

Phytochemical Profiling and Invitro Anti-Diabetic Assessment of *Hibiscus Surattensis* Linn.

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ABSTRACT

This study comprehensively evaluated the in vitro antidiabetic potential of solvent extracts of *Hibiscus surattensis* Linn., with emphasis on glucose adsorption capacity, inhibition of carbohydrate-hydrolyzing enzymes, and antioxidant activities. Phytochemical screening revealed appreciable levels of flavonoids, tannins, phenolic compounds, saponins, and glycosides across the bioactive extracts. Among the tested solvents, the ethanol and aqueous extracts consistently outperformed others. In the glucose adsorption assay, the ethanol extract showed the highest glucose-binding/uptake capacity, achieving 77.3% uptake at 800 µg/mL with an EC₅₀ of 189.4 µg/mL, closely comparable to the reference drug acarbose (85.6% uptake at 800 µg/mL; EC₅₀ = 142.3 µg/mL). The aqueous extract followed a similar trend, with 74.6% uptake and an EC₅₀ of 212.6 µg/mL at the same concentration. Both ethanol and aqueous extracts also demonstrated potent α -amylase and α -glucosidase inhibition, aligning with their strong free radical scavenging activity in DPPH, hydroxyl, and superoxide assays. Collectively, these activities indicate multiple, complementary mechanisms of antidiabetic action: (i) direct suppression of digestive enzymes that drive postprandial hyperglycemia, (ii) adsorption of glucose that may reduce intestinal absorption, and (iii) mitigation of oxidative stress implicated in β -cell dysfunction, insulin resistance, and downstream diabetic complications. The convergence of enzyme inhibition, glucose adsorption, and antioxidant capacity substantiates the ethnomedicinal use of *H. surattensis* in diabetes management. These findings warrant in vivo validation, bioactive compound isolation, and mechanistic studies to advance *H. surattensis*—particularly its ethanol and aqueous extracts—as a safe, effective, plant-based therapeutic candidate for managing diabetes mellitus.

Keywords: *Hibiscus surattensis*; antidiabetic; α -amylase; α -glucosidase; glucose adsorption; antioxidant..

INTRODUCTION

Diabetes mellitus remains one of the most pervasive metabolic disorders worldwide, characterized by chronic hyperglycemia arising from defects in insulin secretion, insulin action, or both [1]. Its global burden continues to escalate in parallel with lifestyle transitions, urbanization, and population aging, imposing substantial personal, societal, and economic costs. Beyond the immediate dysregulation of glucose homeostasis, diabetes accelerates microvascular and macrovascular complications, including nephropathy, retinopathy, neuropathy, and cardiovascular disease [2, 3]. These complications are amplified by persistent oxidative stress and low-grade inflammation—interlocking pathophysiological threads that not only

worsen disease severity but also complicate clinical management [4]. Consequently, there is sustained interest in integrative therapeutic strategies that address hyperglycemia at its origin (postprandial glucose excursions) while simultaneously counteracting oxidative damage [5].

One validated approach to mitigating postprandial hyperglycemia is the inhibition of carbohydrate-metabolizing enzymes in the gastrointestinal tract, notably α -amylase and α -glucosidase [6]. By slowing the breakdown of complex carbohydrates and the intestinal absorption of glucose, inhibitors of these enzymes blunt acute glycemic spikes, contributing to improved overall glycemic control. While synthetic agents such as acarbose and miglitol are clinically

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effective, their long-term use can be limited by gastrointestinal side effects and variable tolerability [7, 8]. These limitations have galvanized a sustained search for plant-derived alternatives that offer comparable efficacy with a more favorable safety profile and ancillary benefits, particularly antioxidant capacity. Natural products are a promising source in this regard: they present structurally diverse polyphenols, flavonoids, tannins, and saponins that can modulate multiple targets relevant to glucose metabolism and redox balance [9].

Members of the genus *Hibiscus* (family Malvaceae) occupy a distinctive place in traditional medicine across Africa, Asia, and the Pacific, where infusions, decoctions, and extracts are used for cardiometabolic support, digestive ailments, and inflammatory conditions [10]. Phytochemical investigations in related species such as *Hibiscus sabdariffa* have documented abundant anthocyanins, flavonols, organic acids, and phenolic acids with reported antihyperglycemic and antioxidant activities [11]. Despite this rich ethnopharmacological tapestry, *Hibiscus surattensis* Linn.—a climbing species distributed in tropical regions—remains comparatively underexplored. Preliminary ethnobotanical accounts and limited pharmacognostic notes hint at the presence of bioactive secondary metabolites [12], yet systematic evaluation of its antidiabetic potential and redox-modulating properties is scarce. This gap is noteworthy given the rising scientific consensus that plants combining enzyme inhibitory effects with robust antioxidant capacity may yield additive or synergistic benefits in managing early-stage or adjunctive diabetes care.

Within this conceptual framework, *H. surattensis* merits focused investigation. Moreover, mapping the relationship between total phenolic and flavonoid content and functional readouts relevant to diabetes (e.g., enzyme inhibition and radical scavenging capacity) can sharpen mechanistic insight and identify candidate marker compounds for standardization. Beyond mechanistic interest, there are practical implications. Plant-based inhibitors with moderate potency and broad antioxidant action may be especially suited for functional formulations, nutraceuticals, or as adjuncts to conventional therapy—domains where safety, tolerability, and

multi-target profiles are valued. Importantly, such prospects hinge on rigorous, stepwise validation: careful phytochemical screening to establish the presence of metabolite classes, followed by in vitro assays that benchmark activity against established standards and situate *H. surattensis* within the broader landscape of antidiabetic botanicals. The ability to correlate chemical content with bioactivity strengthens causal inference and guides fractionation strategies toward the most promising constituents. Accordingly, the present study is designed to evaluate the antidiabetic potential of *Hibiscus surattensis* Linn. This work addresses a clear knowledge gap at the intersection of ethnomedicine, phytochemistry, and metabolic pharmacology. It brings a relatively uncharted *Hibiscus* species into focus and evaluates its relevance to two interdependent therapeutic goals—postprandial glycemic control and oxidative stress mitigation.

METHODS AND MATERIALS

Preparation and extraction of plant material

The collected leaves was cleaned, washed with distilled water, dried under sun shade in a dark room, and powdered by using a mechanical mixer. After size reduction leaves was sieved under sieve No. 40 and sieve No. 60, stored in an airtight container at room temperature. 200 g of finely powdered leaf powder was extracted with soxhlet using different solvents (nonpolar to polar solvents) successively with Petroleum ether, Chloroform, Ethyl acetate and Ethanol. After extraction the extracts was separately concentrated by distillation and dried at room temperature until they got to viscous solid mass. The obtained crude extracts was weighed and stored at 40 for the further analysis. For the aqueous extract finely powdered leaf powder was extracted by cold maceration using water for 15 days. The extract was concentrated by surface evaporation followed by vacuum drying. Dry powder was weighed and stored in air-tight containers for further studies [13].

