



ANTIDIABETIC POTENTIAL OF COMBINED EXTRACTS OF AGERATUM CONYZOIDES AND CYPERUS ROTUNDUS: IN VIVO STUDY

Wahyu Rahmatulloh^{1*}, Laeli Fitriyati¹, Tri Cahyani Widiastuti², Dwiana Rahmi Nur Rofiqoh¹, Aryadi Suryanata Kusuma¹, Muh Fathurahman³

¹Pharmacy Study Program, Faculty of Health Science, Universitas Muhammadiyah Gombong, Jl. Yos Sudarso No. 461, Gombong, Kebumen, Jawa Tengah 54412, Indonesia

²Pharmacist Professional Education Program, Faculty of Health Science, Universitas Muhammadiyah Gombong, Jl. Yos Sudarso No. 461, Gombong, Kebumen, Jawa Tengah 54412, Indonesia

³Pharmacy Study Program, Faculty of Health Science, Universitas Karta Mulia Purwakarta, Jl. Ibrahim Singadilaga No.80, Nagri Kaler, Purwakarta, Jawa Barat 41119, Indonesia

*wahyurahmatulloh@unimugo.ac.id

ABSTRACT

Long-term use of synthetic antidiabetic drugs often causes side effects, prompting the search for safer herbal alternatives. *Ageratum conyzoides* and *Cyperus rotundus* are known for their antihyperglycemic properties, but studies on their combination remain limited. To evaluate the antihyperglycemic effect of combined ethanol extracts of *A. conyzoides* leaves and *C. rotundus* rhizomes in streptozotocin (STZ)-induced diabetic rats. Male white rats were divided into nine groups: control, single extract, and combination treatments (100:200 and 150:500 mg/kg BW). Treatments were given orally for 15 days, and blood glucose levels were measured periodically. Data were analyzed using one-way ANOVA and Tukey's post hoc test. The combination of *A. conyzoides* and *C. rotundus* (150:500 mg/kg BW) significantly reduced blood glucose levels, comparable to glimepiride ($p < 0.05$ vs. negative control; $p > 0.05$ vs. positive control). Single extracts showed moderate effects in a dose-dependent manner. The combination of *A. conyzoides* and *C. rotundus* extracts exhibited synergistic antihyperglycemic activity, suggesting potential as a safe phytotherapeutic alternative for diabetes management.

Keywords: *ageratum conyzoides*; antihyperglycemic; *cyperus rotundus*; diabetes mellitus; synergy

How to Cite (in APA Style)

Rahmatulloh, W., Fitriyati, L., Widiastuti, T. C., Rofiqoh, D. R. N., Kusuma, A. S., & Fathurahman, M. (2026). Antidiabetic Potential of Combined Extracts of *Ageratum Conyzoides* and *Cyperus Rotundus*: in Vivo Study. *Indonesian Journal of Global Health Research*, 8(2), 873–878. <https://doi.org/10.37287/ijghr.v8i2.808>.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by impaired insulin production or function in the pancreas, leading to an inability to meet the body's insulin needs and resulting in hyperglycemia (W. Rahmatulloh, et al, 2023). The global prevalence of DM has shown a significant upward trend. According to data from the International Diabetes Federation (IDF), the number of people living with DM increased from 151 million to 537 million, equivalent to a rise in prevalence from 4.6% to 10.5% among individuals aged 20–79 years. The IDF projects that this figure will reach 783 million (12.2%) by 2045 (IDF, 2021).

The prevalence of DM has also risen in Indonesia, reaching 8.5% of the total population, placing the country fifth among the ten nations with the highest number of diabetes cases worldwide. Estimates suggest that by 2030, the number of people with DM in Indonesia will reach 21.3 million (Badan Litbang Kesehatan, 2018). The 2018 National Health Survey (Riskesdas) reported an increase in diabetes cases across nearly all provinces, including Central Java, where prevalence exceeds the national average. In Kebumen Regency, 12,000 patients were diagnosed with diabetes in 2022 (Dinkes Kebumen, 2022). Currently, DM management largely relies on pharmacological therapy using synthetic drugs such as glimepiride, metformin, and insulin.

