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In-vitro evaluation of antidiabetic and antioxidant activities of lime juice extract of Gossypium herbaceum leaves

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ABSTRACT

Management of diabetes mellitus increasingly focuses on controlling postprandial hyperglycemia through inhibition of carbohydrate-hydrolyzing enzymes responsible for gastrointestinal glucose release and absorption. This study evaluated the in vitro antidiabetic and antioxidant activities of a lime juice extract derived from Gossypium herbaceum leaves. Fresh leaves were extracted using lime juice (Citrus aurantifolia) as a natural solvent medium. Inhibitory activities against α-amylase and α-glucosidase were assessed alongside antioxidant capacity using DPPH, ABTS, and hydroxyl radical scavenging assays at concentrations of 7.8125–1000 µg/mL, conducted in triplicate using standard spectrophotometric protocols. Acarbose served as the reference enzyme inhibitor, while butylated hydroxytoluene (BHT) was used as the antioxidant standard. The extract exhibited concentration-dependent inhibition of both carbohydrate-digesting enzymes and significant free radical scavenging activity. Although reference standards demonstrated higher activity across most concentrations, ABTS radical scavenging increased progressively with dose, approaching BHT performance at higher concentrations. Notably, hydroxyl radical scavenging at 31.25 µg/mL showed no significant difference from BHT, indicating strong antioxidant potential at moderate dosage. In contrast, α-glucosidase inhibition remained significantly lower than acarbose ($p < 0.05$) at all tested concentrations, suggesting moderate regulation of carbohydrate digestion rather than complete enzyme suppression. These findings demonstrate that lime-mediated extraction of Gossypium herbaceum leaves yields appreciable hypoglycemic potential through partial enzyme inhibition combined with enhanced antioxidant defense mechanisms. Synergistic contributions from ascorbic acid and bioactive phytochemicals present in Citrus aurantifolia likely underpin the observed bioactivity. Overall, the extract represents a promising natural candidate for development as an oral antidiabetic phytomedicine aimed at complementary diabetes management.

Keywords: Gossypium herbaceum; Citrus aurantifolia; antidiabetic activity; antioxidant activity; α-amylase inhibition; α-glucosidase inhibition.

INTRODUCTION

Diabetes mellitus (DM) is a chronic, heterogeneous metabolic disorder defined by persistent hyperglycemia arising from defects in insulin secretion, action, or both. This disrupts carbohydrate, lipid, and protein metabolism, with hyperglycemia serving as the hallmark diagnostic biomarker. Untreated, it precipitates long-term complications including micro- and macrovascular damage, nephropathy, neuropathy, retinopathy, cardiovascular disease, and heightened risks of certain cancers and neurodegenerative disorders (Amirqulova et

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al., 2024; Parameswari et al., 2025). Globally, DM imposes a profound public health burden, affecting over 537 million adults—a figure projected to escalate markedly by 2045 (Genitsaridi et al., 2026; Huang et al., 2025).

The two primary forms are type 1 DM, an autoimmune condition impairing pancreatic insulin secretion, and type 2 DM, the predominant variant, characterized by insulin resistance and relative insulin deficiency. Current therapies encompass exogenous insulin and oral hypoglycemics, including α-glucosidase inhibitors (e.g., acarbose, miglitol, voglibose) that delay carbohydrate digestion and attenuate postprandial hyperglycemia (Dash et al., 2018; McKeirnan and Rodin, 2023).

Antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) address oxidative damage, yet prolonged use of these synthetics raises concerns over adverse effects like endocrine disruption, carcinogenicity, and cost, spurring interest in safer natural alternatives.

Hyperglycemia fosters reactive oxygen species (ROS) generation via glucose auto-oxidation, protein glycation, and polyol pathway activation. Excess ROS, such as superoxide anion (O_2^-), hydroxyl radical ($^{\cdot}OH$), and hydrogen peroxide (H_2O_2), inflict damage on proteins, lipids, and DNA, exacerbating pancreatic β-cell dysfunction and insulin resistance. Thus, agents that concurrently lower glycemia and scavenge radicals hold substantial therapeutic promise.

