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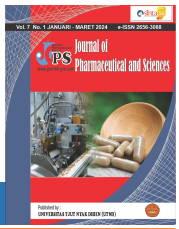

In-Vivo Study of the Potential of Akway Leaf Extract (*Drimys* cf. *piperita*) as an Antihyperglycemia

Studi In-Vivo Potensi Ekstrak Daun Akway (*Drimys* cf. *piperita*) Sebagai Antihiperglikemia

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Abstract

Background : Hyperglycemia is a metabolic condition characterized by an increase in blood glucose levels above normal limits and is the main characteristic of diabetes mellitus, a chronic disease with a prevalence that continues to increase in various countries. The akway plant (*Drimys* cf *piperita.*) is endemic to the Sorong Mountains, Southwest Papua . Its leaves are known to contain various bioactive compounds such as flavonoids, tannins, saponins, and alkaloids. **Purpose:** This study aims to test the ability of akway leaf extract to lower blood sugar levels in vivo in white mice that were previously raised glucose levels through glucose solution. **Method** : This study design is an experimental laboratory with five treatment groups, namely negative control (Na-CMC 0.5 mg), positive control (glibenclamide 3 mg), and three groups of akway leaf extracts, each at doses of 100, 200, and 400 mg/kg BW. Hyperglycemia was induced for 7 days using a glucose solution. Blood glucose measurements were performed at 30, 60, 90, 120, and 150 minutes using a glucometer. Data analysis used One-Way ANOVA followed by *a post* -LSD test. **Results:** Akway leaf extract at a dose of 200 mg/kg BW provided the highest reduction in blood glucose, namely 54.24%, which was statistically significant (<0.05) and even better than glibenclamide (51.15%). Meanwhile, a dose of 400 mg/kBW actually produced lower effectiveness (45.85%). Implications: Akway leaf extract has been proven to have antihyperglycemic activity with an optimal dose of 200 mg/kgBW.

Keywords: Akway leaves, Antihyperglycemic, Mice, Glibenclamide.

Abstract

Background : Hiperglikemia merupakan kondisi metabolik yang ditandai oleh peningkatan kadar glukosa darah di atas batas normal dan menjadi karakteristik utama pada diabetes melitus, suatu penyakit kronis dengan prevalensi yang terus meningkat di berbagai negara. Tanaman akway (*Drimys* cf. *piperita*) tumbuh endemik di Pegunungan Sorong, Papua Barat Daya. Daunnya mengandung flavonoid, tanin, saponin, dan alkaloid. **Tujuan**: Penelitian ini bertujuan menguji efek ekstrak daun akway dalam menurunkan gula darah secara in vivo pada mencit putih yang sebelumnya kadar glukosanya dinaikkan menggunakan larutan glukosa. **Metode**: Rancangan penelitian bersifat eksperimental laboratorium dengan lima kelompok: kontrol negatif (Na-CMC 0,5 mg), kontrol positif (glibenklamid 3 mg), serta tiga kelompok ekstrak daun akway dosis 100, 200, dan 400 mg/kgBB. Hiperglikemia diinduksi selama 7 hari dengan larutan glukosa. Kadar glukosa darah diukur pada menit ke-30, 60, 90, 120, dan 150 menggunakan glukometer. Analisis data memakai One-Way ANOVA dilanjutkan dengan uji LSD. **Hasil**: Ekstrak dosis 200 mg/kgBB memberikan penurunan glukosa darah tertinggi (54,24%), berbeda bermakna secara statistik (p<0,05) dan bahkan lebih baik dari glibenklamid (51,15%). Sementara dosis 400 mg/kgBB justru menghasilkan efektivitas lebih rendah (45,85%). **Kesimpulan**: Ekstrak daun akway terbukti memiliki aktivitas antihiperglikemia dengan dosis optimal 200 mg/kgBB.

Kata kunci: Daun Akway,Antihiperglikemia,Mencit,Glibenklamide.

