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Antidiabetic Activity of *Pulutan* (*Urena lobata* L.) and *Temu Blenyeh* (*Curcuma purpurascens* Blume) Leaf Extracts in Alloxan-Induced Mice

Aktivitas Antidiabetik Ekstrak Daun Pulutan (*Urena lobata* L.) dan Daun Temu Blenyeh (*Curcuma purpurascens* Blume) pada Mencit yang Diinduksi Aloksan

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ABSTRACT

Diabetes mellitus is a disorder of blood glucose regulation caused by impaired insulin secretion. This study aimed to determine the antidiabetic activity of a combination of pulutan leaf extract (*Urena lobata* L.) and temu blenyeh leaf extract (*Curcuma purpurascens* Blume) in reducing blood glucose levels. Twenty-five male white mice were divided into five groups. Diabetes was induced with 120mg/kgBW alloxan, and blood glucose was measured three days later. The groups received metformin 65mg/kgBW, (positive control), distilled water (negative control), or a combination of pulutan (300mg/kgBW) and temu blenyeh (400mg/kgBW) leaf extracts at ratios of 25%:75%, 50%:50%, and 75%:25%. Blood glucose levels were measured on days 1, 3, 5, and 7. All three combinations reduced blood glucose relative to the negative control and did not differ from metformin on day 7; the greatest reduction, 69.85 ± 3.51%, was obtained with pulutan and temu blenyeh leaf extracts at 25%:75%.

ABSTRAK

Diabetes melitus adalah gangguan pengaturan glukosa darah akibat terganggunya sekresi insulin. Studi ini bertujuan mengetahui aktivitas antidiabetik kombinasi ekstrak daun pulutan (*Urena lobata* L.) dan daun temu blenyeh (*Curcuma purpurascens* Blume) dalam menurunkan kadar glukosa darah. Dua puluh lima mencit putih jantan dibagi menjadi lima kelompok. Diabetes diinduksi dengan aloksan 120mg/kgBB selama tiga hari. Kelompok perlakuan menerima metformin 65mg/kgBB, kontrol negatif mempergunakan akuades, kombinasi ekstrak daun pulutan 300 mg/KgBB dan daun temu blenyeh 400 mg/KgBB dengan rasio 25%:75%, 50%:50%, dan 75%:25%. Kadar glukosa darah diukur di hari 1, 3, 5, & 7. Ketiga kombinasi menurunkan kadar glukosa darah dibandingkan kontrol negatif dan tidak berbeda dari metformin pada hari ke-7; penurunan terbesar, yaitu 69,85 ± 3,51%, diperoleh pada perbandingan 25%:75%, satu-satunya kombinasi yang tidak berbeda dari metformin pada seluruh titik pengukuran.

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1. INTRODUCTION

Indonesia ranks third in Southeast Asia in the number of people with diabetes mellitus, reaching a prevalence of 11.3%. This makes Indonesia one of the main contributors to the rising number of diabetes cases in the region (Resti & Cahyati, 2022). Diabetes mellitus is a chronic metabolic disorder that cannot be permanently cured and requires long-term treatment, usually through the use of insulin or oral antidiabetic drugs, the cost of which is fairly high. Long-term use of synthetic drugs can trigger adverse side effects, so there is a need to develop alternative therapies based on natural materials that are safer and more economical (Wenas et al., 2020).

The leaves of *temu blenyeh* and *pulutan* are natural sources with antidiabetic potential. *Temu blenyeh* (*Curcuma purpurascens* Blume), a member of the Zingiberaceae family that to date remains little explored in research, is believed to have efficacy as an antiobesity agent (Pramiastuti et al., 2025), and obesity is one of the diseases that can lead to diabetes. As obesity and insulin resistance continu to develop, the body's need for insulin increases while the ability of the beta cells to produce insulin decreases, resulting in less insulin available and higher levels of glucose in the blood. Therefore, knowing how pancreatic beta cells fail to function properly is essential for early identification and treatment of diabetes (Iida & Watada, 2025). The presence of active constituents such as alkaloids, flavonoids, saponins, tannins, steroids, and triterpenoids supports the antidiabetic potential of this plant (Pramiastuti & Murti, 2022). *C. purpurascens* has long been used in ethnomedicine to treat cough, itching, boils, scabies, wounds, and fever (Pramiastuti et al., 2021). A methanol extract of its rhizome also inhibited α-glucosidase by 64.7 ± 2.0% at 1000 µg/mL (Tunnisa et al., 2022).

On the other hand, the leaf extract of *pulutan* (*Urena lobata* L.) is known to contain phytochemicals such as alkaloids, for example piperidine; phenols, for example mangiferin and gossypetin; and steroids, for example stigmasterol and β-sitosterol (Meiviani et al., 2022). These constituents contribute to suppressing glucose absorption in the digestive tract and to slowing the action of the α-amylase and α-glucosidase enzymes (Kifle et al., 2022). This plant is traditionally known as a remedy for hemorrhoids and also has antimicrobial, antidiabetic, antifertility, and hepatoprotective activity (Silalahi, 2020).