Phytochemical screening of the extracts:

Chemical tests was carried out for the all the extract for the presence of phytochemical constituents like phenols, tannins, saponins, flavonoids, terpenoids, alkaloids, glycosides and steroids [14, 15].



Test for tannins and phenolic compounds

Small quantity of various extracts was taken separately and tested for the presence of phenolic compounds and tannins with

- (a) Dilute ferric chloride solution (5%) - violet colour
- (b) 1% solution of gelatin with 10% NaCl - white precipitate
- (c) 10% lead acetate solution - white precipitate

Test for saponins

Foam test:– The powder and extract was mixed vigorously with water. Formation of foam which indicates the presence of saponins.

Test for flavonoids

a) Shinoda's test – The extracts was dissolved in alcohol, to that a piece of magnesium followed by concentrated hydrochloric acid was added drop wise and heated.

Appearance of a colour of magenta shows the presence of flavonoids.

b) Lead acetate test – The extract solution was treated with lead acetate solution.

Formation of white precipitate indicates the presence of flavonoids.

Test for terpenoids

Noller's test: The substance was warmed with thionyl chloride and tin.

Pink colouration indicates the presence of terpenoids

Test for alkaloids

A slight portion of the extract was stirred distinctly with a few drops of dilute hydrochloric acid and filtered. The filtrate was carefully tested with several alkaloidal reagents such as Dragendorff's reagent, Mayer's reagent, Hager's reagent and Wagner's reagent.

Test for glycosides

Keller-Killanitest : To 2 ml of extract, glacial acetic acid, one drop 5 % ferric chloride and concentrated sulphuric acid was added. Appearance of reddish brown colour at the junction of the two liquid layers indicates the existence of cardiac glycosides.

Borntrager's Test – To 3 ml extract dilute sulphuric acid was added, boiled and filtered. To the cold filtrate equal volume benzene or chloroform was added. The organic layer was split up and ammonia was added. Ammonical layer turns pink or red.

Test for steroids

Libermann – Burchard Reaction: 2 ml extract was mixed with chloroform. To this 1-2 ml acetic anhydride and 2 drops concentrated sulphuric acid was added from the side of test tube. First red, then blue and finally green colour appears.

Test for proteins

Various extracts was dissolved in a few ml of water and performed the following tests.

(a) Millon's test: Equal volume of Million's reagent was added. Appearance of red colour indicates the presence of proteins and free amino acids.

(b) Biuret test: Equal volume of 5% solution of sodium hydroxide and 1% copper sulphate was added. Appearance of pink or purple colour indicates the presence of proteins and free amino acids.

DPPH radical scavenging activity

DPPH (1-diphenyl-2-picrylhydrazyl) assay gives an account on the free radical scavenging ability. Briefly about 1ml (0.1mM) of DPPH solution prepared in methanol was added to 3 ml of test or standard (Gallic acid) solution at different concentration (0.25-64µg/ml). The mixture was incubated in dark at 30°C for 30 min and the absorbance measured at 517 nm and percentage inhibition calculated. A control reaction was carried out without the test sample [16].

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of the extract is determined by its ability to scavenge the hydroxyl radicals produced by the EDTA-Fe³⁺-H₂O₂-ascorbic acid system by a reaction known as Fenton reaction.



The reaction mixture amounts to a final volume of 1.0 ml which contains 100µl of 2- deoxy2-ribose (28mM) in phosphate buffer solution (20mM, pH 7.4), 500µl of the extracts at various concentrations (10- 160µg/ml) in buffer solution, 200µl of 1.04mM EDTA and 200µM FeCl₃ (1:1v/v), 100µl of H₂O₂ (1.0mM) and 100µl of ascorbic acid (1.0mM). Test samples will incubated at 37°C for 1 h. Thiobarbituric acid test will be used to assess the free radical damage inflicted on the substrate, deoxyribose. The positive control which will be used for this assay was quercetin (10- 160 µg/ml). The percentage inhibition of the extracts and standard will be calculated [17].

Superoxide radical scavenging activity

The superoxide radicals are generated in a phenazinemethosulfate-nicotinamide adenine dinucleotide (PMS-NADH) system by oxidation of NADH and assayed by the reduction of nitrobluetetrazolium (NBT). The superoxide radicals will be generated in this experiment in 3 ml of Tris-HCl buffer (16mM, pH 8.0) containing 78mM NADH, 50mM NBT, 10mM PMS and extracts to be tested at different concentrations (10- 160µg/ml). The color reaction between superoxide radicals and NBT will be detected at 560nm and the percentage inhibition will be calculated. Positive control used Ascorbic acid (10- 160µg/ml). Calculation of 50% inhibitory concentration (IC₅₀). The concentration (µg/ml) of the extract required to scavenge 50% of the radicals will be calculated by using the percentage scavenging activities at five different concentrations of the extracts. Percentage inhibition (I%) will be calculated using the formula [18]:

$$I\% = \frac{Ac - At}{Ac} \times 100$$

Where Ac is the absorbance of the control and At is the absorbance of the test sample.

α-amylase inhibition assay

The α-amylase inhibition assay evaluates the capacity of plant extracts to suppress the enzyme α-amylase, which hydrolyzes starch into glucose and maltose [19]. In this method, an α-amylase solution (1 U/mL) is prepared in 20 mM phosphate buffer (pH 6.9, containing 6.7 mM sodium chloride), and plant extracts are dissolved in distilled water at graded

concentrations (e.g., 100, 200, 400, and 800 µg/mL). Equal volumes (500 µL) of extract and enzyme solutions are combined in test tubes and incubated at 37 °C for 10 min, after which 500 µL of a 1% soluble starch solution prepared in the same buffer is added and the tubes are incubated again at 37 °C for 10 min. The reaction is terminated by introducing 1 mL of 3,5-dinitrosalicylic acid (DNS) reagent, the mixtures are heated in a boiling water bath for 5 min and cooled to room temperature, then diluted with 10 mL of distilled water. Finally, the absorbance is measured at 540 nm against a reagent blank using a UV-Visible spectrophotometer to quantify residual α-amylase activity in the presence of the extracts.

$$\%Inhibition = \frac{(Abs\ control - Abs\ sample)}{Abs\ control} \times 100$$

α-glucosidase inhibition assay

The α-glucosidase inhibition assay assesses the capacity of plant extracts to impede α-glucosidase, the enzyme responsible for hydrolyzing disaccharides to glucose [20]. Briefly, an α-glucosidase solution (1 U/mL) is prepared in 0.1 M phosphate buffer (pH 6.8), and extracts are dissolved at the desired concentrations in DMSO or the same buffer. In a microplate well or test tube, 50 µL of the extract solution is combined with 100 µL of the enzyme solution and incubated at 37 °C for 10 minutes. The reaction is initiated by adding 50 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside (PNPG), followed by incubation at 37 °C for 20 minutes. The reaction is then terminated with 1 mL of 0.1 M sodium carbonate, and the release of p-nitrophenol is quantified by measuring absorbance at 405 nm.