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Introduction

Diabetes mellitus is a non-communicable disease whose prevalence continues to skyrocket globally. This disease is characterized by chronic hyperglycemia due to disorders of insulin secretion, insulin action, or both [1]. This condition can trigger various serious complications, such as blood vessel damage, nerve disorders, decreased kidney function, and an increased risk of heart and blood vessel disease [2]. The World Health Organization (WHO) estimates that the incidence of diabetes in the adult population will reach around 8.5%, and this disease causes approximately 1.3 million deaths before a person reaches the age of 70 [3].

Several factors contributing to the surge in DM cases include obesity, unhealthy eating habits (high in fat and low in fiber), lack of physical activity, and age. According to a 2020 report from the West Papua Health Service, there were 640 cases of DM with a prevalence of 17.6 per 1,000 residents across 13 districts and cities [4].

Synthetic antidiabetic drugs such as glibenclamide and metformin remain the mainstay of diabetes therapy [5]. However, some of these synthetic drugs have the potential to cause side effects, are expensive, and are not widely available, especially in remote areas. This situation has prompted the need to explore alternative therapies based on natural ingredients that are safer, more affordable, and more readily available to the public [6].

Papua is known to have abundant biological wealth. One of the plants that has the potential as an antihyperglycemic is akway (*Drimys* cf. *piperita*), a Papuan endemic plant that has long been used by local people as a traditional medicine. Akway is a woody plant with aromatic leaves and belongs to the Winteraceae family [7]. Morphologically, akway has a smooth stem, dark green leaves with orange tips. The average plant height reaches 3.09 meters and grows in primary and secondary tropical forests at an altitude of 1,200–2,400 meters above sea level [8].

Akway leaves contain active compounds such as flavonoids, phenolics, tannins, saponins, and terpenoids. These phytochemicals are known to have various pharmacological activities, including antioxidant, anti-inflammatory, and antidiabetic potential [9]. Flavonoids play a role in lowering blood glucose by increasing insulin sensitivity, inhibiting carbohydrate digestive enzymes, and protecting pancreatic β cells thanks to their antioxidant properties. Meanwhile, tannins work by increasing the process of glycogenesis and acting as an astringent that inhibits glucose absorption in the small intestine [10]. In vivo testing using mice (*Mus musculus*) is a common approach used to evaluate the antidiabetic potential of an herbal extract, considering that their metabolic physiology is similar to that of humans [11]. However, scientific studies on the antidiabetic effects of akway extract, especially those derived from the leaves, are still very limited. Therefore, this study was conducted to examine the antihyperglycemic potential of akway leaf extract through direct testing on white mice while determining the most effective dose [12].

Experimental Section

Sample was in the form of akway leaves (*Drimys* cf. *piperita*) was obtained from the Aimas region, Sorong Regency, Southwest Papua.

Materials and Equipment

The tools used include a glucometer, analytical balance, digital balance, test tube, measuring cup, blender, water bath, vortex, oven, hot plate, 60 mesh sieve, mortar and pestle, aluminum foil, filter paper,

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maceration container, oral probe, stirring rod, 1 cc disposable syringe, gloves, glass jar, scissors, mouse cage, food container, and drinking bottle. Materials needed: male white mice (25), akwa y leaves, glucose (hyperglycemia inducer), glibenclamide (positive control), distilled water, 1% Na-CMC, 96% ethanol, NaCl, sawdust (mouse feed), mouse drinking water, Dragendorff reagent (alkaloid test), magnesium powder (flavonoid test), HCl, chloroform, Lieberman-Burchard reagent (steroid/terpenoid test), $FeCl_3$ (tannin test).

Sampling

The akway leaf samples used in this study were taken from the Aimas area, Sorong, Southwest Papua, and then identified in the Natural Materials and Pharmacology Laboratory of Muhammadiyah Sorong University of Education.

Sample preparation

The 4 kg of akway leaves collected underwent wet sorting. Next, the leaves were washed with running water to remove dirt or foreign materials, then shredded and dried for three days in an oven at 40°C. Once dry, dry sorting was carried out to remove unwanted fragments. The dried simplicia was then blended into powder and sieved with a 60 mesh sieve [13].

