



# Phytochemical Screening of Ethanolic Extract of Kopasanda (*Chromolaena Odorata* (L) RM King and H Rob) Leaves and Its Anti-Hyperglycaemic Activity in the Mouse Model of Hyperglycaemia

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## Abstract

**Background/Aim:** Hyperglycaemia is a defining feature of diabetes mellitus, a chronic metabolic disorder. Complications of diabetes mellitus may include vascular issues both macrovascular and microvascular as well as nervous system abnormalities or neuropathy. The herb “Kopasanda” (*Chromolaena odorata* (L)) has been shown to reduce blood glucose levels. This study aimed to investigate the antihyperglycaemic potential of ethanol-extracted Kopasanda leaves in male mice induced with oral glucose. Phytochemical screening was conducted to confirm the presence of bioactive constituents by identifying secondary metabolites.

**Methods:** The test animals were randomly divided into five groups. Three groups were administered ethanol extract of *C. odorata* leaves at doses of 5, 10 and 20 mg/kg body weight (bw) orally. The positive control group received glibenclamide (0.013 mg/kg bw), while the negative control group was given a 0.5 % w/v sodium carboxymethyl cellulose (Na-CMC) solution at a dose of 2.5 mL/kg body weight orally.

**Results:** Administration of the ethanol leaf extract at a dose of 5 mg/kg bw resulted in a significant 55.21 % reduction in blood glucose levels. A 38.24 % decrease was observed in the group receiving 10 mg/kg bw while a notable reduction was also recorded at the 20 mg/kg. These findings were statistically significant compared to the negative control ( $p < 0.05$ ).

**Conclusion:** Phytochemical analysis confirmed the presence of alkaloids, flavonoids, saponins and tannins in the ethanol extract of *C. odorata* L leaves. The extract demonstrated notable antihyperglycaemic effects.

**Keywords:** Hyperglycaemia; *Chromolaena odorata*; Phytochemicals; Blood glucose; Glucose intolerance; Mice.

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## Introduction

Hyperglycaemia, defined as a sustained elevation in blood glucose concentrations, is a key clinical feature of diabetes mellitus (DM), a chronic and progressive metabolic disorder with a rapidly growing global impact.<sup>1</sup> It has been recognised as

a significant public health concern, with projections indicating a marked increase in its prevalence over the coming decades. According to the World Health Organization (WHO), the global prevalence of diabetes is expected to triple by

2030, underscoring the urgent need for effective measures in prevention, early diagnosis and therapeutic intervention. It is estimated that 21.3 million individuals will be affected by diabetes and data from the International Diabetes Federation (IDF) suggest that this number may rise to 16.7 million by 2045.<sup>1</sup>

In Indonesia, the situation mirrors global trends. The 2018 Basic Health Research (RISKESDAS) reported that approximately 20.4 million Indonesians had been diagnosed with DM, representing a national prevalence of 8.5 %. In addition, individuals with diabetes are at increased risk of developing severe acute and chronic complications, some of which may be life-threatening.<sup>2</sup>

DM may lead to complications such as neuropathy, nervous system abnormalities and vascular disorders, encompassing both macrovascular and microvascular damage. These complications can occur not only in individuals with long-standing type 2 diabetes, but also in those who have recently been diagnosed. Macrovascular complications commonly affect the heart, brain and major blood vessels, while microvascular complications primarily involve the kidneys and eyes. Neuropathic manifestations, including motor, sensory and autonomic neuropathy, are also frequently observed in diabetic patients. These complications substantially impair patients' quality of life and contribute to increased healthcare expenditures.<sup>3</sup>

Currently, synthetic agents such as metformin and glibenclamide are widely used in the management of diabetes mellitus. However, long-term use of these medications is associated with adverse effects, including hepatotoxicity, hypoglycaemia and gastrointestinal disturbances.<sup>4</sup>

In light of these limitations, pharmacological research is increasingly focused on identifying alternative therapies derived from natural sources with fewer side effects. One such candidate is Kopasanda (*Chromolaena odorata*), a tropical plant indigenous to Indonesia, known for its diverse pharmacological properties. The plant is rich in phytochemical constituents such as alkaloids, flavonoids, saponins and tannins, which are recognised for their involvement in various biological activities and have attracted significant interest in natural product research.<sup>5</sup> Kopasanda exhibits a wide range of pharmacological properties, including antidiabetic, anticancer, anti-in-

flammatory, antimicrobial, antiparasitic, antinociceptive and antipyretic activities.<sup>6</sup>

Ethanol extract of *C odorata* leaves is practically non-toxic when administered acutely due to LD<sub>50</sub> value is 15 g/kg bw. Throughout the 14-day observation period, no mortality was recorded among the white rats administered with *C odorata* leaf extract. The only observable toxic manifestation was a decrease in appetite, noted consistently across all treatment groups. This reduction in food intake corresponded with a progressive decline in body weight, which was found to be statistically significant on days 1, 3, 7, 10 and 14. Biochemical analyses revealed that serum levels of AST, ALT and creatinine remained within normal limits and showed no significant differences between treated and control groups. Furthermore, histopathological assessments of liver and kidney tissues indicated no detectable structural alterations, suggesting the absence of overt organ damage at the administered doses. From these outcomes, the median lethal dose (LD<sub>50</sub>) of the extract was estimated at 15 g/kg body weight, classifying it within the range of substances considered practically non-toxic. However, the delayed adverse effect characterised by anorexia and subsequent weight loss highlights the importance of conducting more extensive sub-chronic and chronic toxicity investigations to ensure its long-term safety profile.<sup>7</sup>

