INTEGRATED MOLECULAR DOCKING AND ADMET ANALYSIS OF Hyoscyamus niger METABOLITES TARGETING A-GLUCOSIDASE AND A-AMYLASE FOR ANTIDIABETIC THERAPY

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ABSTRACT

The global diabetes epidemic requires safer therapeutic alternatives to conventional α-glucosidase and α-amylase inhibitors, which cause significant gastrointestinal side effects. Natural products provide structurally diverse scaffolds for antidiabetic drug discovery. This study evaluated the dual enzyme inhibitory potential of *Hyoscyamus niger L.* (black henbane) through integrated experimental and computational approaches. Methanolic extracts of *H. niger* seeds were assessed for α-glucosidase and α-amylase inhibitory activities using enzymatic assays. *H. niger* extract exhibited superior inhibitory activity compared to acarbose, with IC_{50} values of 35.85 ± 5 µg/mL (α-glucosidase) and 44.56 ± 5 µg/mL (α-amylase) versus acarbose's IC_{50} values of 141.0 ± 5 µg/mL and 131.0 ± 10 µg/mL, respectively. 100 phytochemicals were subjected to molecular docking against target enzymes (PDB IDs: 3L4Y and 3BAJ). Lead compounds were evaluated for drug-likeness using Lipinski's Rule of Five and comprehensive ADMET profiling. Molecular docking identified one coumarinolignan called cleomiscosin B as the most promising lead compound, demonstrating excellent binding affinities (-8.1 kcal/mol for both enzymes), complete Lipinski compliance, and favorable safety profiles with no AMES toxicity. In spite to pongamoside D was also founded as the top dual-target inhibitor with binding affinities of -9.3 kcal/mol (α-amylase) and -8.3 kcal/mol (α-glucosidase). However, ADMET analysis suggested that pongamoside D violated Lipinski's rule and showed positive AMES toxicity with possible carcinogenicity risk. This study establishes *H. niger* as a valuable source of novel dual-target antidiabetic compounds, with cleomiscosin B representing a promising alternative to synthetic inhibitors.

Keywords: *Hyoscyamus niger*, α-glucosidase, α-amylase, cleomiscosin B, molecular docking, antidiabetic agents.

INTRODUCTION

Diabetes mellitus represents one of the most formidable health challenges of the 21st century, with its global prevalence reaching alarming proportions [1]. According to the latest International Diabetes Federation (IDF) Atlas, diabetes affects 589 million adults globally – roughly 1 in 9 people. This number has skyrocketed, more than quadrupling since 1990, and is expected to reach 853 million by 2050[2]. In 2024 alone, diabetes caused an estimated 3.4 million deaths, or one death every 9 seconds. The disease also led to massive healthcare costs, amounting to at least USD 1 trillion worldwide [3]. This escalating burden necessitates the urgent development of effective therapeutic strategies that can provide safer, more accessible, and cost-effective management approaches for the millions affected by this condition [4].

The strategic targeting of carbohydrate-digesting enzymes has emerged as a promising therapeutic approach for managing postprandial hyperglycemia, a critical factor in diabetes progression and complications [5]. One of the most effective management strategies to decrease postprandial hyperglycemia is to retard glucose absorption by inhibiting carbohydrate hydrolyzing enzymes, such as α-glucosidase and α-amylase, in the digestive organs. α-amylase initiates starch hydrolysis by cleaving α(1,4)-glycosidic bonds in polysaccharides, while α-glucosidase completes the final step of carbohydrate digestion by releasing glucose from oligosaccharides at the intestinal brush border (see Figure 1) [6]. Currently approved inhibitors for these enzymes are restricted to acarbose, miglitol, and voglibose. However, although these inhibitors retard glucose absorption, undesirable gastrointestinal side effects impede their application.

These limitations, including flatulence, diarrhea, and abdominal discomfort, significantly compromise patient compliance and treatment efficacy, creating an urgent need for alternative therapeutic agents [7].

