

Evaluation of Corn Cob (*Zea mays* L.) Extract and Fractions as Inhibitors of α - and β -Glucosidase

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Abstract

Corn cob (*Zea mays* L.), a common agricultural waste, may act as a natural inhibitor of the α/β -glucosidase enzymes, which are crucial for blood glucose regulation and carbohydrate metabolism. This potential arises from secondary metabolites in corn cob, including phenolics, flavonoids, polysaccharides, and steroids, which have been reported to exhibit α/β -glucosidase inhibitory activity. Despite its abundance, corn cob has been largely underexplored as a source of dual α -/ β -glucosidase inhibitors, particularly through fraction-based bioactivity profiling. This study aims to assess the potential of the methanol extract and fractions (n-hexane, ethyl acetate, and butanol) of corn cob as α - and β -glucosidase inhibitors. The methodology includes drying and grinding corn cob, followed by maceration and fractionation. The inhibitory activities of the extract and fractions against α - and β -glucosidase were evaluated in vitro using spectrophotometry with a microplate reader and thin-layer chromatography (TLC) bioautography. Furthermore, the secondary metabolite content of the bioactive fraction was determined via liquid chromatography-tandem mass spectrometry (LC-MS/MS). The corn cob extract and fractions exhibited inhibitory activity against α - and β -glucosidase, with the n-hexane fraction showing the lowest observed IC_{50} values ($IC_{50}=22.11\pm1.07$ $\mu\text{g/mL}$ for α -glucosidase and $IC_{50}=77.28\pm0.29$ $\mu\text{g/mL}$ for β -glucosidase). LC-MS/MS profiling of the n-hexane fraction led to the detection of 21 compounds; of these, 10 were putatively identified as fatty acids, steroids, terpenoids, and flavonoids, which act as active inhibitors of α - and β -glucosidase. These findings highlight the novel potential of corn cob waste as a dual enzyme inhibitor and a promising natural therapeutic candidate for managing diabetes and hyperglycemia.

Keywords: secondary metabolites, enzyme inhibition, antidiabetic, LC-MS/MS, TLC bioautography

1. Introduction

Diabetes mellitus, an endocrine metabolic disease, has resulted in significant mortality worldwide. Research has indicated that diabetes, especially type 2, can induce major side effects, such as renal failure and cardiovascular disease (Meme *et al.*, 2015; Souza *et al.*, 2011). The α/β -glucosidase enzymes play a direct role in carbohydrate metabolism, which affects blood glucose levels. The hydrolysis of complex carbohydrates into glucose is catalyzed by α - and β -glucosidases, allowing for intestinal absorption (Saliu *et al.*, 2011; Sadat *et al.*, 2023). Controlling the activity of these enzymes can inhibit excessive glucose absorption, offering a promising therapeutic approach in the treatment of diabetes. This results in the reduction of postprandial hyperglycemia, a major contributor to diabetes. Furthermore, oxidative stress contributes to diabetes mellitus by exacerbating β -cell dysfunction and insulin resistance (Okoli *et al.*, 2011). Plant-derived α -glucosidase inhibitors typically exhibit IC_{50} values of 0.012–0.437 mg/mL (acarbose: 0.0013–0.282 mg/mL) (Dirir *et al.*, 2021). Flavonoids, phenolics, and steroids, found in a variety of medicinal plants, exhibit antidiabetic properties via the processes of glycemic control and the modulation of oxidative stress (Ardalani *et al.*, 2021). In particular, corn cob extract is a potential antidiabetic agent, given its composition, which shows an abundance of these compounds.

Corn production in Indonesia reached approximately 14.77 million tons of dry-shelled maize (14% moisture content) in 2023 (Badan Pusat Statistik [BPS], 2024). Further, corn cobs are estimated to account for 40–50% of total corn production (Islam *et al.*, 2023). Thus, the large-scale use of corn as a food source results in a considerable amount of corn cob waste. Due to their low economic value, corn cobs remain largely unused and are typically only employed as animal feed. However, as the site where corn kernels grow, corn cobs naturally contain a rich array of secondary metabolites that can be utilized in various fields.