The ethanol extract of *C odorata* leaves has been shown to reduce blood glucose levels by 27.29 %, 34.68 % and 52.24 % in Swiss-Webster mice with type 1 diabetes induced by alloxan, at administered doses of 125, 250 and 500 mg/kg body weight (bw), respectively.<sup>8</sup> Furthermore, an ethanol extract of *C odorata* leaves formulated using liposomal nanotechnology, administered to rats at a dose of 250 mg/kg bw, demonstrated an antihyperglycaemic effect comparable to glibenclamide at a dose of 0.45 mg/kg bw, with statistically significant results ( $p < 0.05$ ).<sup>9</sup> A study by Elekofehinti et al reported that administration of ethanol extract from *C odorata* leaves at 200 mg/kg bw in streptozotocin-induced type 1 diabetic rats significantly upregulated the expression of GLUT2 and glucokinase mRNA, both of which are crucial in the regulation of blood glucose homeostasis.<sup>10</sup>

The present study aimed to evaluate the antihyperglycaemic effect of an ethanol extract from Kopasanda leaves (*Chromolaena odorata* L) in

male Swiss mice using an acute hyperglycaemia model induced by oral glucose administration. In parallel, a phytochemical screening was conducted to identify the presence of potential bioactive secondary metabolites.

## Methods

### Equipment

The equipment used in this study included an analytical balance (Sartorius®), digital glucometer (Accu-Chek®), Erlenmeyer flasks (Duran®), measuring cylinders (Iwaki®), water bath (Julabo®), blender (Philips®), smartphone camera (iPhone 13®), laboratory gas burner (Panasonic®), Oven (Binder®), rotary vacuum evaporator (Heidolph®), digital animal balance (Camry®), mortar and pestle (standard ceramic laboratory type), animal cages (stainless steel or polypropylene type), filter paper (Millipore®) and syringes (BD Plastipak®).

Chemicals used in this study were: sodium carboxymethyl cellulose (Na-CMC) (Sigma Aldrich®), distilled water, HCl, 70 % ethanol (Brataco®), concentrated sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) (Carlo Erba®), sodium hydroxide (NaOH) (Honeywell®), ferric chloride (FeCl<sub>3</sub>) (Loba Chemie®), *n*-hexane (Fisher Chemical®), amyl alcohol (Loba Chemie®), glacial acetic acid (Fisher Scientific®) and phytochemical reagents such as Mayer's, Dragendorff's and Liebermann-Burchard's (Tokyo Chemical Industry®).

Kopasanda leaves, were obtained from Juang Sub-district, Bireuen Regency, Banda Aceh City, Aceh Province, Indonesia. The plant was taxonomically identified and authenticated at the Herbarium Medanese (MEDA), University of North Sumatra (1486/MEDA/2023).

### Dry leaf powder preparation

Samples of Kopasanda leaves were thoroughly washed to remove residual impurities, then drained and weighed. The samples were subsequently dried at 50 °C using a drying oven. After drying, the plant material was reweighed and homogenised to produce the simplicial.<sup>11</sup>

#### Determination of moisture content

Five grams of dry leaf powder were weighed using a calibrated cup, dried for five hours at 105 °C



Figure 1: *Chomolaena odorata* (L) RM King and H Rob

in an oven, the sample was placed in a desiccator and subsequently weighed until it reached a specified weight. The percentage of moisture content was then calculated.<sup>12, 13</sup>

$$\% \text{ Moisture} = \frac{b-(c-a)}{b} \times 100 \%$$

Description:

a = weight in cup (gram)

b = initial sample weights (gram)

c = weight of cup + sample after heating (gram)

#### Determination of total ash

Two grams of dried leaf powder were placed in a silica crucible and incinerated at 600 °C for one hour in a furnace. Following cooling, the sample was weighed repeatedly until a consistent weight was achieved.<sup>12, 13</sup> The following formula was used to calculate the total ash content:

$$\% \text{ Total ash} = \frac{\text{weight of heating residual ash}}{\text{weight of dry leaf powder}} \times 100 \%$$

#### Determination of acid-insoluble ash

The previously obtained ash sample was boiled in 25 mL of hydrochloric acid for five minutes, filtered through ash-free filter paper, followed by a thorough washing with hot water to ensure purity. Then, the sample was incubated in a water bath at a constant temperature of 80 ± 2.5 °C to maintain its weight. According to Sofia et al and Gurning and Simanjuntak, the concentration of acid-insoluble ash is quantified as a percentage of the total sample.<sup>12, 13</sup>

$$\% \text{ Acid-insoluble ash} = \frac{\text{residual ash weight}}{\text{weight of dry leaf powder}} \times 100 \%$$