Although both plants have been studied individually, has not previously been reported research on the synergistic effect on the reduction of blood glucose levels using a combination of *C. purpurascens* and *U. lobata* leaf extracts. Combinations of natural materials often show a more significant therapeutic effect than single use. This study therefore aimed to determine the ability of the combined leaf extracts of *U. lobata* and *C. purpurascens* to become a safe and efficient herbal treatment option, and to contribute to the advancement of herbal medicines for the management of diabetes.

2. METHODS

2.1. Study Site and Period

The study evaluating the antidiabetic activity of the leaves of *U. lobata* and *C. purpurascens* was carried out in 2025 at the Pharmacology Laboratory and the Pharmaceutical Natural Products Laboratory, Study Program of Pharmacy, Faculty of Health Sciences, Universitas Bhamada Slawi. This research obtained ethical approval under registration number KEPK/UMP/341/VI/2025, issued on 23 June 2025 by the Health Research Ethics Committee of Universitas Muhammadiyah Purwokerto.

2.2. Instruments and Materials

The instruments used in this study were a digital balance (Joanlab, China), a mortar and pestle, a blender (Philips HR2012), glass jars, glass bowls, a *water bath* (Biobase, China), *moisture analyzer* (DSH-50-10, Hinotek, China), a furnace (Zengzhou CY, China), an oven (Getra, China), glassware, glass containers, glass cover plates, TLC plates GF_{254} (Merck, Germany), 1.0 mL injection syringes (Onemed, Indonesia), an oral gavage needle, and a glucometer (Autocheck, Taiwan).

The materials used in this study were *C. purpurascens* and *U. lobata* leaves (collection sites and dates are given in Section 2.3), both of which were authenticated at the Natural Products Laboratory, Faculty of Health Sciences, Universitas Bhamada Slawi under document number 003/F-BMD/Bahan Alam/XII/2024. Male white mice of the Swiss Webster strain were obtained from the Pharmacology Laboratory of the same faculty, weighing 20–35 g and aged 2 to 3 months, and were in healthy physical condition. The chemicals and consumables used were alloxan, distilled water, 96% ethanol, metformin, methanol, flannel cloth, filter paper, glucose test strips (Autocheck), magnesium, 1 N HCl, H_2SO_4, $FeCl_3$, chloroform, acetic anhydride, Mayer's reagent, Dragendorff's reagent, and ethyl acetate.

2.3. Preparation of the Dried Plant Material

Leaf samples of *U. lobata* were collected in August 2024 from Kalisapu Village, Slawi Subdistrict, Tegal Regency, Central Java, at coordinates 6°58'59.9"S 109°07'51.4"E. Meanwhile, leaves of *C. purpurascens* were collected in August 2024 from the Regional Technical Implementation Unit of the Jamu Health Tourism Park (Wisata Kesehatan Jamu), Kalibakung Village, Tegal Regency, Central Java, at an altitude of 650 m above sea level and coordinates 7°06'14.3"S 109°07'53.7"E. The leaves selected for the study were dark green, free from fungal pests or disease, and exposed to sunlight.

The powder preparation procedure began with wet sorting to remove dirt. A total of 4 kg of each leaf was washed with running water, drained, and dried in an oven at 60 °C. Once dry, the dried plant material was sorted again and then milled to obtain a fine powder (T. O. T. Dewi et al., 2021).

2.4. Extraction

The extract was obtained by soaking the powdered plant material in 96% ethanol using the maceration technique. This was done by mixing 500 g of each powdered plant material with 5 L of 96% ethanol at a ratio of 1:10 (Damanik et al., 2024). Extraction was carried out for five days, with stirring once a day at the same time so that the secondary metabolites in the sample dissolved optimally. The macerate was filtered through flannel cloth and filter paper to separate residual dirt from the solution, and the filtrate was then evaporated on a water bath to produce a viscous extract (Riskiyani et al., 2020).

2.5. Extract Quality Parameters

The tests performed determined how much water was lost on drying, how much ash was present, and how moist the extract was. The first test was the determination of loss on drying. The initial step of this test was that an empty weighing bottle with its lid was weighed, heated in an oven at 105 °C for 30 minutes, and then cooled. Then, 1 g of sample was added, spread evenly, heated again with the lid open, and cooled again. This process was repeated until a constant weight was obtained (Departemen Kesehatan Republik Indonesia, 2000), Ash content was determined using a crucible heated in a furnace at 600 °C for 30 minutes, after which the extract was added and heated again to obtain the ash weight (Marpaung & Septiyani, 2020). Moisture content was determined with a moisture analyzer at 105 °C, then 0.5 g of sample was weighed and placed and spread evenly on the metal plate inside the instrument (Rukmawati et al., 2017).

