

**Research Article****Antidiabetic Effectiveness Test of Combination of Soursop Leaf Extract and Red Betel Leaf Extract in Mice Induced By Alloxan**Eka Wahyu Riyadi¹✉, Gunawan Pamudji Widodo¹, Vivin Nopiyanti¹¹Faculty of Pharmacy, Universitas Setia Budi, Surakarta, Central Java, Indonesia✉ ekawahyuriyadi60@gmail.com🌐 <https://doi.org/10.33751/jf.v16i1.72>**Article info:**

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Universitas Pakuan**ABSTRACT**

Soursop leaves contain saponins, terpenoids, steroids, flavonoids, tannins, and alkaloids, while red betel leaves contain flavonoids, polyphenols, saponins, alkaloids, and terpenoids (triterpenoids), which have the potential to lower blood glucose levels. This study aims to determine the effect of the combination of soursop leaf extract and red betel leaf on changes in body weight and reduction of blood glucose levels in alloxan-induced mice, as well as to determine the most effective combination ratio. The study used 40 mice divided into eight groups: normal control, positive control (glibenclamide), negative control (Na-CMC), single soursop leaf extract, single red betel leaf extract, and three combination groups with ratios of 25%:75%, 50%:50%, and 75%:25%. Alloxan was injected intraperitoneally at a dose of 150 mg per kilogram of body weight, followed by treatment after 14 days. Blood glucose levels were measured on days 0, 3, 7, and 14 using a glucometer, and the data were analyzed using SPSS version 27. The results showed that both extracts contained alkaloids, flavonoids, tannins, saponins, and triterpenoids. The combination of extracts showed a tendency to improve body weight and lower blood glucose levels compared to the negative control. The 75%:25% ratio provided the highest blood glucose reduction descriptively, but statistical analysis showed no significant differences between the combination ratios. Thus, all combination ratios had relatively equivalent antidiabetic effectiveness.

Keywords: Antidiabetic; soursop leaves; red betel leaves; alloxan; combination of extracts**INTRODUCTION**

Diabetes mellitus (DM) is a long-term metabolic disease marked by elevated blood glucose levels due to defects in insulin secretion, insulin function, or a combination of both. The prevalence of diabetes continues to increase and has become a major global health concern. According to the IDF, (2024), approximately 589 million people were living with diabetes in 2024, and this number is projected to reach 853 million by 2050. The increasing prevalence of diabetes contributes to severe complications, including nephropathy, neuropathy, retinopathy, and cardiovascular diseases (Kemenkes, 2019). These conditions highlight the need for the development of effective and safe antidiabetic therapies.

Soursop (*Annona muricata* L.) and red betel (*Piper crocatum* Ruiz & Pav.) leaves are medicinal plants with promising antidiabetic properties. Soursop leaves contain alkaloids, flavonoids, tannins, saponins, steroids, and terpenoids that contribute to

antihyperglycemic activity (Asfahani et al., 2022), whereas red betel leaves contain flavonoids, polyphenols, alkaloids, saponins, tannins, and terpenoids that function as antioxidants and inhibitors of glucose absorption (Intami et al., 2024). Wijayanti & Wulandari, (2023) reported that soursop leaf extract at doses of 250, 500, and 750 mg/kg body weight effectively reduced blood glucose levels in glucose-induced mice, whereas Opara et al., (2021) showed significant glucose-lowering effects at doses of 200 and 400 mg/kg body weight in diabetic rats. Adrianto et al., (2023) reported that ethanolic extract of red betel leaves at a dosage of 100 mg/kg body weight showed the greatest reduction in blood glucose levels in diabetic models.

These findings indicate that both plants possess considerable potential for development as natural antidiabetic agents. Soursop leaves contain active compounds such as tannins, flavonoids, and alkaloids which have been proven to have the potential to

regenerate damage to pancreatic beta cells and re-stimulate insulin secretion to improve glycemic control (Dewi et al., 2025). On the other hand, red betel leaves significantly complement this antidiabetic therapy pathway through their bioactive compound content which acts as an inhibitor of the α -glucosidase enzyme in the intestinal mucosa to suppress post-meal carbohydrate digestion and absorption (Mustika et al., 2022). The combination of the multifaceted action mechanisms of these two herbal plants complement each other in controlling blood glucose levels towards homeostasis.

The antidiabetic activities of soursop and red betel leaves have been widely reported individually, but studies evaluating their combination have not yet been reported. Anggraini & Kusuma, (2019) and Kuntari et al., (2019) showed that combining soursop leaves with other medicinal plants produced greater glucose-lowering effects than single-extract treatments. Utami et al., (2020) have reported that a combination of soursop leaves and insulin leaves, administered at a dose of 2.24 mg per 20 g of body weight, successfully reduced blood glucose levels to an average of 93.15 mg/dL. These findings suggest that herbal combinations may produce synergistic effects through complementary mechanisms of action. The purpose of this study is to examine the effect of a combination of soursop and red betel leaf extracts on body weight and blood glucose levels in alloxan-induced mice, as well as to evaluate which combination is most effective.

METHODS

Study Site, Plant Authentication, and Ethical Approval

The research was conducted at the Pharmacognosy and Phytochemistry Laboratory and the Pharmacology Laboratory, Faculty of Pharmacy, Setia Budi University, Surakarta. Soursop leaves (*Annona muricata* L.) and red betel leaves (*Piper crocatum* Ruiz & Pav.) were obtained from Tawangmangu, Central Java. The identity of the plant materials used in this study was verified through examination at the UPT Herbal Medika Laboratory, Batu, East Java numbers 000.9.3/2451/102.20/2025 for soursop leaves and 000.9.3/2452/102.20/2025 for red betel leaves. The research has obtained ethical approval from the Health Research Ethics Commission of Dr. Moewardi Hospital, Surakarta with number 2.401/XI/HREC/2025.

Tools

The equipment used in this study included an analytical balance (Ohaus®), blender (Ossel®), drying oven (Memmert®), a 40-mesh sieve, maceration vessel, vacuum rotary evaporator (IKA®), water bath (Memmert®), a Bidwell–Sterling distillation

apparatus, EasyTouch® glucometer (Biopitik Technology Inc.), UV lamps at 254 and 366 nm, oral gavage equipment, beaker glass (Pyrex®), measuring cylinders (Pyrex®), Erlenmeyer flasks (Pyrex®), volumetric flasks (Pyrex®), glass funnels (Pyrex®), glass stirring rods (Pyrex®), vials, and other supporting laboratory equipment.