However, long-term use of these medications often causes side effects and poses an economic burden on patients. Therefore, exploring safe, affordable, and nature-based alternative therapies has become increasingly important. Indonesia's rich biodiversity provides excellent potential for the development of herbal medicines (Harefa, et al., 2022). Among the plants that have demonstrated significant biological activity are *Ageratum conyzoides* (Bandotan) and *Cyperus rotundus* (Purple Nutsedge or Rimpang Teki). Several local plants have been reported to possess promising antidiabetic properties. Ethanol extracts of *Ageratum conyzoides* leaves have been shown to lower blood glucose levels and improve the function of pancreatic Langerhans islets (C. Pay, et al., 2022), while *Cyperus rotundus* rhizome extract exhibits antihyperglycemic effects through inhibition of the α -glucosidase enzyme (Deng et al., 2019). Although both plants show potential individually, studies investigating the combined effects of their extracts on blood glucose regulation and glycemic profiles remain limited.

This study aims to evaluate the potential of combining *Ageratum conyzoides* leaf extract and *Cyperus rotundus* rhizome extract in reducing blood glucose levels in streptozotocin-induced male rat models. The findings are expected to contribute scientifically to the development of locally sourced herbal therapies as safe and effective alternatives for DM treatment, with the potential to be further developed into national phytopharmaceutical products.

METHOD

This study used an experimental laboratory approach with male white rats (*Rattus norvegicus*) as research subjects. A total of 30 rats were used, each weighing approximately 200 grams. Rats were selected using simple random sampling, ensuring that each eligible animal had an equal probability of inclusion in the experiment. All experimental procedures were approved by the Research Ethics Committee of Universitas Ahmad Dahlan Yogyakarta under approval number REC-UAD/02/02/02-2025/019, ensuring compliance with animal welfare standards and biomedical research guidelines. The initial phase of the study involved extracting plant materials. *Ageratum conyzoides* leaves and *Cyperus rotundus* rhizomes were extracted separately using the maceration method with 70% ethanol as the solvent. The maceration process was carried out for several days, with occasional stirring to ensure optimal solvent saturation with active compounds. The resulting macerate was filtered and evaporated using a rotary evaporator to obtain a thick extract. Before treatment, the test animals were acclimatized for one week to adapt to environmental conditions and ensure uniform health status. Diabetes induction was performed by intraperitoneal injection of a streptozotocin solution. Three to four days after induction, blood glucose was measured with a glucometer to confirm hyperglycemia, defined as a blood glucose concentration ≥ 200 mg/dL.

Rats meeting this criterion were classified as diabetic models, while those with normal glucose levels were assigned to the healthy control group. Treatments were administered orally once daily for fifteen days. The study included a normal control group, a negative control group, a positive control group (glimepiride), treatment groups receiving single extracts of *Ageratum conyzoides* and *Cyperus rotundus* at two dose variations, and two groups receiving combinations of both extracts at different ratios. The combination doses were determined based on preliminary findings to evaluate potential synergistic effects on blood glucose reduction. Blood glucose levels were measured on days 1, 5, 10, and 15 after induction. Blood samples were collected from the tail vein and analyzed using a glucometer. The measured glucose values were used to calculate the percentage change in blood glucose levels during the treatment period to assess the effectiveness of each test group. Data were statistically analyzed using one-way ANOVA. If significant differences were observed between groups ($p < 0.05$), the analysis was followed by a Tukey post hoc test to identify specific intergroup differences.

RESULT

The results showed that the negative control group induced with STZ without treatment experienced

a significant increase in blood glucose levels throughout the observation period. In contrast, the positive control group treated with glimepiride demonstrated a consistent reduction in blood glucose levels from day 5 to day 15.

Administration of single extracts of *Ageratum conyzoides* leaves and *Cyperus rotundus* rhizomes also reduced blood glucose levels, although the degree of effectiveness varied depending on the dose used. The *Ageratum conyzoides* leaf extract at a dose of 150 mg/kg BW produced a greater decrease in blood glucose levels compared to the 100 mg/kg BW dose. Similarly, the *Cyperus rotundus* rhizome extract at 500 mg/kg BW resulted in a more pronounced reduction than the 200 mg/kg BW dose.

The combination of both extracts exhibited the most notable antihyperglycemic effect. The combined extract of *Ageratum conyzoides* leaves and *Cyperus rotundus* rhizomes at a ratio of 150:500 mg/kg BW produced the most significant reduction in blood glucose levels, approaching the effect observed in the positive control group treated with glimepiride. Meanwhile, the 100:200 mg/kg BW combination showed an glucose-lowering moderate impact.