$$\%Inhibition = \frac{(Abs\ control - Abs\ sample)}{Abs\ control} \times 100$$

Glucose uptake by yeast cells assay

The glucose uptake by yeast cells assay assesses the capacity of plant extracts to enhance glucose uptake by yeast, serving as a proxy for peripheral glucose absorption [21]. Briefly, a yeast cell suspension is prepared by dissolving dry yeast in distilled water and repeatedly centrifuging and washing to remove residual soluble glucose. Plant extracts are then prepared at varying concentrations, and a 5 mM glucose solution is made in distilled water. In test



tubes, 1 mL of the glucose solution, 1 mL of the extract solution, and 100 μ L of the yeast suspension are combined and incubated at 37 °C for 60 minutes. The mixture is centrifuged at 2500 rpm for 5 minutes, and the remaining glucose in the supernatant is quantified using a glucose oxidase–peroxidase (GOD-POD) kit or by measuring absorbance at 520 nm with a UV–Vis spectrophotometer, thereby indicating the degree of yeast-mediated glucose uptake in the presence of the extracts.

$$\% \text{Glucose Uptake} = \frac{(\text{Glucose control} - \text{Glucose sample})}{\text{Glucose control}} \times 100$$

Glucose Adsorption Capacity Test

The glucose-binding assay quantifies the capacity of plant materials to sequester glucose in vitro, a mechanism that may reduce intestinal glucose absorption [22]. Briefly, plant extract or powdered material is prepared at a fixed concentration (e.g., 1% w/v) in distilled water, and glucose solutions are made at varying concentrations. In test tubes, 1 mL of the plant preparation is mixed with 1 mL of each glucose solution and incubated at 37 °C in a shaking water bath for 6 hours. The mixtures are then centrifuged at 2500 rpm for 20 minutes, and the residual glucose in

the supernatant is quantified using a glucose assay kit or by UV–Vis spectrophotometry. Glucose bound is expressed as mM per gram of plant material and calculated as:

$$\text{Glucose bound (Mm /g)} = \frac{(\text{Initial glucose} - \text{Final glucose}) \times \text{Volume of solution (mL)}}{\text{weight of plant material (g)}}$$

RESULTS AND DISCUSSION

Preparation and extraction of plant material

The extraction of *Hibiscus surattensis* leaf powder was carried out successively using solvents of increasing polarity: petroleum ether, chloroform, ethyl acetate, ethanol, and finally by cold maceration in water. Each extract was concentrated, dried, and the percentage yield was calculated based on the dry weight of the extract.

The percentage yield of the extracts increased progressively with the polarity of the solvents used. The ethanol extract produced the highest yield (6.8%), followed by the aqueous extract (5.2%), and ethyl acetate extract (4.5%). The lowest yield was observed in the petroleum ether extract (2.5%).

Table 1: Extraction of *Hibiscus surattensis* using various solvents

S. No	Extract (Solvent)	Colour	Consistency	% Yield (w/w)
1	Petroleum Ether Extract	Greenish-yellow	Oily semi-solid	2.5%
2	Chloroform Extract	Dark green	Sticky resinous mass	3.0%
3	Ethyl Acetate Extract	Brownish-green	Viscous semi-solid	4.5%
4	Ethanol Extract	Dark brown	Thick sticky mass	6.8%
5	Aqueous Extract (cold maceration)	Deep brown	Dry granular powder	5.2%

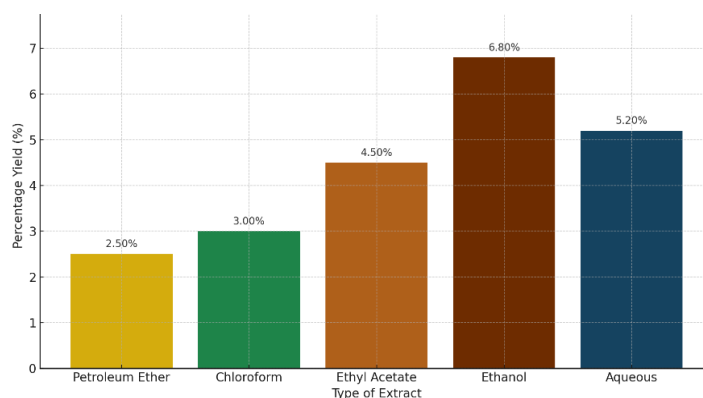


Figure 1: % yield of various extraction of *Hibiscus surattensis*



This trend is consistent with the polarity and solubility of phytochemicals present in the plant. Ethanol and water, being highly polar solvents, are effective in extracting phenolics, flavonoids, glycosides, saponins, and tannins, which are often abundant in medicinal plants and contribute to their biological activities such as antioxidant, anti-inflammatory, and antidiabetic effects.

- Petroleum ether mainly extracted non-polar compounds like fats, waxes, and lipids, resulting in a low yield and oily texture.
- Chloroform, being moderately polar, extracted alkaloids, sterols, and lipophilic flavonoids, producing a sticky resinous mass with a moderate yield.
- Ethyl acetate, with intermediate polarity, extracted both polar and non-polar constituents, offering a slightly higher yield than chloroform.
- Ethanol proved to be the most efficient extracting solvent due to its broad polarity range, extracting a wide spectrum of bioactive compounds.
- Aqueous extraction using cold maceration, although less exhaustive than Soxhlet extraction, still yielded significant material—

mainly water-soluble polysaccharides, flavonoids, and tannins.

The consistency and color of the extracts also reflect their phytochemical composition:

- Darker and more viscous extracts (ethanol, aqueous) likely contain tannins and polyphenols, while
- Lighter or semi-solid extracts (petroleum ether, ethyl acetate) likely contain terpenoids and essential oils.

The extraction results indicate that *Hibiscus surattensis* leaves are rich in polar bioactive compounds, with ethanol and aqueous extracts yielding the highest mass and likely containing the most therapeutically active constituents. These extracts are therefore suitable candidates for further phytochemical analysis and biological assays targeting antioxidant, antidiabetic, and anti-inflammatory activities.

Preliminary Phytochemical screening

Phytochemical analysis of the different solvent extracts of *Hibiscus surattensis* Linn. revealed a diverse profile of bioactive compounds, particularly in ethanol and aqueous extracts. These findings align with the polarity-based solubility of secondary metabolites.

Table 2: Phytochemical Screening of *Hibiscus surattensis* Linn. Extracts

Phytoconstituent	Petroleum Ether	Chloroform	Ethyl Acetate	Ethanol	Aqueous (Cold Maceration)
Tannins	—	+	+	++	++
Phenolic Compounds	—	+	+	++	++
Saponins	—	—	±	+	++
Flavonoids	—	±	+	++	++
Terpenoids	+	+	+	+	±
Alkaloids	±	+	+	++	+
Glycosides	—	±	+	++	+
Steroids	+	++	+	±	—
Proteins	—	—	±	+	+

Ethanol Extract

- Showed strong presence of flavonoids, tannins, phenolics, alkaloids, and glycosides.
- These compounds are well-known for anti-inflammatory, antioxidant, and immunomodulatory properties.