Materials

The materials used in this study included soursop (*Annona muricata* L.) and red betel (*Piper crocatum* Ruiz & Pav.) leaves were locally sourced and authenticated, alloxan monohydrate ($C_4H_2N_2O_4 \cdot H_2O$; Sigma-Aldrich, USA), glibenclamide ($C_{23}H_{28}ClN_3O_5S$; Hexpharm Jaya). The remaining chemical reagents and solvents were procured from Merck (Darmstadt, Germany), which included 70% ethanol (C_2H_5OH), sodium carboxymethyl cellulose (Na-CMC), ethyl acetate ($C_4H_8O_2$), magnesium powder (Mg), hydrochloric acid (HCl), ferric chloride ($FeCl_3$), methanol (CH_3OH), n-butanol ($C_4H_{10}O$), n-hexane (C_6H_{14}), and silica gel GF254 TLC plates. Physiological sodium chloride (0.9% NaCl) was sourced from PT Otsuka Indonesia (Malang, Indonesia), Distilled water (H_2O) was produced locally using a laboratory-grade water purification and distillation system. Meanwhile, the phytochemical screening reagents, including Mayer, Dragendorff, anisaldehyde-sulfuric acid, and citroborate reagents, were analytical-grade solutions provided directly by the laboratory.

Methodology

Preparation of Plant Sample

Fresh soursop and red betel leaves underwent sorting and washing under running water, followed by oven drying at 50°C until a constant weight was achieved. The dried materials were pulverized and sieved through a 40-mesh sieve to obtain a standardized and homogeneous simplicia powder.

Extraction Process

A total of 500 g of powder from each plant was macerated using 5 L of 70% ethanol at a 1:10 ratio for 24 hours with occasional stirring. The remaining marc was then re-macerated using 2.5 L of fresh 70% ethanol at a 1:5 ratio for an additional 24 h. All the filtrates were combined and concentrated using a vacuum rotary evaporator, continued with heating in a water bath until a thick extract was obtained.

Characterization of Simplicia and Extracts

The characterization of simplicia and extracts included determination of drying loss, extraction yield, and moisture content. Drying loss was determined using a gravimetric method in an oven until a constant weight was obtained. Extraction yield was calculated

as the percentage ratio of the obtained extract weight to the initial simplicia weight. Moisture content was determined using the toluene distillation method with a Bidwell–Sterling distillation apparatus based on the volume of water separated during the distillation process.

Phytochemical Screening

Qualitative phytochemical screening was performed on the ethanolic extracts of soursop and red betel leaves to identify the presence of secondary metabolites. Alkaloids were detected using Mayer and Dragendorff reagents based on precipitate formation (Prayitno & Utami, 2024). Flavonoids were identified using magnesium powder and concentrated HCl, indicated by the formation of red to orange coloration (Hasnawati et al., 2025). Saponins were identified by the formation of stable foam after shaking, whereas tannins were detected using FeCl₃ solution, indicated by a dark green or bluish-black color (Purnamasari, 2021). Steroids and triterpenoids were identified using the Liebermann–Burchard reagent, producing bluish-green and reddish-brown coloration, respectively (Kaidun et al., 2022).

Further identification was conducted using thin-layer chromatography (TLC) on silica gel GF254 plates. Alkaloids were analyzed using toluene:ethyl acetate (7:3) and visualized with Dragendorff reagent. Flavonoids were analyzed using n-butanol:acetic acid:water (4:1:5) as the mobile phase and visualized with citroborate reagent. Tannins were analyzed using methanol:water (6:4) mixture and detected with FeCl₃ reagent. Steroids and triterpenoids were analyzed using n-hexane:ethyl acetate (4:1) and visualized with anisaldehyde-sulfuric acid reagent. Chromatographic spots were analyzed under UV light at 254 and 366 nm, and *R_f* values were compared with reference data for compound identification.

Experimental Animals

The test animals used were male mice weighing 20–40 g and aged 2–3 months. Male white mice were acclimatized for 7 days with normal food and water intake. Test animals were considered healthy if they did not experience a change in body weight of more than 10% during the acclimatization period and showed no visual abnormalities.

Antidiabetic Activity Test

After the acclimatization period, the mice were fasted for approximately 16 h prior to the measurement of baseline blood glucose levels and body weight (T₀). Diabetes mellitus was induced by a single intraperitoneal injection of alloxan. The alloxan dose was based on the rat induction protocol reported by Mongi et al., (2019), in which alloxan was administered at 150 mg/kg body weight, and was

subsequently converted to the equivalent mouse dose using the standard interspecies dose-conversion table, resulting in a dose of 4.2 mg/20 g body weight. Three days after alloxan administration, blood glucose levels were remeasured (T₁), and mice with fasting blood glucose levels exceeding 200 mg/dL were considered diabetic. Alloxan takes 48–72 hours to induce full beta-cell necrosis. Measuring immediately (24h) may give false negatives.

The animals were randomly assigned into eight groups, each consisting of five mice: normal control, negative control (0.5% Na-CMC), positive control (glibenclamide 0.013 mg/20 g body weight; equivalent to 0.65 mg/kg body weight, converted from the human therapeutic dose of 5 mg), soursop leaf extract (250 mg/kg body weight), red betel leaf extract (100 mg/kg body weight), and three extract combination groups with ratios of 25%:75%, 50%:50%, and 75%:25%. Treatments were administered orally for 14 days. Fasting blood glucose levels and body weights were measured after approximately 16 h of fasting on day 7 (T₂) and day 14 (T₃). Calculation of blood sugar levels and body weight using equation 1 and 2.

$$\% \text{ Change in induction} = \frac{T_0 - T_1}{T_0} \times 100\% \dots\dots\dots (1)$$

$$\% \text{ Change in treatment} = \frac{T_1 - T_n}{T_1} \times 100\% \dots\dots\dots (2)$$

Where, T₀ = Normal initial value

T₁ = Value after induction

T_n = Value on the respective treatment day (T₂ or T₃)

Interpretation, Negative value = Indicates an increase

Positive value = Indicates a decrease

Statistical Analysis

Statistical analysis was performed using SPSS version 27. Body weight data were initially tested for normality and, because the data were not normally distributed, were further analyzed using the Kruskal–Wallis and Wilcoxon Signed-Rank tests. Blood glucose levels were evaluated using the Shapiro–Wilk normality test and homogeneity test, followed by one-way ANOVA. Post hoc comparisons were performed using Tukey’s HSD or Games–Howell test according to the characteristics of the data distribution and variance.