Previous phytochemical studies of corn cobs have revealed that corn cob extract contains phenolic compounds (Fidrianny *et al.*, 2016), flavonoids (Harahap *et al.*, 2024; Dong *et al.*, 2014; Fidrianny *et al.*, 2016), tannins (Kusriani *et al.*, 2017), polysaccharides (Ma *et al.*, 2016), and steroids (Fidrianny *et al.*, 2016). Considering the presence of these compound groups, corn cobs have been reported to exhibit antioxidant (Suryanto *et al.*, 2017; Silveira *et al.*, 2014), anti-tyrosinase (Kampipit *et al.*, 2023; Harahap *et al.*, 2024), anti-elastase (Harahap *et al.*, 2024), anti-hyaluronidase (Harahap *et al.*,

2024), antiproliferative (Silveira *et al.*, 2014), and sunscreen activities (Wungkana *et al.*, 2013). Several studies have also reported the antidiabetic activity of polysaccharides from sweet corn cobs in inhibiting α -amylase and α -glucosidase, with IC_{50} values of 20.91 and 12.47 mg/mL, respectively (Ma *et al.*, 2016). In addition, an *in vivo* study on the hypoglycemic effect of polysaccharides from sweet corn cobs indicated a significant lowering of blood glucose and lipid levels in type 2 diabetes mellitus mice by regulating glycolipid metabolism and islet β -cell function via several pathways (Wang *et al.*, 2024). The anthocyanin from purple corn cobs also exhibits α -glucosidase inhibitory activity, with an IC_{50} range of 0.45–1.221 mg/mL (Dai *et al.*, 2022). However, to date, studies have not evaluated the antidiabetic activity of the methanol extract of corn cobs, as related to their phenolic, flavonoid, and steroid content. Therefore, this study evaluated the α - and β -glucosidase inhibitory activity of the methanol extract and fractions of corn cobs.

Herein, thin-layer chromatography (TLC) bioautography was employed for the initial screening of corn cob fractions against α - and β -glucosidase, a sensitive technique that detects inhibition zones directly on chromatograms (Simões-Pires *et al.*, 2009). TLC bioautography was applied as a rapid, high-throughput screening method for glucosidase inhibitors, enabling the direct visualization of active fractions on TLC plates, as validated by Simões-Pires *et al.* (2009). While these recent studies (Dai *et al.*, 2022; Wang *et al.*, 2024) have primarily focused on water-soluble polysaccharides or anthocyanins, there is a distinct lack of research concerning the dual α - and β -glucosidase inhibitory potential of the more lipophilic secondary metabolites, such as the phenolics and steroids found in corn cob fractions. Therefore, the current study aimed to screen the methanol extract and fractions of corn cobs for α - and β -glucosidase inhibition, determine IC_{50} values, and characterize the bioactive compounds via LC-MS/MS analysis. Unlike prior corn-based antidiabetic research focusing on silk or pericarp, this work presents a new approach by targeting corn cob waste—the most voluminous corn by-product—for comprehensive enzymatic screening and metabolite characterization, addressing a critical gap in agro-waste valorization. Therefore, the antidiabetic effects of corn cobs are expected to increase the economic value of corn cob waste.

2. Methodology

2.1 Extraction and Fractionation

Following previous work, the total methanol extract and *n*-hexane, ethyl acetate, and butanol fractions were obtained from corn cobs sourced from Tikalak hamlet, Pasaman Regency, West Sumatra (Harahap *et al.*, 2024). Dried corn cob powder (5,000 g) was macerated using methanol (1:5 w/v) for 3 days, yielding 100.015 g of crude extract (2.0003% yield). Furthermore, the concentrated corn cob extract was dissolved in aquadest and partitioned with *n*-hexane (9.948 g), ethyl acetate (17.756 g), and *n*-butanol (20.628 g).

