



Pharmacological assessment of standardized *Curcuma longa* L. and *Centella asiatica* (L.) Urban extracts for diabetes management

[Evaluación farmacológica de extractos estandarizados de *Curcuma longa* y *Centella asiatica* para el manejo de la diabetes]

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Abstract

Context: Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia and insulin resistance, often linked to oxidative stress. *Curcuma longa* (turmeric) and *Centella asiatica* (gotu kola) are traditionally recognized for antidiabetic and antioxidant properties.

Aims: To identify major active compounds and evaluate the antioxidant and antidiabetic activities of these extracts individually and in combination.

Methods: Extracts were standardized using TLC densitometry according to the Indonesian Herbal Pharmacopoeia. Antioxidant activity was assessed via DPPH scavenging and malondialdehyde (MDA) inhibition, while antidiabetic effects were evaluated through α -glucosidase inhibition and *in vivo* testing in alloxan-induced diabetic rats. Insulin resistance was measured by the highest tolerated level of insulin (HTLI).

Results: Both extracts met pharmacopoeial quality standards. The 1:1 combination exhibited markedly stronger α -glucosidase inhibition (IC_{50} = 45.45 μ g/mL) than *C. longa* (135.03 μ g/mL), *C. asiatica* (88.07 μ g/mL), and acarbose (113.92 μ g/mL). Antioxidant activity was also greater in the combination (IC_{50} = 40.68 μ g/mL). *In vivo*, combination treatment reduced blood glucose comparably to glibenclamide and improved insulin sensitivity, with HTLI reaching 1.634 mg/dL versus 1.308 mg/dL for metformin. MDA levels were significantly reduced, indicating lower lipid peroxidation. A moderate correlation between antioxidant and enzyme inhibition (r = 0.685) suggests a mechanistic link between oxidative stress and carbohydrate metabolism.

Conclusions: The combination of *C. longa* and *C. asiatica* demonstrated synergistic antioxidant and antidiabetic effects, meeting quality standards, which supports its potential as an adjunctive therapy for diabetes management.

Keywords: antidiabetic; antioxidant; *Centella asiatica*; *Curcuma longa*; synergistic effect.

Resumen

Contexto: La diabetes mellitus es un trastorno metabólico crónico caracterizado por hiperglucemia y resistencia a la insulina, a menudo asociada al estrés oxidativo. La *Curcuma longa* (cúrcuma) y la *Centella asiatica* (gotu kola) son tradicionalmente reconocidas por sus propiedades antidiabéticas y antioxidantes.

Objetivos: Identificar los principales compuestos activos y evaluar las actividades antioxidantes y antidiabéticas de estos extractos, tanto individualmente como en combinación.

Métodos: Los extractos se estandarizaron mediante densitometría por TLC según la Farmacopea Herbal de Indonesia. La actividad antioxidante se evaluó mediante la eliminación de DPPH y la inhibición del malondialdehído (MDA), mientras que los efectos antidiabéticos se evaluaron mediante la inhibición de la α -glucosidasa y pruebas *in vivo* en ratas con diabetes inducida por aloxano. La resistencia a la insulina se midió mediante el nivel máximo tolerado de insulina (HTLI).

Resultados: Ambos extractos cumplieron con los estándares de calidad de la farmacopea. La combinación 1:1 mostró una inhibición de la α -glucosidasa notablemente mayor (CI_{50} = 45,45 μ g/mL) que la de *C. longa* (135,03 μ g/mL), *C. asiatica* (88,07 μ g/mL) y acarbosa (113,92 μ g/mL). La actividad antioxidante también fue mayor en la combinación (CI_{50} = 40,68 μ g/mL). *In vivo*, el tratamiento combinado redujo la glucemia de forma comparable a la glibenclamida y mejoró la sensibilidad a la insulina, con un HTLI de 1,634 frente a 1,308 para la metformina. Los niveles de MDA se redujeron significativamente, lo que indica una menor peroxidación lipídica. Una correlación moderada entre la inhibición antioxidante y enzimática (r = 0,685) sugiere un vínculo mecanicista entre el estrés oxidativo y el metabolismo de los carbohidratos.

Conclusiones: La combinación de *C. longa* y *C. asiatica* demostró efectos antioxidantes y antidiabéticos sinérgicos, cumpliendo con los estándares de calidad, lo que respalda su potencial como terapia complementaria para el manejo de la diabetes.

Palabras Clave: antidiabético; antioxidante; *Centella asiatica*; *Curcuma longa*; efecto sinérgico.

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INTRODUCTION

About 589 million individuals worldwide were living with diabetes in 2024, corresponding to a global prevalence 1 in 9 among adults aged 20–79 years are living with diabetes, and this number is projected to rise to 853 million by 2050 (IDF, 2025). Prediabetes is also increasingly prevalent, particularly among older populations, with 48.8% of U.S. adults aged ≥ 65 years affected in 2020 (CDC, 2020). These figures highlight the urgent need for effective, safe, and accessible treatments to manage diabetes and its complications.

The increased incidence and consequences of diabetes mellitus have led to an increase in the need for treatment medicines that are both cost-effective and have few adverse effects. Herbal medicines have emerged as promising alternatives, with recent literature indicating that over 60% of pharmaceutical drugs have natural product origins (Newman and Cragg, 2020). Among these, turmeric (*Curcuma longa* L., Zingiberaceae) and gotu kola (*Centella asiatica* (L.) Urb., Apiaceae) have been used traditionally for their medicinal properties.

Curcumin, the major active compound in *C. longa*, possesses potent antioxidant activity, neutralizes free radicals, reduces oxidative stress, enhances insulin sensitivity, regulates blood glucose levels, and modulates fat metabolism and inflammation. Similarly, *C. asiatica* contains triterpenoid compounds such as asiaticoside and asiatic acid, which contribute to antioxidant defense, protect against oxidative damage, and reduce adipogenesis and lipid accumulation, thereby assisting in glycemic control and weight management. Oxidative stress plays a crucial role in the pathogenesis of diabetes, and numerous studies have shown that antioxidants can enhance insulin sensitivity and reduce hyperglycemia (Ceriello, 2000).

To ensure reproducibility and efficacy in herbal studies, the standardization of extracts is essential. Thin-Layer Chromatography (TLC) densitometry was employed in this study to quantify curcumin in *C. longa* and asiaticoside in *C. asiatica*, ensuring consistent active compound content in accordance with the Indonesian Herbal Pharmacopoeia (Nurhayati et al., 2020; Wahyuni et al., 2018). Previous research indicates that *C. longa* extract and its constituents significantly reduce fasting blood glucose levels and improve insulin resistance in diabetic animal models (Leksmi et al., 2014; Sunday and Udoh, 2020), while *C. asiatica* extract has shown comparable effects, largely attributed to asiaticoside (Fitrianda et al., 2017).