RESULTS AND DISCUSSION

Characterization of Simplicia and Extracts

Soursop and red betel leaves simplicia prepared from 12 kg of fresh materials yielded 1.23 kg and 1.14 kg of dried simplicia, corresponding to extraction yields of 10.25% and 9.50%, respectively. Pulverization of the dried materials produced powder yields of 98.37% for soursop leaves and 96.49% for red betel leaves. The drying loss values of soursop and red betel leaf powders were 8.85 ± 0.27 and 8.07 ± 0.07, respectively, which complied with the Indonesian Herbal Pharmacopoeia requirement of not more than

10% (Kemenkes, 2017). These low drying loss values indicate minimal moisture and volatile compound content, contributing to better storage stability. The results suggest that both simplicia possessed adequate quality for further extraction processes.

Extraction was performed by maceration using 70% ethanol to obtain the secondary metabolites present in both plant materials. The extraction yield of soursop leaves was 18%, while that of red betel leaves was 14%. The soursop leaf extract met the Indonesian Herbal Pharmacopoeia requirement of not less than 11.4%, whereas the red betel leaf extract yield was lower than the minimum required value of 17% (Kemenkes, 2017). Variations in extraction yield may be influenced by plant characteristics, particle size, solvent properties, and extraction efficiency (Salamah & Widayari., 2015 in Wijaya et al., 2018). These findings indicate that 70% ethanol was effective in extracting bioactive constituents from both plant materials.

Moisture content determination using the toluene distillation method showed that the moisture content of soursop leaf extract was 6.33 ± 0.58 , while that of red betel leaf extract was 3.65 ± 0.58 . Both values complied with the Indonesian Herbal Pharmacopoeia requirement, which specifies a moisture content of not more than 10%. Low moisture content is important for preventing microbial growth and minimizing degradation of active compounds during storage. Therefore, the extracts obtained in this study can be considered of good quality and sufficiently stable for pharmacological evaluation.

Phytochemical Screening

Phytochemical screening and TLC showed that soursop and red betel leaf extracts contained alkaloids, flavonoids, tannins, saponins, and triterpenoids (Table 1). These results support the theoretical basis for the potential antidiabetic properties of both extracts.

The results of compound identification in this study are consistent with previous reports demonstrating that soursop and red betel leaves contain various secondary metabolites with potential antihyperglycemic activity. Thin Layer

Chromatography (TLC) analysis (Table 2) was performed to confirm the presence of the metabolites detected during the preliminary phytochemical screening. Alkaloid identification showed that the piperine standard had an Rf value of 0.514, while the soursop leaf extract exhibited Rf values of 0.514, 0.604, and 0.716, and the red betel leaf extract showed Rf values of 0.402 and 0.718. The appearance of brownish orange spots after Dragendorff reagent spraying and the similarity of the Rf values to the reference standard indicated the presence of alkaloid compounds. Flavonoid identification using quercetin as the reference standard Rf 0.808 produced fluorescent spots under UV 366 nm. The soursop leaf extract showed an Rf value of 0.900, whereas the red betel leaf extract exhibited Rf values of 0.802 and 0.808, confirming the presence of flavonoids with chromatographic characteristics comparable to the standard.

Tannin identification produced dark spots after $FeCl_3$ spraying, indicating the formation of phenolic complexes. The gallic acid standard showed an Rf value of 0.808, while the soursop leaf extract exhibited Rf values of 0.912, 0.604, and 0.516, and the red betel leaf extract showed an Rf value of 0.806. Saponin identification produced characteristic spots after derivatization, with the sapogenin standard showing an Rf value of 0.716, whereas the soursop leaf extract exhibited Rf values of 0.600 and 0.700, and the red betel leaf extract showed Rf values of 0.208, 0.316, 0.600, and 0.700. In the triterpenoid analysis, the stigmasterol standard had an Rf value of 0.716, while the soursop leaf extract exhibited Rf values of 0.318 and 0.514, and the red betel leaf extract showed Rf values of 0.100, 0.316, and 0.512. Although the triterpenoid spots obtained after anisaldehyde sulfuric acid spraying were less distinct than those of the other metabolite groups, the observed chromatographic profiles and Rf values still supported the presence of triterpenoid compounds. Overall, the agreement between the Rf values and spot characteristics of the extracts and the reference standards confirmed the presence of alkaloids, flavonoids, tannins, saponins, and triterpenoids in both soursop and red betel leaf extracts.

Table 1. Phytochemical screening

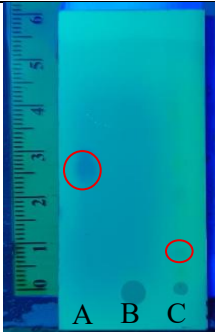
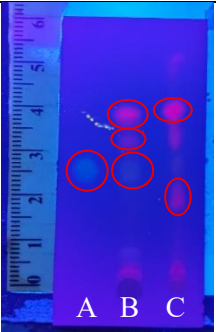

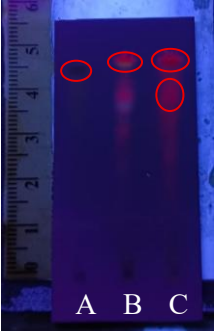
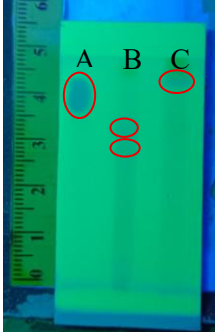
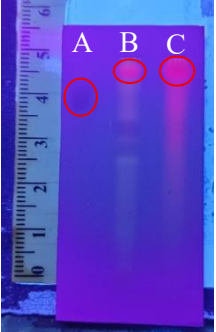

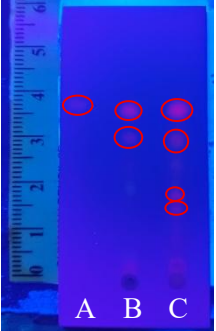

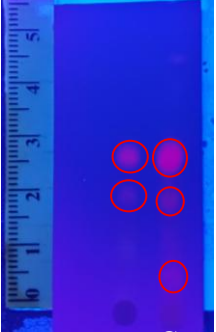
Compound	Soursop Leaves	Red Betel Leaves	Interpretation
Alkaloids	+	+	Detected in test tubes and supported by TLC
Flavonoids	+	+	Detected by its characteristic fluorescence characteristics
Tannins	+	+	Detected by dark color change
Saponins	+	+	Detected through foam formation and TLC
Triterpenoids	+	+	Detected in tube test and TLC indication

Note:

(+) : Positive secondary metabolite compounds

(-) : Negative secondary metabolite compounds

Table 2. Thin Layer Chromatography

	254 nm	366 nm	TLC Systems
Alkaloid			Plate : Silika Gel Gf254 Eluent : Toluene : ethyl acetate (7:3) A : Piperin B : Soursop leaf extract C : Red betel leaf extract
Flavonoid			Plate : Silika Gel Gf254 Eluent : ethyl acetate : methanol : Water (4:1:5) A : Quercetin B : Soursop leaf extract C : Red betel leaf extract
Tannin			Plate : Silika Gel Gf254 Eluent : ethyl acetate : methanol : Water (4:1:5) A : Gallic acid B : Soursop leaf extract C : Red betel leaf extract
Saponin			Plate : Silika Gel Gf254 Eluent : n-hexane : ethyl acetate (6:4) A : Sapogenin B : Soursop leaf extract C : Red betel leaf extract
Steroid/ Triterpenoid			Plate : Silika Gel Gf254 Eluent : n-hexane : ethyl acetate (7:3) A : Stigmasterol B : Soursop leaf extract C : Red betel leaf extract

Effect of Treatment On Body Weight Of Mice

Measuring the body weight of mice is an important physiological parameter in antidiabetic tests to assess the general health condition of test animals and to detect metabolic disorders due to treatment (Adeyemi et al., 2009). Integration of descriptive data in Table 3 and the curve graph in Figure 1 shows that the normal control group exhibited a stable weight gain trend, while all alloxan-induced groups experienced a sharp weight loss in the early phase. This post-induction weight loss (T0-T1) occurred due to the cytotoxic effect of alloxan, which damages pancreatic beta cells through increased Reactive Oxygen Species (ROS), thereby reducing insulin secretion (Lerrick et al., 2025 ; Panjaitan & Novitasari, 2021). This insulin deficiency forces the body to accelerate the catabolism of fat tissue and structural proteins to generate replacement energy. Consequently, in the negative control group, the curve continued to decline steadily without any signs of recovery until the end of the observation period due to the persistence of the metabolic damage (Alam et al., 2022; Jusup, 2016).

The direction of the development of the body weight of the test animals was proven to reverse and increase (metabolic recovery phase) after the administration of standard drug interventions and herbal extract preparations after the induction phase (T1). The positive control group (Glibenclamide) showed the most responsive and significant growth curve reversal pattern until the end of observation (T3), which is in line with its pharmacodynamic mechanism in stimulating endogenous insulin secretion from functional pancreatic beta cells to suppress the degradation pathway of structural proteins and tissue lipids (Panjaitan & Novitasari, 2021). The herbal

therapy group went through a phase that tended to be stable first in the first week of treatment (T2) before finally increasing progressively in the second week (T3). The combination group of DS 50% : DSM 50% and DS 75% : DSM 25% showed a more optimal weight recovery curve compared to the single extract preparation from all variations of herbal treatments tested. This synergistic effect is mediated by the presence of secondary antioxidant metabolite compounds that work complementary in reducing oxidative stress due to free radicals (Reactive Oxygen Species / ROS), protecting the cytological integrity of pancreatic cells, limiting glucose absorption flux in the small intestine, and stabilizing cellular energy homeostasis towards the normal physiological range (Adeyemi et al., 2009 ; Guevara-Vásquez et al., 2021 ; Jusup, 2016; Lerrick et al., 2025).

Statistically, cross-sectional analysis using the Kruskal-Wallis test at each observation point (T0, T1, T2, T3) provided an Asymp. Sig. value > 0.05. This indicates that the biological variation between individual mice at the same time interval point is descriptively still within the range of equality or homogeneity. However, analysis of internal longitudinal differences in groups using the Wilcoxon Signed-Rank test detected a highly significant change in values across all pairs of measurement times ($p < 0.001$). The results of the inter-temporal statistical evaluation empirically prove that the intervention of administering herbal extract products has a significant effect on the dynamics of the test animals. Thus, the combination therapy intervention of soursop leaf extract and red betel leaf is declared effective in increasing body weight.

Table 3. Mean body weight of mice

Treatment group	Body weight (g) ± SD			
	T0	T1	T2	T3
Normal Control	26.4 ± 1.14	26.6 ± 1,67	26.2 ± 1,09	27 ± 1.58
Positive Control	26.4 ± 1.34	25.8 ± 1,09	26.6 ± 1,14	29.4 ± 1.81
Negative Control	27.2 ± 0.83	26.8 ± 1,09	26.8 ± 0,83	26.6 ± 0.89
Soursop Leaves	26.2 ± 0.83	25.8 ± 1,30	25.8 ± 0,83	27 ± 0.70
Red Betel Leaves	27 ± 0.83	26.6 ± 0.54	26.8 ± 0.83	27.6 ± 1.14
DS 25% : DSM 75%	26.4 ± 0.89	26.2 ± 1.30	26.2 ± 1.30	27.2 ± 0.83
DS 50% : DSM 50%	27 ± 0.70	26.2 ± 0.83	27 ± 0.70	28.2 ± 0.83
DS 75% : DSM 25%	27.8 ± 0.83	27 ± 0.70	27.8 ± 0.83	29 ± 0.70

Note:

DS : Soursop Leaves

DSM : Red Betel Leaves

T0: Measurement time before alloxan induction on day 0

T1: Measurement time after alloxan induction on day 3

T2: Measurement time of body weight after treatment on week 1

T3: Measurement time of body weight after treatment on week 2

a : Significantly different from the normal control

b : Significantly different from the negative control

c : Significantly different from the positive control

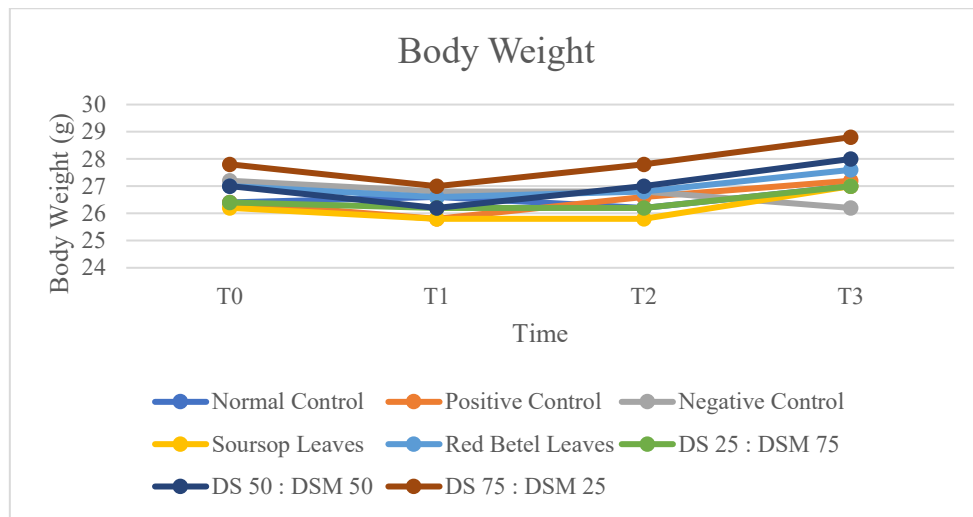


Figure 1. Graph of the relationship between mean body weight (g) and time.