2.6. Phytochemical Screening

Phytochemical testing, covering flavonoids, tannins, saponins, and alkaloids, was performed by placing 0.1 g of each extract into a test tube and then adding the reagent appropriate to the compound of interest. For flavonoids, the sample was treated with 1–2 mL of methanol, 0.05 mg of magnesium powder, and 4 drops of HCl. If the solution changed to red or orange, the sample was considered positive for flavonoids (Pramiastuti & Murti, 2022). For tannins, the sample was treated with 5 drops of $FeCl_3$ (Ameliani et al., 2024). If the solution changed to dark green or dark blue, the sample was considered positive for tannins (Pramiastuti & Murti, 2022). For saponins, the sample was treated with distilled water and then shaken vigorously for 1 minute; if foam appeared, 1 N HCl was then added (Pramiastuti & Murti, 2022). The sample was positive for saponins when the foam was stable at a height of 1–3 cm (Ameliani et al., 2024). For alkaloids, the sample was treated with 0.5 mL of HCl and then divided into two separate tubes. Two drops of Mayer's reagent were added to the first tube and two drops of Dragendorff's reagent to the second tube. If a brick-red, red, or orange precipitate formed with Dragendorff's reagent, or a white to yellowish precipitate with Mayer's reagent, the sample was positive for alkaloids (Pramiastuti & Murti, 2022).

2.7. Phytochemical Screening by Thin-Layer Chromatography

Phytochemical testing by thin-layer chromatography used silica gel GF_{254} plates as the stationary phase, and 10 mL of mobile phase solution was prepared. The mobile phase for flavonoids was ethyl acetate and methanol at a ratio of 3:2, and for alkaloids chloroform and methanol at a ratio of 1:4 (Pramiastuti & Murti, 2022), for tannins methanol and water at a ratio of 6:4(Yuda et al., 2017), and for saponins chloroform and acetone at a ratio of 4:1(Pramiastuti & Murti, 2022). To compare the Rf values of the samples, quercetin (flavonoid), catechin (tannin), saponin (saponin), and piperine (alkaloid) were used as reference standards (Mulyani et al., 2024). The spots were observed under UV 254, UV 366, and as visible spots. The visualizing reagents used were $AlCl_3$ for flavonoid spots (Ferdinan & Natasa, 2024), Dragendorff's reagent for alkaloid spots (Mulyani et al., 2024), $FeCl_3$ for tannin spots (Yuda et al., 2017), and Liebermann-Burchard reagent for saponin spots (Mulyani et al., 2024).

2.8. Antidiabetic Activity Test

The antidiabetic activity test used 25 male white mice (*Mus musculus*) of the Swiss Webster strain, aged 2 to 3 months and weighing 20–35 g. The mice were divided into five groups, with five mice in each cage. Before the test began, the mice first underwent an acclimatization period of one week. The animals were then fasted for at least 8 hours, and their body weight was recorded. Diabetes was induced by an intraperitoneal injection of alloxan solution. Thereafter, the animals were left for three days with free access to food and a 10% (w/v) glucose solution to perform an oral glucose tolerance test in the test animals and to prevent early-phase hypoglycemia resulting from the sudden release of insulin from lysed pancreatic beta cells (Ighodaro et al., 2017; Noena et al., 2020).n

On day 0 (pre-induction), the average blood glucose level of the mice ranged from 63 to 154 mg/dL, whereas the threshold for classifying a mouse as diabetic is a blood glucose level exceeding ≥200 mg/dL. Three days after induction, the blood glucose levels of the mice ranged from 226 to 358 mg/dL were measured, and the mice were then given the suspension at the predetermined doses, orally and once daily. The negative control received distilled water, the positive control received metformin solution, and the remaining groups received doses of *U. lobata* leaf extract 300 mg/kg BW and *C. purpurascens* leaf extract 400 mg/kg BW at the ratio of the combined doses of *U. lobata* leaf extract and *C. purpurascens* leaf extract 25% (75mg/kgBW):75% (300mg/kgBW):, 50% (150mg/kgBW):50% (200mg/kgBW), and 75%(225mg/kgBW):25% (100 mg/kgBW). The treatments were given in the fasted state, that is, after the mice had not been fed for at least 8 hours (Wati & Fadhilah, 2023). Blood glucose was then measured on days 1, 3, 5, and 7, and the mean reduction in blood glucose level and its standard deviation were calculated (Yani, 2024).

Due to the scarcity of scientific data regarding the antidiabetic effects of *Pulutan* and *C. purpurascens* leaves, the extract dosages were established using the optimal therapeutic doses of phylogenetically related species within the same families. Specifically, the dosage for *C. purpurascens* was referenced from *C.*

longa (Zingiberaceae) (Damanik et al., 2024), whereas the dosage for *Pulutan* leaves was adapted from hibiscus species (Malvaceae) (Meilina et al., 2022).

2.9. Data Analysis

Data were analyzed using IBM SPSS Statistics version 32. Normality and homogeneity of variance tests were performed before conducting a one-way analysis of variance (ANOVA) to compare mean blood glucose levels among groups at significance level of 0.05. Significant ANOVA results were followed by Tukey's HSD or Games-Howell post hoc tests, as appropriate. Effect size was estimated using eta squared (η^2).

