



# Phytochemical analysis, antioxidant potential, and in vitro antidiabetic activity of *Grewia lasiodiscus* (K Schum) leaves extract

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## ABSTRACT

**Introduction:** *Grewia lasiodiscus* is recognized for its therapeutic potential in addressing emesis, diabetes, hypertension, and oxidative stress. This research evaluated the in vitro antioxidant and antidiabetic activities of *G. lasiodiscus* leaf extract using *Saccharomyces cerevisiae* yeast in a glucose absorption model.

**Methods:** Phytochemical screening, quantification of phenols, flavonoids, and condensed tannins, in conjunction with in vitro antioxidant evaluations using the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) test and Ferric-reducing power test (FRAP) were conducted on the hydroethanolic extract. The assessment of the antidiabetic impact involved the measurement of yeast cell glucose absorption, with an additional investigation into their glucose adsorption capabilities.

**Results:** Phytochemical analysis showed the presence of tannins, steroids, flavonoids, terpenes, glycosides, and phenols in the hydroethanolic extract, while alkaloids and saponins were absent. Quantitative analysis revealed substantial levels of phenols ( $200.92 \pm 0.00$  milligrams equivalent to catechin per gram (mgEqC/g)), flavonoids ( $31.75 \pm 0.25$  mgEqC/g), and condensed tannins ( $2740.44 \pm 142.66$  mgEqC/g). The extract demonstrated noteworthy antioxidant activity, corresponding to an  $IC_{50}$  value of  $91.11 \pm 0.11$   $\mu$ g/mL in the DPPH test and ferric reducing power of  $11.35 \pm 1.42$  mmol/L. Furthermore, the extract enhanced glucose absorption by yeast cells, reaching up to 75%, in a manner directly proportional to glucose concentration and extract weight, indicating dose-dependent effects across glucose levels.

**Conclusion:** *G. lasiodiscus* leaf extract exhibited significant antioxidant and antidiabetic properties probably attributed to its phenolic compounds. Further research, including in vivo studies and toxicity assessments, is imperative to ascertain its therapeutic potential in managing diabetes mellitus.

### Implication for health policy/practice/research/medical education:

*Grewia lasiodiscus* showed potent antioxidant and antidiabetic properties, which have implications for medical practices and ongoing research. Further preclinical and clinical validation can solidify its potential role in diabetes management.

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## Introduction

Diabetes represents a metabolic disorder arising from either a complete or relative insufficiency of insulin, insulin resistance, or a combination of both factors.

Diabetes mellitus is marked by an extended period of elevated blood sugar levels (1). The burgeoning diabetes epidemic in developing nations underscores the pressing challenges surrounding access to healthcare, treatment,

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education, and the management of individuals with diabetes and its associated complications (2). In both type 1 and type 2 diabetes, the chronic elevation of glucose levels leads to diabetic complications in various target organs due to impaired glucose absorption (3). The pathogenic consequences of hyperglycemia are thought to involve heightened generation of reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) (3). Furthermore, maintaining glucose homeostasis in diabetic patients can potentially mitigate the diverse complications linked to the condition (4).

Current pharmacological therapies employed in diabetes treatment encompass a range of oral hypoglycemic agents and insulin. Nevertheless, these pharmaceutical interventions fall short of fully restoring normal glucose balance and are also associated with adverse effects. Therefore, given the drawbacks associated with existing drug regimens, exploring the potential of medications derived from traditional antidiabetic plants appears promising in terms of safety, affordability, and efficacy (5). The utilization of natural products abundant in antioxidants and hypoglycemic agents can play a pivotal role in preventing diabetic complications, owing to their effectiveness and the accessibility of medicinal plants (4,6). Despite the widespread utilization of various plants for treating diabetes in Sub-Saharan Africa, scientific investigations into their efficacy remain limited (7).