There is increasing evidence that combining multiple herbal extracts can lead to synergistic effects, wherein the pharmacological efficacy of each component is enhanced when used together. Such

combinations often yield greater antioxidant, anti-inflammatory, and antidiabetic effects than individual herbs. Additionally, they may lower the required dose of each component, potentially reducing the risk of toxicity. Although no specific synergy study has been conducted on *C. longa* and *C. asiatica*, the traditional use of their combination in herbal preparations suggests potential complementary mechanisms worthy of investigation.

Considering the antidiabetic and antioxidant properties of *C. longa* and *C. asiatica*, this study aimed to evaluate the effects of their single and combined extracts in reducing blood glucose levels and oxidative stress in streptozotocin-induced diabetic mice. We hypothesized that co-administration of standardized *C. longa* and *C. asiatica* extracts would exert synergistic antioxidant and antidiabetic effects exceeding those of the individual extracts.

MATERIAL AND METHODS

Plant materials and preparation

Rhizomes of *Curcuma longa* L. and aerial parts (leaves and stems) of *Centella asiatica* (L.) Urb. were collected from a local garden in Manoko, Lembang, West Java, Indonesia. The plant species were taxonomically identified at the Herbarium, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran (GPS: -6.9270° S, 107.7700° E). Plant materials were washed, cut into smaller pieces, and dried in a laboratory oven at 40°C until a constant weight was achieved. Dried samples were ground using a mechanical grinder and sieved through mesh No. 12 and 18 to obtain a uniform particle size.

Chemicals and reagents

All chemicals and reagents used were of analytical grade and obtained from Sigma-Aldrich (USA), Merck (Germany). These included ethanol (96%), methanol, chloroform, 2,2-diphenyl-1-picrylhydrazyl (DPPH) for antioxidant assay, α -glucosidase enzyme from *Saccharomyces cerevisiae*, p-nitrophenyl- α -D-glucopyranoside (pNPG), and acarbose (positive control) for α -glucosidase inhibitory activity. For lipid peroxidation analysis, reagents such as thiobarbituric acid (TBA), trichloroacetic acid (TCA), hydrochloric acid (HCl), and butylated hydroxytoluene (BHT) were used in the malondialdehyde (MDA) assay. Induction of insulin deficiency was performed using alloxan monohydrate dissolved in normal saline to induce diabetes in rats, while insulin resistance was induced by administration of a 25% fructose solution. Additional chemicals included sodium carboxymethyl cellulose (Na-CMC) as a suspending agent, glibenclamide as a reference antidiabetic drug, potassium oxonate, glucose reagent,

Tris-glycine chloride buffer, and ferrous sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$). All reagents were of analytical grade.

Animal models and ethical approval

All animal procedures were approved by the Health Research Ethics Committee, Bhakti Kencana University (Approval No. 091/09.KEPK/UBK/VI/2024) and conducted in accordance with international guidelines for animal care and use. Rats were randomly assigned to experimental groups to minimize selection bias under controlled temperature ($22 \pm 2^\circ\text{C}$), humidity ($55 \pm 10\%$), and a 12-hour light/dark cycle, with free access to standard feed and water. At the end of the study, euthanasia was performed using CO_2 inhalation followed by cervical dislocation to minimize suffering.

Extraction

The powdered *C. longa* rhizome and *C. asiatica* powders (each 1000 g) were extracted by maceration using 96% ethanol at a ratio of 1:10 (w/v) for 24 hours per cycle, and the process was repeated three times with fresh solvent for each cycle. The resulting extract was concentrated under reduced pressure using a rotary evaporator. Each extract, as well as its combinations, was then tested for antidiabetic and antioxidant properties.

Determination of curcumin and asiaticoside levels

Curcumin determination using TLC densitometry

A stock solution of 200 ppm was made by dissolving 10 mg of standard curcumin in 50 mL of ethanol. Then, standard solutions of various concentrations were prepared. 500 mg of *C. longa* extract was dissolved in 5 mL of ethanol for the sample solution. Sample and standards were applied (5 μL in triplicate) onto a TLC plate. The plate was developed in a chamber with a chloroform:methanol (95:5) mixture as the mobile phase. The plate was dried and scanned with a densitometer. The curcumin content in the sample was calculated using the peak area and the linear regression equation from the standard curve (Ministry of Health RI, 2017; Wahyuni et al., 2018).

Asiaticoside determination with TLC densitometry

A stock solution containing 2500 ppm of a standard asiaticoside was made by dissolving 5 mg of the asiaticoside in 2 mL of methanol. After that, the stock solution was diluted to create asiaticoside solutions in different concentrations. With a stationary phase of silica GF254 and a mobile phase of ethyl acetate: formic acid: water (8:1:1) optimized beforehand, the asiaticoside concentration was ascertained using TLC-

densitometry. *C. asiatica* herb extract was applied, 5 μL of each extract in triplicate, which is how spotting was done. The plates were heated on a hotplate after being eluted and sprayed with 10% H_2SO_4 , a universal solvent. The heating process was performed to enhance the visibility of the spots compared to their appearance before spraying and heating. The asiaticoside spots were measured using a densitometer. The asiaticoside content in the extract was calculated using a calibrated standard curve (Ministry of Health RI, 2017; Pratiwi et al., 2023).

Antioxidant activity assay

The DPPH radical scavenging activity was determined according to the method of Molyneux (2004) with slight modification. A 0.1 mM DPPH solution was prepared in methanol. Various concentrations of the extract (20–100 $\mu\text{g}/\text{mL}$) were mixed with an equal volume of DPPH solution and incubated for 30 min at room temperature in the dark. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The percentage of inhibition was calculated, and IC_{50} values were obtained by linear regression of concentration versus % inhibition (Jhansi and Kola, 2019; Molyneux, 2004).

Alpha-glucosidase inhibitory activity

The inhibitory activity of the extracts against α -glucosidase was evaluated using a modified method, which employed α -glucosidase from *Saccharomyces cerevisiae* and pNPG as the substrate, with acarbose serving as the positive control. Extract samples (*C. longa*, *C. asiatica*, and their combination) were prepared at concentrations of 50–350 $\mu\text{g}/\text{mL}$. In a 96-well plate, 50 μL of sample solution was mixed with 50 μL of α -glucosidase (0.5 U/mL in phosphate buffer, pH 6.8) and incubated at 37°C for 10 min. Subsequently, 50 μL of 5 mM pNPG was added, and the mixture was incubated for an additional 30 min at 37°C . The reaction was terminated by adding 100 μL of 0.2 M Na_2CO_3 .

The absorbance of the released p-nitrophenol was measured at 405 nm using a microplate reader. A blank (without enzyme) and a control (enzyme without inhibitor) were processed under identical conditions. The percentage inhibition was calculated as per equation [1].

$$\%I = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100\% \quad [1]$$

IC_{50} values were determined by linear regression of percentage inhibition versus concentration using five concentrations of each sample. Acarbose served as a positive control and was analyzed under the same conditions for comparison.