#### Determination of water extract water

Five g of dried leaf powder was carefully weighed before being added to a 100 mL solution of water saturated with chloroform contained within a sealed flask. Extraction lasted 24 h with occasionally shaking. Twenty millilitres the filtrate was subjected to drying through the process of evaporation in a water bath. The remaining material in the cup was then heated to 105 °C before being weighed at a consistent weight.<sup>12, 13</sup> The following formula was used to determine the water-soluble essence content:

$$\frac{\text{amount of water soluble}}{\text{weight of dry leaf powder}} = \frac{\text{weight of essence}}{\text{weight of dry leaf powder}} \times \frac{100}{20} \times 100 \%$$

#### Determination of ethanol soluble extract

In a corked tube that was periodically shaken, 5 g of dry leaf powder was macerated with 100 mL of 95 % ethanol during 24 hours. The sample is rapidly filtered and 20 mL of filtrate was positioned in a water bath to dry. The remaining material in the cup is heated to 105 °C and weighed at a constant weight.<sup>12, 13</sup> The following formula is used to determine the amount of ethanol soluble essence:

$$\frac{\text{amount ethanol soluble essence}}{\text{weight of dry leaf powder}} = \frac{\text{weight of essence}}{\text{weight of dry leaf powder}} \times \frac{100}{20} \times 100 \%$$

### Extraction

*C odorata* leaves were extracted using the maceration method, with 70 % ethanol as the solvent. Five hundred g of powdered dried leaves were placed in a macerator and enough solvent was added to completely immerse the powder (1:10). The mixture was then agitated and swirled intermittently while remaining in place 72 hours. After this time, the filtrate was separated and the residual pulp was placed in a dark bottle, followed by another rinse with 70 % ethanol. This extraction technique was repeated twice, with the same solvent type and volume. The liquid extract was then concentrated using a rotary evaporator set to 50 °C to produce a concentrated extract of substantial consistency. A concentrated extract was prepared and the yield was calculated.<sup>11</sup>

### Screening of phytochemicals

Presence of alkaloids, flavonoids, glycosides, tannins and steroids/triterpenoids are all examined

during the phytochemical screening of *C odorata* leaf extract.<sup>14</sup>

#### Alkaloids

The solution was subjected to heating in a water bath for a duration of two minutes, then allowed to cool and filtered after incorporating 0.5 g of dry leaf powder into 1 mL of 2 N hydrochloric acid, followed by add 9 mL of water. The resulting filtrate was used for alkaloid testing. To each of three test tubes, 0.5 mL of the filtrate was added, followed by two drops of Mayer's, Bouchardat's and Dragendorff's reagents. The emergence of turbidity served as an indication of the presence of alkaloids. A positive result for alkaloids was considered when at least two of the three reagents produced a positive reaction.<sup>14</sup>

#### Flavonoids

Twenty millilitres of boiling water were added to 2 g of dry leaf powder and the mixture was filtered while still hot after five minutes of boiling. Subsequently, a quantity of 0.1 grams of magnesium powder, along with 1 millilitre of concentrated hydrochloric acid and 2 millilitres of amyl alcohol was introduced into the reaction mixture. The mixture was then mixed and left to separate. The emergence of yellow, orange, or red hues in the amyl alcohol layer indicated the presence of flavonoids.<sup>14</sup>

#### Saponins

A total of 0.5 g of dry leaf sample was placed into a test tube with 10 millilitres of hot water, resulting in a mixture that was subsequently analysed. After being cooled and shaken for a duration of 10 seconds, if a significant amount of foam develops that persists for a minimum of 10 minutes, achieving a height between 1 and 10 centimetres, The presence of saponins remains evident and does not diminish with the introduction of 1 mL of 2 N HCl.<sup>14</sup>

#### Tannins

After three minutes of boiling 1 g of dry leaf powder in 10 mL of distilled water, it was cooled and filtered and one or two drops of FeCl<sub>3</sub> were added. Tannins were indicated by a light blue or light green tint.<sup>14</sup>

#### Analysis of terpenoids and steroids

Twenty millilitres of *n*-hexane were macerated with 1 g of dry leaf powder for two hours. Following the evaporation of the filtrate, concentrated H<sub>2</sub>SO<sub>4</sub> and anhydrous acetic acid were added. Ste-

roids or triterpenoids were indicated by a purple or red tint that turns purple blue or greenish blue.<sup>13</sup>