2.2 α - and β -Glucosidase Inhibitor Profiling via TLC-bioautography

The inhibitory profiles of the extract and fractions against α - and β -glucosidase were evaluated via TLC bioautography according to the procedure described by Simões-Pires *et al.* (2009), with slight modifications (Simões-Pires *et al.*, 2009). First, 5 μ L aliquots of the extract and fractions, with a concentration of 5,000 μ g/mL, were spotted on a TLC silica gel 60 F₂₅₄ plates (20 x 20 cm, 1.05554.0001, Merck KGaA, Germany) using a mobile phase of toluene, ethyl acetate, and formic acid (all reagents obtained from Merck KGaA, Germany) with a composition of 70:25:5—this was selected based on preliminary trials optimizing the separation of corn cob metabolites. Subsequently, 30 U of α -glucosidase from *Saccharomyces cerevisiae* (G5003, Sigma-Aldrich, USA) and β -glucosidase from almonds (492900, Sigma-Aldrich, USA) were dissolved in 100 mL of an acetate buffer solution (10.25 g sodium acetate, 1.06268.0250, Merck KGaA, Germany), with 0.1 M acetic acid (1.00062.2500, Merck KGaA, Germany) added to reach pH 7.5, and the solution was kept at 4°C. The TLC plate was then sprayed with an enzyme solution and incubated by placing it flat on a plastic plug in a plastic tank containing a small amount of water to prevent direct contact between the plate and the water. To maintain humidity, the tank was covered, and incubation was carried out at room temperature for 60 minutes for α -glucosidase and 20 minutes for β -glucosidase (Simões-Pires *et al.*, 2009). Furthermore, enzyme activity was measured by reactions with the substrates 2-naphthyl- α -D-glucopyranoside (Sigma-Aldrich, USA) for α -glucosidase and 2-naphthyl- β -D-glucopyranoside (Sigma-Aldrich, USA) for β -glucosidase at a concentration of 2 mg/mL in ethanol (Merck KGaA, Germany), and with Fast Blue B Salt (S461873, Sigma-Aldrich, USA) at 2.5 mg/mL in water. The ratio between the substrate and reagent for α -glucosidase was 1:1, while that for β -glucosidase was 1:4. The reaction mixture was incubated for 2–5 min to yield

a purple background (from the formation of the *p*-nitrophenol product), while the inhibition zones appeared as white spots (Simões-Pires *et al.*, 2009).

2.3 Determination of Inhibitory Concentration 50 (IC₅₀) of Extract and Fractions

The α - and β -glucosidase inhibitor activity of the extract and fractions was evaluated *in vitro* to obtain the IC₅₀ value, using a previous method with slight modifications (Zhang *et al.*, 2023). First, 40 μ L of a phosphate buffer solution (pH = 6.86, 0.1 M), 20 μ L of the sample solution, and 40 μ L of the α - and β -glucosidase enzyme solution (0.2 U/mL in PBS) were added to a 96-well plate and allowed to react for 15 min at 37°C. Subsequently, 20 μ L of 2.5 mM *p*-nitrophenyl- α -D-glucopyranoside (N1377, Sigma-Aldrich, USA) for α -glucosidase and 2.5 mM *p*-nitrophenyl- β -D-glucopyranoside (N1006, Sigma-Aldrich, USA) for β -glucosidase were added and allowed to react for 20 min at 37°C. Finally, 80 μ L of 0.2 M Na₂CO₃ (Merck KGaA, Germany) was added to stop the reaction. The absorbance was measured at 405 nm, and the inhibition percentage (%) was calculated using Equation 1. All assays were performed in triplicate (n = 3), and the results are presented as mean $\pm$ standard deviation (SD). IC₅₀ values were determined using linear regression analysis in Microsoft Excel between the sample concentration (x) and % enzyme inhibition (y), with the equation $y = a + bx$. The IC₅₀ was obtained as the x-value when y = 50% was substituted into Equation 1.

$$\text{Inhibitor percentage} = \frac{A_0 - (A_1 - A_2)}{A_0} \times 100\% \quad (1)$$

where *A1* is the reaction absorbance of the combined solution (enzyme + substrate + sample), *A0* is the absorbance of the PBS buffer substituted for the sample, and *A2* is the blank absorbance—the absorbance of only the sample and PBS buffer substituted for the α/β -glucosidase solution and pNPG (*p*-nitrophenyl- α -D-glucopyranoside and *p*-nitrophenyl- β -D-glucopyranoside) solutions.

2.4 LC-MS/MS Analysis of Bioactive Fraction of Corn Cob

The bioactive fraction of the corn cobs was examined via LC-MS/MS, using a C18 column set to a temperature of 50°C. Two solutions—acetonitrile + 0.05% formic acid (B) and water + 0.05% formic acid (A)—comprised the mobile phase for the LC analysis. The injection volume was 5 μ L, and the flow rate was set to 0.2 mL/min for 23 min. Electrospray ionization (ESI) in

positive mode was employed for the mass spectrometry (MS) analysis, using a m/z scan range of 50–1200 and desolvation temperatures of 100 and 350°C. The Masslynx program (version 4.1, Waters Corporation) was used to analyze the data (Waters Corporation, 2005).

