.5	42±15.5	66±15.5	29±7.8^{b}
Shallot bulb	150	3±15.5	20±15.5	26±15.5	50±15.5	25±7.8^{b}
Mean±SEM		-2±7.8^{p}	-2±7.8^{p}	10±7.8^{p}	46±7.8^{q}	

Values are presented as mean ± SEM. Different letters in the column (a, b) indicate a significant difference between the negative control and treatment groups ($p < 0.05$). Different letters in the row (p, q) indicate a significant difference between day 1 and other day groups ($p < 0.05$), based on Duncan's Multiple Range Test.

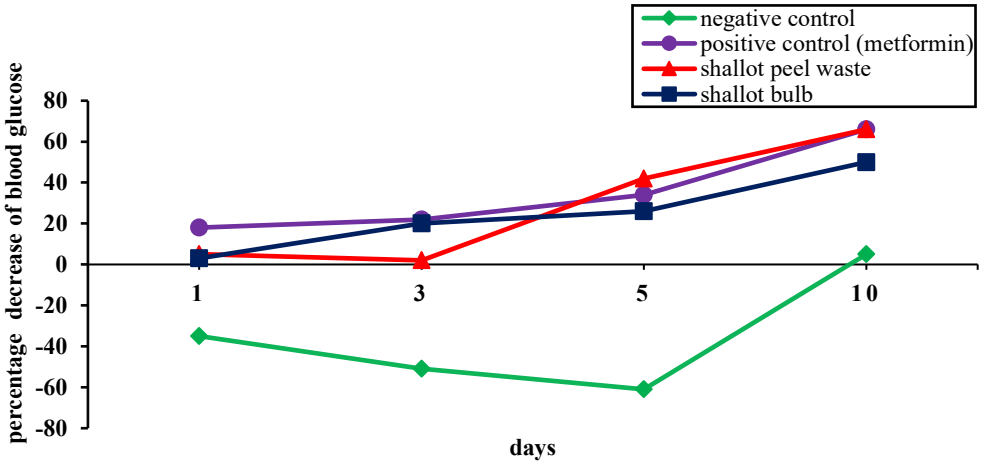

Figure 2. Effect of treatment in percentage decrease of fasting blood glucose

Another *in vivo* study involving *Allium ascolanicum* L. bulb methanol extract in alloxan-induced diabetes Wistar rats demonstrated a significant 32% reduction in postprandial blood glucose levels after a three-week treatment period. Moreover, the previous study provided evidence of increased regulation of GLUT-4 and Insulin genes (Moradabadi et al., 2013; Moldovan et al., 2022). These findings strongly support the potential of *Allium ascolanicum* L. as an effective antidiabetic treatment.

Shallot contains various phytochemicals and has been associated with promoting health and reducing disease risks, such as lowering cancer risk in different tissues and preventing cardiovascular and neurodegenerative disorders (Albishi et al., 2013). The peel and bulb extracts exhibited robust antioxidant profiles, with values of 124.814 µg/mL and 1,712 µg/mL, respectively. These findings suggest that the extracts possess antioxidant activity, which is beneficial in treating diabetes. Antioxidants help reduce oxidative stress by scavenging free radicals, binding metals and enhancing the activity of key enzymes such as superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT). These actions prevent free radical damage and protein glycosylation and protect endothelial and neuronal cells from glucose-induced harm (Zatalia & Sanusi, 2013; Asmat et al., 2016). By decreasing the formation of harmful compounds like superoxide and peroxynitrite, antioxidants help mitigate complications such as diabetic neuropathy, contributing to overall protection against diabetes-related oxidative damage (Shafras et al., 2024).

The experimental plant may also exert its antidiabetic activity by enhancing the activity of endogenous free radical scavenging enzymes (Kamtekar et al., 2014). Quercetin and kaempferol, which were reported to be contained in shallots (Maryuni et al., 2022), have demonstrated activity as antihyperglycemic agents. They play pivotal roles as inhibitors of α-amylase and α-glucosidase in intestinal glucose absorption. Additionally, these compounds exhibit insulin-secretory and insulin-sensitizing activities and can improve glucose utilization in peripheral tissues (Eid & Haddad, 2017; Ansari et al., 2022).

Quercetin exhibits potential as an inhibitor of glucose transport through intestinal GLUT2 and GLUT5 transporters, leading to reduced glucose absorption. This mechanism contributes to its capacity to lower blood glucose levels in experimental models (Kwon et al., 2007; Hamilton et al., 2018). Moreover, quercetin's antioxidative properties enable it to bind and neutralize free radicals, enhancing β-pancreatic cell function and insulin secretion (Golovinskaia & Wang, 2023). Notably, quercetin therapy supports β-pancreatic cell integrity by stimulating pancreatic progenitor cells to form new islets of Langerhans cells (Al-Ishaq et al., 2019).

In a 10-day treatment period, all groups displayed decreased body weight compared to the control group. While our study noted a significant increase in body weight within the negative control groups over time, the experimental and positive groups exhibited a reduction in body weight. Despite identifying notable differences between the groups, the decline in body weight among the experimental groups implied a potential blood sugar-lowering effect attributed to the ethanol extract of shallot bulb and peel. This decrease in body weight aligned with enhanced insulin resistance and improved glycemic control as highlighted in prior research (Aucott et al., 2016).