Medicinal plants abundant in polyphenols, flavonoids, and antioxidants offer viable antidiabetic sources. Gossypium herbaceum L. (Malvaceae), known as cotton, features prominently in African and Asian traditional medicine for infections, inflammation, wounds, reproductive issues, and gastrointestinal disorders (Olanrewaju et al., 2025; Catherine et al., 2023).

Its leaf extracts exhibit antimicrobial, anti-inflammatory, antifertility, and antispermatogenic effects, with phytochemical profiling revealing flavonoids, phenolics, alkaloids, tannins, saponins, terpenes, and other bioactives (Mili et al., 2025; Larayetan et al., 2021). Emerging evidence suggests metabolic benefits, including pancreatic β-cell regeneration post-streptozotocin injury (Roy et al., 2025), highlighting its antidiabetic relevance.

Lime juice (Citrus aurantifolia Swingle), replete with vitamin C, organic acids, and flavonoids, imparts antioxidant activity and enhances extraction of polar and non-polar phytochemicals (Karki et al., 2024; Vig et al., 2026).

Acidic media like lime juice boost phenolic and flavonoid yields from plant matrices, amplifying radical-scavenging efficacy. While G. herbaceum and lime juice have been examined separately, their synergy via lime-based extraction for antidiabetic and radical-scavenging effects remains underexplored.

This study thus evaluates the in vitro antidiabetic and radical-scavenging activities of a lime juice extract from G. herbaceum leaves, focusing on α-amylase and α-glucosidase inhibition (key to carbohydrate digestion) and free radical scavenging via DPPH, ABTS, and hydroxyl radical assays. Results are benchmarked against acarbose (enzyme inhibition) and BHT (antioxidant activity) to gauge the extract's promise as a natural adjunct for DM management.

MATERIALS AND METHODS

Plant materials and authentication

Fresh, mature leaves of Gossypium herbaceum L. (Figure 1) and ripe Citrus aurantifolia (lime) fruits were collected from a farm in Offa, Kwara State, Nigeria, during the dry season. The plant was identified by its local name and formally authenticated at the Department of Plant Biology, University of Ilorin, Nigeria, where a voucher specimen was deposited for future reference.

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Figure 1: Image of Gossypium herbaceum plant taken from a farm site in Offa, Kwara State, Nigeria

Chemicals and reagents

All chemicals, reagents, and commercial assay kits used in this study were of analytical grade and high purity. Porcine pancreatic α-amylase, α-glucosidase from Saccharomyces cerevisiae, 4-nitrophenyl-β-D-glucopyranoside (PNPG), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), acarbose, and butylated hydroxytoluene (BHT) were purchased from Sigma-Aldrich (St. Louis, USA). Iron (II) sulfate, hydrogen peroxide, and sodium phosphate were obtained from Biophore, India (pharmaceutical division). All stock and working solutions were freshly prepared with distilled water, which was used consistently throughout the study.

Preparation of lime-juice extract of Gossypium herbaceum leaves

Fresh leaves of Gossypium herbaceum were separated from stems and thoroughly rinsed with clean water to remove surface contaminants. The leaves were air-dried in a shaded, well-ventilated area in the laboratory to preserve phytochemical integrity, avoiding direct sunlight and overheating. The dried leaves were pulverized into a fine powder using an electric blender and stored in airtight containers at room temperature until use.

Lime fruits (Citrus aurantifolia) were washed with distilled water, sliced, and manually squeezed using a sterile juice extractor. The crude juice was filtered through a muslin cloth followed by Whatman No. 1 filter paper to obtain a clear filtrate, which was used immediately as the extraction solvent.