Sample extraction

Extraction was carried out using the maceration method. A total of 300 grams of powdered simplicia was placed in a glass jar, then soaked in 96% ethanol while stirring occasionally. The soaking was left for three days [14]. After that, re-maceration was carried out, then filtered to separate the filtrate from the residue. The filtrate obtained was concentrated using a water bath [15].

Phytochemical screening

Alkaloids : 2 grams of extract + 2 drops of Dragendorff's reagent → positive if an orange precipitate forms. Flavonoids : 1 gram of extract + 2 mg of Mg powder + 3 drops of concentrated HCl → positive if the color changes to yellow, red, or orange. Saponins : 1 gram of extract + 10 mL of water, shaken for 30 seconds → positive if foam forms that does not disappear after the addition of 1 drop of 2N HCl. Tannins : 1 gram of extract + a few drops of $FeCl_3$ → positive if a dark blue or greenish black color forms.

Test Solution

Na-CMC 1 g : Na-CMC is dissolved in hot distilled water (70°C) up to 100 mL. Glucose Solution: Refers to WHO standards for glucose tolerance test in humans (75 grams in 250 mL of water). Glibenclamide : 3 mg tablets are converted for 20 grams of mice (conversion factor 0.0026) → 3 mg × 0.0026 = 0.0078 mg per 20 gBW. The suspension is dissolved with Na-CMC up to 10 mL. Test Extract: Weigh the thick extract according to the dose (100, 200, 400 mg/kgBW), then dissolve it in 1% Na-CMC.

Test Animals and Treatment

Male white mice (aged 2–3 months, BW 20–30 grams) were fasted for 8–12 hours before treatment, but were still given drinking water [16]. Mice were divided into 5 groups (5 mice each): negative control (Na CMC), positive control (glibenclamide 3 mg), group 1 (extract 100 mg/kgBW), group 2 (extract 200 mg/kgBW), group 3 (extract 400 mg/kgBW) [17]. Hyperglycemia induction was carried out using a glucose solution for 7 days. Blood glucose levels were measured at 30, 60, 90, 120, and 150 minutes using a glucometer. Blood was taken from the tip of the mice's tail, which had been cut to a length of 0.1 cm [18]. Data were tested for normality using the Shapiro-Wilk test and for homogeneity using Levene's test [19]. Differences between groups were analyzed using One-Way ANOVA, followed by the LSD post hoc test [20].

Results and Discussion

Table 1. Akway Leaf Yield

Sample	Simple Weight (g)	Extract Weight (g)	Yield (%)
Akway Leaves	330	30	9%

Source: Primary data, 2026

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From 330 grams of Akway leaf simple powder extracted with 96% ethanol by maceration, 30 grams of thick extract was obtained, so the yield obtained was 9% (Table 1). The results of the phytochemical test showed that the akway leaf extract contained positive alkaloids, flavonoids, tannins, and saponins (table 2).

Table 2. Phytochemical Screening

Compound Groups	Reagent	Observation	Note
Flavonoids	Pb II Acetate	Reddish yellow	+
Alkaloids	Dragendorf	Orange colored sediment	+
	Bourchardat	Orange colored sediment	+
Tannins	$FeCl_3$	Greenish black	+
Saponins	Aquadest	Formation of foam	+

Source : Primary data, 2026

Data analysis techniques

Table 3. Effect of Glucose Induction on Increasing Blood Glucose Levels in Mice

Group	Pre-induction Glucose (g/dl)	Post-Induction Glucose (g/dl)	Δ(%)	95% CI	p-value
Control +(Glibenclamide)	143.8 ± 23.45	299.6 ± 51.98	110.05 ± 35.61	100.80 ± 210.80	0.001*
Control – (Na. CMC)	146 ± 19.54	208 ± 46.02	43.63 ± 31.84	8.41 ± 115.59	0.033*
EDKA 100 mg/kgBW	132.2 ± 19.61	225.2 ± 47.11	73.49 ± 46.16	28.55 ± 157.45	0.016*
EDKA 200 mg/kg BW	154.4 ± 42.93	246 ± 58.88	61.39 ± 20.89	58.54 ± 124.66	0.002*
EDKA 400 mg/kg BW	142.2 ± 12.28	294.8 ± 93.72	108.66 ± 68.77	34.46 ± 270.74	0.023*

Source : Primary data, 2026

Note: EDKA = Kayu Akway Leaf Extract; Data analysis using paired-samples t-test; *: declared significant, if p-value < 0.05.