3. RESULTS AND DISCUSSION

3.1. Extract Yield of *U. lobata* and *C. purpurascens* Leaves

The extraction results reveal a significant difference in extract yield between *U. lobata* and *C. purpurascens* (Table 1). *U. lobata* produced a yield of 14.8%, whereas *C. purpurascens* yielded a lower 5.6%. Yield percentage serves as crucial parameter for assessing extraction efficiency (Hasnaeni & Wisdawati, 2019). This nearly threefold difference is primarily attributed to variations in anatomical characteristics, cell wall thickness, and the concentration of soluble secondary metabolite. *U. lobata* likely possesses a higher proportion of polar macromolecules that facilitate solvent penetration, while *C. purpurascens* may have a more rigid cellular architecture. Furthermore, these results align with existing literature, such as the reported range of 3.24% (per 100g sample) to 16.20% (per 500g sample) for *U. lobata* (Putri et al., 2024) and the 4.18% yield observed in *C.longa* a species belonging to the same family as *C. purpurascens* (Nurcahyani et al., 2025).

Overall, total extract content is influenced by the plant species, chemical composition, leaf maturity, and the polarity of the solvent used. A low yield value does not necessarily indicate poor raw material quality, as there is currently no reference standard etablishing minimum compound content limits for *C. purpurascens* leaves. Additionally, external factors-such as presence of impurities in the sample-can affect water content and the efficiency of the dissolution process (Riamadhanty et al., 2026; Zahara et al., 2024). The extract yield obtained by maceration is shown in **Table 1**.

Table 1. Extract yield of *U. lobata* and *C. purpurascens* leaves

Sample	Powder weight (g)	96% ethanol solvent (L)	Extract weight (g)	Yield (%)
U. lobata leaves	500	5	74	14.8
C. purpurascens leaves	500	5	28	5.6

The yield can be influenced by the duration of extraction. Maceration that is too short risks not dissolving the active constituents optimally, whereas a duration that is too long does not always give a significant increase because the solvent can reach saturation (Asworo & Widwiastuti, 2023). The amount of yield obtained is influenced by the extraction process. In addition, the stirring and filtration techniques can also affect the yield. Uneven stirring can hinder the release of compounds from the dried plant material (L. K. Dewi et al., 2024). A large yield reflects a high content of usable active substances present in the sample (Nahor et al., 2020).

3.2. Extract Quality Parameters

The main purpose of parameter testing, or standardization, is to ensure the quality, safety, and consistency of the extract (Mewar & As'ad, 2023). The three parameters measured were moisture content, ash content, and loss on drying (Mokodompit et al., 2023). The extract parameter tests were carried out without replication. The extract parameter values are shown in **Table 2**.

Table 2. Results of the extract quality parameter tests

Extract sample	Result (%)		
	Ash content	Loss on drying	Moisture content
U. lobata leaves	0.43	14.20	19.54
C. purpurascens leaves	0.22	7.00	5.9

n = 1; the parameter tests were performed without replication, so no standard deviation is reported.

Both extracts met the requirements for all three quality parameters. Ash content analysis reflects the content of heat-resistant inorganic material, and the result was that the ash content of the *U. lobata* leaf extract was ≤12.2% (Kementerian Kesehatan Republik Indonesia, 2022) and that of the *C. purpurascens* leaf extract was ≤16.6% (Dayanti et al., 2023). A low ash level indicates low contamination by heavy metals or other undesirable residues (Smith et al., 2023). A high ash content can reduce the quality of the extract and may cause toxic effects in the body owing to excessive inorganic substances (Kuswanti et al., 2023).

As presented in **Table 2**, the loss on drying value for the *U. lobata* leaf extract was 14.20%, which exceeds the maximum limit of ≤10% established by (Kemenkes RI, 2017). Conversely, the *C. purpurascens* leaf extract met this requirement with a value of 7.00%. These values reflect the total amount of water and volatile compounds that evaporate during the heating process (Maryam et al., 2020). A high loss on drying value in *U. lobata* may stem from insufficient drying time during production (Anggriani et al., 2024; Kemenkes RI, 2013) or improper storage condition that render the extract hygroscopic (Ratnani et al., 2015). Furthemore, elevates drying values often coreellate with a greater loss of active comppunds (Sutomo et al., 2021).

The moisture content results show that both extracts met the requirements according to the respective literature, namely ≤20.6% for the *U. lobata* leaf extract (Kementerian Kesehatan Republik Indonesia, 2022) and ≤10% for the *C. purpurascens* leaf extract (Dayanti et al., 2023). Although the moisture content of the *U. lobata* leaf extract was still below the optimal limit of 20.6% set by Kementerian Kesehatan Republik Indonesia, (2022), the value approached that limit. The high value was caused by the

sample not being completely dry during drying, so that the water in the sample had not been fully removed. The high moisture content arose from an imperfect drying process, so that water remained. A high moisture content must be avoided because it can trigger the growth of fungi and harmful microorganisms (Mewar & As'ad, 2023). The better the drying process, the less water remains in the sample, which helps to reduce the possibility of fungal growth (Sutomo et al., 2021). Microbial or fungal contamination can shorten the shelf life of medicinal plants and thus accelerate spoilage (Ulfah et al., 2020).