The percentage changes in body weight in Table 4 show the response of blood glucose levels in test animals in various treatment groups during the observation periods ΔT_0 , ΔT_1 , and ΔT_2 . Negative values indicate an increase in body weight, while positive values indicate a decrease in body weight. The glibenclamide positive control group showed a value of -13.99 ± 5.91 , indicating a significant percentage increase in body weight at the end of the second week of the study. In addition to being influenced by the recovery of glucose homeostasis after therapy, this increase in body weight at the end of the observation phase is closely related to the main side effect of glibenclamide which pharmacologically stimulates massive insulin secretion, thereby triggering the accumulation of fat tissue (lipogenesis) and increasing the appetite of the test animals (Alfaqeeh et al., 2024).

All combination groups showed increased

body weight when compared to the negative control group after 14 days of treatment. The DS 25% : DSM 75% combination group showed increased body weight, which may be associated with the antioxidant activity and α -amylase and α -glucosidase inhibitory effects of red betel leaf extract (Afsari et al., 2016 ; (Widiana & Marianti, 2022). Optimal antioxidant activity protects the mitochondrial electron transport chain complex from free radical damage, thereby increasing the efficiency of oxidative phosphorylation in producing ATP (Bottje et al., 2002). This efficient biological energy supply prevents cellular calorie waste due to proton leakage, thereby redirecting nutrient allocation optimally to support tissue formation and body weight gain (Bottje et al., 2009). This improved glucose utilization reduces the body's need to break down fat and protein reserves for energy, thus preventing weight loss.

Table 4. Percentage change in body weight of mice

Treatment group	Body weight(%) \pm SD		
	ΔT_0	ΔT_1	ΔT_2
Normal Control	-0.71 ± 3.23	1.30 ± 5.09	-1.57 ± 3.39^c
Positive Control	2.20 ± 3.47	-3.84 ± -3.11	-13.99 ± 5.91^{ab}
Negative Control	1.48 ± 2.03	-0.05 ± 2.72	0.62 ± 4.80^c
Soursop Leaves	1.54 ± 3.37	-0.08 ± 2.67	-4.76 ± 3.35
Red Betel Leaves	0.71 ± 1.60	-0.74 ± 1.65	-3.70 ± 2.61^c
DS 25% : DSM 75%	0.77 ± 3.22	-0.02 ± 2.66	-3.91 ± 2.96^c
DS 50% : DSM 50%	2.97 ± 1.67	-3.07 ± 1.72	-7.67 ± 2.78^{ab}
DS 75% : DSM 25%	2.87 ± 1.60	-2.96 ± 1.66	-6.67 ± 1.67^b

Note:

DS : Soursop Leaves

DSM : Red Betel Leaves

ΔT_0 : Percentage of body weight T0 to T1 (influent of induction)

ΔT_1 : Percentage of body weight T1 to T2 (influent of treatment on 7 days)

ΔT_2 : Percentage of body weight T1 to T3 (influent of treatment on 14 days)

a : Significantly different from the normal control

b : Significantly different from the negative control

c : Significantly different from the positive control

The DS 50% : DSM 50% combination group showed improved body weight, which may be associated with the combined activity of secondary metabolites from both extracts, potentially contributing to pancreatic β -cell protection, improved insulin sensitivity, and inhibition of glucose absorption (Afsari et al., 2016 ; Fadel & Besan, 2020). The DS 75% : DSM 25% combination group also showed relatively good weight gain, which may be related to the dominance of the pharmacological activity of soursop leaf extract. Flavonoids in soursop leaves are known to increase glucose utilization by tissues, while alkaloids play a role in helping pancreatic β -cell regeneration, thus improving insulin production (Fadel & Besan, 2020 ; Meriem & Fakta, 2021). Furthermore, saponins and tannins contribute to increasing insulin sensitivity and block glucose absorption in the intestine.

The increased effectiveness of glucose utilization as an energy source causes a reduction in protein and fat catabolism, thus maintaining or increasing the mice's body weight during the treatment period. These findings indicate that the combination of soursop leaf extract and red betel leaf extract can improve metabolic disorders caused by diabetes, as reflected in the recovery of the mice's body weight.

Effect Of Treatment on Blood Glucose Levels

Initial blood glucose levels before alloxan induction ranged from 63.2 - 79.6 mg/dL, still within the normal range. According to Rahmawati & Candra, (2015), normal levels of fasting blood range from 50 - 109 mg/dL. After alloxan induction, blood glucose levels elevated in all groups except normal controls. The average blood glucose levels after induction ranged from 132.6 – 151.8 mg/dL, indicating that alloxan induction successfully induced hyperglycemia (Widiana & Marianti, 2022).

According to Maharani et al., (2023), alloxan exerts its diabetogenic effect through the production of free radicals that cause damage to insulin-producing pancreatic cells. Mice were considered diabetic when their fasting blood glucose levels exceeded 126 mg/dL. (Sasmita et al., 2024).

Table 5 showed, after treatment, the positive control group, single extract, and combination of extracts showed a decrease in blood glucose levels at T2 and T3. The negative control still showed high blood glucose levels, indicating that 0.5% Na-CMC did not have an antihyperglycemic effect. The use of Na-CMC as a control is based on its nature that cannot be metabolized by mice due to the absence of the cellulase enzyme, so it does not affect glycemic conditions (Indrawati et al., 2015). At T3, all herbal treatment groups differed significantly from the negative control, but not significantly from the normal control or the positive control. These results indicate that soursop leaf extract, red betel leaf extract, and their combination have antidiabetic activity after administration for 14 days.

The percentage of decrease in blood glucose levels (Table 6) showed that the combination group had a tendency to have a higher effect than the single extract, especially on $\Delta T2$. The combination of DS 25% : DSM 75% reduced blood glucose by 43.70%, the combination of DS 50% : DSM 50% by 44.83%, and the combination of DS 75% : DSM 25% by 47.76%. Descriptively, the ratio of DS 75% : DSM 25% provided the highest reduction and was close to the positive control of glibenclamide at 51.90%. Comparison with a single extract showed that the combination group tended to have a higher effect, especially on $\Delta T2$.