Extraction was carried out using the maceration method described by Franz et al. (2018). Briefly, 100 g of G. herbaceum leaf powder was macerated in 500 mL of freshly prepared lime juice. The mixture was kept at room temperature (25 ± 2 °C) for 72 h with intermittent shaking to facilitate diffusion of phytochemicals into the solvent. After 72 h, the extract was filtered first through muslin cloth and then through Whatman No. 1 filter paper to obtain a clear filtrates. The filtrate was concentrated under reduced pressure using a rotary evaporator at a temperature below 40 °C. The concentrated extract was further evaporated to dryness on a water bath to yield a semi-solid crude extract, which was stored in a sterile, airtight bottle in a refrigerator at 4 °C until further

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use. For each assay, the extract was reconstituted in an appropriate solvent (e.g., distilled water or assay buffer) and serially diluted prior to testing.

In vitro antidiabetic activity assays

α-Amylase inhibition assay

The α-amylase inhibitory activity of the lime-juice extract was determined spectrophotometrically according to the method of Bernfeld (1955), with minor modifications. Briefly, 40 µL of the extract at various concentrations (7.8125, 15.625, 31.25, 62.5, 125, 250, 500, and 1000 µg/mL) was incubated with 40 µL phosphate buffer (pH 6.9) and 40 µL α-amylase solution (porcine pancreatic α-amylase) at 37 °C for 10 min. After pre-incubation, 40 µL of 1% (w/v) starch solution was added, and the mixture was further incubated at 37 °C for 15 min. The reaction was stopped by adding 100 µL of glucose reagent, and the mixture was incubated for another 15 min at 37 °C. Acarbose (1–5 mg/mL) was used as the positive control. Absorbance was measured at 505 nm in a UV–visible spectrophotometer. A blank was prepared for each concentration by replacing the enzyme with 100 µL of distilled water at the start of the reaction, and absorbance was read at 540 nm. Percentage inhibition was calculated using Equation 1:

$$\text{Inhibition } (\%) = \frac{A_{control} - A_{sample}}{A_{control}} x100 \qquad (1)$$

where $A_{control}$ is the absorbance of the control (enzyme + substrate, no inhibitor) and

A_{sample} is the absorbance of the test sample (enzyme + substrate + extract).

α-Glucosidase inhibition assay

The α-glucosidase inhibitory activity was assessed using the method of Apostolidis et al. (2007), adapted for in vitro conditions. Briefly, 1 mL of 2% (w/v) maltose or sucrose solution was mixed with 50 µL of the extract at the same concentration range (7.8125–1000 µg/mL). The reaction was initiated by adding 100 µL of α-glucosidase solution (1 U/mL, S. cerevisiae), and the mixture was incubated at 37 °C for 10 min. Twenty microliters of 4-nitrophenyl-β-D-glucopyranoside (PNPG) was then added, and the reaction continued for 20 min at 37 °C. The reaction was terminated by adding 100 µL of 1 M Na_2CO_3. Acarbose (1–10 mg/mL) was used as the standard inhibitor. The mixture was incubated for an additional 5 min at 25 °C, and absorbance was measured at 405 nm. Liberated glucose was quantified using the glucose oxidase–peroxidase method, and inhibitory activity was expressed as percentage inhibition using Equation 1.

In vitro antioxidant activity assays

DPPH radical scavenging assay

The free-radical scavenging capacity of the extract was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, following the method of Blois (1958) as modified by Brand-Williams et al. (1995). Briefly, 20 µL of the extract at various concentrations was incubated with 200 µL of 0.1 mM DPPH in methanol, while the control contained 20 µL of solvent (distilled water) instead of extract. The reaction mixtures were incubated in the dark at room temperature for 20 min, and the reduction in absorbance was measured at 517 nm in a UV–visible spectrophotometer. BHT was used as the positive control. The percentage DPPH radical scavenging activity was calculated using Equation 1.

ABTS radical scavenging assay

The ABTS radical cation ($ABTS^+$) scavenging capacity of the extract was determined according to the method described by Re et al. (1999). $ABTS^+$ was generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate

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and allowing the mixture to stand in the dark at room temperature for 12–16 h. The stock solution was diluted with phosphate-buffered saline to an absorbance of 0.70 ± 0.02 at 734 nm. In triplicate, 20 µL of the extract at different concentrations was mixed with 200 µL of the $ABTS^{+}$ working solution and incubated in the dark for 30 min. The decrease in absorbance was measured at 734 nm. BHT was used as the standard antioxidant. The percentage ABTS radical scavenging activity was calculated using Equation 1.