3. Results and Discussion

3.1 TLC Bioautographic Profile of α -/ β -glucosidase Inhibitory Compounds

TLC bioautography combines TLC-based separation with bioactivity analysis, providing rapid access to data on the activity and localization of active compounds in complex plant matrices (Marston *et al.*, 2002). The TLC bioautography assay in the detection of α - and β -glucosidase inhibitors is based on the hydrolysis of *p*-nitrophenyl- α -D-glucopyranoside (for the α -glucosidase inhibitor) and *p*-nitrophenyl- β -D-glucopyranoside (for the β -glucosidase inhibitor) to form 2-naphthol; this, in turn, reacts with Fast Blue B Salt to yield a purple diazonium dye (Simões-Pires *et al.*, 2009). In this work, a TLC bioautography assay was conducted on the methanol extract and *n*-hexane, ethyl acetate, and butanol fractions of the corn cobs. Separation was performed using a TLC plate with the eluant toluene:ethyl acetate:formic acid (70:25:5). The inhibition spots of each sample can be noted on the TLC plates, as shown in Figure 1.

In the TLC bioautography assay (Figure 1), the *n*-hexane fraction shows six inhibition spots: two spots that only inhibit α -glucosidase (R_f = 0.35 and 0.63), one spot that only inhibits β -glucosidase (R_f = 0.13), and three spots that inhibit both enzymes (R_f = 0.46, 0.51, and 0.75). Inhibitory spots emerge under UV light of 365 nm following citroborate spot detection (Figure 1d). Since citroborate is a specific reagent for identifying flavonoid compounds, it is suspected that the inhibitory spots on the TLC bioautogram belong to the flavonoid compound group. Other inhibitory spots were also detected on the TLC plate using the ANS reagent (Figure 1c); these spots are blue, which may include terpenoid- or steroid-like metabolites. However, the ANS reagent is not selective enough to permit definitive classification of the compounds. While not as prevalent as in the *n*-hexane fraction, inhibition spots are also present in the other fractions; these are suspected to be polar compounds, possibly phenolics.