Table 5. Mean blood glucose levels of mice

Treatment group	Mean (mg/dL) \pm SD			
	T0	T1	T2	T3
Normal Control	66 \pm 12.06	63.2 \pm 8.11 ^{bc}	67 \pm 7.21 ^{bc}	75.2 \pm 12.81 ^b
Positive Control	66 \pm 11.22	147.4 \pm 20.84 ^a	93.2 \pm 7.12 ^a	69.6 \pm 5.03 ^b
Negative Control	63.2 \pm 13.08	135.2 \pm 8.07 ^a	142.2 \pm 15.85 ^a	144.8 \pm 8.29 ^{bc}
Soursop Leaves	79.6 \pm 4.88	146 \pm 12.90 ^a	119.2 \pm 16.39 ^a	85 \pm 11.60 ^b
Red Betel Leaves	77.4 \pm 12.11	132.6 \pm 7.16 ^a	106 \pm 10.37 ^a	79.6 \pm 11.01 ^b
DS 25% : DSM 75%	77.8 \pm 9.42	151.8 \pm 8.17 ^a	118.8 \pm 11.41 ^a	85.2 \pm 9.96 ^b
DS 50% : DSM 50%	70.6 \pm 9.40	136.8 \pm 12.28 ^a	107.4 \pm 13.50 ^a	75 \pm 10.52 ^b
DS 75% : DSM 25%	65.8 \pm 13.37	143.4 \pm 19.33 ^a	102.2 \pm 6.80 ^a	73.4 \pm 6.11 ^b

Note:

DS : Soursop Leaves

DSM : Red Betel Leaves

T0: Measurement time before alloxan induction on day 0

T1: Measurement time after alloxan induction on day 3

T2: Measurement time of blood glucose levels after treatment on week 1

T3: Measurement time of blood glucose levels after treatment on week 2

a : Significantly different from the normal control

b : Significantly different from the negative control

c : Significantly different from the positive control

Table 6. Percentage change in blood glucose levels

Treatment group	Blood glucose levels (%) ± SD		
	ΔT0	ΔT1	ΔT2
Normal Control	3,06 ± 11,73 ^c	-7,16 ± 16,10 ^c	1,75 ± -20,14 ^c
Positive Control	-125,79 ± 32,59 ^a	35,93 ± 8,98 ^{ab}	51,90 ± 8,71 ^{ab}
Negative Control	-123,55 ± 55,09	-5,02 ± 7,42 ^c	-7,25 ± 6,28 ^c
Soursop Leaves	-83,40 ± 11,22 ^a	18,54 ± 6,36 ^{ab}	41,76 ± 7,64 ^{ab}
Red Betel Leaves	-75,39 ± 33,67 ^a	22,98 ± 8,40 ^{ab}	39,80 ± 9,13 ^{ab}
DS 25% : DSM 75%	-96,71 ± 18,46 ^a	21,50 ± 9,18 ^{ab}	43,70 ± 7,52 ^{ab}
DS 50% : DSM 50%	-95,32 ± 19,66 ^a	21,04 ± 11,13 ^{ab}	44,83 ± 9,60 ^{ab}
DS 75% : DSM 25%	-131,50 ± 86,36 ^a	28,18 ± 5,24 ^{ab}	47,76 ± 10,43 ^{ab}

Note:

DS : Soursop Leaves

DSM : Red Betel Leaves

ΔT0: Percentage of blood glucose levels T0 to T1 (influent of induction)

ΔT1: Percentage of blood glucose levels T1 to T2 (influent of treatment on 7 days)

ΔT2: Percentage of blood glucose levels T1 to T3 (influent of treatment on 14 days)

a : Significantly different from the normal control

b : Significantly different from the negative control

c : Significantly different from the positive control

This finding indicates that the combination of the two extracts has the potential to provide a better effect than single use, although this increase has not shown a clear difference between the various combination ratios. Although the combination groups showed descriptively higher glucose reduction than single extracts, the lack of statistical significance ($p > 0.05$) suggests an additive rather than a true synergistic effect under the current experimental conditions. The analysis results showed no significant difference between the combination groups ($p > 0.05$), so that each ratio provided a relatively equivalent effect. This condition indicates that the active compounds from both extracts likely work together in producing an antihyperglycemic effect, but have not produced a specific difference in effectiveness based on the ratio used.

The mechanism of action of secondary metabolites as antidiabetics involves an integrated multifaceted pathway, where flavonoid compounds work to protect pancreatic beta cells from damage due to oxidative stress, restore insulin receptor sensitivity, and inhibit intestinal mucosal GLUT2 to suppress glucose absorption (Hasan et al., 2024). This pathway is strengthened by alkaloids that are able to regenerate damaged pancreatic beta cells, increase glucose transport in the blood, inhibit gluconeogenesis, and stimulate the sympathetic nerves to trigger insulin secretion (Firdaus et al., 2024 ; Hasan et al., 2024). Tannin compounds focus their action on inhibiting digestive enzymes such as α -amylase and α -glucosidase, as well as stimulating glucose transport through phosphorylation of insulin receptors (Budianto et al., 2022). Saponin compounds then improve glucose metabolism by increasing insulin signaling, triggering the expression of the GLUT-4 glucose transporter, stimulating glycogen accumulation, and

actively improving insulin resistance conditions (Firdaus et al., 2024 ; Budianto et al., 2022). The combination of soursop leaf extract and red betel leaf showed potential in lowering blood glucose levels with a pattern of increasing effect over time, but variations in the extract combination ratios produced comparable effects, with no statistically significant differences detected.

CONCLUSION

The combination of ethanol extracts of soursop leaves and red betel leaves significantly affected alloxan-induced weight changes in mice. The treatment group showed a tendency toward weight gain or stabilization in comparison to the negative control group. The extract combination also exhibited antidiabetic activity, showed by reduced blood glucose levels in alloxan-induced mice. The DS 75% : DSM 25% ratio indicates the highest decrease in blood glucose levels descriptively. However, there were no significant differences between the various combination ratios. Therefore, the most effective combination ratio has not yet been statistically determined.

Further research should utilize a wider dose range and combination ratio, a larger number of test animals, and a longer observation period. Histopathological examination of the pancreas is also necessary to visualize pancreatic beta cells so that the mechanism of action of the extract combination as an antidiabetic agent can be more robustly elucidated.

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CONFLICT OF INTEREST

All author declared that there was no conflict of interest.