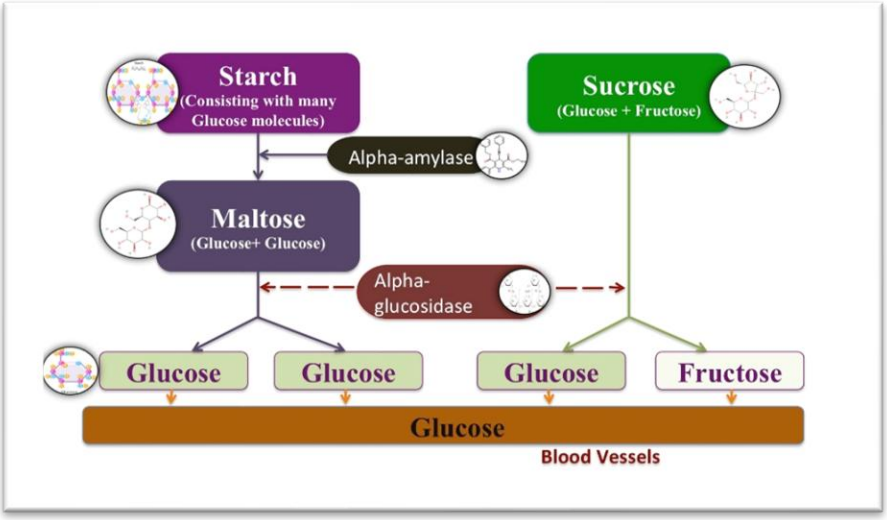

Figure 1. Figure illustrating the biochemical working of a-amylase & a-glucosidase enzymes.

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Natural products continue to serve as treasure troves for drug discovery, offering unique structural diversity and biological activity that synthetic chemistry often struggles to replicate [8]. Plant-derived enzyme inhibitors present compelling alternatives to synthetic drugs, combining therapeutic efficacy with reduced side effects and enhanced patient tolerance [9]. Among the vast pharmacopeia of medicinal plants, *H. niger* L. (black henbane) emerges as a particularly fascinating candidate worthy of systematic investigation. This member of the Solanaceae family has earned recognition not merely as a traditional remedy, but as a biochemically rich reservoir of bioactive compounds with demonstrated therapeutic potential across multiple pathological conditions [10].

H. niger represents a pharmaceutical powerhouse, primarily renowned for its abundant tropane alkaloids—particularly hyoscyamine and scopolamine, which exhibit potent anticholinergic, mydriatic, antispasmodic, and antiemetic effects [11]. Scopolamine, the most valued tropane alkaloid, demonstrates fewer side effects and finds clinical application in anesthetic premedication and motion sickness management [12]. Beyond these well-characterized alkaloids, recent investigations have unveiled the plant's remarkable pharmacological versatility. Chemical and biological validation studies have established *H. niger* seeds' anti-inflammatory properties, while essential oil analyses have revealed significant antimicrobial and antioxidant activities [13]. The plant's therapeutic repertoire extends to pain management, where its alkaloids provide analgesic relief for muscle spasms, neuralgia, menstrual cramps, and various localized pain conditions. Additionally, these compounds demonstrate efficacy against ear and eye inflammation, rheumatism, ulcers, cough, asthma, and bronchitis, underscoring the plant's broad-spectrum therapeutic potential [14].

While *H. niger's* extensive pharmacological profile spans numerous therapeutic domains, its potential as a modulator of glucose homeostasis through carbohydrate-digesting enzyme inhibition remains an intriguing yet unexplored frontier. This research gap presents a compelling opportunity to investigate whether this biochemically diverse medicinal plant harbors compounds capable of targeting the key enzymes responsible for dietary carbohydrate breakdown and subsequent glucose absorption. Given the plant's demonstrated anti-inflammatory and antioxidant properties—factors intimately linked to diabetes pathophysiology, there exists a strong biological rationale for exploring its antidiabetic potential through enzyme inhibition mechanisms [15].

The present investigation was conceived to bridge this knowledge gap through a comprehensive, multidisciplinary approach that combines experimental validation with computational prediction. Our research strategy encompassed systematic *in vitro* enzymatic assays to quantitatively assess the α-amylase and α-glucosidase inhibitory potential of *H. niger* extracts. This was complemented by sophisticated molecular docking studies utilizing a meticulously curated library of known *H. niger* bioactive compounds, enabling identification of the most promising molecular candidates through virtual screening against both target enzymes. The computational predictions were subsequently validated through targeted *in vitro* assays of individual compounds, creating a robust evidence-based framework for understanding the mechanistic basis of any observed inhibitory activity. This integrated approach not only promises to reveal novel aspects of *H. niger's* therapeutic potential but also contributes valuable insights to the expanding field of natural product-based enzyme inhibitors, potentially offering safer, more accessible alternatives to conventional synthetic medications for managing postprandial hyperglycemia in diabetic patients.

EXPERIMENTAL

Sample collection

1.0 kg of *H. niger* seed were purchased from local seeds market in District Multan of Province Punjab, Pakistan in April 2023 (Figure 2). The identification of these seeds was done by the Department of Botany, Government College University in Faisalabad (GCUF), Punjab, Pakistan.

Figure 2. Dried seeds of *H. niger* L (black henbane).