## Assessment of antihyperglucaemic effects

A study was carried out to assess the antihyperglucaemic properties of the tested substance using the oral glucose tolerance test (OGTT) methodology. The study involved male Swiss mice weighing 20-30 g and aged 3-4 months. Animals were randomly assigned into five groups. The first group served as the negative control, receiving 2.5 mL/kg BW CMC Na 0.5 % wv orally (*per os* - po)), which utilised as a suspending agent. The positive control, providing a benchmark for comparison in the experimental design of 0.013 mg/kg bw of glibenclamide po was administered, while the other three groups received the test substance. Before the experiment commenced, the mice were allowed to acclimatise to their surroundings for a duration of seven days, The test extract was given at dosages to the remaining three groups at doses of 5, 10 and 20 mg/kg bw, respectively. At the outset of the study, baseline blood glucose levels ( $T_0$ ) were recorded prior to the administration of the test substance following 16 hours of fasting. The test substance was administered to the animal half an hour before glucose po induction. A glucose solution (0.195 g/kg bw) was then given orally to each mouse. Blood glucose concentrations were assessed at intervals of 30, 60, 90, 120 and 150 minutes following the intervention by oral. The blood specimen was applied to a testing strip. The blood specimen was applied to a testing strip and the blood glucose level was measured using testing strips and a commercial glucometer. The concentration measured in milligrams per decilitre (mg/dL) was utilised to compute the area under the curve (AUC) through established methodologies:<sup>13, 15</sup>

$$AUC_{0-150} = \frac{t_1 - t_0}{2} (C_0 + C_1) + \frac{t_1 - t_0}{2} (C_1 + C_2) + \frac{t_1 - t_0}{2} (C_n + C_{n-1})$$

In this context, 't' represents time measured in minutes, while 'C' denotes the concentration of blood glucose expressed in mg/dL and  $AUC_{0-150}$  = area under the curve for 150 minutes. Once the  $AUC_{0-150}$  value has been computed, the subsequent equation can be employed to determine percentage decrease in blood glucose levels (% reduction):

$$\% \text{ reduction} = \frac{AUC_{0-150} \text{ (negative control)} - AUC_{0-150} \text{ (treatment control)}}{AUC_{0-150} \text{ (negative control)}} \times 100 \%$$

## Statistical analysis

The assessment of data normality and homogeneity can be conducted using the Shapiro-Wilk test and Levene's test, which are essential statistical methods for evaluating these assumptions in research. Subsequently, data analysis was performed using the SPSS 20 software, employing a one-way ANOVA. The subsequent section presents the findings of the Post Hoc Tukey test, along with a comprehensive discussion that centres on the implications of these results.

## Results

The maceration technique was employed, utilising a solvent composed of 70 % ethanol, to achieve extraction results for fresh materials. The extraction outcomes for fresh *C odorata* leaves are presented in Table 1. From a total of 1.8 kilograms of freshly harvested leaves that had been thoroughly washed and dried, a concentrated extract was produced with a yield of 4.68 %. From a total of 1.8 kilograms of freshly harvested leaves, concentrated extract was produced 4.68 % yield.

An evaluation of non-specific parameters pertaining to the dried leaves has been conducted standards by the *Herbal Pharmacopoeia* 2017 2nd edition, as indicated in Table 2.

Table 2 displays the results of determination of *C odorata* leaves non-specific parameters. High content of moisture (water) in dry samples can have significant impact on the substance's stability, shelf life and quality. The development of germs and chemical processes that can harm the plant material constituents are influenced by a high moisture level. The complete amount of inorganic material left over after burning the material is ascertained by looking at the total amount of ash. This comprises pollution (such dust, sand, or heavy metals) and naturally occurring mineral compounds. Finding the complete amount of inorganic material left over after the material has burned is the goal of measuring the total amount of ash. This comprises pollution (such dust, sand, or heavy metals) and naturally occurring mineral compounds. The amount of active chemicals

that dissolve in diluted acid (often diluted HCl) is measured using an acid soluble essence test. It is employed to identify certain substances that dissolve in acid, such as metal oxides or carbonates. In the meantime, the amount of active chemicals that dissolve in ethanol from the substance or simplisia is ascertained by examining the ethanol soluble essence. The findings of the analysis of simplisia's characteristics indicate levels that meet the standards specified by the *Indonesian Pharmacopoeia VI edition*.<sup>16</sup>

Phytochemical analysis indicated that Kopasanda leaves contain components such as alkaloids, flavonoids, saponins and tannins (Table 3).

The mean blood glucose level (Table 4) for each treatment group at a given minute was used to create the curve in Figure 2 that depicts the relationship between mean alteration in blood glucose (mg/dL) (Table 5) versus time which was used to calculate the AUC value.

Table 1: Extraction yield data

| Duration of extraction (hour) | Green leaf (g) | Dried sample weight (g) | Extract mass (g) | Extraction yield (%) |
|-------------------------------|----------------|-------------------------|------------------|----------------------|
| 72                            | 5000           | 500                     | 70               | 4.68                 |

Table 2: An evaluation of non-specific parameters in dry foliag

| N | Parameter                         | Result  | Herbal pharmacopeia of Indonesia 2 <sup>th</sup> Ed (Kemenkes RI, 2017) | Criteria  |
|---|-----------------------------------|---------|---|-----------|
| 1 | Moisture content                  | 9.90 %  | ≤ 10 %  | Qualified |
| 2 | Amount of total ash               | 9.54 %  | ≤ 10 %  | Qualified |
| 3 | Amount of acidinsoluble ash       | 5.16 %  | ≤ 7 %   | Qualified |
| 4 | Amount of water-soluble essence   | 14.27 % | ≥ 10 %  | Qualified |
| 5 | Amount of ethanol soluble essence | 13.45 % | ≥ 12 %  | Qualified |