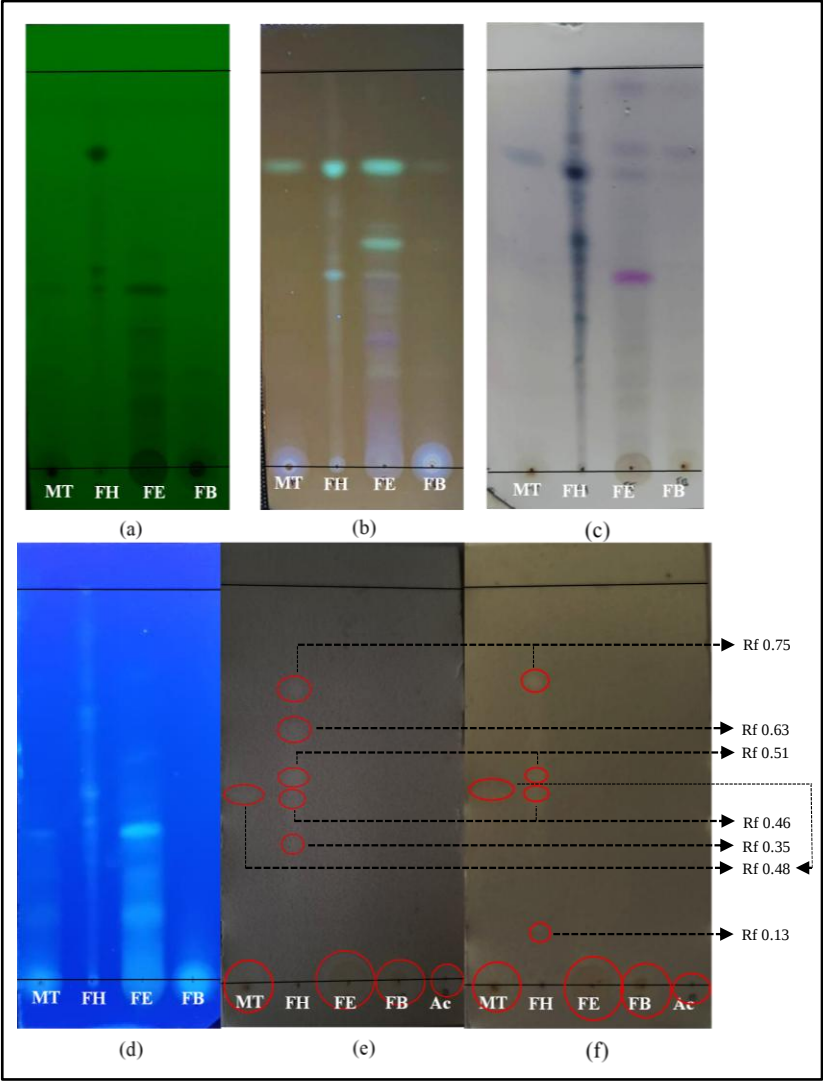


Figure 1. TLC bioautograms of the extract and fractions of the corn cobs using the mobile phase toluene : ethyl acetate : formic acid (70:25:5). MT = methanol extract, FH = *n*-hexane fraction, FE = ethyl acetate fraction, FB = butanol fraction, and Ac = acarbose (positive control). Chromatograms involving UV 254 nm (a), UV 365 nm (b), anisaldehyde-sulfuric acid (c), UV 365 nm following citroborate reagent spraying (d), the α -glucosidase inhibitor (e), and the β -glucosidase inhibitor (f)

3.2 Inhibitory Potency of Corn Cob Extract and Fractions against α -/ β -glucosidase

The α - and β -glucosidase inhibitor activity was tested spectrophotometrically using a microplate reader. The results are based on the absorbance values (at 405 nm) of *p*-nitrophenol, the final product of the hydrolysis of *p*-nitrophenyl- α -D-glucopyranoside and *p*-nitrophenyl- β -D-glucopyranoside by α -glucosidase and β -glucosidase, respectively. The IC_{50} values of the corn cob extract and fractions were calculated using acarbose as the positive control. Table 1 shows that both the methanol extract and the fractions of corn cobs exhibit inhibitory effects on the two enzymes. The *n*-hexane fraction exhibits the strongest activity among the samples, with IC_{50} values of 22.11 ± 1.07 and 77.28 ± 0.29 $\mu\text{g/mL}$ against α - and β -glucosidase, respectively. These are followed by the activities of the ethyl acetate fraction against α -glucosidase ($IC_{50} = 24.1 \pm 0.66$ $\mu\text{g/mL}$) and the methanol extract against β -glucosidase ($IC_{50} = 138.63 \pm 0.31$ $\mu\text{g/mL}$). The *n*-hexane and ethyl acetate fractions display lower IC_{50} values than acarbose (positive control, $IC_{50} = 198.96 \pm 3.29$ $\mu\text{g/mL}$) in regard to the α -glucosidase inhibitor assay. Only the *n*-hexane fraction demonstrates superior inhibitory activity over acarbose for β -glucosidase ($IC_{50} = 104.91 \pm 0.81$ $\mu\text{g/mL}$). This is consistent with the earlier TLC bioautography tests, which revealed the greatest number of inhibitory spots in the *n*-hexane fraction. These activities align with those of known plant-derived α -glucosidase inhibitors, which typically show IC_{50} values of 0.012–0.437 mg/mL (comparable to acarbose: 0.0013–0.282 mg/mL) (Dirir *et al.*, 2021). The high inhibitory activity of the *n*-hexane fraction may also reflect its complexity, in which synergistic/additive effects among several weak inhibitors may contribute more than the sum of their individual effects. While strong IC_{50} values were established for the corn cob fractions, the mechanisms of inhibition remain undetermined, representing a key limitation of this study.

Future kinetic studies should elucidate competitive and noncompetitive inhibition modes, guiding the optimization of lead compounds.