Insulin deficiency assay

White male Wistar rats aged 2–3 months and weighing 150–250 g (obtained from Bandung Institute of Technology) were used in this study. Prior to testing, the animals were adapted to new cage environments, including feeding routines. Rats were fasted for 12 hours to stabilize baseline blood glucose (T₀), then induced with alloxan monohydrate (200 mg/kg BW, intraperitoneal) dissolved in normal saline. After 3 days, fasting blood glucose was measured from the tail vein using a validated glucometer; rats with glucose levels greater than 200 mg/dL were considered diabetic (T₁) (Igodharo et al., 2017). Eight groups (n = 5 per group) were assigned: normal control, negative control (Na-CMC), positive control (glibenclamide), single extracts (*C. longa* and *C. asiatica* at 50 mg/kg BW), and combination extracts (1:1, 2:1, 1:2 ratios). All treatments were given orally for 14 days. Blood glucose was measured on days 0, 3, 7, and 14 (Banda et al., 2018).

Insulin resistance

Insulin resistance was induced by administering Lipofundin® mixed with 25% fructose at 0.42 mL/20 g BW orally once daily for 14 days (Susilawati et al., 2024). After induction, insulin sensitivity was evaluated using the Insulin Tolerance Test (ITT). Rats were fasted for 6 hours, then injected intraperitoneally with insulin (0.75 U/kg BW). Blood glucose levels were measured at 0, 15, 30, 45, and 60 minutes using an Easy Touch® glucometer (Susilawati et al., 2024). The rate constant for glucose disappearance (KITT, %/min) was calculated as equation [2].

$$KITT = \frac{0.693}{t_{1/2}} \quad \text{where} \quad t_{1/2} = \frac{\ln 2}{\text{Slope of } \ln(\text{glucose decline})} \quad [2]$$

Glucose data were log-transformed and plotted

against time, and the slope was determined by linear regression. The highest tolerance level of insulin (HTLI) was recorded as the maximum blood glucose level before and after therapy and used in conjunction with KITT to assess insulin sensitivity.

MDA measurement

After 14 days of treatment, rats were euthanized, and liver tissues were collected for oxidative stress analysis. A 20% liver homogenate was prepared in 0.01 M Tris-HCl buffer (pH 7.4). Lipid peroxidation was determined using the thiobarbituric acid reactive substances (TBARS) assay adapted from Nagababu et al. (2010) and Espín et al. (2017). Briefly, homogenates were incubated with FeSO₄ to initiate peroxidation, treated with the TBA reagent, and heated at 100 °C for 10 minutes. After cooling and centrifugation, the absorbance of the supernatant was measured at 532 nm using a UV-Vis spectrophotometer. MDA concentration was calculated from a standard curve using 1,1,3,3-tetraethoxypropane and expressed as nmol MDA per gram of liver tissue.

Statistical analysis

All data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using one-way ANOVA followed by Tukey's post hoc test to determine significance between groups. A p-value of less than 0.05 was considered statistically significant. In addition, Pearson's correlation analysis was performed to evaluate the relationship between antioxidant activity and antidiabetic parameters (α-glucosidase inhibition, insulin deficiency, and insulin resistance).

A schematic summary of the experimental design is presented in Fig. 1.

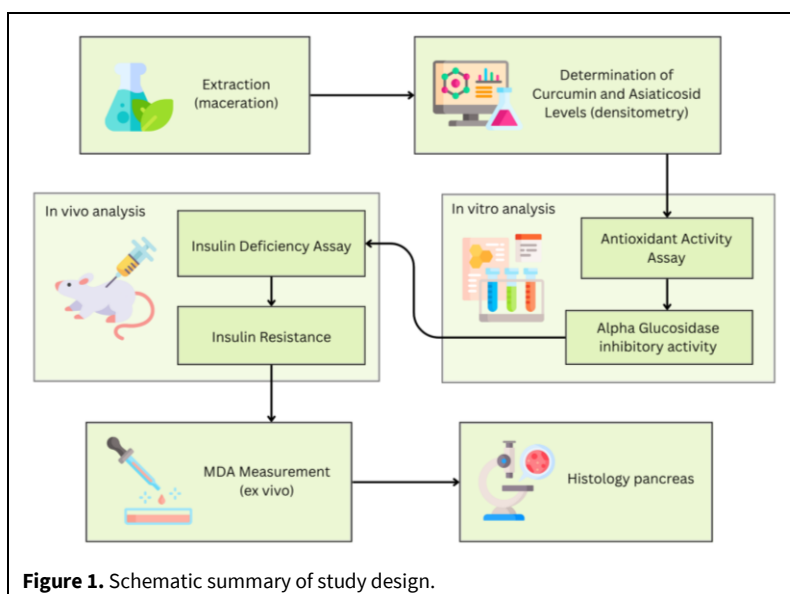


Table 1. Characterization of *Curcuma longa* rhizome and *Centella asiatica* herb.

Characterization parameters	IHP requirement (% w/w)	IHP result (% w/w)	IHP requirement (% w/w)	IHP result (% w/w)
Loss on drying	<10	7.33 ± 0.28	<10	7.83 ± 1.25
Total ash content	<8.2	6.67 ± 1.41	<11.6%	4.56 ± 0.51
Acid-insoluble ash content	<0.9	0.83 ± 0.57	<2.3%	1.93 ± 1.00
Water-soluble extract content	>11.5	18.33 ± 1.52	>15.4%	26.5 ± 1.80
Ethanol-soluble extract content	>11.4	7.66 ± 1.15	>4.4%	27.86 ± 1.02
Moisture content	<10	5 ± 0.1	<10	1.33 ± 1.44

Data are presented as mean ± standard deviation (n = 3). IHP: Indonesian Herbal Pharmacopoeia; SD: Standard deviation.

RESULTS

Characterization and extract yields

To ensure the quality and identity of raw materials, standardization of *C. longa* and *C. asiatica* crude drug materials were performed according to the Indonesian Herbal Pharmacopoeia. The evaluation included organoleptic characteristics, moisture content, ash values, and extractive values to confirm compliance with pharmacopeial requirements. Both crude drug materials met the standard quality parameters (Table 1) and yielded 19.8% and 16.6% crude extract, respectively.

TLC densitometry-based quantitative analysis of curcumin and asiaticoside content

Quantification of marker compounds is crucial for standardizing extracts. Therefore, the curcumin content in *C. longa* and the asiaticoside content in *C. asiatica* were determined using validated TLC-densitometry methods, as described by Wahyuni et al. (2018) with slight modifications. Standard curcumin and sample solutions were prepared in methanol and spotted (5 µL each, in triplicate) on silica gel 60 F254 plates (Merck). The chromatographic development was performed using chloroform:methanol (95:5 v/v) as the mobile phase. After development, the plates were visualized under UV light at 254 nm, and the curcumin bands were identified by comparison with the standard ($R_f \approx 0.65$). The plates were scanned using a TLC scanner (CAMAG TLC Scanner 4), and the peak areas were recorded. A calibration curve was constructed ($y = 6.915x + 482.9$; $R^2 = 0.996$), and the curcumin content was calculated as 11.642%, meeting the Indonesian Herbal Pharmacopoeia standard of $\geq 11.17\%$ (Ministry of Health of the Republic of Indonesia, 2017).