3.3. Phytochemical Screening

This test was designed to detect the presence of secondary metabolites in the leaf extracts of *U. lobata* and *C. purpurascens*. The secondary metabolites observed were flavonoids, tannins, saponins, and alkaloids. The screening was qualitative, that is, by observing the changes in the sample when mixed with the reagent appropriate to the secondary metabolite to be analyzed. Each test used 0.1 g of extract, to which the reagent was added (Pramiastuti & Murti, 2022). The phytochemical screening results are shown in **Table 3**.

Table 3. Results of phytochemical screening

Extract sample	Secondary metabolite	Result
U. lobata leaves	Flavonoids	+
	Tannins	+
	Saponins	+
	Alkaloids	+
C. purpurascens leaves	Flavonoids	+
	Tannins	+
	Saponins	+
	Alkaloids	+

(+), secondary metabolite detected; (−), secondary metabolite not detected.

The phytochemical test results show that the extracts of *C. purpurascens* and *U. lobata* contain active constituents, including tannins, flavonoids, saponins, and alkaloids, which are able to help control blood glucose levels. Flavonoids are known to have an antidiabetic effect related to their ability to form hydrogen bonds, to interact with residues on enzymes, and to their -OH groups, which can slow the degradation of starch in the digestive tract (Damanik et al., 2024). Alkaloids act by reducing glucose absorption in the digestive tract and thus contribute to the control of glucose levels (Hasan et al., 2022). Tannins act by slowing the α-glucosidase and α-amylase enzymes, enhance cell recovery propagation, and also interfere with glucose absorption, which indirectly reduces blood glucose levels (Kifle et al., 2022). As phytochemicals, saponins prevent elevated blood glucose levels by slowing gastric emptying and reducing glucose absorption in the small intestine (Sutomo & Purwanto, 2023).

3.4. Phytochemical Screening by Thin-Layer Chromatography

Thin-layer chromatography (TLC) is a method for separating liquids using two main phases, a stationary phase and a mobile phase. The mobile phase consists of a solvent mixture with a high dissolving capacity, so that it can facilitate the separation of compounds through elution. The success of this separation is strongly influenced by the polarity of the solvent and by the chemical characteristics of the substances in the sample. In addition to serving as a separation method, TLC is also used to strengthen the findings of phytochemical screening through observation (Pratiwi et al., 2023). The first step in this procedure is saturation of the chamber with solvent vapor, which aims to equalize the vapor pressure inside the vessel so that the separation of compounds on the chromatographic plate can proceed optimally. To make the results easier to observe, and to reveal flavonoids, the plate was sprayed with various visualizing reagents, such as $AlCl_3$ (Ferdinan & Natasa, 2024), Dragendorff's solution to detect alkaloid spots on the plate (Mulyani et al., 2024), $FeCl_3$ to mark tannin spots on the plate (Yuda et al., 2017), and Liebermann-Burchard reagent to mark saponin spots on the plate (Mulyani et al., 2024). The plate was then air-dried. The TLC plate was subsequently analyzed by visual observation under ultraviolet light at wavelengths of 366 nm and 254 nm. The Rf values of the samples and the reference standards were then calculated. The thin-layer chromatography results for *U. lobata* leaves are shown in **Figure 1** and **Table 4**, and those for *C. purpurascens* leaves in **Figure 2** and **Table 5**.

Table 4. Thin-layer chromatography results for *U. lobata* leaves

Secondary metabolite	Reference standard	Rf sample	Rf standard
Flavonoids	Quercetin	0.85	0.85
Tannins	Catechin	0.82	0.87
Saponins	Saponin	0.92	0.93
Alkaloids	Piperine	0.81	0.81

Mobile phase — flavonoids, ethyl acetate:methanol (3:2); tannins, methanol:water (6:4); saponins, chloroform:acetone (4:1); alkaloids, chloroform:methanol (1:4). Stationary phase, silica gel GF_{254}. Rf = distance travelled by the spot / distance travelled by the solvent front. One plate per compound; no replication. Plates are shown in Figure 1. GF_{254}, silica gel containing a fluorescent indicator active at 254 nm; Rf, retention factor.

Table 5. Thin-layer chromatography results for *C. purpurascens* leaves

Secondary metabolite	Reference standard	Rf sample	Rf standard
Flavonoids	Quercetin	0.81	0.82
Tannins	Catechin	0.82	0.87
Saponins	Saponin	0.93	0.92
Alkaloids	Piperine	0.86	0.83

Mobile phase — flavonoids, ethyl acetate:methanol (3:2); tannins, methanol:water (6:4); saponins, chloroform:acetone (4:1); alkaloids, chloroform:methanol (1:4). Stationary phase, silica gel GF_{254}. Rf = distance travelled by the spot / distance travelled by the solvent front. One plate per compound; no replication. Plates are shown in Figure 2. GF_{254}, silica gel containing a fluorescent indicator active at 254 nm; Rf, retention factor.