Table 1. IC_{50} values for fractions and corn cob extract

Sample	IC_{50} ($\mu\text{g/mL}$)	
	α -glucosidase inhibitor	β -glucosidase inhibitor
Methanol Extract	204.08 ± 1.46	138.63 ± 0.31
<i>n</i> -hexane fraction	22.11 ± 1.07	77.28 ± 0.29
Ethyl acetate fraction	24.10 ± 0.66	152.24 ± 1.33
Butanol fraction	246.74 ± 0.17	159.68 ± 3.31
Acarbose (positive control)	198.96 ± 3.29	104.91 ± 0.81

3.3 Metabolite Profile of Bioactive Fraction of Corn Cobs Revealed via LC-MS/MS

The *n*-hexane fraction constitutes the bioactive fraction based on the tests of α - and β -glucosidase inhibitor activity via both TLC bioautography and spectrophotometry (using a microplate reader). To explore the diversity of available phytochemicals in the bioactive fraction with respect to α - and β -glucosidase inhibitor activity, the *n*-hexane fraction was analyzed by LC-MS/MS. The chromatogram indicates at least 21 compounds (Figure 2). Among these, 10 are known compounds from various groups, including fatty acids, steroids, terpenoids, and flavonoids, and 11 are compounds that cannot be identified based on their mass spectral patterns (Table 2). The metabolites were putatively annotated based on accurate mass and MS/MS fragmentation patterns compared with published literature.

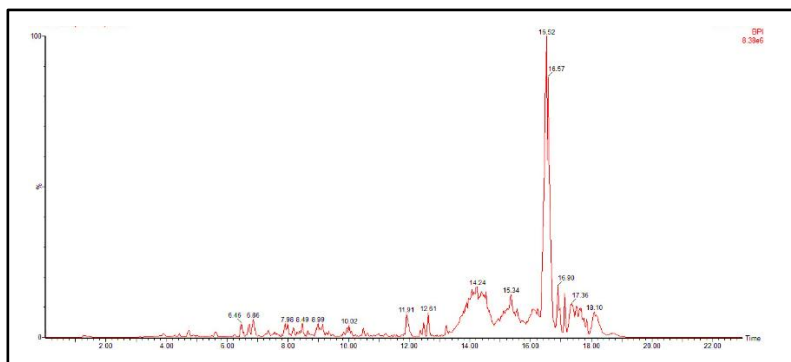


Figure 2. LC Chromatogram of corn cob *n*-hexane fraction

The LC-MS/MS analysis of the *n*-hexane fraction reveals the presence of antidiabetic bioactive compounds, such as catechins. According to earlier research, the IC_{50} value of catechin in inhibiting α -glucosidase is 51.7 μ M (the value for acarbose as a positive control is 300.1 μ M) (Kim *et al.*, 2014). Molecular dynamics simulations have also indicated α -glucosidase inhibition via the stabilization of the catechin- α -glucosidase complex, which induces ligand repositioning and disrupts the enzyme's hydrolytic activity at the active site (Choudhary *et al.*, 2020). Other substances with antidiabetic properties include abscisic acid (Daliu *et al.*, 2019), tangeretin (Qin & Jiang, 2020), stigmaterol (Lolok *et al.*, 2022), and ergosterol (Xiong *et al.*, 2017). These have been extensively isolated from various plants, particularly catechin, which has previously been discovered in corn cobs (Harahap *et al.*, 2024). This

indicates that α - and β -glucosidases are significantly inhibited by these substances. Figures 3 and 4 display spectral interpretations of the ion chromatograms generated via tandem mass spectrometry.

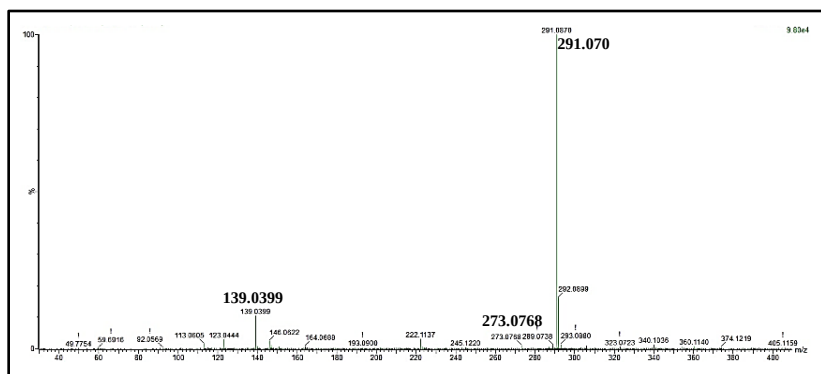


Figure 3. Mass spectrum of catechin from n-hexane fraction of corn cob, m/z 291.0870