Table 3: Outcomes of *Chromolaena odorata* phytochemical screening test

| N | Metabolite             | Chemical reagent                          | Observation       | Result |
|---|------------------------|---|-------------------|--------|
| 1 | Alkaloids              | Dragendorff                               | brown sediment    | (+)    |
|   |                        | Bouchardat                                | brown sediment    | (+)    |
|   |                        | Mayer                                     | no white sediment | (-)    |
| 2 | Flavonoids             | Zn + HCl(p)                               | red orange        | (+)    |
|   |                        | Mg + HCl(p)                               | yellow            | (+)    |
| 3 | Tannin                 | FeCl 5 %                                  | dark green        | (+)    |
| 4 | Saponins               | Mixed with hot water and concentrated HCl | foam present      | (+)    |
| 5 | Steroids/Triterpenoids | Liebermann-Burchard                       | green             | (-)    |

Remarks: The component under examination is contained in (+); whereas it is absent from (-);

**Table 4:** Mean values (mg/dL) and deviation of standard glucose level at different time intervals

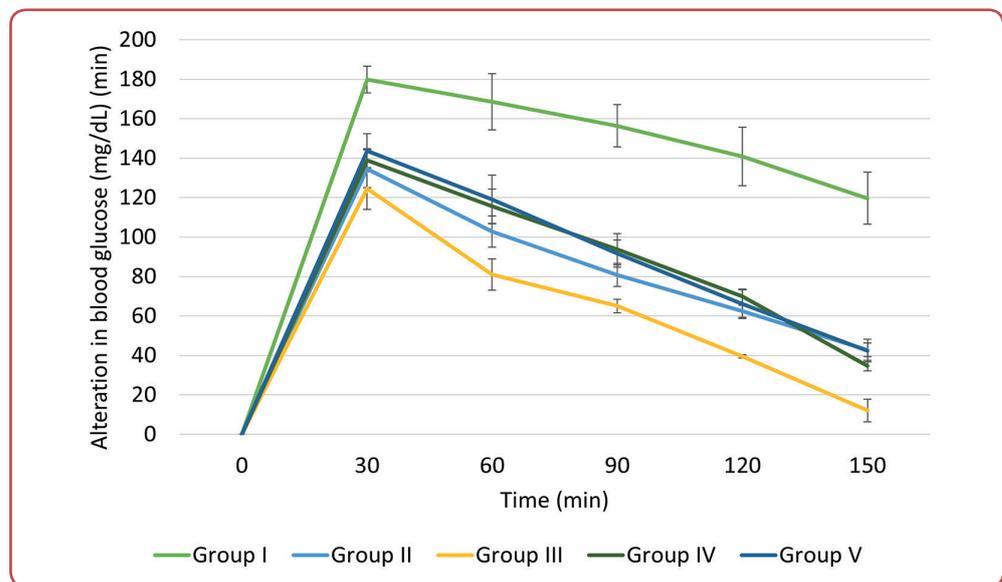
| Group | Mean ± SD glucose level (mg/dL) (min) |                |                |                |                |                |
|-------|---------------------------------------|----------------|----------------|----------------|----------------|----------------|
|       | 0                                     | 30             | 60             | 90             | 120            | 150            |
| I     | 74.00 ± 5.66                          | 253.80 ± 12.43 | 242.60 ± 19.87 | 230.37 ± 16.34 | 214.86 ± 20.44 | 193.65 ± 18.87 |
| II    | 72.45 ± 8.90                          | 207.04 ± 18.42 | 175.22 ± 16.88 | 153.23 ± 14.65 | 134.82 ± 12.01 | 115.23 ± 12.32 |
| III   | 80.82 ± 9.45                          | 205.32 ± 20.06 | 161.80 ± 17.34 | 145.85 ± 12.85 | 120.22 ± 10.31 | 92.87 ± 15.17  |
| IV    | 76.65 ± 8.36                          | 215.62 ± 14.23 | 192.25 ± 17.05 | 170.42 ± 16.30 | 146.60 ± 12.08 | 111.43 ± 10.98 |
| V     | 76.23 ± 6.63                          | 219.86 ± 15.35 | 195.27 ± 18.90 | 167.86 ± 13.55 | 142.22 ± 13.86 | 118.67 ± 12.32 |

I = the negative control (carboxymethyl cellulose (CMC) Na 0.5 % w/v, po); II = the positive control (glibenclamide 0.013 mg/kg BW); III = Ethanol extract of *C odorata* 5 mg/kg BW; IV = Ethanol extract of *C odorata* L 10 mg/kg BW; Ethanol extract of *C odorata* 20 mg/kg BW;