Similarly, asiaticoside content in *C. asiatica* extract was analyzed using TLC-densitometry following Pharmacopoeia guidelines (Pratiwi et al., 2023). Standard asiaticoside and sample solutions were applied (5 µL each, in triplicate) on silica gel 60 F254 plates. The

plates were developed in ethyl acetate:formic acid:water (8:1:1 v/v/v). After development, the plates were sprayed with 10% H_2SO_4 and heated at 105°C for 5 minutes to visualize the bands. Detection was performed at 366 nm after derivatization. Asiaticoside was identified based on $R_f \approx 0.32$, as compared to the reference standard. The calibration curve ($y = 3.4784x + 2345.4$; $R^2 = 0.9996$) was used to calculate the asiaticoside content, which was 0.984%, above the minimum requirement of 0.90% (Ministry of Health of the Republic of Indonesia, 2017).

Antioxidant activity assay with the DPPH method

To evaluate antioxidant potential, the radical scavenging activity of single and combined extracts was assessed using the DPPH assay. This method measures the ability of the samples to neutralize free radicals, expressed as IC_{50} values. This study found that combining extracts of *C. longa* rhizome and *C. asiatica* herb results in stronger antioxidant activity than using the individual extracts alone (Table 2).

The 1:1 combination of *C. longa* and *C. asiatica* extracts demonstrated the strongest DPPH radical scavenging activity, with an IC_{50} value of 40.68 µg/mL. This value was significantly lower than those of the individual extracts (60.15 µg/mL for *C. longa* and 67.67 µg/mL for *C. asiatica*) ($p < 0.05$), indicating a synergistic antioxidant effect. According to the classification described by Kedare and Singh (2011), substances with IC_{50} values below 50 µg/mL are considered to have 'very strong' antioxidant activity. For comparison, the positive control, vitamin C, exhibited an IC_{50} of 4.22 µg/mL.

Alpha-glucosidase inhibitory activity

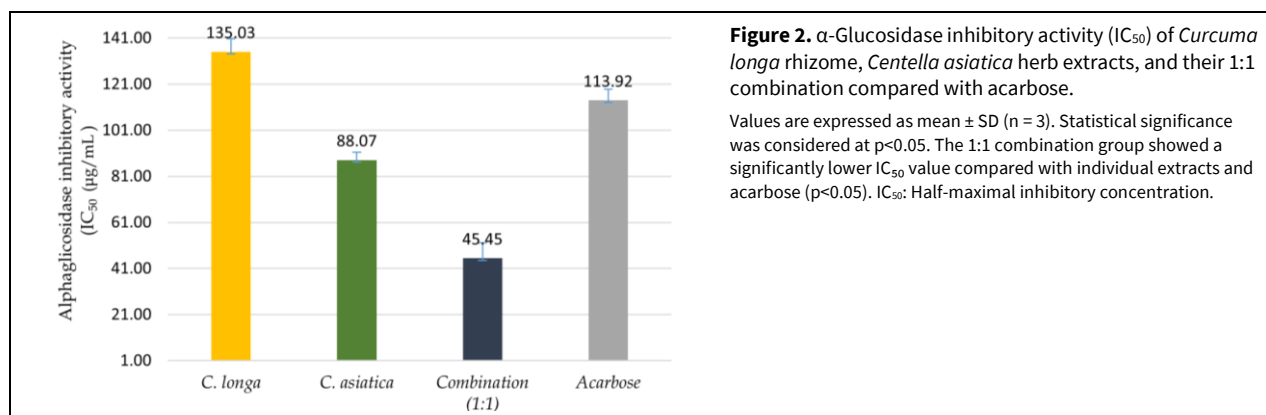
The α -glucosidase inhibitory activity was tested *in vitro* to determine the potential of the extracts to control postprandial hyperglycemia by delaying carbohydrate digestion. The α -glucosidase inhibitory activity assay showed that *C. longa* and *C. asiatica* extracts

Table 2. Antioxidant activity using the DPPH reduction method.

Sample	IC ₅₀ (µg/mL)
<i>Curcuma longa</i> extract	60.15 ± 1.16
<i>Centella asiatica</i> extract	67.67 ± 0.90
Combination (<i>Curcuma longa</i> : <i>Centella asiatica</i>) (1:1)	40.68 ± 0.75
Combination (<i>Curcuma longa</i> : <i>Centella asiatica</i>) (2:1)	47.14 ± 0.64
Combination (<i>Curcuma longa</i> : <i>Centella asiatica</i>) (1:2)	56.75 ± 0.54
Vitamin C	4.22 ± 0.32

IC₅₀ values were analyzed with linear regression. Data are presented as the mean ± SD of triplicate measurements.

IC₅₀: Half-maximal inhibitory concentration.



exhibited IC₅₀ values of 135.03 µg/mL and 88.07 µg/mL, respectively. Interestingly, the 1:1 combination of both extracts resulted in a significantly lower IC₅₀ value of 45.45 µg/mL (p<0.05), suggesting a synergistic effect. For comparison, the positive control, acarbose, showed an IC₅₀ of 113.92 µg/mL. Both extracts and their combinations inhibited α-glucosidase in a dose-dependent manner, with combinations showing stronger effects than single extracts (Fig. 2).