Table 1.
Average blood glucose levels of rats in each treatment group (mg/dL)

Treatment Group	Day 1	Day 5	Day 10	Day 15
Normal control (CMC-Na 0,5%)	16.66±2.776	12.45±12.140	28.91±10.736	31.77±4.317
Negative control (STZ+CMC-Na 0,5%)	33.02±7.540	17.86±10.786	21.70±11.825	22.30±4.630
Positif control (Glimepiride 0,036 mg/kg BB)	59.47±0.719	73.61±2.028	80.26±2.931	78.34±3.571
<i>Ageratum conyzoides</i> extract (100 mg/kg BB)	33.68±1.847	36.81±13.108	53.04±2.022	61.87±3.892
<i>Ageratum conyzoides</i> extract (150 mg/kg BB)	36.57±0.935	56.84±12.126	64.77±4.489	65.94±1.012
<i>Cyperus rotundus</i> extract (200 mg/kg BB)	34.87±11.120	49.69±16.994	62.85±4.187	64.63±4.372
<i>Cyperus rotundus</i> extract (500 mg/kg BB)	27.00±1.685	55.59±1.967	50.33±8.361	63.06±1.101
Combination EKB:EKRT (100:200 mg/kg BB)	48.18±15.162	55.60±11.589	75.57±5.650	71.20±3.436
Combination EKB:EKRT (150:500 mg/kg BB)	52.11±6.973	55.57±10.964	78.23±4.558	79.44±1.277

Note: EKB (*Ageratum conyzoides* extract), EKRT (*Cyperus rotundus* extract)

The Shapiro–Wilk normality test indicated that all data were normally distributed ($p > 0.05$). Furthermore, the one-way ANOVA test revealed a significant difference among the treatment groups ($p < 0.05$). The Tukey post hoc test indicated that the combination of EKB: EKRT extracts (150:500 mg/kg BW) showed a significant difference compared with the negative control group, but no significant difference compared with the positive control group (glimepiride).

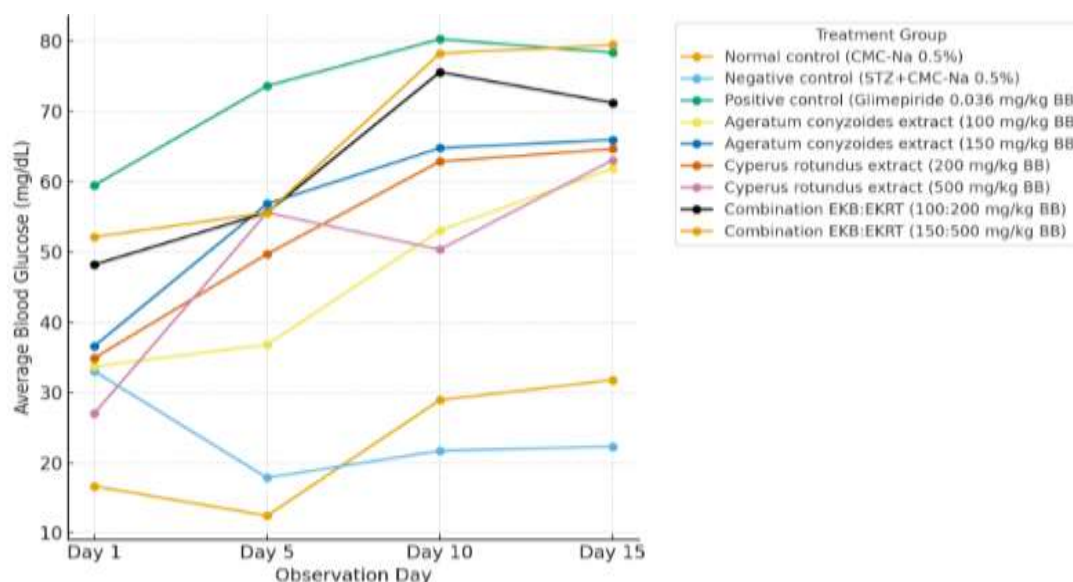


Figure 1. Graph of changes in blood glucose levels of rats during 15 days of treatment.

In general, the trend of decreasing blood glucose levels showed a positive correlation between higher extract combination dosage and its antihyperglycemic effectiveness. The pattern of blood glucose changes also indicated that the combination extract groups showed a steady decline from day 10 to day 15, whereas the untreated group maintained elevated glucose levels throughout the observation period.