Hydroxyl radical scavenging assay

The hydroxyl (·OH) radical scavenging activity of the extract was evaluated using the deoxyribose degradation assay based on the Fenton reaction, as described by Apak et al. (2011). In triplicate, 50 µL of the extract at different concentrations (and 50 µL of BHT solution as standard) were added to the reaction mixture containing 50 µL of 2-deoxy-D-ribose, 20 µL of $Fe_2(SO_4)_3$, and 50 µL of H_2O_2, with 100 µL of salicylic acid used to trap the radicals. The mixture was incubated at 37 °C for 1 h. Malondialdehyde (MDA) formed was measured as thiobarbituric acid reactive substances (TBARS) by adding 1 mL of 1% thiobarbituric acid in 0.05 M NaOH and heating at 95 °C for 45 min, followed by cooling and measurement of absorbance at 532 nm. The percentage hydroxyl radical scavenging activity was calculated using Equation 2:

$$\% \text{ Hydroxyl radical scavenging} = \frac{[A_0 - (A_1 - A_2)]}{A_0} \text{x } 100 \qquad (2)$$

where A_0 is the absorbance of the control (without sample), A_1 is the absorbance of the reaction mixture with sample and 2-deoxy-D-ribose, and A_2 is the absorbance of the sample without 2-deoxy-D-ribose. Dose-response curves were plotted as percentage inhibition versus concentration.

Statistical analysis

All assays were performed in triplicate, and results are presented as mean ± standard deviation (SD). Statistical analysis was carried out using Microsoft Excel and GraphPad Prism version 8.0 (GraphPad Software, USA). Comparisons among groups were performed using one-way analysis of variance (ANOVA) followed by appropriate post-hoc tests when required. A p-value less than 0.05 was considered statistically significant.

RESULTS

Enzyme inhibition assays

α-Amylase inhibitory activity

The in vitro α-amylase inhibitory activity of the lime-juice extract of G. herbaceum leaves increased in a concentration-dependent manner, with no statistically significant difference compared with acarbose at $p < 0.05$ (Figure 2a). However, acarbose consistently showed slightly higher inhibitory activity across all concentrations. This suggests that the G. herbaceum lime extract contains bioactive compounds capable of slowing carbohydrate digestion, albeit with lower potency than the standard drug.

α-Glucosidase inhibitory activity

The α-glucosidase inhibitory activity of both the lime extract and acarbose increased with concentration, but acarbose exhibited significantly higher inhibition ($p < 0.05$) at each concentration compared with the extract (Figure 2b). Despite this difference, the lime extract of G. herbaceum showed a clear concentration-dependent increase in α-glucosidase inhibition. This indicates the presence of biologically relevant compounds that can contribute to the regulation of carbohydrate digestion, supporting its potential role in diabetes management.