**Table 5:** Mean alteration in blood glucose (mg/dL) at different time intervals

| Group | Mean ± SD alteration in blood glucose (mg/dL) (min) |                |                |                |                |                |
|-------|---|----------------|----------------|----------------|----------------|----------------|
|       | 0   | 30             | 60             | 90             | 120            | 150            |
| I     | 0.00  | 179.80 ± 6.77  | 168.60 ± 14.21 | 156.37 ± 10.68 | 140.80 ± 14.78 | 119.65 ± 13.21 |
| II    | 0.00  | 134.59 ± 9.52  | 102.77 ± 7.98  | 80.78 ± 5.75   | 62.37 ± 3.11   | 42.78 ± 3.42   |
| III   | 0.00  | 124.50 ± 10.61 | 80.98 ± 7.89   | 65.03 ± 3.40   | 39.40 ± 0.86   | 12.05 ± 5.72   |
| IV    | 0.00  | 138.97 ± 5.87  | 115.60 ± 8.69  | 93.77 ± 7.94   | 69.95 ± 3.72   | 34.78 ± 2.62   |
| V     | 0.00  | 143.63 ± 8.72  | 119.04 ± 12.27 | 91.63 ± 6.92   | 65.99 ± 7.23   | 42.44 ± 5.69   |

I = the negative control (carboxymethyl cellulose (CMC)Na 0.5 % w/v, po); II = the positive control (glibenclamide 0.013 mg/kg BW); III = Ethanol extract of *C odorata* 5 mg/kg BW; IV = Ethanol extract of *C odorata* L 10 mg/kg BW; Ethanol extract of *C odorata* 20 mg/kg BW;



**Figure 2:** Average variation in blood glucose concentration

Group I = the negative control (carboxymethyl cellulose (CMC) Na 0.5 % w/v, po); II = the positive control (glibenclamide 0.013 mg/kg BW); III = Ethanol extract of *C odorata* 5 mg/kg BW; IV = Ethanol extract of *C odorata* L 10 mg/kg BW; Ethanol extract of *C odorata* 20 mg/kg BW;

The AUC<sub>0-150</sub> value for each treatment group reflects the level of blood glucose fluctuations over a 150-minute period, reflecting the effects of each treatment. The AUC value is inversely related to the antihyperglycemic efficacy of a substance; a lower AUC value signifies a stronger antihyperglycemic effect. The group receiving the negative control (CMC Na 0.5 % w/v) exhibited

the highest AUC value, while the ethanol extract derived from the leaves of *Chromolaena odorata* was administered at doses of 20 and 10 mg/kg BW, in addition to the positive control group receiving glibenclamide at a dosage of 0.013 mg/kg BW, showed progressively lower AUC values. The group receiving the 5 mg/kg BW extract dose had the lowest AUC value. This suggests

that the decrease in blood glucose levels is negatively correlated with the AUC value; higher AUC values correspond to smaller reductions in blood glucose levels.

The ethanol extract derived from the leaves of *C odorata* at a dosage of 5 mg/kg BW produced the most pronounced decrease in blood glucose levels, achieving a reduction of 55.21 %. This was followed by glibenclamide at 43.02 %, the extract at 10 mg/kg BW with a reduction of 38.24 % and

the extract at 20 mg/kg BW which resulted in a 37.40 % decrease (Table 6, Figure 3).

Following the positive control group (glibenclamide 0.013 mg/kg BW, with a reduction of 43.02 %), the group treated with *C odorata* leaf ethanol extract at a dose of 10 mg/kg BW showed a 38.24 % decrease and the 20 mg/kg BW dosage resulted in a 37.40 % reduction. However, the group that received the 5 mg/kg BW dose of the ethanol extract exhibited the most substantial reduction in blood glucose levels (55.21 %). Significant differences in blood glucose levels were observed between the positive control group and the negative control group (CMC Na 0.5 % w/v) ( $p < 0.05$ ) after the ethanol extract of *C odorata* leaves was administered at doses of 5, 10 and 20 mg/kg BW.

Post Hoc Tukey's HSD test, which further examined intergroup differences. These findings indicate that the ethanol extract of *C odorata* leaves effectively reduces blood glucose levels in mice, as evidenced by the substantial decrease in glucose levels in the groups treated with the extract compared to the negative control group (CMC Na 0.5 % w/v).

Table 6: Level glucose reduction

| Group | AUC <sub>0-150</sub> (mg/min/dL) mean $\pm$ SD | Glucose reduction as a percentage (%) |
|-------|--|---------------------------------------|
| I     | 21161.85 $\pm$ 1433.21                         | 0.00                                  |
| II    | 12057.00* $\pm$ 8654.90                        | 43.02                                 |
| III   | 9478.05* $\pm$ 812.54                          | 55.21                                 |
| IV    | 13070.40* $\pm$ 1256.77                        | 38.24                                 |
| V     | 13245.30* $\pm$ 9807.32                        | 37.40                                 |

Post Hoc Tukey's HSD \* = markedly different from the negative control; I = the negative control (carboxymethyl cellulose (CMC) Na 0.5 % w/v, po); II = the positive control (glibenclamide 0.013 mg/kg BW); III = Ethanol extract of *C odorata* 5 mg/kg BW; IV = Ethanol extract of *C odorata* L 10 mg/kg BW; Ethanol extract of *C odorata* 20 mg/kg BW;;