In our findings, metformin, as the positive control, demonstrated a decrease in body weight over the treatment period. Previous studies have shown that metformin primarily causes weight loss by reducing hunger through its direct effects on the brain, leading to lower calorie consumption. Additionally, it indirectly influences appetite control (Haddad et al., 2023). Intriguingly, a prior study mirrored our findings, reporting a significant decrease in body weight among diabetic animal models treated with aqueous extracts and compounds from diverse medicinal plants in comparison to normal and diabetic control groups (Sharma & Gupta, 2017; Zhao et al., 2022). This suggests that these extracts may have similar activity to metformin in controlling diabetes, reducing body weight, and altering appetite, thereby reducing food consumption and improving polyphagia, especially in the case of shallot bulb extract.

Quercetin has emerged as a multifaceted bioactive compound capable of preventing and/or suppressing hyperglycemia. Its mechanisms include inhibiting sugar digestion and uptake, and potentially enhancing insulin secretion through its interaction

with the GLP-1 receptor as an agonist. This GLP-1 receptor effect extends to its impact on body weight (Niisato & Marunaka, 2023). GLP-1 receptor agonists contribute to reduced food intake and consequent weight loss. The reduction in body weight is primarily linked to decreased energy intake, possibly complemented by altered food preferences and sustained energy expenditure despite the weight loss (Knudsen, 2010).

Significant reductions in food consumption, water intake, and urine volume were observed in the bulb extracts group, indicating notable improvements in diabetes symptoms such as polyphagia, polydipsia, and polyuria after ten days, compared to the negative control group (Table 4). The bulb group, in particular, showed promising results with reductions of 56.85±2.75 mL/animal/day for water intake and 16.9±2.5 mL/animal/day for urine volume. In contrast, the peel extract group showed reductions of 111.15±2.21 mL/animal/day and 51.35±8.13 mL/animal/day, respectively, which were not as significant. These findings underscore that antidiabetic activity can vary significantly among different plant varieties and their parts. Despite containing similar phytochemicals, distinct plant parts may play different roles in alleviating diabetes symptoms (Flieger et al., 2021). This highlights the importance of identifying and utilizing the most effective plant parts for diabetes management and suggests a need for further research to explore the underlying mechanisms and potential applications of these findings.

Table 4. Body weight, food consumption, water intake, and urine volume of rats after ten days of treatment

Treatment	Negative Control	Positive Control	Shallot Peel	Shallot Bulb
Body weight change (g)	14.08 ± 5.90^{b}	-5.82 ± 3.89^{a}	-5.76 ± 5.95^{a}	-14.36 ± 3.8^{a}
Food Consumption (g/animal/day)	16.31 ± 1.79^{a}	16.36 ± 1.32^{a}	21.41 ± 2^{b}	15.22 ± 1.3^{a}
Water intake (mL/animal/day)	85.40 ± 7.24^{b}	$64.65\pm9.24^{a,b}$	111.15 ± 8.37	56.85 ± 7.10^{a}
Urine volume (mL/animal/day)	$28.45\pm4.23^{a,b}$	37.70 ± 9.76^{b}	$51.35\pm8.13^{b,c}$	16.9 ± 2.5^{a}

Body weight change was measured by differences between the beginning and end of the 10-day treatment. All values are presented in mean±SEM; Different letters in the row (a, b, c) indicate a significant difference between the negative control and treatment groups ($p < 0.05$), based on Duncan's Multiple Range Test.

Although the study showed promising results, further investigation is needed to assess the long-term effects of shallot extracts on diabetes management. The study's limitations include its relatively short duration, which restricted the evaluation of antihyperglycemic effectiveness and its impact on diabetes symptoms such as polyuria and polydipsia, particularly for the peel extract. Additionally, the variability in phytochemical content among different plant samples was not thoroughly examined, which could influence the outcomes. Therefore, a detailed analysis of the phytochemical profile and standardization of the extracts are recommended.

While the study highlights the antioxidant and antihyperglycemic properties of the extracts, the precise mechanisms underlying these effects remain unclear. Future research should focus on a comprehensive exploration of this plant's *in vivo* antioxidant activities. It should also aim to standardize the extracts and investigate the relationship between

individual phenolic compounds and their antioxidant mechanisms. Isolation, screening, and characterization of specific compounds responsible for the antioxidant and antihyperglycemic effects are essential to validate their suitability as natural antioxidants. Exploring their synergistic effects with antidiabetic drugs, such as metformin and sulfonylureas, could provide further insights into their potential therapeutic applications. Moreover, the safety of shallot extract supplementation should be evaluated to ensure its suitability for long-term use. These efforts will support their traditional medicinal uses and enhance their development as novel therapeutic agents.

4. Conclusions

The ethanol extracts derived from the bulb and peel of *Allium ascalonicum* L. exhibited notable levels of total phenolic and total flavonoid content, indicating their potential as rich sources of natural antioxidants. Furthermore, these extracts demonstrated significant scavenging effects on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, highlighting their antioxidant properties. Additionally, both extracts effectively reduced hyperglycemia in alloxan-induced diabetic rats, with the bulb extract showing superior efficacy in ameliorating diabetic symptoms such as polyphagia, polydipsia, and polyuria. Consequently, *Allium ascalonicum* L. holds promising potential for preventing and managing various detrimental human diseases, including diabetes mellitus.

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6. Authors' Contribution

M. Rifqi Efendi: performed research, analyzed data and wrote the paper. Fadila Dwiyanti: performed research and contributed reagents/ analytic tools. Oktavionita: performed research and analyzed data. Mesa Sukmadani Rusdi: analyzed data and wrote the paper. Armenia: designed research, coordinated research and wrote the paper.

7. Conflicts of Interest

The authors declared no conflict of interest.

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