REFERENCES

- Adeyemi, D. O., Komolafé, O. A., Adewole, O. S., Obuotor, E. M., & Adenow, T. K. (2009). Anti Hyperglycemic Activities Of *Annona muricata* (Linn). *Afr. J. Trad. CAM*, 6(1), 62–69. <https://doi.org/10.4314/ajtcam.v6i1.57075>
- Adrianto, M., Widodo, G. P., & Herowati, R. (2023). Aktivitas Antidiabetes Ekstrak Etanol Daun Sirih Merah (*Piper crocatum*) Pada Tikus Putih Retinopati. In *Medical Sains: Jurnal Ilmiah Kefarmasian* (Vol. 8, Number 1). <https://doi.org/10.37874/ms.v8i1.719>
- Afsari, R., Kusmiyati, & Merta, I. W. (2016). Pengaruh Pemberian Ekstrak Daun Sirih Merah (*Piper Crocatum*) Terhadap Penurunan Kadar Gula Darah Mencit (*Mus musculus*). *Jurnal Biologi Tropis*, Volume 16. <https://doi.org/10.29303/jbt.v16i1.216>
- Alam, S., Sarker, M. M. R., Sultana, T. N., Chowdhury, M. N. R., Rashid, M. A., Chaity, N. I., Zhao, C., Xiao, J., Hafez, E. E., Khan, S. A., & Mohamed, I. N. (2022). Antidiabetic Phytochemicals From Medicinal Plants: Prospective Candidates for New Drug Discovery and Development. In *Frontiers in Endocrinology* (Vol. 13). Frontiers Media S.A. <https://doi.org/10.3389/fendo.2022.800714>
- Alfaqeeh, M., Khairinisa, M. A., & Permana, H. (2024). Pharmacokinetics and Pharmacodynamics of Glyburide: Insights for Optimizing Treatment in Type 2 Diabetes. *Indonesian Journal of Clinical Pharmacy*, 13(2). <https://doi.org/10.15416/ijcp.2024.13.2.48713>
- Anggraini, M. D., & Kusuma, E. W. (2019). Uji Efek Antidiabetes Kombinasi Ekstrak Herba Sambiloto (*Andrographis paniculata* (Burm. F.) Nees.) Dan Daun Sirsak (*Annona muricata* L.) Pada Tikus Jantan Yang Diinduksi Aloksan. *As-Syifaa Jurnal Farmasi*, 11(01), 24–29. <https://doi.org/10.56711/jifa.v11i1.498>
- Asfahani, F., Halimatussakdiah, & Amna, U. (2022). Analisis Fitokimia Ekstrak Daun Sirsak (*Annona muricata* Linn.) dari Kota Langsa. *Jurnal Kimia Sains Dan Terapan*, Volume 4, Nomor 2. <https://doi.org/10.33059/jq.v4i2.6530>
- Botlje, W., Brand, M. D., Ojano-Dirain, C., Lassiter, K., Toyomizu, M., & Wing, T. (2009). Mitochondrial Proton Leak Kinetics and Relationship With Feed Efficiency Within a Single Genetic Line of Male Broilers. *Poultry Science*, 88(8), 1683–1693. <https://doi.org/10.3382/ps.2009-00100>
- Botlje, W., Tang, Z. X., Iqbal, M., Cawthon, D., Okimoto, R., Wing, T., & Cooper, M. (2002). Association of Mitochondrial Function with Feed Efficiency within a Single Genetic Line of Male Broilers. *Poultry Science*, 81(4), 546–555. <https://doi.org/10.1093/ps/81.4.546>
- Budianto, R. E., Linawati, N. M., Arijana, I. G. K. N., Wahyunari, I. A. I., & Wiryawan, I. G. N. S. (2022). Potensi Senyawa Fitokimia pada Tumbuhan dalam Menurunkan Kadar Glukosa Darah pada Diabetes Melitus. *Jurnal Sains Dan Kesehatan*, 4(5), 548–556. <https://doi.org/10.25026/jsk.v4i5.1259>
- Dewi, K. F. R., Landra, I. komang G., Setiawan, K. H., & Mahiswari, K. W. I. (2025). Tinjauan Literatur: Potensi Antidiabetik Ekstrak Daun Sirsak Untuk Kontrol Glikemik Diabetes Melitus. *Jurnal Kesehatan Ilmiah Indonesia*, 10. <https://doi.org/10.51933/health.v10i2.2263>
- Fadel, M. N., & Besan, E. J. (2020). Uji Aktivitas Antidiabetes Ekstrak Daun Sirsak (*Annona muricata* L.) Pada Mencit Yang Diinduksi Aloksan. *Indonesia Jurnal Farmasi*, Volume 5 Nomor 2. <https://doi.org/10.26751/ijf.v5i2.1170>
- Firdaus, I. N., Hidayati, T. K., & Wardati, Y. (2024). Uji Aktivitas Antidiabetes Ekstrak Metanol Daun Kesum Pada Mencit Yang Diinduksi Glukosa. *Sabdariifarma*, 12(2). <https://doi.org/10.53675/jsfar.v12i2.1418>
- Guevara-Vásquez, A. M., Campos-Florián, J. V., & Dávila-Castillo, J. H. (2021). *Annona muricata* L. extract decreases intestinal glucose absorption and improves glucose tolerance in normal and diabetic rats. *Journal of HerbMed Pharmacology*, 10(3), 359–366. <https://doi.org/10.34172/jhp.2021.42>
- Hasan, H., Djuwamo, E. N., Hiola, F., Ramadhani, F. N., & Halada, I. O. (2024). Penapisan Fitokimia Dan Uji Efek Antidiabetes Ekstrak Metanol Daun Brotowali (*Tinospora crispa* L.) Pada Mencit (*Mus musculus*). *Journal of Pharmacology and Natural Products*, 1(1). <https://doi.org/10.70075/jpnp.v1i1.8>
- Hasnawati, Yamin, Sartinah, A., & Selvi. (2025). Uji Aktivitas Antioksidan dari Daun Sirsak (*Annona Muricata* L.) dengan Menggunakan Metode DPPH dan FRAP. *Lansau: Jurnal Ilmu Kefarmasian*, 3(2), 116–127. <https://doi.org/10.33772/lansau.v3i2.48>