Table 4. Effect of Akway Leaf Extract on Reducing Blood Glucose Levels in Mice and Comparison of Effectiveness Between Treatment Groups

Variables	Control + (Glibenclamide)	Control – (Na. CMC)	EDKA 100 mg/kgBW	EDKA 200 mg/kg BW	EDKA 400 mg/kg BW
Pre-test	299.6 ± 51.98	208 ± 46.02	225.2 ± 47.11	246 ± 58.88	294.8 ± 93.72
Post-test					
30th minute	232.6 ± 35.81	174.8 ± 22.09	202.8 ± 40.03	207.2 ± 52.66	245.8 ± 78.97
60th minute	191 ± 20.01	159.8 ± 14.37	175.2 ± 22.97	176 ± 30.11	206.6 ± 30.53
90th minute	149.8 ± 28.87^{b*}	160.6 ± 19.73^{a*}	161 ± 22.34^{a*}	121.4 ± 20.67^{b*}	178.4 ± 18.30^{b*}
120th minute	147.4 ± 27.91^{b*}	152.4 ± 19.01^{a*}	153.4 ± 26.16^{a*}	114.4 ± 15.99^{b*}	161.2 ± 11.65^{b*}
150th minute	144 ± 25.15^{b*}	151.6 ± 19.63^{a*}	146.8 ± 22.55	109.6 ± 16.18^{b*}	147.6 ± 8.56^{b*}
Δ(%)					
30th minute	21.43 ± 11.41	10.34 ± 32.83	9.62 ± 4.68	14.97 ± 15.22	15.70 ± 12.17
60th minute	35.29 ± 8.21	19.20 ± 23.98	20.88 ± 8.54	26.52 ± 15.69	26.73 ± 14.80
90th minute	49.34 ± 9.09^{b*}	18.73 ± 24.90^{a*}	26.77 ± 12.42^{a*}	49.56 ± 8.75^{b*}	36.12 ± 14.76
120th minute	50.09 ± 9.15^{b*}	23.59 ± 21.31^{a*}	30.06 ± 14.23^{a*}	52.18 ± 9.09^{b*}	41.97 ± 14.42^{b*}
150th minute	51.15 ± 8.96^{b*}	24.09 ± 20.43^{a*}	32.99 ± 13.42	54.24 ± 8.65^{b*}	45.85 ± 16.99^{b*}

Source: Primary Data, 2026

Note: Values are expressed as mean ± SD, Δ (%) = percentage difference between pre-induction with glucose and post-induction with glucose, (*) sig < 0.05 using one-way ANOVA. (a) sig < 0.05, there is a significant difference compared to the positive control, (b) sig < 0.05, there is a significant difference compared to the negative control. EDKA = Akway Leaf Extract.

Discussion

The success of glucose induction in significantly increasing blood glucose levels in mice indicates that the hyperglycemia model has been successfully created. This finding is in line with previous research reports stating that repeated oral glucose administration can cause persistent increases in blood glucose levels in experimental animals. This hyperglycemic condition is important to ensure that the experimental animals are truly in a state that represents diabetes mellitus before being given the test extract intervention [21].

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The results of the study showed that akway leaf extract has good antihyperglycemic potential. The highest effectiveness was shown by a dose of 200 mg/kgBW with a reduction percentage reaching 54.24%, which is even superior to glibenclamide as a positive control which only provided a reduction of 51.15%. This finding is very interesting because it shows that herbal extracts can provide pharmacological effects that are equal to or even better than synthetic antidiabetic drugs. This superiority is due to the content of bioactive compounds in akway leaves contained therein [22].