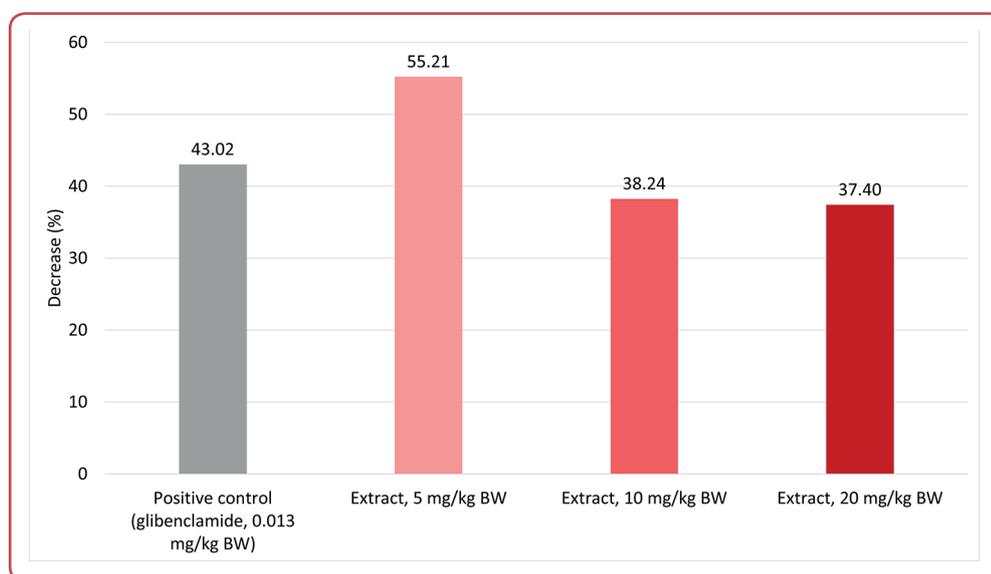


Figure 3: Decrease of blood glucose levels (%); Extract: ethanol extract of *C odorata*;

## Discussion

Phytochemical analysis indicated that Kopasanda leaves contain components such as alkaloids, flavonoids, saponins and tannins. The results of this

investigation align with those of other researchers who found that *C odorata* contain the same secondary metabolite compounds.<sup>17, 18</sup>

The AUC provides a more comprehensive evaluation of the blood glucose response than a single-point measurement because it encompasses all fluctuations, including peak values, declines and the duration of glucose level changes over time. In contrast, a single blood glucose measurement only reflects glucose levels at a specific moment. The ethanol extract derived from the leaves of *C odorata* at a dosage of 5 mg/kg BW produced the most pronounced decrease in blood glucose levels, achieving a reduction of 55.21 %. This was followed by glibenclamide at 43.02 %, the extract at 10 mg/kg BW with a reduction of 38.24 % and the extract at 20 mg/kg BW which resulted in a 37.40 % decrease. These findings suggest that the AUC is a more precise measure of the overall impact of the treatments on blood glucose levels, as it accounts for the variations in glucose over the full duration of the study period.<sup>19</sup>

In this investigation, there was no correlation between rising extract dosage and falling blood glucose levels; in other words, the impact did not rise linearly with rising extract dosage. The discrepancy between increasing dose and pharmacological response is explained by the non-monotonic dose-response relationship (NMDR). Inter-receptor competition, cytotoxicity, receptor selectivity, endocrine negative feedback and receptors and cofactors specific to cells and tissues are examples of NMDR processes.<sup>20</sup> The mechanism by which dosage and pharmacologic response are related in this investigation is not well understood.

In traditional medicine, *C odorata* is used to cure a variety of ailments, including diabetes mellitus. The chemical mechanism behind this plant's activity, however, has not yet been studied. An oral application of 200 mg/kg BW *C odorata* leaf ethanol extract for three weeks was shown to suppress erythroid 2-related factor 2/Kelch-like ECH-associated protein 1 (Nrf2/keap1) gene expression, while increasing Glut2 and glucokinase mRNA gene expression in type 1 diabetic rats.<sup>10</sup> Glutamate 2 is a transporter protein that facilitates the transfer of glucose from the blood to the hepatocytes. Additionally, it helps glucose enter the pancreatic beta cells, where the enzyme glucokinase phosphorylates it, this contributes to the processes of glycogenesis and glycolysis that reduce blood glucose levels. The active ingredients found in *C odorata*; luteolin and 5,7-dihydroxy-6-4-dimethoxyflavanone are implicated in this action, according the findings of molecular docking.<sup>10</sup>

Research on the medicinal effects *C odorata* leaf water extract in Wistar rats given alloxan by Egwuatu et al, showed that administering extracts during 21 days at dosages of 100, 200 and 400 mg/kg BW can considerably reduce blood glucose levels. Furthermore, in contrast to the adverse effects in control group, the outcomes of the histological investigation indicated regeneration of pancreatic beta cells ( $p < 0.05$ ).<sup>21</sup> In streptozotocin-induced diabetic rats, oral administration of *C odorata* leaf ethanol extract demonstrated that at doses of 100, 200 and 400 mg/kg BW it significantly increased insulin levels, decreased blood glucose levels and increased body weight in comparison to diabetic rat groups.<sup>22</sup>