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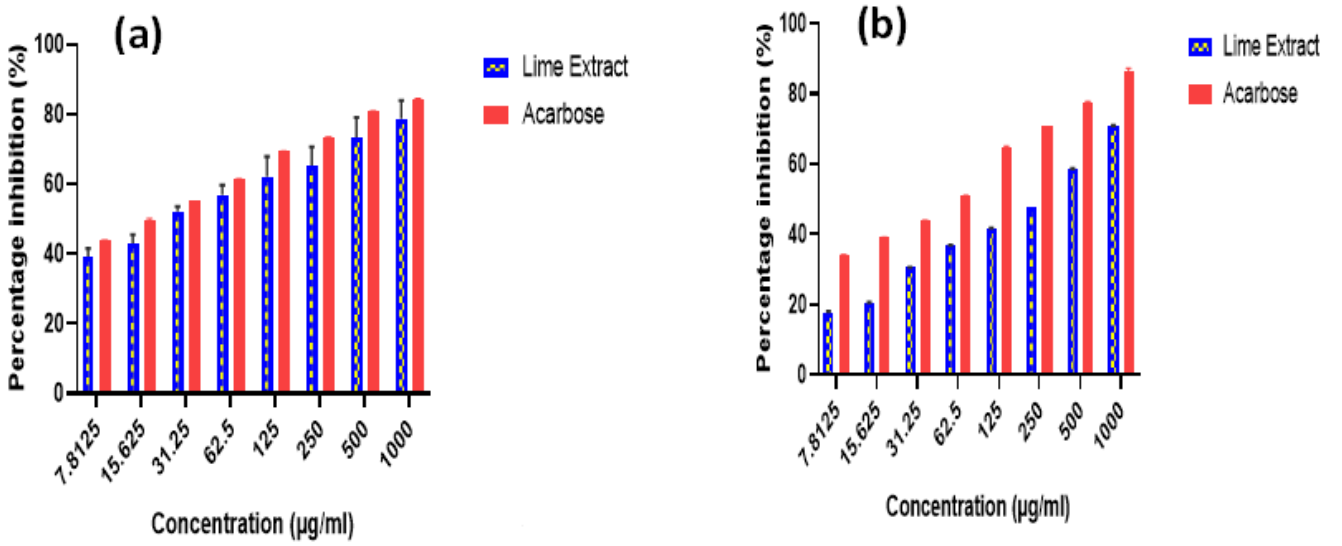

Figure 2: Inhibitory effect of lime-juice extract of Gossypium herbaceum leaves and acarbose on (a) α-amylase activity, and (b) α-glucosidase activities. Results are expressed as mean ± SD (n = 3).

In vitro antioxidant activity assays

DPPH radical scavenging activity

Both the lime-juice extract of G. herbaceum and the synthetic antioxidant BHT exhibited dose-dependent increases in DPPH radical scavenging activity (Figure 3a). BHT showed higher percent inhibition at all concentrations, reflecting its strong synthetic antioxidant capacity. The lime extract, while slightly less potent, demonstrated a progressive rise in activity with increasing concentration, particularly at higher doses. This suggests that the extract possesses significant natural antioxidant potential that may be beneficial in the management of oxidative stress associated with diabetes.

ABTS radical scavenging activity

The ABTS radical scavenging activity of the lime extract and BHT increased in a dose-dependent manner, with BHT consistently showing slightly higher percent inhibition across all concentrations, consistent with its strong synthetic antioxidant profile (Figure 3b). The lime extract of G. herbaceum exhibited mild but significant activity, with its scavenging capacity approaching that of BHT at higher concentrations. This indicates that the extract contains phytochemicals capable of effectively neutralizing the pre-formed $ABTS^{+}$ radical and supports its potential as a natural antioxidant agent.

Hydroxyl (·OH) radical scavenging activity

The hydroxyl radical scavenging activity of both the lime extract and BHT increased with concentration, indicating a concentration-dependent antioxidant effect (Figure 3c). BHT consistently exhibited higher percent inhibition, reflecting its greater synthetic antioxidant efficiency. The lime extract of G. herbaceum leaves showed progressive scavenging capacity, but at 31.25 µg/mL its activity declined and became comparable to BHT, with no significant difference ($p < 0.05$) at that concentration, suggesting a transient reduction in therapeutic effect at this intermediate dose. Nonetheless, overall the extract demonstrated appreciable hydroxyl-radical scavenging potential, indicating its ability to mitigate oxidative damage associated with diabetes.