The investigation of natural substances for their potential medicinal properties has garnered substantial attention within the pharmaceutical and medical research arenas. Natural compounds often offer distinct advantages compared to synthetic drugs, including a higher degree of biocompatibility and a reduced likelihood of adverse effects (8). In this context, *Grewia lasiodiscus*, a plant renowned for its therapeutic properties in addressing conditions such as emesis, diabetes, hypertension, and oxidative stress, emerges as a promising avenue for research. Despite the historical utilization of various *Grewia* species, including *G. lasiodiscus* in traditional medicine, a significant research void exists in the scientific literature regarding their pharmacological attributes (9). This study aims to bridge this knowledge gap by investigating the phytochemical screening, the in vitro antioxidative potential, and the antidiabetic potential of leaf extracts from *G. lasiodiscus*, employing innovative methodologies.

## Materials and Methods

### Plant materials

In August 2021, the leaves of *G. lasiodiscus* were collected from the Sara-Kawa region (9.64954 N, 9°38'5835552; 1.19571 E, 1° 11'44.55312), situated approximately 40 km from Kara in the Kozah prefecture of Togo. The collection was conducted during the flowering and ripening phase of the plant. Authentication and botanical identification of

the *G. lasiodiscus* leaves were carried out at the Botany and Plant Ecology Laboratory, Faculty of Sciences, University of Lomé, and assigned reference number TOGO 15873. The leaves underwent a thorough wash with water and were subsequently dried naturally at ambient room temperature. Following this, the dried leaves were finely powdered using a grinding mill. The obtained powder was carefully placed into a labeled plastic pouch, safeguarded in a well-ventilated environment, and shielded from direct sunlight until further analysis.

### Chemicals and reagents

This study employed analytical-grade chemicals and equipment. The following chemicals and reagents were utilized: ascorbic acid, quercetin, aluminum trichloride ( $\text{AlCl}_3$ ), iron III trichloride ( $\text{FeCl}_3$ ), 1,1-diphenyl-2-picryl-hydroxyl (DPPH), sodium carbonate ( $\text{Na}_2\text{CO}_3$ ), Folin-Ciocalteu reagent, gallic acid, metronidazole, 2,4,6-Tripyridyl-S-triazine (TPTZ), catechin, glucose, Fehling liquor A and B, and acetic acid.

All these chemicals and reagents were procured from Merck (Merck KGaA, Darmstadt, Germany). Additionally, other chemicals such as ethanol, sulfuric acid, hydrochloric acid, soda ash, and chloroform were sourced from the same supplier.

### Extraction

A 100 g portion of *G. lasiodiscus* powder underwent maceration in 1 L of a hydroethanolic solvent (95:5 v/v) with intermittent agitation over a 72-hour duration. After completing the maceration process, the mixture passed through Whatman paper filtration, and the resulting filtrate was subsequently concentrated via vacuum evaporation at a temperature of 45 °C until a dry extract was achieved. The dry extract was carefully preserved at a temperature of 4 °C for subsequent experiments. The extraction yield, based on the initial weight of the plant material, was found to be 8.76% (w/w).

### Determination of major phytochemical groups in *Grewia lasiodiscus* extract

The *G. lasiodiscus* extract was subjected to characterization for major chemical groups, which included phenols, flavonoids, tannins, saponins, terpenes, alkaloids, reducing sugars, free quinones, cardiac glycosides, condensed tannins, sterols, and triterpenes. These characterizations were conducted through coloration and precipitation reactions, following well-established methods (10,11).

### Total phenolic content

Quantification of the total phenolic content was performed spectrophotometrically with Folin-Ciocalteu reagent based on a colorimetric approach, as described in the literature (12). This method was designed to assess the concentration of hydroxyl groups present within the

extract. For the assay, 200 µL of the extract was added to a test tube containing 1 mL of Folin-Ciocalteu reagent and diluted tenfold. After a 4-minute incubation period, 800 µL of a 7.5% sodium carbonate solution was introduced. The tubes were gently mixed and then placed in a dark environment at room temperature for 30 minutes. The absorbance was measured at 765 nm using a UV-visible 5100B spectrophotometer. A calibration curve was constructed using gallic acid, spanning concentrations ranging from 10 to 100 µL. Each concentration was tested in triplicate. The findings were reported as milligram equivalent of gallic acid per gram of dry extract (mg EqGA/g).