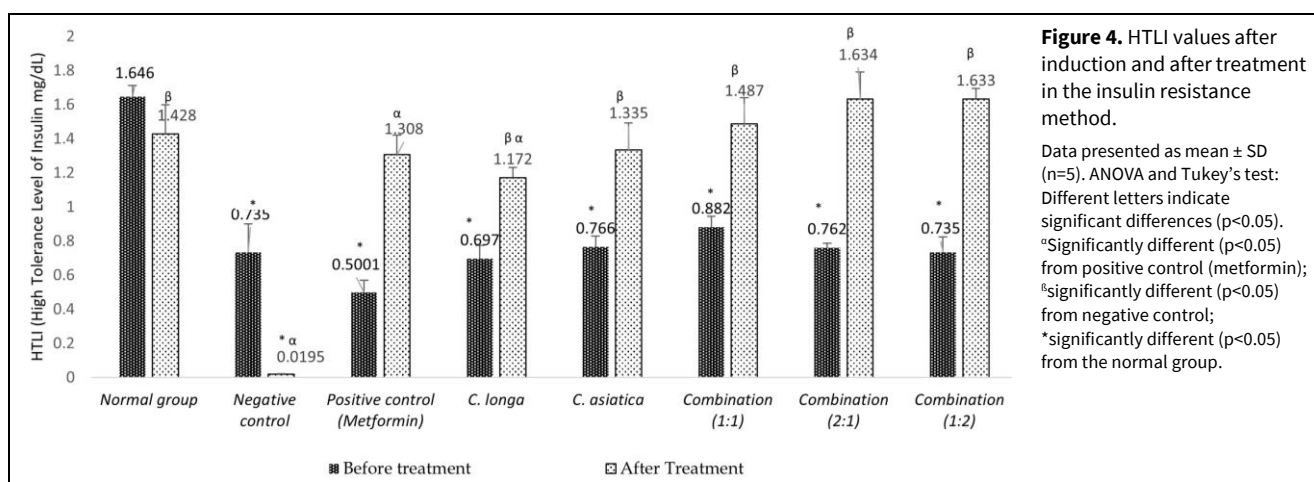
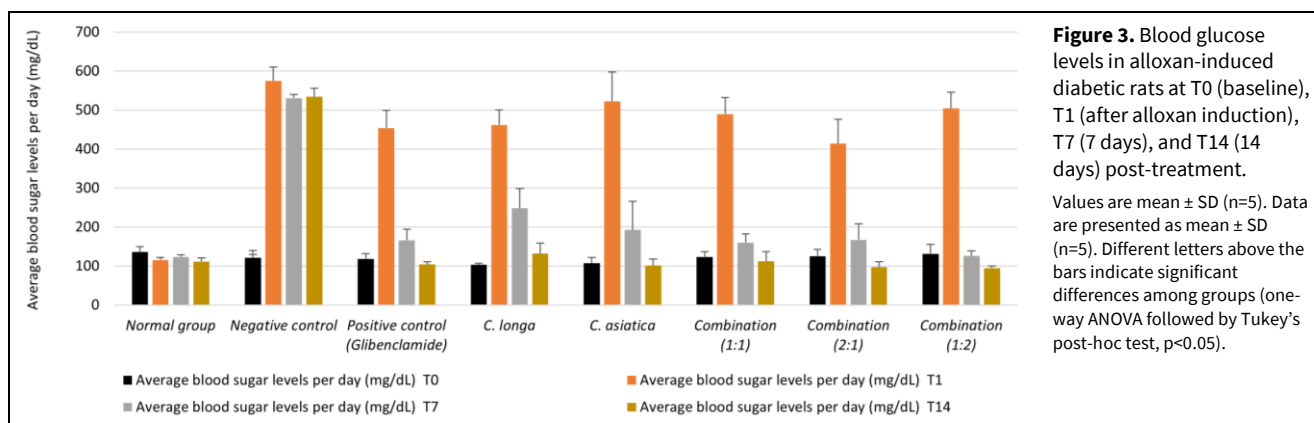
Antidiabetic activity in insulin deficient model

The antidiabetic activity of the *C. longa* and *C. asiatica* extracts was induced with alloxan (200 mg/kg BW) to simulate diabetes, and their fasting blood glucose levels were measured before and after treatment (Fig. 3). Fasting blood glucose levels at baseline (T0) ranged from 103.3 mg/dL to 136.3 mg/dL across all groups. After alloxan induction (T1), glucose levels increased significantly in all diabetic groups, with the negative control reaching 575.3 mg/dL. On day 14 (T14), treatment with *C. longa* and *C. asiatica* extracts reduced

blood glucose levels to 132 mg/dL and 101.3 mg/dL, corresponding to reductions of 71.4% and 80.6% from T1, respectively. The 1:1, 2:1, and 1:2 combinations further decreased glucose levels to 112.3, 97.3, and 94.7 mg/dL, respectively, which were statistically comparable to the glibenclamide group (104 mg/dL). Notably, the 1:2 combination demonstrated the highest percentage reduction (81.2%) from T1 to T14. These results indicate a synergistic antihyperglycemic effect of the combination treatments, particularly the 1:2 ratio of *C. longa* and *C. asiatica*.

Insulin resistance method

To assess insulin sensitivity, insulin resistance was induced using a high-fructose and lipid emulsion model. The HTLI was measured before and after treatment to reflect the rate of insulin-mediated glucose clearance. After induction, HTLI levels significantly declined in all diabetic groups, with the negative control group showing the lowest value (0.0195). Treatment with *C. longa* and *C. asiatica* extracts moderately improved HTLI levels to 1.308 and 1.335, respectively.



Notably, the 1:1 and 1:2 combination treatments restored HTLI values to 1.634 and 1.633, which were significantly higher than the positive control group treated with metformin (1.308) ($p < 0.05$).

These results indicate that the extract combinations, particularly in 1:1 and 1:2 ratios, enhanced insulin sensitivity more effectively than metformin, as reflected by higher HTLI values post-treatment. A higher HTLI value indicates a better response to insulin and improved glucose tolerance, suggesting enhanced insulin sensitivity (Fig. 4).

Malondialdehyde inhibitory activity

Liver MDA levels were measured in all experimental groups of the metabolic syndrome mouse model using TCA and TBA reagents, with absorbance recorded at 532 nm. MDA, a key end product of lipid peroxidation, is widely used as a biomarker of oxidative stress. All treatment groups exhibited significantly lower liver MDA levels compared to the untreated diabetic group ($p < 0.05$), though no significant differences were observed between single and combination extract treatments (Fig. 5). Among the combination groups, the 1:1 ratio yielded an MDA level of 3.98 $\mu\text{g/mL}$, which was approximately 231.7% higher

than the normal group, indicating partial but not complete restoration to baseline oxidative status.

Pearson correlation analysis between antioxidant and antidiabetic parameters

To further explore the relationship between antioxidant and antidiabetic activities of the tested herbal extracts, Pearson correlation analysis was performed between antioxidant IC_{50} values (DPPH method), alpha-glucosidase inhibitory IC_{50} , and HTLI values. The results are summarized in Table 3.

The correlation matrix indicates a moderate positive correlation between antioxidant activity and alpha-glucosidase inhibition ($r = 0.685$), suggesting a potential synergistic mechanism of action. However, the correlation between antioxidant activity and insulin sensitivity is weak ($r = 0.084$), implying that other factors, such as direct modulation of insulin signaling pathways, may play a more critical role in improving insulin sensitivity. This analysis supports the hypothesis that the antioxidant and antidiabetic effects observed are partially linked through enzyme inhibition, while improvements in insulin sensitivity are likely mediated through different mechanisms.