*Chromolaena odorata* has an anti-diabetic effect because it includes secondary metabolite compounds such alkaloids, flavonoids, saponins, tannins, phenolics, triterpenoids and steroids. These compounds can act as antioxidants, reducing the generation of free radicals that harm the pancreatic beta cells as a result of hyperglycaemia. This antioxidant effect will maintain blood glucose levels within the normal range<sup>23</sup> because adequate insulin is generated. Steroid, alkaloid, flavonoid and tannin components found in *C odorata* ethanol extract may have anti-diabetic properties.<sup>24</sup>

The presence of alkaloids in *C odorata* leaves, as confirmed in this study, aligns with a growing body of evidence supporting the antidiabetic potential of alkaloid compounds. Alkaloids have been shown in several *in vivo* studies to exert antihyperglycaemic effects by enhancing insulin secretion and improving glucose uptake. For example, the alkaloids isolated from *Vitex doniana* and *Ficus thonningii* have been reported to increase insulin production and promote peripheral glucose utilisation, resulting in lowered blood glucose levels in diabetic animal models.<sup>25</sup> Furthermore, specific alkaloids from *Piper longum* have been shown to inhibit protein tyrosine phosphatase 1B (PTP1B), a negative regulator of insulin signalling. PTP1B dephosphorylates insulin receptor substrate-1 and -2 (IRS-1 and IRS-2), thereby impairing insulin signalling and contributing to insulin resistance. By inhibiting this pathway, alkaloids may enhance insulin sensitivity. Although the specific alkaloid profile of *C odorata* remains to be fully elucidated, the presence of this compound class in the extract suggests a possible shared mechanism with other antidiabetic plants. Further phytochemical and mecha-

nistic studies are needed to determine whether *C odorata* alkaloids exhibit similar PTP1B inhibitory activity or act through alternative antidiabetic pathways.<sup>26</sup>

The hydroxyl group at position C atom number 3 plays a significant part in the process by which flavonoids exhibit anti-diabetic activity through an inhibitory mechanism against the alpha glucosidase enzyme.<sup>27, 28</sup> Additionally, alpha amylase, an enzyme crucial to controlling blood glucose levels, is inhibited by some flavonoids. The enzyme alpha amylase is in charge of converting carbs into glucose and maltose. By postponing the absorption of glucose, inhibition of alpha amylase activity can lower blood glucose levels.<sup>29</sup> Additionally, flavonoids reduce hyperglycaemia and insulin resistance by enhancing the activation of the of the gene peroxisome proliferator-activated receptor gamma (PPAR- $\gamma$ ) in the liver and adipose tissue.<sup>30</sup> Flavonoids have been shown to repair cells in the islets of Langerhans and exhibit anti-diabetic effects. The presence of flavonoids plays a beneficial role in managing diabetes mellitus, whether the condition is due to insulin receptor dysfunction or insulin deficiency, as flavonoid compounds can help address insulin shortages.<sup>31</sup> Flavonoids may mimic insulin by altering insulin-related processes, enhancing glucose absorption in peripheral tissues and regulating blood glucose levels. Giang et al investigation into the pharmacological impacts of flavonoids found in *C odorata* leaves identified the flavonoid molecule isosakuranetin as an inhibitor of the alpha-glucosidase enzyme, with an IC<sub>50</sub> value of  $55.99 \pm 1.25$   $\mu\text{g/mL}$ .<sup>32</sup>

The molecular mechanisms through which saponins prevent diabetes involve regulating various PPAR- $\gamma$  is one of the signalling pathways, nuclear factor erythroid 2-related factor 2 (Nrf2), AMP-activated protein kinase (AMPK), IRS-1 and phosphatidyl inositol 3-kinase (PI3K).<sup>33</sup> In a 2022 study, Sahakyan et al found that several tannins, including gallic acid, ellagic acid, catechins, epicatechins and procyanidins from different plant extracts, contribute to improved peripheral glucose uptake, thereby reducing blood glucose levels.<sup>34, 35</sup>

## Conclusion

Phytochemical screening of the ethanol extract of *Chromolaena odorata* L leaves confirmed the presence of key bioactive compounds, including alkaloids, flavonoids, saponins and tannins. These constituents are known for their pharmacological properties and may act synergistically to produce therapeutic effects. Notably, the extract demonstrated significant antihyperglycemic activity, suggesting its potential utility as a complementary agent in the management of diabetes mellitus. These findings provide a scientific basis for the traditional use of *Chromolaena odorata* in managing blood sugar levels. Future investigations should focus on isolating and characterising the specific active constituents responsible for the observed activity, elucidating their mechanisms of action and conducting *in vivo* efficacy and toxicity studies. Clinical trials may also be warranted to validate its safety and effectiveness in human subjects.

## Ethics

The study protocol was examined and approved by the Prima University Faculty of Medicine's Ethics Committee, approval No 080/KEPK/UN-PR1/11/2024, dated 27 February 2024.

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## Conflicts of interest

The authors declare that there is no conflict of interest.

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## Data access

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

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