Based on the results of phytochemical screening, akway leaf extract showed the presence of flavonoids, alkaloids, tannins, and saponins. The presence of flavonoids is indicated by the formation of a reddish yellow color after the addition of Pb(II) acetate reagent [23]. This reaction occurs due to the formation of a complex between metal ions and hydroxyl groups in the flavonoid structure, which indicates the presence of polyphenol compounds in the extract [24]. Alkaloids are detected positively through Dragendorff and Bouchardat reagents, which are characterized by the formation of an orange precipitate, indicating the presence of an organic base compound that is able to bind with the iodide complex ion from the reagent [25]. Meanwhile, the color change to greenish black in the $FeCl_3$ reagent confirms the presence of tannin due to the formation of a complex between the Fe^{3+} ion and the phenolic group in the tannin compound [26]. The presence of saponins is indicated by the formation of stable foam after shaking with distilled water, which is related to the amphiphilic nature of saponins which are able to reduce the surface tension of the solution [27].

Flavonoids, tannins, saponins, and alkaloids contained in akway leaves contribute to antihyperglycemic potential through various mechanisms. Flavonoids increase insulin sensitivity through activation of the insulin signaling pathway, inhibit α-amylase and α-glucosidase enzymes thereby slowing glucose absorption, protect pancreatic β cells from oxidative stress, and increase GLUT4 translocation to increase glucose uptake by peripheral tissues [28] . Tannins also inhibit the activity of carbohydrate digestive enzymes, reduce glucose absorption in the intestine, and increase glucose storage in the form of glycogen in the liver [29].

Meanwhile, saponins are known to stimulate insulin secretion, increase glucose utilization by peripheral tissues, and inhibit α-glucosidase activity [30]. Alkaloids play a role by increasing insulin secretion and sensitivity, inhibiting hepatic gluconeogenesis, and by antioxidant activity that helps maintain pancreatic β-cell function [31]. The presence of these various compounds underlies the potential of akway leaf extract in lowering blood glucose levels, especially in conditions of postprandial hyperglycemia [32], [33].

An interesting phenomenon to observe is the decrease in effectiveness at a dose of 400 mg/kgBW, which is actually lower (45.85%) compared to a dose of 200 mg/kgBW. This pattern indicates a bell-shaped dose-response curve effect or an inverse dose-response effect. A similar phenomenon has also been reported in studies of other herbal extracts, where increasing the dose beyond the optimal limit is not always followed by an increase in pharmacological effects [34].

The decrease in blood glucose levels observed in the negative control group can be explained by physiological responses occurring during the observation period and does not solely reflect the effects of the treatment. Fasting before testing increases glucose utilization by tissues and activates homeostatic mechanisms to maintain metabolic balance [35]. In addition, repeated handling procedures of test animals, such as restraint during blood sampling, can trigger a stress response that affects the glycemic profile [36] . Fluctuations in circadian rhythms, which play a role in the regulation of glucose metabolism, also contribute to changes in blood glucose levels during observation [37]. Therefore, the decrease in glucose in the negative control group should be viewed as a physiological variation that can occur in acute test models, so that the interpretation of the antihyperglycemic effect of the extract is more appropriately based on a comparison of changes in blood glucose levels to the control group [38].

This study has several limitations that need to be acknowledged. First, the active compound levels of the extract were not measured, so the chemical compound content remains unknown quantitatively. Second, insulin levels were not measured, so the extract's mechanism of action in influencing insulin secretion cannot be directly confirmed. Third, pancreatic histopathology was not performed to assess the beta-cell protective effect. Fourth, oxidative parameters such as malondialdehyde or superoxide dismutase were not measured, so the contribution of antioxidant mechanisms cannot be empirically proven. Fifth, the relatively short observation duration (150 minutes) only captured the acute effects, not the long-term effects of extract administration. Sixth, toxicity testing was not performed, so its safety cannot be confirmed.

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Conclusion

Based on the results of the research conducted, it can be concluded that akway leaf extract exhibits significant antihyperglycemic activity in hyperglycemic mice. This ability to lower blood sugar levels is gradually apparent from the 30th to the 150th minute. The dose that produced the best effect was 200 mg/kgBW, with a blood sugar reduction percentage reaching 54%, which is slightly superior to the comparison drug glibenclamide. This effect comes from the synergistic action of the four main bioactive compounds (flavonoids, tannins, saponins, and alkaloids) through various complementary mechanisms.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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