DISCUSSION

The results showed that the combination of ethanol extracts of *Ageratum conyzoides* leaves and *Cyperus rotundus* rhizomes significantly reduced blood glucose levels in male rats induced with streptozotocin (STZ). The most significant decrease in blood glucose levels was observed with the combination dose of 150:500 mg/kg BW, which was comparable to that of the glimepiride-positive control group. Meanwhile, the groups receiving single extracts of *Ageratum conyzoides* or *Cyperus rotundus* showed lower reductions in blood glucose levels than the combination treatment, although still better than the negative control group. The difference in effectiveness between the combination and single extracts suggests a synergistic effect of the bioactive compounds present in both plants. *Ageratum conyzoides* is known to contain flavonoids, alkaloids, saponins, and terpenoids, which exhibit antidiabetic activity through several mechanisms, including enhancing insulin secretion, repairing pancreatic β -cell damage, and acting as potent antioxidants that counteract oxidative stress induced by STZ (Siti Sakinah, 2018; Nyemb Nyunai ab, 2009; Johnes Kalfari, 2025). Similarly, Pay et al. (2022) reported that the ethanol extract of *Ageratum conyzoides* leaves improved the morphology of Langerhans cells and reduced blood glucose levels in alloxan-induced diabetic rats.

Meanwhile, *Cyperus rotundus* rhizomes contain phenolic compounds, flavonoids, cyperene, and rotundone, which inhibit the activities of α -glucosidase and α -amylase, thereby reducing glucose absorption in the small intestine (Al-awar & Alqabbani, 2021; Pichetkun et al., 2024). In addition, the active compounds in *Cyperus rotundus* exhibit hepatoprotective and anti-inflammatory effects, helping maintain pancreatic and liver function under hyperglycemic conditions (Al-awar & Alqabbani, 2021). When both extracts were combined, the blood glucose-lowering effect increased significantly compared with single-extract administration. The combination of EKB: EKRT (150:500 mg/kgBW) reduced blood glucose levels to nearly the same level as the glimepiride group, whereas the 100:200 mg/kgBW combination produced a moderate effect. These findings support the hypothesis that combining bioactive compounds with different mechanisms of action can produce a synergistic effect. Flavonoids and alkaloids from *Ageratum conyzoides* contribute to enhancing insulin secretion and protecting pancreatic β -cells,

while phenolic and terpenoid compounds from *Cyperus rotundus* aid in inhibiting carbohydrate digestion and improving insulin sensitivity. The combination of these mechanisms results in more stable and comprehensive glycemic control compared to monotherapy.

These findings are consistent with previous studies reporting that combining phytotherapeutic agents from two or more plants with antihyperglycemic activity can enhance blood glucose regulation more effectively than single extracts (Woyessa et al., 2024; Ariastuti et al., 2020). Such combinations also tend to reduce the risk of hypoglycemia commonly associated with synthetic drugs like sulfonylureas, as their effects occur more physiologically and gradually. Furthermore, the stable decline in blood glucose levels observed from day 10 to day 15 suggests that the extract combination acts not only through acute mechanisms but also exerts regenerative effects on pancreatic β -cells. The antioxidant properties of flavonoids from *Ageratum conyzoides* likely help mitigate oxidative damage induced by STZ. At the same time, the phenolic compounds from *Cyperus rotundus* help reduce glucose absorption in the gastrointestinal tract. Together, these effects complement each other, resulting in a more effective and sustained reduction in blood glucose levels.

Therefore, the combination of *Ageratum conyzoides* and *Cyperus rotundus* extracts has strong potential to be developed as an alternative phytotherapy for the management of diabetes mellitus, particularly in Indonesia, which is rich in medicinal plant resources. Compared to single extracts, the combination demonstrated a more substantial, more stable antihyperglycemic effect that closely approached the efficacy of standard drugs without causing significant side effects. This approach supports the concept of multi-target phytotherapy, in which multiple active compounds act synergistically across various pathophysiological pathways of diabetes mellitus.

CONCLUSION

This study aimed to evaluate the antihyperglycemic effect of combined ethanol extracts of *Ageratum conyzoides* leaves and *Cyperus rotundus* rhizomes in streptozotocin-induced diabetic rats. The results showed that the combination, particularly at a dose ratio of 150:500 mg/kg BW, significantly reduced blood glucose levels, comparable to the standard drug glimepiride. These findings confirm that combining both extracts enhances their antihyperglycemic efficacy through a synergistic interaction of bioactive compounds. Therefore, the combination of *A. conyzoides* and *C. rotundus* has strong potential to be developed as a safe and effective phytotherapeutic alternative for the management of diabetes mellitus.

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