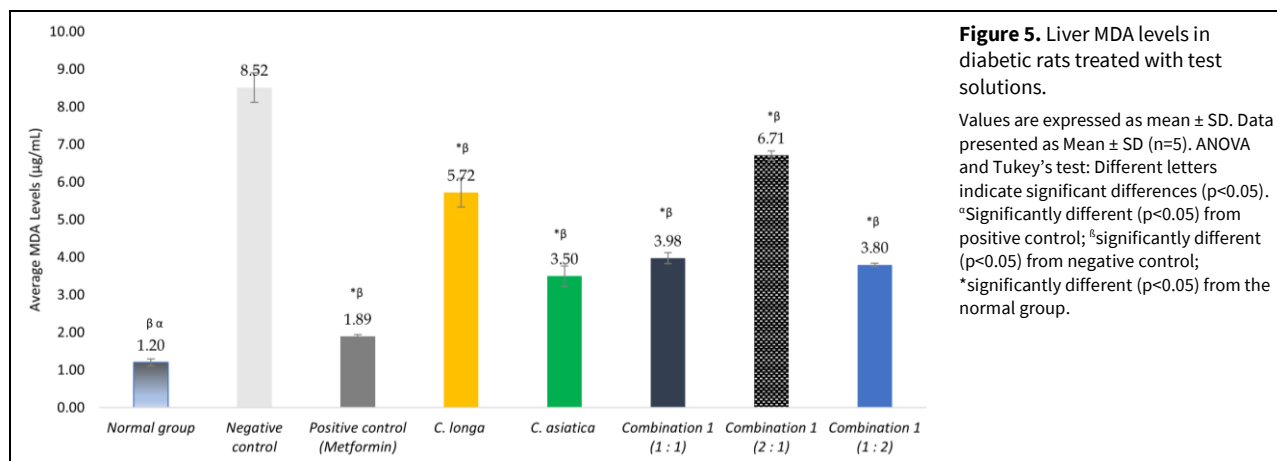


Figure 5. Liver MDA levels in diabetic rats treated with test solutions.

Values are expressed as mean $\pm$ SD. Data presented as Mean $\pm$ SD (n=5). ANOVA and Tukey's test: Different letters indicate significant differences ($p < 0.05$). ^aSignificantly different ($p < 0.05$) from positive control; ^bsignificantly different ($p < 0.05$) from negative control; *significantly different ($p < 0.05$) from the normal group.

Table 3. Pearson correlation between antioxidant and antidiabetic parameters.

Parameters	Antioxidant IC ₅₀	Alpha-glucosidase IC ₅₀	HTLI
Antioxidant IC ₅₀	1.000	0.685	0.084
Alpha-glucosidase IC ₅₀	0.685	1.000	-0.668
HTLI	0.084	-0.668	1.000

Statistical significance was set at $p < 0.05$. HTLI: Highest tolerated level of insulin.

Histology of pancreas with HE staining

Histological examination of pancreatic tissues (Fig. 6) revealed marked structural damage in the islets of Langerhans of untreated diabetic rats, with evident shrinkage and disruption of β -cell architecture. In contrast, treatment groups showed varying degrees of islet preservation. The turmeric extract and the 1:1 combination group notably demonstrated more intact islet morphology with increased β -cell presence compared to the diabetic control.

Quantitative cell counts supported these observations: the normal group had 148 β -cells and 82 α -cells, while the negative control had only 70 β -cells and 44 α -cells. The turmeric group restored β -cells to 100%, while the 1:1 and 2:1 combinations showed 95% and 105% β -cells, respectively, approaching near-normal levels. Among the treatment groups, the 2:1 combination produced the highest β -cell count, suggesting improved regeneration potential.

DISCUSSION

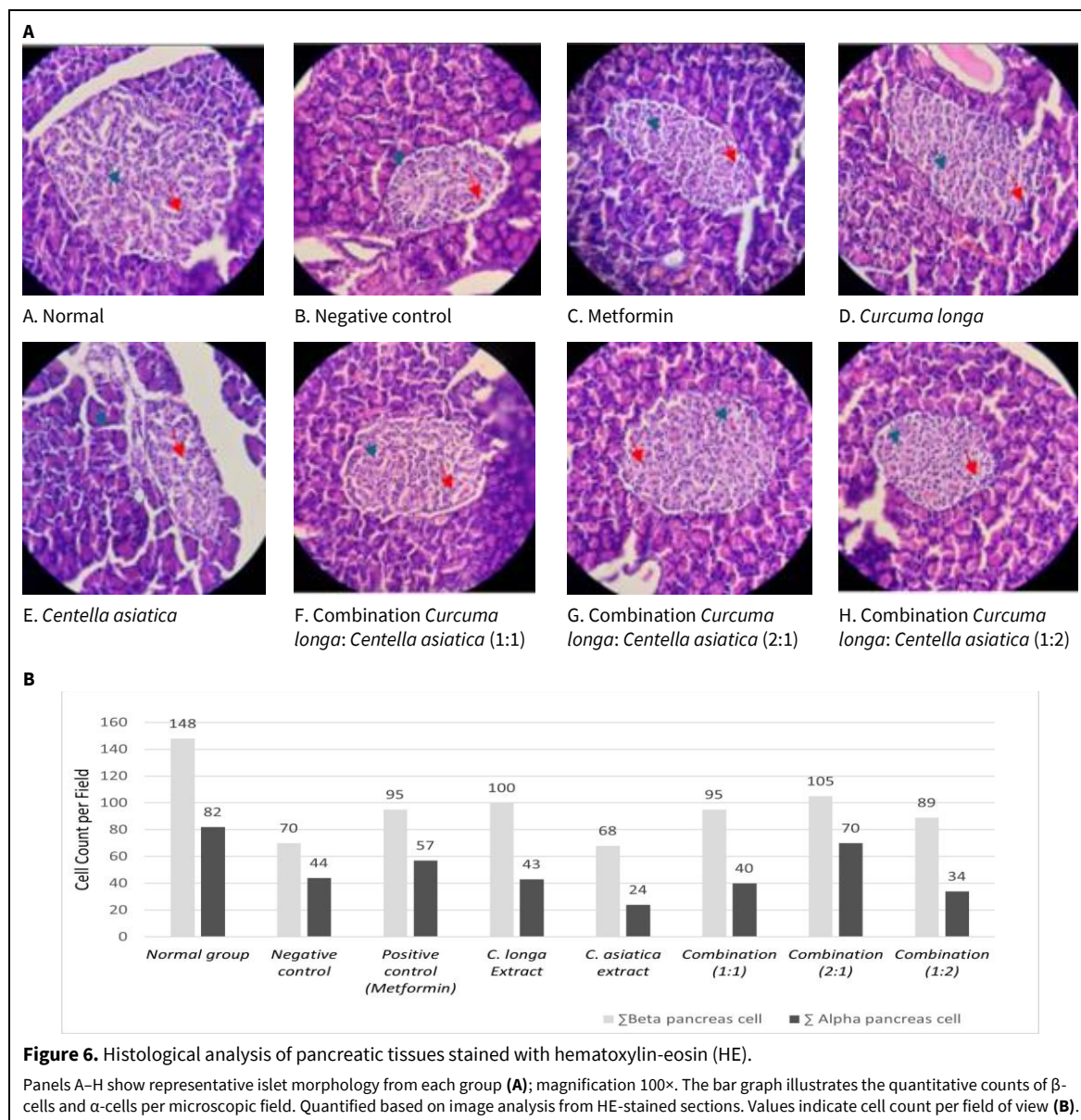
This study confirmed that *C. longa* and *C. asiatica* extracts met the Indonesian Herbal Pharmacopoeia standards, with curcumin and asiaticoside contents of 11.6% and 0.98%, respectively. These results confirm the suitability of both plants as sources for standardized herbal antioxidant therapy.