#### Total flavonoids content

The total flavonoid content of the extract was assessed using the aluminum chloride (AlCl<sub>3</sub>) assay (13). The assay protocol included combining 1 mL of a 2% (w/v) AlCl<sub>3</sub> solution in pure ethanol with 1 mL of the extract, which was also dissolved in ethanol at a concentration of 1 mg/mL. Following a 10 minutes incubation in the absence of light, the absorbance of the mixture was assessed at 415 nm using a UV-visible spectrophotometer, with a blank used for reference. To create the standard range, quercetin solutions with concentrations spanning from 10 to 100 µg/mL were prepared using the same procedures as for the extract. A calibration curve was established using these diverse quercetin concentrations. The assay results were reported as milligram equivalent of quercetin per gram of dry extract (mg EqQ/g) based on the calibration curve.

#### Condensed tannins determination

The method employed for determining condensed tannins relied on a combination of the vanillin method and hydrochloric acid. This method is based on the interaction between vanillin and the terminal flavonoid group of condensed tannins, resulting in the formation of red-colored complexes. This color change, where tannins transform into red anthocyanins upon reacting with vanillin, serves as the basis for the formation of these complexes. The evaluation of condensed tannin content followed the vanillin method as detailed in Julkunen-Titto (14). For the assay, a 50 µL portion of the extract was vigorously mixed with 1.5 mL of a 4% vanillin/methanol solution. Subsequently, 750 µL of concentrated hydrochloric acid (HCl) was introduced to the mixture. The resulting solution underwent a 20 minutes reaction at room temperature. The absorbance was then measured at 550 nm using a blank for reference. A calibration curve was established using various concentrations ranging from 0 to 1000 µg/mL, derived from a catechin stock solution. The assay results were quantified as milligrams of catechin equivalent per gram of the sample (mg EqC/g), based on the calibration curve.

#### In vitro antioxidant assays

##### DPPH free radical scavenging activity assay

The extract ability to scavenge free radicals was assessed by employing DPPH as a stable free radical, following the procedure outlined by Bakoma et al (15) with minor modifications. For the assay, 100 µL of the extract, dissolved in methanol at a concentration of 1 mg/mL, was introduced to 2 mL of a methanolic solution containing DPPH (0.004%). The mixture was vigorously vortexed to ensure homogeneity, and its absorbance was measured at 517 nm using a spectrophotometer following a 30 minutes incubation at room temperature, with protection from light. Three independent tests were conducted for each individual sample. As a positive control, a solution of a standard antioxidant, quercetin (with concentrations ranging from 0 to 1000 mg/mL), was prepared, and its absorbance was assessed under the same conditions as the sample. The DPPH radical scavenging activity was quantified as the inhibition percentage using the following formula:

$$\text{Inhibition percentage (\%)} = [(Abs\ control - Abs\ sample) / Abs\ control] \times 100$$

where Abs control is the absorbance of the control and Abs sample is the absorbance of the sample.

The inhibition concentration 50 (IC<sub>50</sub>) values of quercetin and extract were generated by GraphPad Prism 8 (USA).

##### Ferric reducing antioxidant power (FRAP) assay

The iron III reducing capability of the extract was assessed using established methods (16,17). The FRAP test involves assessing the extract capacity to release an electron, thereby converting Fe<sup>3+</sup> into Fe<sup>2+</sup>. This conversion can be quantified by measuring the resulting formation of Fe<sup>2+</sup> ions. The FRAP solution was prepared by mixing 25 mL of acetate buffer, 2.5 mL of Fe<sup>3+</sup>-TPTZ (10 mM) in HCl (40 mM), and 2.5 mL of FeCl<sub>3</sub>·6H<sub>2</sub>O (20 mM/L). To 900 µL of the FRAP solution, 30 µL of the extract (1 mg/mL) and 90 µL of distilled water were incorporated. The optical density was measured at 593 nm when the intense blue color became apparent. Calibration was conducted using an iron sulfate solution (FeSO<sub>4</sub>). Each concentration was tested three times, and the results were expressed in millimoles of FeSO<sub>4</sub> per gram (mM of FeSO<sub>4</sub>/g).