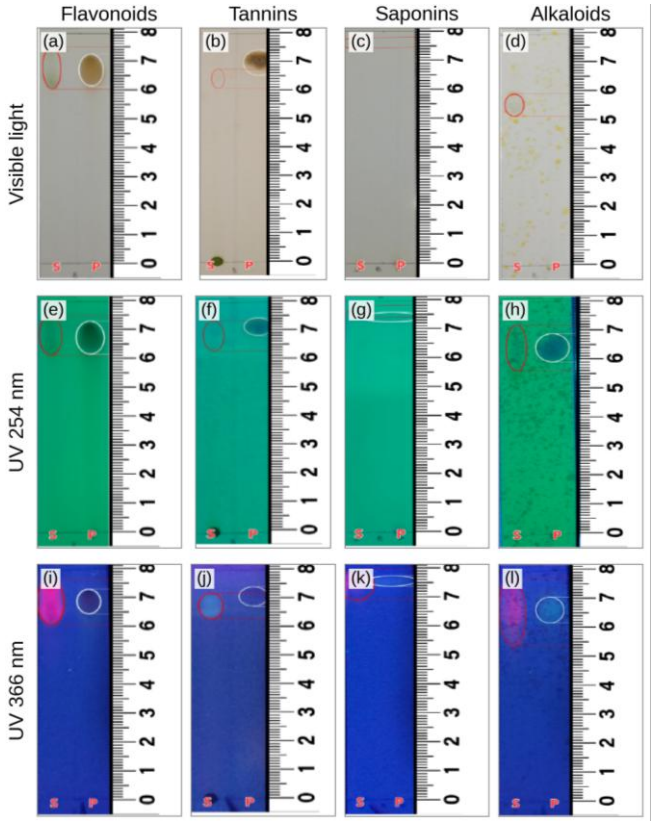

Figure 1. Thin-layer chromatograms of the *U. lobata* leaf extract and the corresponding reference standards. Columns, secondary metabolite tested; rows, viewing condition. Panels (a)–(d), visible light; (e)–(h), UV 254 nm; (i)–(l), UV 366 nm. In each panel the left lane (S) is the sample and the right lane (P) is the reference standard; the ruler beside each plate is in centimetres. Visualising reagents: $AlCl_3$ (flavonoids), $FeCl_3$ (tannins), Liebermann–Burchard (saponins), and Dragendorff's reagent (alkaloids). Rf values are given in Table 4.

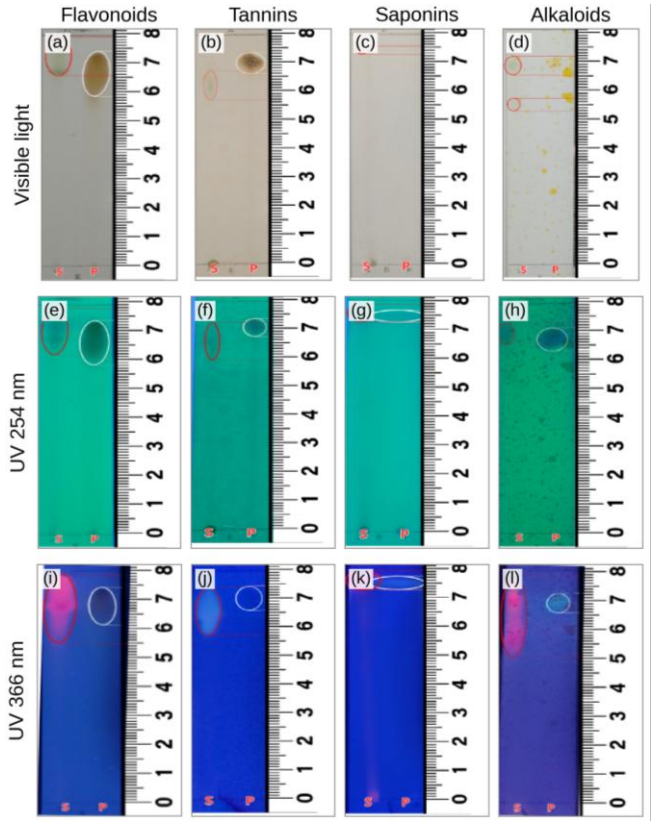

Figure 2. Thin-layer chromatograms of the *C. purpurascens* leaf extract and the corresponding reference standards. Columns, secondary metabolite tested; rows, viewing condition. Panels (a)–(d), visible light; (e)–(h), UV 254 nm; (i)–(l), UV 366 nm. In each panel the left lane (S) is the sample and the right lane (P) is the reference standard; the ruler beside each plate is in centimetres. Visualising reagents: $AlCl_3$ (flavonoids), $FeCl_3$ (tannins), Liebermann–Burchard (saponins), and Dragendorff's reagent (alkaloids). Rf values are given in Table 5.