- IDF. (2024). *IDF Diabetes Atlas*. <https://diabetesatlas.org>
- Indrawati, S., Yuliat, & Ihwan. (2015). Efek Antidiabetes Ekstrak Air Kulit Buah Pisang Ambon (*Musa paradisiaca* L.) Terhadap Mencit (*Mus musculus*) Model Hiperglikemia. *Galenika Journal of Pharmacy*, Vol. 2 (1), 133 - 140. <https://doi.org/10.22487/j24428744.2015.v1.i2.6245>
- Intami, E., Girsang, E., & Napiah, A. (2024). Studi Literature : Manfaat Ekstrak Daun Sirih Merah (*Piper crocatum*) Sebagai Obat Tradisional Diabetes Mellitus. <https://doi.org/10.30633/jsm.v7i1.2478>
- Jusup, S. (2016). Antidiabetic and Antioxidant Activities of 70% Ethanol-Diluted Extract of *Piper crocatum* Leaves in Streptozotocin Induced Diabetic Rats. *Jurnal Kedokteran Brawijaya*, 29(1). <https://doi.org/10.21776/ub.jkb.2016.029.01.1>
- Kaidun, R. C., Tombuku, J. L., Sumalong, F. P., & Sangande, F. (2022). Skrining Fitokimia Fraksi Methanol, Etil Asetat, N-Heksan Ekstrak Kulit Buah Sirsak *Annona muricata* L. *Jurnal Biofarmasetikal Tropis*. 2022, 5(1), 73–78. <https://doi.org/10.55724/jbiofarmasetikal.v5i1.372>
- Kemenkes. (2017). FARMAKOPE HERBAL INDONESIA EDISI II (II). Kementerian Kesehatan RI.
- Kemenkes. (2019). Pedoman Pelayanan Kefarmasian pada Diabetes Mellitus. Kementerian Kesehatan Republik Indonesia RI.
- Kuntari, D. N. A., Ifada, A. S., & Hadi, S. (2019). Pengaruh Pemberian Kombinasi Metformin dan Ekstrak Etanol Daun Sirsak (*Annona muricata* L.) Terhadap Kadar Glukosa Darah Mencit Jantan (*Mus musculus*). *Jurnal Ilmu Kesehatan Dan Farmasi*, 7.
- Lerrick, R. A., Damanik, E. M. B., Amat, A. L. S., & Nurina, Rr. L. (2025). The Effect of Red Betel Leaf Ethanol Extract (*Piper crocatum*) to Histopathological Image of Rat (*Rattus norvegicus*) Cardiac Muscle Induced by Alloxan. *Cendana Medical Journal (CMJ)*, 13(2), 386. <https://doi.org/10.35508/cmj.v13i2.22056>
- Maharani, A. D. K., Santoso, P., & Udayani, W. (2023). Efek Ekstrak Etanol Buah Dewandaru (*Eugenia uniflora* L) terhadap Penurunan Kadar Glukosa Darah dan Perbaikan Sel Beta Pankreas pada Mencit yang Diinduksi Aloksan. *Jurnal Integrasi Obat Tradisional*, 2. <http://dx.doi.org/10.36733/usadha.v2i3.7384>
- Meriem, M. S., & Fakta, A. H. (2021). Analisis efektifitas penggunaan daun sirsak (*Annona muricata* linn) terhadap penurunan kadar gula darah pada pasien diabetes melitus. *Holistik Jurnal Kesehatan*, 15(2). <https://doi.org/10.33024/hjk.v15i2.4321>
- Mongi, R. E., Simbala, H. E. I., & Queljoe, E. de. (2019). Uji Aktivitas Penurunan Kadar Gula Darah Ekstrak Etanol Daun Pinang Yaki (*Areca vestiaria*) Terhadap Tikus Putih Jantan Galur Wistar (*Rattus norvegicus*) Yang Diinduksi Aloksan. *PHARMACON*, 8. <https://doi.org/10.35799/pha.8.2019.29312>
- Mustika, W., Mega, & Safithri. (2022). Studi In Vitro Senyawa Bioaktif Ekstrak dan Fraksi Daun Sirih Merah (*Piper crocatum*) Sebagai Inhibitor α -Glukosidase. *Tunas Medika Jurnal Kedokteran & Kesehatan*, 8.
- Opara, P. O., Enemor, V. H. A., Eneh, F. U., & Emengaha, F. C. (2021). Blood Glucose – Lowering Potentials of *Annona muricata* Leaf Extract in Alloxan-Induced Diabetic Rats. *European Journal of Biology and Biotechnology*, 2(2), 106 - 113. <https://doi.org/10.24018/ejbio.2021.2.2.178>
- Panjaitan, R. G. P., & Novitasari. (2021). Anti-diabetic Activity of the Red Dragon Fruit Peel (*Hylocereus polyrhizus*) in Ethanol Extract Against Diabetic Rats. *Pharmacognosy Journal*, 13(5), 1079–1085. <https://doi.org/10.5530/pj.2021.13.140>
- Prayitno, S. A., & Utami, D. R. (2024). Identifikasi Senyawa Fitokimia Secara Kualitatif dari Ekstrak Etanol Daun Sirih Merah. *Journal of Herbal, Clinical and Pharmaceutical Science (HERCLIPS)*, 6(01), 1. <https://doi.org/10.30587/herclips.v6i01.8213>
- Purnamasari, F. (2021). Identifikasi Senyawa Aktif dari Ekstrak Daun Sirsak (*Annona muricata* L.) dengan Perbandingan Beberapa Pelarut pada Metode Maserasi. *Window of Heath*, 04, 231–237. <https://doi.org/10.33096/woh.v4i03.234>
- Rahmawati, R. D., & Candra, A. K. (2015). Pengaruh Pemberian Sari Buah Belimbing Wuluh (*Averrhoa bilimbi* L.) Terhadap Kadar Glukosa Darah Tikus Sprague Dawley. *Journal of Nutrition College, Volume 4, Nomor 2*, 486–491. <https://doi.org/10.14710/jnc.v4i4.10152>
- Sasmita, N. A., Kuswanti, N., & Khaleyra, F. (2024). Efek Ekstrak Daun Kedondong pada Kadar Gula Darah, Diameter Pulau Langerhans, dan Hepatosomatic Index Mencit Diabetes Mellitus Tipe 2. *LenteraBio*, 13(1), 150 - 159. <https://doi.org/10.26740/lenterabio.v13n1.p150-159>
- Utami, N. F., Mbeti, F. N., & Effendi, E. M. (2020). Efektivitas Kombinasi Ekstrak Etanol 96% Daun Insulin (*Tithonia diversifolia*) dan Daun

- Sirsak (*Annona muricata*) Sebagai Antihiperlikemik Pada Mencit Putih Jantan. *FITOFARMAKA: Jurnal Ilmiah Farmasi*, 10(2), 170–177. <https://doi.org/10.33751/jf.v10i2.2153>
- Widiana, H., & Marianti, A. (2022). Aktivitas Antihiperlikemia dan Antioksidan Ekstrak Daun Sirih Merah Pada Tikus Hiperlikemia Induksi Aloksan. *Life Science*, 11.
- Wijaya, H., Novitasari, & Jubaidah, S. (2018). Perbandingan Metode Ekstraksi Terhadap Rendemen Ekstrak Daun Rambai Laut (*Sonneratia caseolaris* L. Engl). *JURNAL ILMIAH MANUNTUNG*, 4(1), 79–83.
- Wijayanti, A. N., & Wulandari, A. D. (2023). Pengaruh Pemberian Ekstrak Daun Sirsak (*Annona Muricata* L) Sebagai Antihiperlikemia Terhadap Mencit Yang Diinduksi Glukosa. *An-Najat: Jurnal Ilmu Farmasi Dan Kesehatan*, 1, 302–315. <https://doi.org/10.59841/an-najat.v1i2.34>