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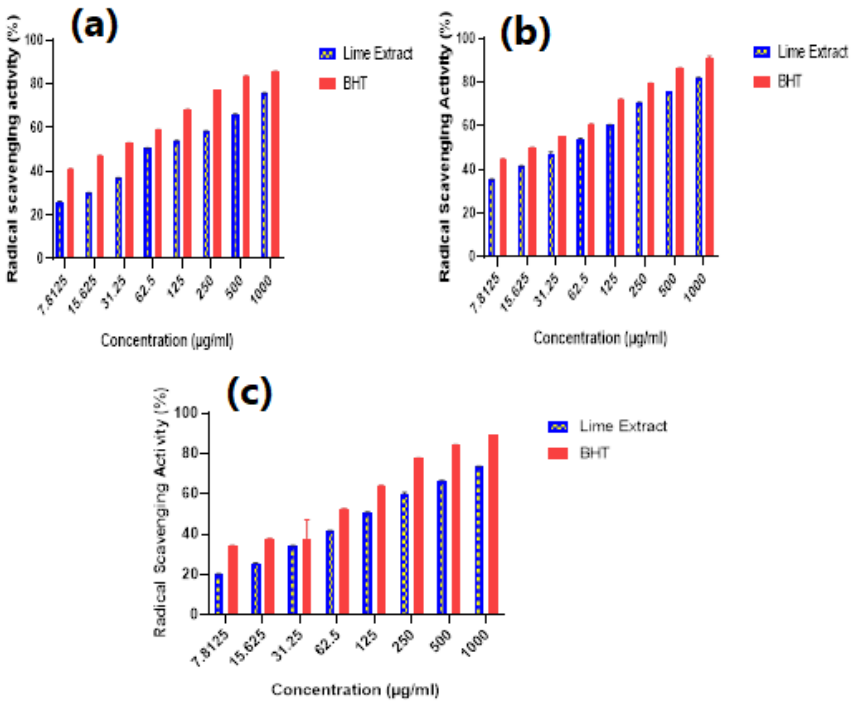

Figure 3: Inhibitory effect of lime extract of Gossypium herbaceum leaves and BHT against (a) DPPH radical scavenging activity (b) ABTS radical scavenging activity (c) hydroxyl radical scavenging activity. Results are expressed as mean ± SD ($p < 0.05$; $n = 3$).

DISCUSSION

The rising global burden of diabetes mellitus and its associated micro- and macrovascular complications has intensified the search for alternative therapeutic agents with improved safety and tolerability profiles. Thus, plant-derived extracts such as those from Gossypium herbaceum have attracted attention due to reported hypoglycemic, antioxidant, and β-cell–protective effects in both in vitro and in vivo models (Okunlola et al., 2021; Olanrewaju et al., 2025). The present study demonstrates that a lime-juice-based extract of G. herbaceum leaves inhibits key carbohydrate-digesting enzymes and scavenges reactive oxygen species in a concentration-dependent manner, supporting its potential role as an adjunct in diabetes management.

The α-amylase and α-glucosidase inhibitory activities of the lime extract are consistent with earlier reports showing that G. herbaceum leaf extracts inhibit these enzymes in a dose-dependent fashion (Ogunyinka et al., 2016; Mili et al., 2025). The extract appears to exert stronger inhibition on α-amylase than on α-glucosidase, a pattern that may be advantageous for modulating postprandial glucose excursions. In contrast, acarbose and related α-glucosidase inhibitors primarily target disaccharidase activity in the small intestine, which often leads to undigested carbohydrates reaching the colon, promoting fermentation-related side effects such as abdominal distension, flatulence, and diarrhea (Kawami et al., 2026; Pathak et al., 2025). While these gastrointestinal adverse effects are less relevant in in vitro systems, the relatively milder α-glucosidase inhibition observed in the present work may suggest a more moderate impact on intestinal carbohydrate breakdown, potentially reducing the risk of such complications if translated to in vivo settings.

Compared with other plant-based α-amylase inhibitors recently reported for species such as Moringa oleifera and Cola nitida (Fidyasari et al., 2026; Ebulue, 2024), the lime-juice extract of G. herbaceum showed comparable but generally lower potency than acarbose. This is not unexpected, as most phytomedicines act via multiple, often weaker, mechanisms rather than a single high-affinity interaction. However, the concentration-dependent inhibition demonstrated here aligns well with the growing body of evidence that plant polyphenols and flavonoids can effectively modulate carbohydrate-digesting enzymes, especially when optimized by extraction method and solvent composition (Indriyani et al., 2023; McKeirnan and Rodin, 2023).