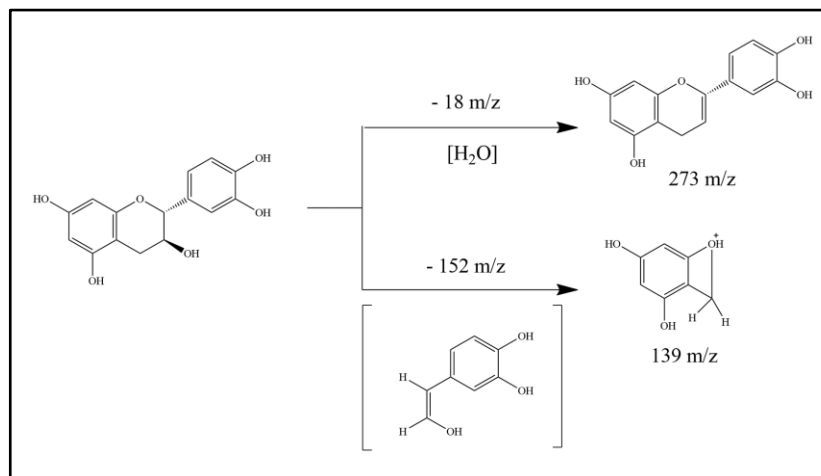
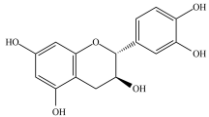
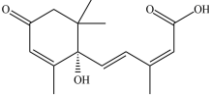
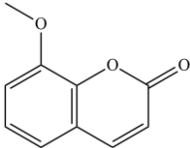
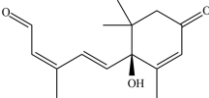
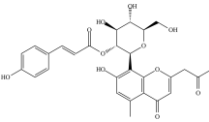
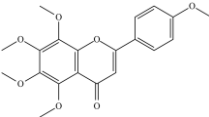
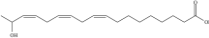
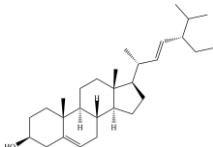
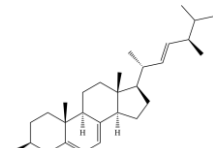
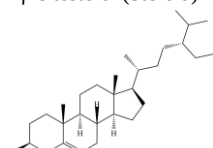


Figure 4. Proposed MS/MS fragmentation pattern for catechin (Chang & Wu, 2011)

Figure 3 displays the mass spectrum of catechin obtained from the *n*-hexane fraction of corn cobs in the positive ion mode. Two fragment ions are generated by the $[M+H]^+$ ion at m/z values of 273 and 139. The loss of an H_2O molecule from catechin is represented by the fragment ion with $m/z = 273$. According to the suggested fragmentation process shown in Figure 4, the fragment ion at $m/z = 139$ results from the loss of a species of $m/z = 152$.

Table 2. Compounds detected in the n-hexane fraction of corn cob via LC-MS/MS

			Known Compounds		Matching metabolite	Ref
Rt (min)	[M+H] ⁺ , m/z	Fragment ion, m/z	Molecular Formula	% peak area		
3.90	291.0870	273.0768 139.0399	C ₁₅ H ₁₄ O ₆	0.42%	Catechin (Flavanol) 	Chang and Wu, 2011
6.46	265.1441	263.1286 219.1386 201.1278	C ₁₅ H ₂₀ O ₄	0.69%	Absciscic acid (sesquiterpenoid) 	Ross et al., 2004
8.37	177.0549	147.0443 115.0541 89.0388	C ₁₀ H ₈ O ₃	0.16%	8-Methoxychromen-2-one (coumarins) 	Cissé et al., 2009
8.49	249.1499	231.1391 177.0557	C ₁₅ H ₂₀ O ₃	0.67%	Absciscic aldehyde (sesquiterpenoid) 	Andersson 2012
9.06	541.1708	517.1837 478.2093 369.1337	C ₂₈ H ₂₈ O ₁₁	0.58%	Aloeresin A (cinnamate ester) 	Ismail et al., 2021
10.48	373.1282	343.0814	C ₂₀ H ₂₀ O ₇	0.45%	Tangeretin (Flavonoid) 	Wang et al., 2019
12.48	295.2272	279.2208	C ₁₈ H ₃₀ O ₃	0.64%	17-Hydroxylinolenic acid (Fatty acid) 	Razgonov a et al., 2022