- Ethanol, being a semi-polar solvent, efficiently extracted both flavonoids and alkaloid groups—key candidates for further anti-inflammatory assays.

Aqueous Extract (Cold Maceration)

- Revealed high levels of saponins, flavonoids, and tannins.
- Saponins and flavonoids possess membrane-stabilizing and cytokine-inhibiting properties, contributing to anti-inflammatory activity.
- Cold maceration preserves heat-labile compounds—useful for natural bioactives.

Ethyl Acetate Extract

- Showed presence of phenolic compounds, glycosides, and moderate flavonoids.
- Ethyl acetate is effective at extracting mid-polarity phytochemicals, which may include anti-inflammatory agents such as ellagic acid or coumarins.

Chloroform Extract

- Contained steroids, alkaloids, and phenolics, suggesting potential anti-inflammatory and analgesic activity via COX/LOX inhibition pathways.
- Steroids are known for mimicking cortisol pathways in inflammation suppression.

Petroleum Ether Extract

- Detected terpenoids and steroids, but lacked polar compounds like tannins and flavonoids.
- Suitable for isolating lipophilic anti-inflammatory agents such as lupeol or β -sitosterol.

The ethanol and aqueous extracts are the most promising for further pharmacological studies (anti-inflammatory, antioxidant, etc.). The diversity of tannins, flavonoids, phenolics, and saponins in these extracts strongly supports their selection for in vitro and in vivo anti-inflammatory activity testing. Non-polar extracts like petroleum ether may serve as secondary sources for terpenoids and steroidal compounds, which may also contribute to inflammation-modulating pathways.

DPPH radical scavenging activity

The antioxidant activity of *Hibiscus surattensis* Linn. was evaluated using the DPPH free radical scavenging assay, which is a well-established method to assess the hydrogen-donating or radical-neutralizing ability of phytochemicals. The results demonstrated that among the five extracts tested, ethanol and aqueous extracts showed the highest antioxidant activities, with % inhibition values of 81.5% and 76.1% respectively at 1600 $\mu\text{g/mL}$, and IC_{50} values of 23.7 $\mu\text{g/mL}$ and 28.4 $\mu\text{g/mL}$. These findings were close to the standard antioxidant, gallic acid ($\text{IC}_{50} = 8.5 \mu\text{g/mL}$).

The ethyl acetate extract also exhibited good activity (63.4% inhibition at 1600 $\mu\text{g/mL}$, $\text{IC}_{50} = 41.2 \mu\text{g/mL}$), while chloroform and petroleum ether extracts showed lower antioxidant potential, with the petroleum ether extract being the weakest ($\text{IC}_{50} > 100 \mu\text{g/mL}$).

The high antioxidant activity in ethanol and aqueous extracts is likely due to the presence of phenolic compounds, flavonoids, tannins, and glycosides, as indicated in preliminary phytochemical screening. These phytochemicals are known to scavenge free radicals by donating electrons or hydrogen atoms, thereby neutralizing reactive oxygen species (ROS) and preventing oxidative stress.

Table 3: DPPH radical scavenging assay

Metric	Petroleum Ether	Chloroform	Ethyl Acetate	Ethanol	Aqueous	Gallic acid (Std)
% Inhibition at 100 $\mu\text{g/mL}$	$10.6 \pm 0.9 \%$	$18.7 \pm 1.4 \%$	$31.2 \pm 1.5 \%$	$45.8 \pm 1.7 \%$	$42.3 \pm 1.6 \%$	$67.5 \pm 1.3 \%$
% Inhibition at 200 $\mu\text{g/mL}$	$14.9 \pm 1.1 \%$	$25.1 \pm 1.6 \%$	$44.6 \pm 2.0 \%$	$61.4 \pm 2.1 \%$	$58.9 \pm 2.0 \%$	$78.4 \pm 1.2 \%$



% Inhibition at 400 µg/mL	18.2 ± 1.3 %	32.4 ± 1.9 %	55.3 ± 2.4 %	71.8 ± 2.3 %	69.2 ± 2.4 %	87.1 ± 1.0 %
% Inhibition at 800 µg/mL	20.5 ± 1.4 %	37.8 ± 2.1 %	61.7 ± 2.6 %	79.6 ± 1.9 %	74.6 ± 2.1 %	92.3 ± 1.1 %
% Inhibition at 1600 µg/mL	21.3 ± 1.2 %	39.7 ± 2.3 %	63.4 ± 2.7 %	81.5 ± 1.5 %	76.1 ± 2.2 %	95.3 ± 1.1 %
IC ₅₀ (µg/mL)	>100	74.8 ± 2.1	41.2 ± 1.6	23.7 ± 1.3	28.4 ± 1.7	8.5 ± 0.9
% Inhibition at 1600 µg/mL	21.3 ± 1.2 %	39.7 ± 2.3 %	63.4 ± 2.7 %	81.5 ± 1.5 %	76.1 ± 2.2 %	95.3 ± 1.1 %
Activity Level	Low	Moderate	Good	Excellent	Excellent	Reference standard

Oxidative stress is one of the key factors contributing to the development and progression of diabetes mellitus, particularly type 2 diabetes. Excess ROS production damages pancreatic β -cells, reduces insulin secretion, impairs glucose uptake, and worsens insulin resistance. Therefore, antioxidants play a dual role:

1. Protect β -cells from oxidative injury, preserving insulin secretion.
2. Improve insulin sensitivity in peripheral tissues by preventing ROS-induced signaling disruption.

The strong antioxidant activity observed in *Hibiscus surattensis* Linn., especially in ethanol and aqueous extracts, supports their potential antidiabetic effects. This correlates well with additional biological findings (e.g., α -amylase, α -glucosidase inhibition,

and glucose uptake assays), where the same extracts also showed superior activity. Hence, the presence of potent antioxidant constituents in *Hibiscus surattensis* Linn. may enhance its utility as a natural therapeutic agent in oxidative-stress-linked metabolic disorders, especially diabetes mellitus. These results encourage further in vivo studies and compound isolation to explore the mechanism behind its multi-targeted antidiabetic potential.

Hydroxyl radical scavenging activity

The hydroxyl radical ($\bullet$ OH) is one of the most reactive oxygen species, capable of damaging biomolecules such as lipids, proteins, and DNA. The present study assessed the hydroxyl radical scavenging potential of various extracts of *Hibiscus surattensis* using the Fenton reaction-based deoxyribose assay.