#### In vitro antihyperglycemic activity

##### Glucose uptake in yeast cells

To evaluate glucose uptake in yeast cells, a suspension of commercial baker yeast in distilled water was prepared through repeated centrifugation at 3000 x g for 5 minutes until clear supernatant was obtained. A 1% (v/v) yeast suspension was subsequently prepared in distilled water. In the experimental arrangement, different concentrations of

plant extracts, spanning from 50 to 2000 µg/mL in distilled water, were introduced to 1 mL of glucose solutions with concentrations of 5, 10, and 25 mM. The mixture was then incubated at 37 °C for 10 minutes. The reaction was triggered by adding 100 µL of the yeast suspension, followed by vortexing, and continued incubation at 37 °C for 60 minutes. After this 60 minutes period, the tubes were centrifuged at 2500 × g for 5 minutes, and the glucose content in the supernatant was quantified (18). Metronidazole served as the standard drug for comparison, as it is known to facilitate glucose absorption in a dose-dependent manner without impairing yeast activity (19–23).

The percentage increase in glucose uptake by yeast cells was computed using the following formula:

$$\text{Increase in glucose uptake (\%)} = \frac{ABS \text{ sample} - ABS \text{ control}}{ABS \text{ control}} \times 100$$

With ABS control: The absorbance of the control reaction (containing all reagents except the test sample);  
ABS sample: The absorbance of the test sample.

#### Glucose adsorption capacity of the extract

The glucose adsorption capacity of the extract was assessed following the procedure described by Ou et al (24). About 1 g of the extract was combined with 100 mL of glucose solution at five different concentrations: 5, 10, 15, 20, and 30 mM. Each mixture was meticulously blended, agitated, and subsequently placed in a shaking water bath set at 37 °C for a duration of 6 hours. After the incubation period, the mixtures were centrifuged for 20 minutes at 4800 rpm. The glucose concentration in the supernatant was measured using a glucose oxidase peroxidase diagnostic kit. The amount of bound glucose per gram of dry extract was calculated using the following formula:

$$\text{Glucose bound} = \frac{B1 - B2}{\text{Weight of the extract}} \times \text{Volume of the sample}$$

where B1 represents the glucose concentration of the original solution and B2 the glucose concentration after 6 hours.

#### Statistical analysis

Statistical analyses were conducted using GraphPad Prism 8 software, based in the USA. The results obtained from the assays, which included flavonoids, total phenols, condensed tannins (using the MetaSpecPro standard curve), and antioxidant tests (FRAP and DPPH) were reported as mean ± SEM (Standard Error of the Mean). For the anti-diabetic test results, a one-way ANOVA analysis was carried out with a significance level established at  $P < 0.05$ . This statistical approach enabled the assessment of significant differences and the determination of the statistical significance of the observed results.

## Results

### Phytochemicals determination

The phytochemical screening conducted on our hydroethanolic extract confirmed the presence of various chemical constituents, as summarized in Table 1. The analysis indicated the presence of tannins, sterols, triterpenes, phenols, flavonoids, cardiac glycosides, reducing sugars, free quinones, and condensed tannins in the extract.

### Determination of phenols, flavonoids, and condensed tannins contents

The quantities of major components, including phenols, flavonoids, and condensed tannins are displayed in Table 2.

### Antioxidant assay

The results of the DPPH free radical reduction test were determined from regression curves. The extract exhibited promising antioxidant activity with an  $IC_{50}$  value of  $66.46 \pm 0.08$  µg/mL for quercetin, in contrast to  $91.11 \pm 0.11$  µg/mL for the extract (Figure 1). Additionally, utilizing the FRAP method, we measured a ferric reducing power of  $11.35 \pm 1.42$  mmol Equivalent  $Fe^{2+}$ /g extract.