Extract Preparation of *H. niger*

The test seeds were cleaned with water to remove contaminants before being baked in an oven at 50ºC to remove moisture. The sample was ground using an electric grinder into a fine powder. Then, 150 mL of methanol was used to extract 10 g of the ground material using the Soxhlet apparatus & thimble. A dark green extract was produced after the mixture was refluxed for 24 hours. Using the modified procedure developed by Kadan et al. [16], the extract was then put into a rotating vacuum evaporator and maintained there at 50ºC until all the solvents evaporated, yielding 1.05 g (10.5%). For further investigation, the dried extract was kept in a refrigerator at 4ºC. The dried extracts were weighed, and a stock solution was prepared using 90% of the methanol.

α -Glucosidase inhibition activity

The inhibitory activity against α-glucosidase was evaluated spectrophotometrically, following an established protocol adapted from Kim et al. [17]. Briefly, test samples (plant extracts) and the reference inhibitor (acarbose) were prepared at varying concentrations in 0.1 M sodium phosphate buffer (pH 6.9). A 100 µL aliquot of each sample solution was combined with 15 µL of α-glucosidase solution (0.1 U/mL, pre-incubated at 37°C for 10 min) in a microplate well. The enzymatic reaction was initiated by adding 250 µL of 20 mM p-nitrophenyl-α-D-glucopyranoside (pNPG) substrate. After incubating the reaction mixture at 37°C for exactly 10 minutes, the reaction was terminated by adding 100 µL of 0.1 M sodium carbonate (Na_2CO_3). The amount of p-nitrophenol liberated by enzymatic hydrolysis was quantified by measuring the absorbance at 405 nm using a microplate reader. The percentage inhibition of α-glucosidase activity was calculated using the following formula:

$$\text{α-Glucosidase inhibition} = \frac{[\text{ABSblank} - \text{ABStest}]}{[\text{ABSblank}} \times 100$$

α-Amylase inhibition activity

Pancreatic α-amylase inhibitory activity was evaluated according to an adapted protocol [18]. Test samples (plant extracts) and acarbose standards (positive control) were prepared at multiple concentrations in 0.1 M sodium phosphate buffer (pH 6.9). For each assay, 250 µL of α-amylase solution (4 U/mL in buffer) was combined with sample solutions and pre-incubated at 37°C for 10 min. The enzymatic reaction was initiated by adding 500 µL of 1% starch substrate (in buffer). Following a 10-min incubation at 37°C, reactions were terminated by adding 1.0 mL of 96 mM 3,5-dinitrosalicylic acid (DNS) reagent. The mixtures were then incubated at 37°C for 5 min, heated at 100°C for 10 min, and immediately cooled in an ice-water bath. After tenfold dilution with distilled water, absorbance was measured at 540 nm. α-Amylase inhibition was calculated as:

$$\alpha\text{-amylase inhibition} = \frac{[\text{ABSblank} - \text{ABStest}]}{[\text{ABSblank}} \times 100$$

Statistical Analysis

All assays were performed in triplicate. Data are presented as mean ± standard deviation (SD), calculated using Microsoft Excel 2016 (Microsoft Corp., USA). Graphical representations of *in vitro* results were generated with GraphPad Prism 8.0 (GraphPad Software, San Diego, CA).

Molecular Docking

PyRx software was utilized for molecular docking to investigate the binding patterns of ligand-receptor interactions in the context of treating or suppressing diabetes. Specifically, the docking focused on phytochemicals present in plant extract and their interactions with target proteins, namely, a-glucosidase and a-amylase. These proteins play significant roles in diabetes management, and understanding their binding interactions with the phytochemicals could provide valuable insights for potential therapeutic applications.

Collection and Optimization of Bioactive Compounds

For the current study, a total of more than 100 biologically important active phytochemicals present in *H. niger* were collected from the literature and different databases. The IMPPAT (Indian medicinal plants, phytochemistry and therapeutics) database (Mohanjra et al., 2018) (https://cb.imsc.res.in/imppat/; accessed on 30 June 2024) [19], and TCMSP (the traditional Chinese medicine systems pharmacology) database (Zhou et al., 2014) (https://tcmsp-e.com/tcmsp.php; accessed on 30 June 2024) were used to search the active phytochemicals with reported antidiabetic activity [20]. PubChem database (Kim et al., 2019) (https://pubchem.ncbi.nlm.nih.gov/; accessed on 10 August 2024) was used to retrieve the chemical structures of these plant phytochemicals in .sdf format. The phytochemicals' energy was minimized before the molecular docking study[21].