The DPPH assay revealed that the antioxidant activity of each extract, as measured by IC₅₀ values, was

strong (*C. longa* = 60.15 ± 1.16 $\mu\text{g/mL}$; *C. asiatica* = 67.67 ± 0.90 $\mu\text{g/mL}$), while combinations, especially in a 2:1 ratio, showed significantly enhanced activity (IC₅₀ = 47.14 ± 0.64 $\mu\text{g/mL}$). This suggests a synergistic effect between curcumin and asiaticoside, which may enhance radical scavenging capacity.

The 2:1 combination demonstrated an antioxidant activity approaching that of vitamin C (IC₅₀ = 4.22 ± 0.32 $\mu\text{g/mL}$), a benchmark antioxidant, highlighting its potential therapeutic relevance. The enhanced effect likely arises from the complementary antioxidant mechanisms of both compounds. These findings align with previous studies that demonstrated DPPH scavenging activity of *C. asiatica* (Harun-Or-Rashid et al. 2023) and curcumin-rich *C. longa* (Razavi et al., 2021). The enhanced effect in our study likely stems from the complementary antioxidant mechanisms of both compounds.

Curcumin exerts its antioxidant effects by scavenging reactive oxygen species (ROS) through hydrogen or electron donation from its phenolic OH group, stabilizing free radicals (Ghasemzadeh and Ghasemzadeh, 2011). It also chelates transition metal ions (Fe²⁺, Cu²⁺), thereby preventing the Fenton reaction and subsequent ROS generation (Menon and Sudheer, 2007). Additionally, it inhibits oxidative enzymes and regenerates endogenous antioxidants, such as vitamins C and E (Gupta et al., 2013).



Several processes are involved in the antioxidant action of asiaticoside, a triterpenoid that is present in *C. asiatica*. It stabilizes these dangerous compounds by directly scavenging free radicals such as ROS and reactive nitrogen species (RNS) by donating electrons (Cheng et al., 2004). Additionally, by activating the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway and upregulating the expression of important enzymes such as glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD), Asiaticoside modifies endogenous antioxidant defense systems (Jiang et al., 2022). Furthermore, it prevents oxidative stress-induced lipid, protein, and DNA damage by raising glutathione (GSH) levels and decreasing MDA levels. These combined mechanisms make asiaticoside a promising agent in mitigating oxidative damage and related pathologies.

The IC₅₀ values of the individual extracts fall within the strong antioxidant category, whereas the combination exhibited significantly greater activity, classified as very strong. (Table 2). This enhancement suggests a synergistic interaction, likely mediated by curcumin and asiaticoside, which act through radical scavenging and inhibition of lipid peroxidation, thereby reducing oxidative stress. Additionally, the combination of *C. longa* rhizome and *C. asiatica* demonstrated antioxidant activity comparable to that of vitamin C, a well-established and powerful antioxidant, highlighting the potential for natural antioxidant formulations in health applications (Jhansi and Kola, 2019).

The α-glucosidase enzyme plays a pivotal role in carbohydrate metabolism by catalyzing the final step in the digestive process of carbohydrates into absorbable monosaccharides. Inhibiting this enzyme

effectively delays glucose absorption and reduces postprandial blood glucose spikes, a strategy widely employed in the management of type 2 diabetes using drugs such as acarbose (Chuengsamarn et al., 2012). In this study, the individual extracts of *C. longa* ($IC_{50} = 135.03 \pm 5.66 \mu\text{g/mL}$) and *C. asiatica* ($IC_{50} = 88.07 \pm 3.31 \mu\text{g/mL}$) exhibited moderate inhibitory activity against α -glucosidase (Fig. 2). However, the 1:1 combination of both extracts showed a significantly stronger inhibitory effect, with an IC_{50} of $45.45 \pm 6.76 \mu\text{g/mL}$ —approximately 60% lower than that of acarbose ($IC_{50} = 113.92 \pm 4.77 \mu\text{g/mL}$). This suggests that the combination has a synergistic effect, potentially due to the interaction of bioactive compounds such as curcumin and asiaticoside. Curcumin has been reported to inhibit carbohydrate-digesting enzymes and suppress postprandial hyperglycemia, likely by interfering with enzyme active sites and exerting antioxidant protection on pancreatic β -cells (Menon and Sudheer, 2007). Asiaticoside, a major compound in *C. asiatica*, also contributes to enzyme inhibition while modulating oxidative pathways through Nrf2 activation and enhancing endogenous antioxidant systems (Jiang et al., 2022; Loushambam and Singh, 2024). The superior activity of the combination may result from complementary modes of action: curcumin contributes through enzyme binding and radical scavenging, while asiaticoside enhances cellular antioxidant responses and may indirectly reduce enzyme activity by mitigating oxidative stress. These findings are consistent with previous reports showing enhanced bioactivity from polyherbal formulations (Jhansi and Kola, 2019). Thus, the 1:1 combination of *C. longa* and *C. asiatica* not only outperformed their individual extracts but also demonstrated higher potency than the reference drug acarbose. This supports their potential development as a natural α -glucosidase inhibitor, either as a standalone therapy or as an adjunct to existing antidiabetic medications.

In insulin deficiency, the body is unable to effectively regulate blood glucose levels. Alpha-glucosidase inhibitors play a role in slowing the digestion of carbohydrates into glucose in the intestine, thereby helping to reduce post-meal blood glucose spikes (Matsui and Yoshimoto, 2021). Curcumin contributes to pancreatic β -cell protection by enhancing endogenous antioxidant defenses, exerting glucose-lowering effects, and stimulating insulin secretion through its antioxidant and anti-inflammatory mechanisms. The antioxidant content in both supports beta cell regeneration (Li et al., 2020; Menon and Shudeer, 2007). Rats with blood glucose levels exceeding 200 mg/dL were considered diabetic, indicating successful induction by alloxan, which selectively destroys pancreatic beta cells, impairing insulin production (Banda et al., 2018). After 7 and 14 days of treatment with the extracts, a significant

reduction in blood glucose levels was observed in the groups treated with *C. longa* and *C. asiatica* extracts, either individually or in combination, as well as in the group treated with glibenclamide (positive control) (Fig. 3). This reduction in blood glucose is attributed to the presence of bioactive compounds in the extracts, curcumin has been shown to lower blood glucose levels in diabetic animals by improving pancreatic function and asiaticoside exhibited significant antidiabetic effects, as reported in previous studies where it reduced blood glucose levels in alloxan-induced diabetic rats (Fitrianda et al., 2017; Leksmi et al., 2014; Sunday et al., 2020).