### In vitro evaluation of antihyperglycemic activity

#### Glucose uptake in yeast cells

The in vitro antidiabetic activity of the hydroethanolic

**Table 1.** Phytochemical screening of *Grewia lasiodiscus* leaves extract

Chemicals groups	Leaves extract
Alkaloids	-
Flavonoids	+
Phenols	+
Tannins	+
Terpenes	+
Reducing sugars	+
Cardiac glycosides	+
Free quinones	+
Condensed tannins	+
Sterols and triterpenes	+
Saponosides	-

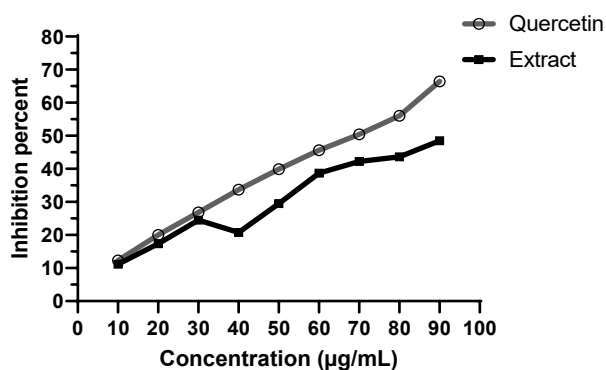
(+) Presence; (-) Absence

**Table 2.** Quantification of phenols, flavonoids, and condensed tannins

Groups	Contents*
Phenols	200.92 ± 0.00 mgEqGA/g
Flavonoids	31.75 ± 0.25 mgEqQ/g
Condensed tannins	2740.44 ± 142.66 mgEqC/g

\*Values are expressed as mean ± SD (n=3) in milligrams equivalent to gallic acid per gram (mgEqGA/g), milligrams equivalent to quercetin per gram (mgEqQ/g), and milligrams equivalent to catechin per gram (mgEqC/g).





**Figure 1.** Scavenging radical DPPH inhibition percentage of quercetin and *Grewia lasiodiscus* extract tested at different concentrations (Values are expressed as mean with n=3).

extract obtained from *G. lasiodiscus* leaves demonstrated its ability to promote glucose uptake through the yeast cell plasma membrane at initial glucose concentrations of 5 mM (Figure 2A), 10 mM (Figure 2B), and 25 mM (Figure 2C). The *G. lasiodiscus* leaf extracts exhibited the ability to augment glucose uptake across the yeast cell membrane (Figure 2). Specifically, at glucose concentrations of 5 mM and 10 mM, the glucose uptake by the extract was not significantly different from that of the glucose absorption enhancer standard, metronidazole ( $P < 0.05$ ) (Figures 2A and 2B). However, at a glucose concentration of 25 mM, metronidazole exhibited a slightly greater effect (Figure 2C).

Furthermore, the glucose uptake capacity of *G. lasiodiscus* at a concentration of 1 mg/mL exceeded 50% (Figures 2A and 2B), and it nearly reached 75% at a 5 mg/mL concentration of the extract (Figure 2A). This suggests that as the extract concentration increases, yeast cells become more proficient at absorbing glucose from their surroundings. Conversely, a negative correlation was noted in relation to glucose molar concentration. This pattern was apparent when comparing glucose uptake by yeast cells at concentrations of 5 mM and 25 mM, while keeping the extract quantity constant (Figures 2A-C). Based on these findings, it can be inferred that yeast

cells tend to absorb more glucose in solutions with lower glucose concentrations, highlighting the dose-dependent nature of the extract. Conversely, this dose-dependent pattern was not observed in the case of metronidazole.