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The DPPH and ABTS radical scavenging activities of the lime juice-based extract of G. herbaceum exhibited clear dose-dependent increases, indicating appreciable antioxidant capacity. The extract effectively reduced DPPH radical color intensity, consistent with the presence of phenolic compounds and flavonoids that can donate hydrogen atoms or electrons to stabilize free radicals (Tumilaar et al., 2024; Kyada et al., 2023). In the ABTS assay, the extract showed moderate but significant scavenging activity, though lower than that of the synthetic antioxidant BHT. This relative difference is typical, as many plant extracts have antioxidant potency in the same order of magnitude as, but rarely exceeding, optimized synthetic standards (Flieger et al., 2021; Gulcin, 2020). The incorporation of lime (Citrus aurantifolia) as the extraction medium may further enhance free-radical scavenging, given its rich content of ascorbic acid, organic acids, and flavonoids, which act synergistically to improve solvent polarity and solubilization of phenolic constituents (Karki et al., 2024; Vig et al., 2026).

The hydroxyl radical scavenging activity of the extract also increased with concentration, with a notable plateau or mild reduction at 31.25 µg/mL, after which the activity approached that of BHT. This transient attenuation may reflect complex interactions between different phytochemical classes or limited availability of specific radical-scavenging sites at intermediate doses. Nonetheless, the overall capacity to neutralize hydroxyl radicals supports the extract's potential to mitigate oxidative damage associated with hyperglycemia. Reactive oxygen species such as superoxide anion, hydroxyl radical, hydrogen peroxide, and peroxyl radicals contribute directly to pancreatic β-cell dysfunction and insulin resistance, and plant-derived antioxidants have been shown to attenuate these processes in experimental models (Indriyani et al., 2023; Saeedi et al., 2019). The observed antioxidant profile of the lime-juice extract of G. herbaceum is therefore consistent with findings from several other medicinal plants, including Moringa oleifera and Cola nitida, which have demonstrated combined antidiabetic and antioxidant properties (Fidyasari et al., 2026; Ebulue, 2024).

It is important to recognize that the current study is limited to in vitro assays, which provide valuable mechanistic insights but do not account for pharmacokinetics, bioavailability, tissue distribution, or systemic toxicity. Enzyme inhibition and radical scavenging observed in cell-free systems may not fully translate to in vivo efficacy, especially given factors such as gastrointestinal degradation, hepatic metabolism, and dose-dependent toxicity. Therefore, while the results are promising, conclusions regarding therapeutic application should be treated as preliminary and indicative, rather than definitive.

CONCLUSION

This study demonstrates that a lime-juice-based extract of Gossypium herbaceum leaves exhibits in vitro antidiabetic and antioxidant activities, as evidenced by concentration-dependent inhibition of α-amylase and α-glucosidase and significant scavenging of DPPH, ABTS, and hydroxyl radicals. These findings support the traditional use of G. herbaceum in diabetes-related ethnomedicine and highlight the potential benefit of lime-mediated extraction in enhancing the release and solubilization of bioactive phytochemicals. However, given the inherent limitations of in vitro models, the observed effects must be interpreted cautiously and should not be extrapolated directly to clinical outcomes.

Availability of Data and Materials

Materials used and data associated with this research are freely available upon reasonable request through the corresponding author.

Declaration of competing interests

The authors claim there are no competing interests.

Authors contribution

Olufemi Ayoade Ajibade: conceptualization; methodology; data acquisition; formal analysis and investigation; writing—original draft preparation, review and editing. **Rasheed B. Ibrahim**: conceptualization; methodology;

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supervision; validation. **Adewale T. Irewale:** writing—review and editing; data analysis, validation, resources. **Shalom Tijesuni Oluyori:** writing—review and editing; data analysis.

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