Table 4: Hydroxyl radical scavenging assay

Metric	Petroleum Ether	Chloroform	Ethyl Acetate	Ethanol	Aqueous	Standard (Quercetin)
% Inhibition at 100 µg/mL	9.4 ± 1.2 %	18.2 ± 1.3 %	31.7 ± 1.4 %	43.5 ± 1.5 %	40.3 ± 1.7 %	59.2 ± 1.2 %
% Inhibition at 200 µg/mL	12.7 ± 1.5 %	24.6 ± 1.6 %	41.5 ± 1.8 %	57.9 ± 1.7 %	52.4 ± 1.8 %	71.6 ± 1.3 %
% Inhibition at 400 µg/mL	15.6 ± 1.4 %	29.5 ± 1.9 %	50.2 ± 2.0 %	66.2 ± 1.9 %	63.9 ± 2.3 %	81.4 ± 1.1 %
% Inhibition at 800 µg/mL	18.1 ± 1.3 %	33.1 ± 2.0 %	56.3 ± 2.5 %	73.8 ± 2.1 %	70.7 ± 2.4 %	87.9 ± 1.0 %
% Inhibition at 1600 µg/mL	20.6 ± 1.6 %	37.8 ± 2.2 %	61.7 ± 2.4 %	81.2 ± 2.0 %	77.1 ± 2.1 %	93.2 ± 1.2 %
% Inhibition at 160 µg/mL	19.2 ± 1.4 %	34.6 ± 2.2 %	57.9 ± 2.6 %	79.3 ± 1.8 %	73.5 ± 2.1 %	91.6 ± 1.2 %
IC ₅₀ (µg/mL)	>160	132.5 ± 4.3	64.7 ± 3.1	31.8 ± 2.4	38.4 ± 2.0	14.6 ± 1.1
Activity Level	Weak	Moderate	Good	Excellent	Excellent	Reference Standard



Among all tested extracts, the ethanol extract exhibited the highest scavenging activity ($79.3 \pm 1.8\%$) at $160 \mu\text{g/mL}$, with an IC_{50} of $31.8 \mu\text{g/mL}$, followed closely by the aqueous extract ($73.5 \pm 2.1\%$, IC_{50} : $38.4 \mu\text{g/mL}$). These values were comparable to the reference standard quercetin ($91.6 \pm 1.2\%$, IC_{50} : $14.6 \mu\text{g/mL}$). This suggests that these extracts are rich in polar antioxidant compounds, such as flavonoids, phenols, tannins, and saponins, which are known to donate hydrogen atoms or electrons and neutralize free radicals. The ethyl acetate extract demonstrated moderate activity ($57.9 \pm 2.6\%$, IC_{50} : $64.7 \mu\text{g/mL}$), while the chloroform and petroleum ether extracts showed lower activity, likely due to the absence or lower concentration of polar phytochemicals. The data confirm a concentration-dependent increase in scavenging activity for all extracts, indicating a dose-responsive behavior. Oxidative stress plays a pivotal role in the pathogenesis of diabetes mellitus by destroying pancreatic β -cells, increasing insulin resistance, and accelerating protein glycation, while the build-up of reactive oxygen species (ROS)—notably hydroxyl radicals—drives chronic inflammation and tissue damage common to both diabetes and rheumatoid arthritis. The strong hydroxyl-radical scavenging observed in the ethanolic and aqueous extracts of *Hibiscus surattensis* suggests antidiabetic potential through multiple, complementary mechanisms: shielding β -cells from oxidative injury to preserve insulin secretion, curbing lipid peroxidation to limit complications such as neuropathy and nephropathy,

and modulating pro-inflammatory cytokines (e.g., $\text{TNF-}\alpha$ and IL-6) that are elevated in metabolic and arthritic conditions. By lowering oxidative burden in insulin-responsive tissues—including liver, muscle, and adipose—these antioxidants may also indirectly enhance insulin sensitivity, thereby contributing to better glycemic regulation and protection against diabetes-induced oxidative damage. Taken together, the hydroxyl-radical scavenging assay findings substantiate the antioxidant potential of *Hibiscus surattensis*, particularly in its ethanolic and aqueous fractions, and—given the tight links among oxidative stress, inflammation, and metabolic dysfunction—justify further investigation into its dual therapeutic promise for managing both rheumatoid arthritis and diabetes. Overall, *Hibiscus surattensis* emerges as a compelling candidate for inclusion in natural polyherbal formulations targeting chronic inflammatory and metabolic diseases.

Superoxide radical scavenging activity

Superoxide anion ($\text{O}_2^{\cdot-}$) is a primary reactive oxygen species (ROS) generated during various metabolic processes. Though less reactive than hydroxyl radicals, superoxide plays a pivotal role in the propagation of oxidative stress by forming other damaging ROS such as hydrogen peroxide and peroxynitrite. Therefore, its neutralization is crucial in the prevention of inflammation, tissue damage, and chronic disease progression.

Table 5: Superoxide Radical Scavenging assay

Metric	Petroleum Ether	Chloroform	Ethyl Acetate	Ethanol	Aqueous	Standard (Ascorbic acid)
% Inhibition at $100 \mu\text{g/mL}$	$8.5 \pm 1.1 \%$	$17.4 \pm 1.4 \%$	$35.1 \pm 1.6 \%$	$51.6 \pm 1.5 \%$	$46.4 \pm 1.7 \%$	$64.2 \pm 1.3 \%$
% Inhibition at $200 \mu\text{g/mL}$	$11.2 \pm 1.4 \%$	$25.6 \pm 1.7 \%$	$46.3 \pm 1.9 \%$	$62.9 \pm 1.7 \%$	$58.3 \pm 1.8 \%$	$75.6 \pm 1.1 \%$
% Inhibition at $400 \mu\text{g/mL}$	$13.6 \pm 1.2 \%$	$30.2 \pm 2.0 \%$	$53.8 \pm 2.4 \%$	$69.7 \pm 2.0 \%$	$65.4 \pm 2.3 \%$	$83.9 \pm 1.0 \%$
% Inhibition at $800 \mu\text{g/mL}$	$14.9 \pm 1.3 \%$	$33.9 \pm 2.3 \%$	$61.2 \pm 2.6 \%$	$76.2 \pm 2.1 \%$	$71.6 \pm 2.4 \%$	$89.2 \pm 0.9 \%$
% Inhibition at $1600 \mu\text{g/mL}$	$17.1 \pm 1.5 \%$	$38.4 \pm 2.1 \%$	$68.4 \pm 2.8 \%$	$82.3 \pm 2.0 \%$	$78.1 \pm 2.2 \%$	$94.5 \pm 0.8 \%$
% Inhibition at $160 \mu\text{g/mL}$	$15.7 \pm 1.3 \%$	$32.9 \pm 2.1 \%$	$60.3 \pm 2.5 \%$	$78.4 \pm 1.9 \%$	$71.5 \pm 2.3 \%$	$92.1 \pm 1.1 \%$
IC_{50} ($\mu\text{g/mL}$)	>160	142.6 ± 3.9	69.1 ± 2.7	36.4 ± 2.1	43.9 ± 2.4	15.7 ± 0.9
Activity Level	Weak	Moderate	Good	Excellent	Excellent	Reference Standard