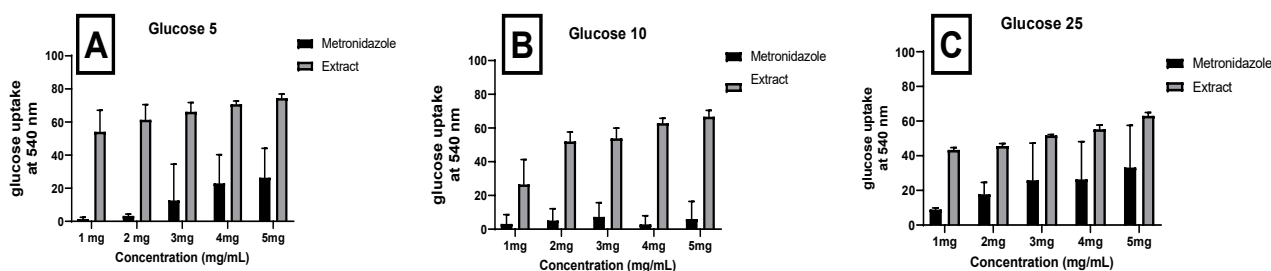
#### Glucose adsorption capacity

The results of in vitro glucose adsorption by the *G. lasiodiscus* extract are illustrated in Figure 3. This study unveiled significant disparities in glucose adsorption between the extract and the control group ( $P < 0.05$ ), underscoring the effectiveness of the extract in adsorbing glucose molecules. At the highest glucose concentration of 30 mM, glucose adsorption of  $0.78 \pm 0.16$  mmol per gram of dry extract was observed. A clear dose-dependent relationship of the extract was demonstrated across nearly all tested glucose concentrations (Figure 3).

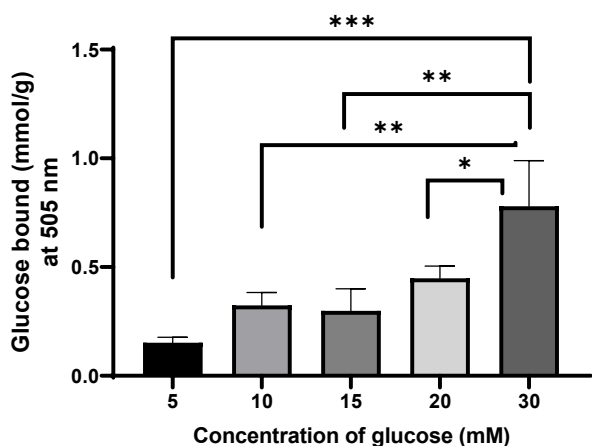
#### Discussion

The phytochemical screening of the hydroethanolic extract of *G. lasiodiscus* unveiled the presence of several significant groups of active compounds, as delineated in Table 1. These included tannins, sterols, triterpenes, phenols, flavonoids, cardiac glycosides, reducing sugars, free quinones, and condensed tannins. Notably, alkaloids and saponins were absent in the extract. The identification of phenols, flavonoids, and condensed tannins within our extract signifies its richness in phenolic compounds.

The presence of these phytochemical constituents in the extract serves as a basis for understanding and validating the traditional use of *G. lasiodiscus* in the treatment of various ailments such as emesis, diabetes, and cancer (9,25). Moreover, it suggests the extract potential applicability in addressing a range of pathologies, considering the well-documented antioxidant activity associated with phenolic compounds. Each of these compound groups is linked to diverse biological activities and potential therapeutic effects (9). For instance, tannins are recognized for their antioxidant and anti-inflammatory properties. The existence of these bioactive compounds in the extract hints at *G. lasiodiscus* potential for a wide spectrum of pharmacological activities, which may elucidate its traditional usage in treating various diseases (26,27).



**Figure 2.** Glucose uptake percentage by yeast cells in the presence of *Grewia lasiodiscus* extract or metronidazole standard at glucose initial concentrations of (A) 5 mM, (B) 10 mM, and (C) 25 mM. Values are expressed as mean  $\pm$  SD (n=3).



**Figure 3.** Glucose adsorption capacity expressed in millimole per gram of dry extract of *Grewia lasiodiscus*. Values are expressed as means  $\pm$  SEM. Adsorptions were compared at different concentrations of glucose with significant difference (\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ).

Hyperglycemia is intricately linked to oxidative stress, characterized by an imbalance between the production of free radicals, ROS, peroxides, and their enzymatic neutralization, as well as cellular defense provided by antioxidants (28). Diabetic individuals often experience heightened production of ROS and lipid peroxidation, both contributing to the pathophysiology of diabetes (29). Antioxidant treatments have demonstrated significant improvements and preventive effects against cardiovascular complications and diabetes (30). These findings underscore the potential therapeutic value of our extract, given its rich phenolic compound content, including condensed tannins, which suggests beneficial effects in combating oxidative stress and managing hyperglycemia associated with diabetes.