The results of the analysis carried out by thin-layer chromatography on the extracts of *U. lobata* and *C. purpurascens* leaves and on the reference standards met the criteria described by Pratiwi et al., (2023), as seen from the sharp, well-defined spots without *tailing* and consistent with the phytochemical screening results. A separated compound is regarded as a single component when it produces one sharp, well-defined spot without tailing (Pratiwi et al., 2023). Tailing generally occurs because of suboptimal separation of the compounds or a high concentration of the compound in the sample (Naselia et al., 2020). The difference in Rf value between the two sample extracts and the reference compounds was less than 0.2, showing a similarity in the types of compounds present, such as alkaloids, flavonoids, saponins, and tannins. Conversely, if the difference in Rf value reaches or exceeds 0.2, the spot in the sample is unlikely to correspond to the reference compound (Suriyanti et al., 2025).

3.5. Antidiabetic Activity Test

In the antidiabetic activity test, the mice were induced with 120mg/kgBB alloxan via intraperitoneal injection of 0.1 mL. They were then left for three days but given food and drink. Blood glucose levels were observed three days after alloxan induction. This is because about half an hour after receiving alloxan there is a rapid fall in blood glucose owing to a temporary rise in insulin. Then, about an hour later, blood glucose begins to rise again because the body produces less insulin and an insulin deficiency develops in the blood. After 4 to 8 hours, blood glucose falls again for several hours. Usually, the insulin-producing cells stop working completely within 12 to 48 hours, marking the transition to a sustained state of high blood glucose (Fajarwati et al., 2023; Rita et al., 2024). The suspension was given orally once a day for one week, in the morning before breakfast, after at least eight hours without food. Blood glucose levels were then measured, the percentage reduction was calculated, and the mean difference for each group was calculated (Yani, 2024). The mean reduction in blood glucose level and the standard deviation are shown in **Figure 3**.

A one-way ANOVA was performed to assess the effect of combined *U. lobata* and *C. purpurascens* leaf extract on blood glucose levels in diabetic mice during a 7-day observation period. The homogeneity of variance test demonstrated that the data on days 1,5, and 7 satisfied the assumption of homoscedasticity ($p > 0.05$), supporting the use of parametric analysis. The analysis revealed significant differences in the reduction in blood glucose among the experimental groups on day 1 ($F(4, 20) = 157.001$), day 3 ($F(4, 20) = 111.091$), day 5 ($F(4, 20) = 281.753$), and day 7 ($F(4, 20) = 505.386$), with $p < 0.001$ throughout. Because Levene's test indicated heterogeneous variances on day 3 ($p = 0.001$), Welch's ANOVA was also run for that day and confirmed the result (Welch's $F = 40.585$, $p < 0.001$).

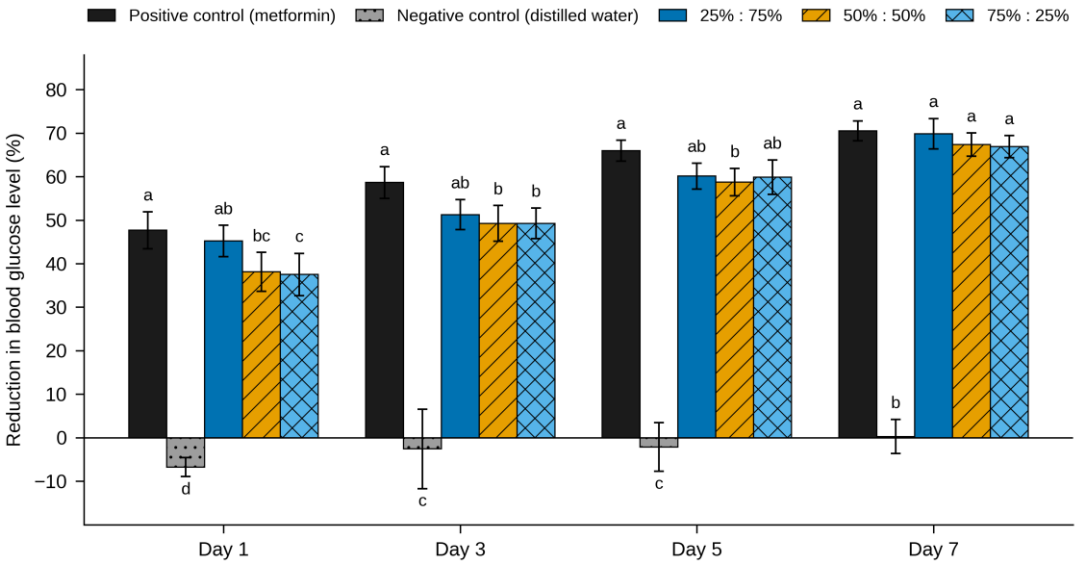

Figure 3. Mean reduction in blood glucose level (%) in alloxan-induced mice over seven days of treatment. Ratios refer to *Urena lobata* : *Curcuma purpurascens* leaf extract. The positive control received metformin and the negative control received distilled water. Bars are means and error bars are standard deviations; n = 5 mice per group. Reduction is expressed relative to the blood glucose level on day 0, that is, three days after alloxan induction. Negative values indicate an increase in blood glucose. Within each day, bars that share a letter do not differ significantly (one-way ANOVA followed by Tukey HSD, or Games-Howell on day 3, $p < 0.05$). SD, standard deviation.