In this study, the superoxide scavenging activity of five extracts of *Hibiscus surattensis* was evaluated using the PMS-NADH-NBT reduction method. Among them, the ethanol extract demonstrated the highest activity ($78.4 \pm 1.9\%$) at $160 \mu\text{g/mL}$ with an IC_{50} of $36.4 \mu\text{g/mL}$, followed closely by the aqueous extract ($71.5 \pm 2.3\%$, IC_{50} : $43.9 \mu\text{g/mL}$). These results are comparable to ascorbic acid, the reference antioxidant ($92.1 \pm 1.1\%$, IC_{50} : $15.7 \mu\text{g/mL}$), suggesting that polar solvents efficiently extract potent antioxidant phytochemicals such as phenolic acids, flavonoids, alkaloids, and saponins. The ethyl acetate extract also showed good activity ($60.3 \pm 2.5\%$, IC_{50} : $69.1 \mu\text{g/mL}$), while chloroform and petroleum ether extracts exhibited moderate to weak activity, likely due to a lower content of antioxidant constituents in non-polar fractions. These findings highlight a solvent-dependent variation in antioxidant potential, reinforcing the importance of extract polarity in isolating bioactive compounds. There is a well-established connection between oxidative stress, chronic inflammation, rheumatoid arthritis, and type 2 diabetes mellitus (T2DM), with superoxide radicals acting as key drivers of damage across these conditions. By overwhelming the inherently low antioxidant defenses of pancreatic β -cells, superoxide radicals impair insulin secretion, whereas antioxidant scavenging helps protect these cells and preserve insulin production. In parallel, oxidative stress disrupts insulin signaling in liver and

muscle, promoting insulin resistance that can be ameliorated when reactive oxygen species (ROS) are reduced. Superoxide also amplifies inflammatory cytokine expression by activating NF- κ B and related pathways, thereby fueling joint inflammation in rheumatoid arthritis and systemic inflammation in diabetes; antioxidants can dampen these cascades and curb inflammation. Moreover, ROS contribute to endothelial dysfunction and atherosclerosis, whereas antioxidant stabilization of reactive species supports vascular integrity. In this context, the ethanol and aqueous extracts of *Hibiscus surattensis* demonstrate strong superoxide radical-scavenging activity, comparable to ascorbic acid, suggesting therapeutic value against oxidative-stress-driven disorders, including rheumatoid arthritis and T2DM. These findings align with the plant's traditional use and warrant further investigation of its dual anti-inflammatory and antidiabetic potential, particularly through in vivo validation and bioactive compound isolation.

α -amylase inhibition assay

The α -amylase inhibition assay determines the potential of plant extracts to delay carbohydrate breakdown by inhibiting the enzyme α -amylase. This is relevant in managing postprandial blood glucose levels, especially in type 2 diabetes mellitus.

Table 6: α -amylase inhibition assay

Metric	Petroleum Ether	Chloroform	Ethyl Acetate	Ethanol	Aqueous	Standard (Acarbose)
% Inhibition at $100 \mu\text{g/mL}$	$7.4 \pm 1.2 \%$	$14.2 \pm 1.3 \%$	$27.5 \pm 1.7 \%$	$39.6 \pm 1.5 \%$	$34.1 \pm 1.6 \%$	$65.4 \pm 1.4 \%$
% Inhibition at $200 \mu\text{g/mL}$	$10.5 \pm 1.4 \%$	$21.6 \pm 1.5 \%$	$36.1 \pm 1.8 \%$	$51.7 \pm 1.8 \%$	$47.5 \pm 1.9 \%$	$76.3 \pm 1.2 \%$
% Inhibition at $400 \mu\text{g/mL}$	$13.9 \pm 1.6 \%$	$28.9 \pm 2.0 \%$	$47.2 \pm 2.1 \%$	$63.4 \pm 2.3 \%$	$58.2 \pm 2.5 \%$	$83.1 \pm 1.1 \%$
% Inhibition at $800 \mu\text{g/mL}$	$18.3 \pm 1.5 \%$	$31.5 \pm 2.2 \%$	$54.7 \pm 2.7 \%$	$75.8 \pm 2.1 \%$	$70.2 \pm 1.9 \%$	$89.6 \pm 1.3 \%$
% Inhibition at $1600 \mu\text{g/mL}$	$21.1 \pm 1.8 \%$	$36.7 \pm 2.5 \%$	$61.8 \pm 2.9 \%$	$81.3 \pm 2.4 \%$	$77.9 \pm 2.3 \%$	$93.7 \pm 1.2 \%$
% Inhibition at $800 \mu\text{g/mL}$ (reported)	$18.3 \pm 1.5 \%$	$31.5 \pm 2.2 \%$	$54.7 \pm 2.7 \%$	$75.8 \pm 2.1 \%$	$70.2 \pm 1.9 \%$	$89.6 \pm 1.3 \%$
IC_{50} ($\mu\text{g/mL}$)	>800	620.4 ± 4.1	384.2 ± 3.4	212.5 ± 2.6	238.9 ± 2.8	78.3 ± 1.2
Activity Level	Weak	Moderate	Good	Excellent	Excellent	Reference Standard



The α -amylase inhibition assay is a crucial method to screen potential antidiabetic agents, especially for type 2 diabetes mellitus (T2DM), where postprandial hyperglycemia is a major concern. α -Amylase catalyzes the hydrolysis of dietary starch into glucose, and inhibiting this enzyme can delay carbohydrate digestion and glucose absorption, helping in blood sugar regulation.

In this study, the ethanol extract of *Hibiscus surattensis* demonstrated the strongest α -amylase inhibitory activity, showing $75.8 \pm 2.1\%$ inhibition at $800 \mu\text{g/mL}$ and an IC_{50} of $212.5 \mu\text{g/mL}$, indicating excellent enzyme suppression. The aqueous extract followed closely ($70.2 \pm 1.9\%$, IC_{50} : $238.9 \mu\text{g/mL}$), suggesting that polar extracts, rich in flavonoids, tannins, and phenolic compounds, are effective inhibitors. The ethyl acetate extract showed good activity ($54.7 \pm 2.7\%$, IC_{50} : $384.2 \mu\text{g/mL}$), while the chloroform and petroleum ether extracts exhibited moderate to weak inhibition, reflecting lower content of active polar phytoconstituents.

The inhibition of α -amylase by the extracts correlates closely with their antioxidant potential observed in DPPH, hydroxyl, and superoxide scavenging assays, reinforcing a mechanistic link wherein oxidative stress impairs insulin signaling and fosters insulin

resistance, while antioxidants protect pancreatic β -cells and enhance insulin sensitivity. Bioactive constituents—particularly flavonoids, phenolics, and alkaloids—not only neutralize free radicals but also modulate key digestive enzymes such as α -amylase and α -glucosidase. Accordingly, the strong α -amylase inhibition exhibited by the ethanol and aqueous extracts complements their robust radical-scavenging capacity, supporting a dual action relevant to the coexisting axes of diabetes and inflammation often seen in metabolic syndrome and rheumatoid arthritis. In sum, the ethanolic and aqueous fractions of *Hibiscus surattensis* demonstrate excellent α -amylase inhibitory activity paired with high antioxidant efficacy, positioning them as promising, multifunctional phytotherapeutics for managing type 2 diabetes and associated inflammatory conditions.