The *in vitro* anti-diabetic activity of the *G. lasiodiscus* leaf extract was evidenced by its ability to enhance glucose uptake across the plasma membrane of yeast cells. The process of glucose uptake in yeast cells is influenced by various factors, including intracellular glucose concentration and its subsequent metabolism by yeast enzymes (31). When a significant portion of internal glucose is converted into other metabolites, the internal glucose concentration decreases, leading to increased glucose uptake into the cells. The uptake of glucose by yeast cells in the presence of the *G. lasiodiscus* extract may involve facilitated diffusion along the concentration gradient and efficient glucose metabolism (31). Tannins and flavonoids may play a role in this diffusion (32-34).

Moreover, the adsorption of glucose by the extract displayed a direct correlation with the glucose concentration at the same extract level. The lowest adsorption was observed at a glucose concentration of 5 mM, while the highest adsorption occurred at 30 mM. This finding underscores the extract significant binding

capacity for glucose, indicating its potential as a glucose-binding agent. This adsorption capacity may be attributed to the presence of tannins in the extract, as prior studies have demonstrated that polyphenols and the porous surface structure of tannins contribute to their high glucose binding capacity (35). Phenolic groups, commonly found in tannins, exhibit a relatively high affinity for binding glucose (36). Tannins also possess the capability to absorb glucose in peripheral tissues, which can stimulate insulin secretion or reduce protein glycation (37). The adsorption of glucose by the extract within the intestinal lumen of a patient may contribute to the reduction of postprandial blood glucose levels (38).

Overall, these findings suggest that *G. lasiodiscus* leaf extract demonstrates promising anti-diabetic activity by promoting glucose uptake and adsorbing glucose, potentially mediated by its phenolic compounds, particularly tannins. *G. lasiodiscus* extract could play a role in managing postprandial hyperglycemia and hold potential therapeutic applications in diabetes treatment.

## Conclusion

In conclusion, our study investigated the antioxidant and anti-diabetic properties of the *G. lasiodiscus* leaf extract. The results demonstrated a robust antioxidant capacity, which was associated with the presence of key chemical groups such as phenols, flavonoids, and tannins. This antioxidant capacity translated into enhanced glucose uptake by yeast cells when treated with the extract, highlighting its potential as an anti-diabetic agent. Future research should prioritize the isolation and identification of the specific compounds responsible for these observed activities. This endeavor could pave a way for the development of cost-effective and efficient treatments for diabetes management.

## Authors' contribution

**Conceptualization:** Eloh Kodjo and Oudjaniyobi Simalou.

**Data curation:** Biham Koza and Sabrina Chris Janiba Sanvee.

**Formal analysis:** Biham Koza and Sabrina Chris Janiba Sanvee.

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**Investigation:** Biham Koza, Sabrina Chris Janiba Sanvee and Marie France Bakäi.

**Methodology:** Eloh Kodjo and Oudjaniyobi Simalou.

**Project administration:** Eloh Kodjo and Oudjaniyobi Simalou.

**Resources:** Biham Koza, Sabrina Chris Janiba Sanvee and Marie France Bakäi.

**Software:** Biham Koza and Sabrina Chris Janiba Sanvee.

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**Validation:** Eloh Kodjo and Oudjaniyobi Simalou.

**Visualization:** Eloh Kodjo, Oudjaniyobi Simalou and Sabrina Chris Janiba Sanvee.

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**Writing–review & editing:** Eloh Kodjo, Oudjaniyobi Simalou, Biham Koza and Sabrina Chris Janiba Sanvee.

### Conflict of interests

The authors declare that there is no conflict of interest.

### Data availability statement

All data is included in this manuscript; however, additional information may be obtained from the authors upon request.

### Ethical considerations

This study does not involve the use of animal or human models. Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, and redundancy) have been ultimately observed by the authors.

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