Post hoc analysis using Tukey's HSD, and Games-Howell on day 3, demonstrated that the combined *U. lobata* and *C. purpurascens* leaf extract 50%:50% and 75%:25% groups produced a significantly smaller reduction in blood glucose than the positive control group on days 1 and 3 (day 1, $p = 0.009$ and $p = 0.005$; day 3, $p = 0.031$ and $p = 0.020$). In contrast, the 25%:75% group did not differ from the positive control on either day ($p = 0.864$ and $p = 0.064$). On day 5 the treatment groups approached the positive control, with only the 50%:50% group still differing from it ($p = 0.048$). By day 7, no significant differences were detected among the treatment groups or between the treatment groups and the

positive group ($p > 0.05$), indicating a comparable antihyperglycemic response among all extract formulations.

The effect size analysis showed a large treatment effect, with eta squared ($\eta 2$) values of 0.969, 0.957, 0.983, and 0.990 on days 1, 3, 5, and 7, respectively. These values indicate that the treatments accounted for more than 95% of the observed variation in blood glucose levels. Collectively, the findings suggest that the combination of *U. lobata* leaf extract and *C. purpurascens* leaf extract significantly modulated blood glucose levels in mice, with all formulations exhibiting comparable efficacy by the end of the observation period.

Based on the mean reduction in blood glucose level, the group receiving the combination of 25% *U. lobata* leaf extract and 75% *C. purpurascens* leaf extract, with a reduction in blood glucose on day 7 of 69.85% and a standard deviation of 3.51, was the group closest to the positive control and the only combination that did not differ from it on any measurement day. This combination also contained the larger proportion of *C. purpurascens* leaf extract compared with *U. lobata* leaf extract, although the present design, which lacks single-extract arms, does not allow the contribution of each species to be separated.

A previous study showed that *C. purpurascens* rhizome extract inhibited α-glucosidase by 64.7 ± 2.0% in vitro, which suggests that the leaves may also possess antidiabetic constituents (Tunnisa et al., 2022). A study by Damanik et al. (2024) reported that turmeric (*Curcuma domestica* Val.) leaf extract, which also comes from the Zingiberaceae family like *C. purpurascens* leaves, contains active constituents including tannins, saponins, flavonoids, and alkaloids, which were shown to reduce blood glucose at a dose of 400 mg/kg body weight. In this study, *U. lobata* leaves, which come from the Malvaceae family, were used as part of the extract combination (Winda, 2022), and hibiscus leaves (*Hibiscus rosa-sinensis* L.), which are also from the Malvaceae family, are likewise known to have an antidiabetic effect. This taxonomic relationship suggests a possible similarity in phytochemical content, such as polyphenols, saponins, and flavonoids, compounds that contribute to the reduction of blood glucose levels. In addition, a previous study showed that hibiscus leaf extract at 300 mg/kg body weight in mice was shown to reduce blood glucose levels (Meilina et al., 2022).

4. CONCLUSION

The combination of the leaf extracts of *U. lobata* and *C. purpurascens* reduced blood glucose levels in alloxan-induced mice at all three ratios tested. On day 7 the three ratios did not differ from one another or from the metformin control; the greatest reduction was obtained at a ratio of 25% *U. lobata* leaves to 75% *C. purpurascens* leaves, which was also the only combination that did not differ from the metformin control on any measurement day. That combination reduced blood glucose by 69.85 ± 3.51% on day 7. Based on the results of this study, further research needs to be carried out to examine toxicity and possible side effects, in order to determine the combination with the most favorable safety profile and the greatest potential for use in the manufacture of medicines.

AUTHOR CONTRIBUTIONS

Conceptualization, O.P.; methodology, O.P. and N.F.S.; validation, O.P and EI; formal analysis, O.P. and N.F.S; investigation, N.F.S.; resources, N.F.S; data curation, O.P. and N.F.S; writing—original draft preparation, N.F.S; writing—review and editing, O.P. and E.I; visualization, N.F.S; supervision, E.I.; project administration, N.F.S. and E.I; funding acquisition, O.P., N.F.S and E.I.

INSTITUTIONAL REVIEW BOARD STATEMENT

The animal study protocol was approved by the Health Research Ethics Committee of Universitas Muhammadiyah Purwokerto (registration number KEPK/UMP/341/VI/2025, approved on 23 June 2025).

INFORMED CONSENT STATEMENT

Not applicable.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ROLE OF FUNDERS

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DECLARATION OF GENERATIVE ARTIFICIAL INTELLIGENCE (AI) USE

During the preparation of this manuscript, the authors used ChatGPT (Open AI) to assist with language editing, text translation, and improvement of scientific writing. After using this tool, the authors thoroughly reviewed, edited, and verified the manuscript to ensure that it accurately represented their data, ideas, and scientific interpretations. The authors take full responsibility for the integrity, accuracy, and originality of the published work.

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