Insulin resistance is a hallmark of type 2 diabetes mellitus, characterized by the inability of peripheral tissues to respond adequately to insulin, resulting in compensatory hyperinsulinemia and impaired glucose homeostasis. The HTLI is a functional parameter that quantifies the body's capacity to utilize exogenous insulin to maintain normoglycemia; thus, higher HTLI values indicate improved insulin sensitivity (Susilawati et al., 2024). In this study, alloxan-induced diabetic rats exhibited a significant decline in HTLI, with the negative control group showing a p-value of 0.0195 compared to the normal group (1.646), confirming the successful induction of insulin resistance. Treatment with metformin, used as the positive control, moderately improved insulin sensitivity, increasing the HTLI to 0.500. Interestingly, the administration of single extracts of *C. longa* and *C. asiatica* resulted in higher HTLI values (0.690 and 0.764, respectively) than metformin (Fig. 4). These findings align with previous reports demonstrating the insulin-sensitizing effects of curcumin through anti-inflammatory and antioxidant pathways (Menon and Sudheer, 2007) and the β -cell protective actions of asiaticoside (Jiang et al., 2022).

Notably, the combination extracts produced further enhancement in insulin sensitivity. The 1:1 ratio exhibited the highest HTLI value ($p=0.887$), followed by 2:1 ($p=0.764$) and 1:2 ($p=0.735$). These values were all greater than the standard drug, indicating that the combined administration of *C. longa* and *C. asiatica* resulted in a synergistic effect. This synergy may be attributed to the complementary mechanisms of action: curcumin improves peripheral insulin sensitivity through the PI3K/Akt pathway, while asiaticoside contributes to β -cell regeneration and antioxidant defense. The data strongly support the potential of this herbal combination as a promising natural adjuvant in improving insulin sensitivity and managing insulin resistance, surpassing the efficacy of either individual extract or standard treatment with metformin (Fig. 4).

The mice were euthanized on the final day of the trial, and their livers and pancreas were isolated. Hepatic MDA levels were measured to evaluate oxidative

stress and lipid peroxidation status. As shown in Fig. 5, MDA levels were significantly elevated in the negative control group (alloxan-induced diabetic rats), indicating oxidative damage to liver tissues. All treatment groups, including *C. longa*, *C. asiatica*, and their combinations, demonstrated a significant reduction in hepatic MDA compared to the untreated diabetic group ($p < 0.05$), suggesting effective antioxidant action. However, no statistically significant difference was observed between the combination and the single-extract groups ($p > 0.05$), indicating that both curcumin and asiaticoside, the main bioactive compounds, already exert potent antioxidant effects independently.

MDA levels, a measure of cell damage caused by lipid peroxidation, were found to be significantly higher in mice in the potassium oxonate induction group on average. Potassium oxonate induction in mice can increase MDA levels in tissues through enhanced oxidative stress and lipid peroxidation. Lipid peroxidation occurs as a result of the generation and propagation of free radicals, which primarily target unsaturated fatty acids within the cell membrane. This process leads to the formation of MDA, a major by-product that significantly contributes to membrane damage (Espín et al., 2017). These findings align with earlier reports that curcumin (Menon and Sudheer, 2007) and asiaticoside (Mahapatra et al., 2021) can attenuate lipid peroxidation by scavenging ROS and stabilizing cell membranes.

Histological analysis of pancreatic tissues was conducted using hematoxylin and eosin (H&E) staining to evaluate the structural integrity of the islets of Langerhans. As shown in Fig. 6, notable morphological differences were observed between treatment groups. The normal control group exhibited well-preserved and compact islets, while the negative control group (alloxan-induced) showed disrupted architecture with vacuolated and shrunken islets, indicating significant β -cell damage. In contrast, the metformin-treated group displayed partial restoration of islet structure. This suggested that therapy had improved the Langerhans islet. Histological examination with H&E staining provides a clear visualization of the pancreatic tissue architecture, including the islets of Langerhans, where alpha (α) and beta (β) cells are located (Kusriani et al., 2023). Among the herbal treatments, *C. longa* extract showed the highest β -cell preservation, with a 42% increase in β -cell count compared to the negative control, suggesting its strong cytoprotective effect. This finding is consistent with previous studies, which report that curcumin protects pancreatic tissue and enhances β -cell regeneration through its antioxidant and anti-inflammatory mechanisms (Menon and Sudheer, 2007). Although *C. asiatica* alone showed modest improvements in islet morphology, the 1:1 combination

of *C. longa* and *C. asiatica* yielded islet integrity comparable to metformin, highlighting a potential synergistic effect. The histological findings align with biochemical data on glucose reduction, further supporting the therapeutic potential of these extracts in preserving pancreatic function under diabetic conditions.

The Pearson correlation analysis presented in this study supports the hypothesis that antioxidant activity may contribute to enzyme inhibition, which in turn plays a role in regulating postprandial blood glucose levels. The moderate correlation between DPPH IC_{50} and alpha-glucosidase IC_{50} ($r = 0.685$) aligns with earlier research suggesting that oxidative stress and carbohydrate metabolism are interconnected. In contrast, the lack of strong correlation between antioxidant levels and HTLI values ($r = 0.084$) highlights the complex, multifactorial nature of insulin resistance, where anti-inflammatory and cellular signaling pathways beyond oxidative status may dominate (Jiang et al., 2022; Susilawati et al., 2024). The combination of *C. longa* and *C. asiatica* extracts demonstrated synergistic antioxidant and antidiabetic effects, with their combination exhibiting superior performance in both *in vitro* and *in vivo* assays compared to single extracts and standard drugs. These results suggest a promising potential for the integration of herbal therapy in diabetes management.

Although this study demonstrated the antidiabetic and antioxidant potential of the combination of *C. longa* and *C. asiatica*, further investigations are needed. Future studies should focus on long-term toxicity evaluations to ensure safety, conduct clinical trials in humans to confirm efficacy, and perform mechanistic studies, such as gene expression analysis, to elucidate the molecular pathways involved in the observed effects.

CONCLUSION

The combined extracts of *Curcuma longa* and *Centella asiatica* met pharmacopeial quality standards and demonstrated higher antioxidant capacity and stronger α -glucosidase inhibitory activity than individual extracts. These findings suggest a synergistic effect that may contribute to improved glycemic control, supporting the potential use of these compounds in diabetes management. Future studies focusing on long-term safety, clinical evaluation, and mechanistic insights are warranted to facilitate the translational application of this combination therapy.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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GENERATIVE ARTIFICIAL INTELLIGENCE (AI)

The authors used ChatGPT (Open IA) to improve the language and readability of the manuscript. The authors reviewed and edited the content and take full responsibility for its accuracy. No images were generated or manipulated using AI tools.

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Contribution	Kusriani H	Mulyani Y	Santoso R	Jafar G	Ishmah FZ	Nadhira AZ
Concepts or ideas	x	x	x			
Design	x	x	x			
Definition of intellectual content	x	x	x			
Literature search	x	x	x	x	x	x
Experimental studies	x	x	x	x	x	x
Data acquisition	x			x	x	x
Data analysis	x	x		x	x	x
Statistical analysis	x		x			x
Manuscript preparation	x			x	x	
Manuscript editing	x	x	x			
Manuscript review	x	x	x	x	x	x

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