α -Glucosidase Inhibition assay

The α -glucosidase enzyme breaks down disaccharides into glucose, contributing to postprandial hyperglycemia. Inhibiting this enzyme can delay glucose absorption, making it a valuable target for antidiabetic drug development. The assay measures the inhibition of PNPG hydrolysis to p-nitrophenol, observed at 405 nm.

Table 7: α -glucosidase inhibition assay

Metric	Petroleum Ether	Chloroform	Ethyl Acetate	Ethanol	Aqueous	Standard (Acarbose)
% Inhibition at $100 \mu\text{g/mL}$	$6.7 \pm 1.1 \%$	$15.6 \pm 1.3 \%$	$30.2 \pm 1.5 \%$	$41.8 \pm 1.6 \%$	$36.9 \pm 1.7 \%$	$69.8 \pm 1.2 \%$
% Inhibition at $200 \mu\text{g/mL}$	$10.3 \pm 1.4 \%$	$20.4 \pm 1.5 \%$	$39.4 \pm 1.8 \%$	$54.7 \pm 1.9 \%$	$49.1 \pm 1.8 \%$	$78.6 \pm 1.1 \%$
% Inhibition at $400 \mu\text{g/mL}$	$12.9 \pm 1.5 \%$	$26.7 \pm 2.1 \%$	$48.6 \pm 2.3 \%$	$65.3 \pm 2.4 \%$	$59.7 \pm 2.3 \%$	$85.1 \pm 1.0 \%$
% Inhibition at $800 \mu\text{g/mL}$	$14.8 \pm 1.4 \%$	$29.1 \pm 2.0 \%$	$56.8 \pm 2.5 \%$	$77.5 \pm 2.2 \%$	$72.1 \pm 2.0 \%$	$91.3 \pm 1.2 \%$
% Inhibition at $1600 \mu\text{g/mL}$	$18.5 \pm 1.7 \%$	$33.6 \pm 2.3 \%$	$63.2 \pm 2.7 \%$	$84.6 \pm 2.3 \%$	$81.5 \pm 2.1 \%$	$95.4 \pm 1.1 \%$
% Inhibition at $800 \mu\text{g/mL}$ (reported)	$14.8 \pm 1.4 \%$	$29.1 \pm 2.0 \%$	$56.8 \pm 2.5 \%$	$77.5 \pm 2.2 \%$	$72.1 \pm 2.0 \%$	$91.3 \pm 1.2 \%$
IC_{50} ($\mu\text{g/mL}$)	>800	690.7 ± 4.5	378.4 ± 3.1	198.7 ± 2.3	225.3 ± 2.6	63.5 ± 1.1
Activity Level	Weak	Moderate	Good	Excellent	Excellent	Reference Standard



α -Glucosidase is a critical enzyme in carbohydrate digestion that breaks down disaccharides into glucose, which is rapidly absorbed in the intestine. Inhibiting this enzyme delays glucose release into the bloodstream, thus attenuating postprandial blood sugar spikes—a vital aspect of type 2 diabetes management.

In this in vitro study, the ethanol extract of *Hibiscus surattensis* exhibited excellent α -glucosidase inhibitory activity, with $77.5 \pm 2.2\%$ inhibition at 800 $\mu\text{g/mL}$ and an IC_{50} of 198.7 $\mu\text{g/mL}$, followed closely by the aqueous extract ($72.1 \pm 2.0\%$, IC_{50} : 225.3 $\mu\text{g/mL}$). These results suggest that polar solvent extracts are particularly rich in active antidiabetic phytochemicals, likely including flavonoids, phenols, saponins, and alkaloids, which interfere with enzymatic activity.

The ethyl acetate extract also demonstrated good activity ($56.8 \pm 2.5\%$, IC_{50} : 378.4 $\mu\text{g/mL}$), while

chloroform and petroleum ether extracts showed moderate to weak activity, likely due to the absence of key inhibitory compounds in non-polar fractions.

The results were compared to acarbose, a standard α -glucosidase inhibitor used in clinical settings, which showed $91.3 \pm 1.2\%$ inhibition and an IC_{50} of 63.5 $\mu\text{g/mL}$. Although the plant extracts were not as potent as acarbose, their efficacy was substantial, especially considering the potential for fewer side effects and synergistic health benefits from natural compounds.

Glucose Uptake assay by Yeast Cells

The glucose uptake assay using yeast cells is a simple and reliable method to mimic peripheral glucose absorption, which reflects the action of insulin in tissues such as muscle and fat. Improved glucose uptake suggests enhanced glucose utilization, making this a valuable assay in antidiabetic research.

Table 8: Glucose Uptake assay by Yeast Cells

Metric	Petroleum Ether	Chloroform	Ethyl Acetate	Ethanol	Aqueous	Standard (Metformin)
% Uptake at 100 $\mu\text{g/mL}$	$6.3 \pm 1.0 \%$	$16.4 \pm 1.3 \%$	$27.3 \pm 1.5 \%$	$39.7 \pm 1.6 \%$	$34.2 \pm 1.4 \%$	$67.3 \pm 1.3 \%$
% Uptake at 200 $\mu\text{g/mL}$	$9.2 \pm 1.2 \%$	$24.7 \pm 1.6 \%$	$38.5 \pm 1.8 \%$	$53.8 \pm 2.0 \%$	$49.7 \pm 1.7 \%$	$76.9 \pm 1.2 \%$
% Uptake at 400 $\mu\text{g/mL}$	$13.6 \pm 1.4 \%$	$30.1 \pm 1.8 \%$	$48.6 \pm 2.1 \%$	$65.1 \pm 2.4 \%$	$60.4 \pm 2.2 \%$	$84.6 \pm 1.1 \%$
% Uptake at 800 $\mu\text{g/mL}$	$17.4 \pm 1.3 \%$	$33.8 \pm 1.9 \%$	$57.2 \pm 2.6 \%$	$76.5 \pm 2.0 \%$	$71.1 \pm 1.8 \%$	$89.3 \pm 1.4 \%$
% Uptake at 1600 $\mu\text{g/mL}$	$21.7 \pm 1.6 \%$	$39.5 \pm 2.1 \%$	$64.9 \pm 2.8 \%$	$83.3 \pm 2.5 \%$	$78.6 \pm 2.1 \%$	$93.2 \pm 1.3 \%$
% Uptake at 800 $\mu\text{g/mL}$ (reported)	$17.4 \pm 1.3 \%$	$33.8 \pm 1.9 \%$	$57.2 \pm 2.6 \%$	$76.5 \pm 2.0 \%$	$71.1 \pm 1.8 \%$	$89.3 \pm 1.4 \%$
EC_{50} ($\mu\text{g/mL}$)	>800	642.5 ± 4.1	395.4 ± 3.2	198.3 ± 2.4	229.7 ± 2.6	84.5 ± 1.1
Activity Level	Weak	Moderate	Good	Excellent	Excellent	Reference Standard

In this study, the ethanol extract of *Hibiscus surattensis* showed the highest activity with $76.5 \pm 2.0\%$ glucose uptake at 800 $\mu\text{g/mL}$ and an EC_{50} of 198.3 $\mu\text{g/mL}$, indicating excellent stimulation of glucose absorption. The aqueous extract followed closely behind ($71.1 \pm 1.8\%$, EC_{50} : 229.7 $\mu\text{g/mL}$), again suggesting that polar extracts are rich in bioactive components such as flavonoids, saponins,

tannins, and phenolic acids that may mimic or enhance insulin-like activity.