16.52	413.3778	395.3665 375.3042	C ₂₉ H ₄₈ O	41.43%	Stigmasterol (Steroid)	Orabi et al., 2022
						
17.63	397.3826	379.3356 313.2737	C ₂₈ H ₄₄ O	2.03%	Ergosterol (steroid)	Mocan et al., 2016
						
17.67	415.3575	397.3823 313.2737	C ₂₉ H ₅₀ O	3.01%	β-sitosterol (Steroid)	Mocan et al., 2016
						

Unknown Compounds						
Rt (min)	[M+H] ⁺ , m/z	Fragment ion, m/z	Molecular Formula	% peak area	Matching metabolite	Ref
6.86	441.2050	-	C ₂₉ H ₂₈ O ₄	0.84%	Unknown	-
8.16	177.0552	-	C ₆ H ₈ O ₆	0.39%	Unknown	-
7.98	527.1567	-	C ₂₇ H ₃₀ O ₁₁	0.61%	Unknown	-
11.91	319.3026	-	C ₁₈ H ₃₉ O ₄	1.70%	Unknown	-
12.61	520.3408	-	C ₂₄ H ₂₃ O ₁₃	1.01%	Unknown	-
14.24	411.3611	-	C ₂₉ H ₄₆ O	15.34%	Unknown	-
14.31	309.2413	-	C ₁₉ H ₃₂ O ₃	8.77%	Unknown	-
16.90	507.4062	-	C ₃₁ H ₅₄ O ₅	2.57%	Unknown	-
17.12	391.2841	-	C ₂₄ H ₃₈ O ₄	1.45%	Unknown	-
17.51	313.2726	-	C ₁₉ H ₃₆ O ₃	3.56%	Unknown	-
17.85	427.3578	-	C ₂₉ H ₄₆ O ₂	3.97%	Unknown	-

Particular consideration should be given to the 11 unknown peaks visible in the *n*-hexane fraction as possible new glucosidase inhibitors. These unknowns, notably those with RT values of 14.24 min (*m/z* = 411.3611, abundance of 15.34%), 14.31 min (*m/z* = 309.2413, 8.77%), 17.51 min (*m/z* = 313.2726, 3.56%), and 17.85 min (*m/z* = 427.3578, 3.97%), match the robust bioautography zones (*R*_f = 0.46–0.75) and superior IC₅₀ value of *n*-hexane. Notably, approximately half of the detected chromatographic peaks remained unidentified, including one relatively abundant peak (RT = 14.24). These unidentified compounds may contribute substantially to the observed α- and

β -glucosidase inhibitory activities. Therefore, further studies involving the isolation and structural elucidation of these unknown compounds are warranted to clarify their chemical identities and evaluate their contribution to the overall bioactivity.

4. Conclusion and Recommendation

In this study, the methanol extract and fractions of corn cobs were evaluated in terms of their inhibitory activity against α - and β -glucosidase. The *n*-hexane fraction showed the lowest observed IC_{50} values against both enzymes, with IC_{50} values of 22.11 ± 1.07 $\mu\text{g/mL}$ for α -glucosidase and 77.28 ± 0.29 $\mu\text{g/mL}$ for β -glucosidase. The phytochemical content of the *n*-hexane fraction (catechin, abscisic acid, tangeretin, stigmasterol, and ergosterol) contributes to the inhibitory activity against α - and β -glucosidase. These findings highlight the *n*-hexane fraction of corn cobs as a promising antidiabetic candidate, given its α - and β -glucosidase inhibition. However, while *in vitro* results are encouraging, translational relevance requires further validation. Future research should employ bioassay-guided fractionation to isolate pure glucosidase inhibitors from the active *n*-hexane fraction (1), kinetic analyses to elucidate competitive/noncompetitive inhibition modes (2), and cell-based assays to confirm antidiabetic potential beyond enzymatic screening (3). This should be followed by *in vivo* evaluations, including toxicity profiling, assessing metabolic stability, and pharmacokinetic studies.

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