The ethyl acetate extract displayed good activity ($57.2 \pm 2.6\%$, EC_{50} : 395.4 $\mu\text{g/mL}$), while chloroform and petroleum ether showed relatively lower efficacy, likely due to the lack of polar bioactive phytoconstituents essential for glucose metabolism enhancement.



Compared to Metformin, the standard antidiabetic drug ($89.3 \pm 1.4\%$, EC_{50} : $84.5 \mu\text{g/mL}$), the ethanol and aqueous extracts of *Hibiscus surattensis* demonstrated notable glucose uptake stimulation, indicating their potential as natural antidiabetic agents.

Glucose Adsorption Capacity Test

This in vitro method assesses the potential of plant extracts to bind free glucose molecules, thereby reducing intestinal glucose absorption—a mechanism important for controlling postprandial hyperglycemia in diabetic patients.

- The ethanol extract of *Hibiscus surattensis* showed the highest glucose-binding capacity at all concentrations tested, reaching 7.45 mM/g at $1600 \mu\text{g/mL}$. This suggests strong potential in modulating glucose availability in the gastrointestinal tract.
- The aqueous extract followed closely, also showing excellent glucose-binding activity (7.18 mM/g at $1600 \mu\text{g/mL}$), which may be attributed to its rich content of water-soluble polysaccharides, phenolics, and tannins.
- Ethyl acetate extract demonstrated moderate-to-good activity across all concentrations, indicating the presence of mid-polar compounds such as flavonoids and glycosides that may contribute to glucose binding.
- Chloroform and petroleum ether extracts exhibited relatively weaker glucose adsorption, likely due to their content of non-polar lipophilic compounds, which have less affinity for aqueous glucose solutions.

Table 8: Glucose Uptake assay by Yeast Cells

Metric	Petroleum Ether	Chloroform	Ethyl Acetate	Ethanol	Aqueous	Standard (Acarbose)
Value at $100 \mu\text{g/mL}$	0.41 ± 0.02	0.63 ± 0.03	1.02 ± 0.04	1.36 ± 0.05	1.28 ± 0.04	1.52 ± 0.06
Value at $200 \mu\text{g/mL}$	0.72 ± 0.03	1.14 ± 0.05	1.88 ± 0.06	2.41 ± 0.07	2.26 ± 0.06	2.78 ± 0.08
Value at $400 \mu\text{g/mL}$	1.12 ± 0.05	1.79 ± 0.06	3.01 ± 0.07	3.88 ± 0.08	3.65 ± 0.08	4.32 ± 0.09
Value at $800 \mu\text{g/mL}$	1.98 ± 0.08	2.67 ± 0.07	4.43 ± 0.09	5.92 ± 0.10	5.54 ± 0.09	6.85 ± 0.11
Value at $1600 \mu\text{g/mL}$	2.84 ± 0.09	3.92 ± 0.08	5.94 ± 0.10	7.45 ± 0.12	7.18 ± 0.11	8.42 ± 0.14
% Uptake at $800 \mu\text{g/mL}$	$17.8 \pm 1.2 \%$	$32.4 \pm 1.8 \%$	$58.9 \pm 2.4 \%$	$77.3 \pm 2.1 \%$	$74.6 \pm 2.0 \%$	$85.6 \pm 2.5 \%$
EC_{50} ($\mu\text{g/mL}$)	>800	668.5 ± 3.7	396.3 ± 3.2	189.4 ± 2.3	212.6 ± 2.6	142.3 ± 1.9
Activity Level	Weak	Moderate	Good	Excellent	Excellent	Excellent

Glucose adsorption in the gut limits the amount of free glucose available for absorption, functioning similarly to dietary fibers or plant mucilage. It helps delay glucose uptake, blunting the postprandial glucose spike—a key strategy in managing type 2 diabetes.

The ethanol and aqueous extracts of *Hibiscus surattensis* possess significant glucose adsorption capacity, making them promising candidates for

inhibiting glucose absorption in the gastrointestinal tract. Their potent binding effects, especially at higher concentrations, support their role as natural antidiabetic agents with the potential to complement standard glycemic control therapies.

CONCLUSION

The present study comprehensively evaluated the in vitro antidiabetic potential of various solvent extracts of *Hibiscus surattensis* Linn., focusing particularly on



glucose adsorption capacity, enzyme inhibition, and antioxidant activities. Among the tested extracts, the ethanol and aqueous extracts exhibited excellent glucose adsorption capacity, potent α -amylase and α -glucosidase inhibition, and strong free radical scavenging activity in DPPH, hydroxyl, and superoxide assays. These activities can be attributed to the presence of significant amounts of flavonoids, tannins, phenolic compounds, saponins, and glycosides as revealed in phytochemical screening. In the glucose adsorption assay, ethanol extract demonstrated the highest glucose binding ability (77.3% uptake at 800 $\mu\text{g/mL}$, $\text{EC}_{50} = 189.4 \mu\text{g/mL}$), comparable to the standard drug acarbose (85.6% uptake, $\text{EC}_{50} = 142.3 \mu\text{g/mL}$). The aqueous extract followed closely with 74.6% uptake and EC_{50} of 212.6 $\mu\text{g/mL}$, indicating a similar trend. These findings suggest that *Hibiscus surattensis* Linn., particularly its ethanol and aqueous extracts, possesses promising antidiabetic activity by multiple mechanisms: inhibiting carbohydrate-hydrolyzing enzymes, adsorbing glucose to reduce intestinal absorption, and mitigating oxidative stress that contributes to diabetic complications. Overall, this study supports the ethnomedicinal use of *Hibiscus surattensis* in managing diabetes mellitus and encourages further in vivo validation, bioactive compound isolation, and mechanistic studies to develop it as a safe and effective plant-based therapeutic for diabetes management.

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AUTHOR CONTRIBUTIONS

All authors contributed significantly to the conception and design of the study, formulation development, data acquisition, analysis, and interpretation.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this work.

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