Retrieval and Preparation of Receptor Proteins

a-Glucosidase and *a*-amylase were selected as receptor proteins and used for molecular docking studies. The three-dimensional (3D) structures of receptor proteins (i.e., *a*-glucosidase with PDB ID: 3L4Y and *a*-amylase with PDB ID: 3BAJ as mentioned in figure 3) were downloaded from Protein Data Bank in .pdb format (https://www.rcsb.org/; accessed on 10 July 2023) [22]. The binding pockets of the receptor proteins were predicted using BIOVIA Discovery Studio [23]. To further prepare the receptor proteins for molecular docking, already bound ligand(s) were removed (if any), the water molecules were deleted, hydrogen atoms were added, and 3D protonation and energy minimization was performed to further prepare the receptor proteins for molecular docking.

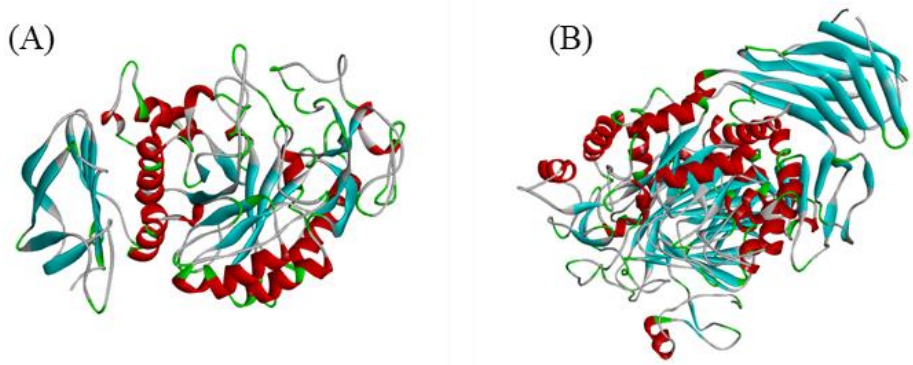

Figure 3. The structures of enzymes (A) α-amylase (PDB Code: 3BAJ) and (B) α-glucosidase (PDB Code: 3L4Y).

Molecular Docking

To identify potential drug candidates for diabetes treatment, a molecular docking study was conducted. PyRx software was employed for docking ligands to the active amino acids within the binding pocket of the receptor proteins. For visualizing the interactions between the receptor proteins and essential active compounds, the BIOVIA Discovery Studio software was utilized. Following the docking process, conformations with the most favorable docking scores and RMSD (root-mean-square deviation) values were carefully chosen for further investigation in subsequent studies. These selected conformations hold promise as potential lead compounds for the development of diabetes treatments [24].

Phytochemicals Scanning through Pharmacokinetics Parameters

Assessing druggability is a crucial step in identifying potential drug candidates with drug-like behavior. To evaluate the druggability of the top phytochemicals, SwissADME was employed [25]. Furthermore, an online server called ADMETLab 2.0 was utilized to perform a comprehensive assessment of pharmacokinetics and pharmacodynamics properties. The ADMET profiling involved checking various parameters, including blood-brain barrier permeability, carcinogenicity, human intestinal absorption, Ame's toxicity, and CaCo-2 permeability. These assessments help predict the drug-like behavior of a candidate from a clinical biochemistry perspective.

In addition to the ADMET profiling, the compounds were tested for Lipinski's rule of five (Ro5) compliance. According to Lipinski's rule, a drug candidate should have a molecular mass below 500 g/mol, no more than five hydrogen bond donors, no more than ten hydrogen bond acceptors, a logP value of less than or equal to five, and a molecular refractivity index within the range of 40–130 [26]. If the compounds successfully met all these parameters, they could be considered potential leading drug candidates and might be further processed for development as potential treatments for diabetes.

RESULTS AND DISCUSSION

Enzymatic assay-based inhibition

The enzymatic inhibition assays revealed that *H. niger* extract demonstrated substantial inhibitory activity against both target carbohydrate-digesting enzymes shown in **Table a**. For α-glucosidase inhibition, the extract exhibited an IC_{50} value of 35.85 ± 5 µg/mL, which was notably lower than the standard inhibitor acarbose (IC_{50} = 141.0 ± 5 µg/mL). Similarly, against α-amylase, *H. niger* extract showed an IC_{50} of 44.56 ± 5 µg/mL, again demonstrating superior inhibitory potency compared to acarbose (IC_{50} = 131.0 ± 10 µg/mL). These quantitative results indicate that the plant extract possesses significant enzyme inhibitory capacity, with particularly pronounced activity against α-glucosidase.

The dose-response curves presented in Figures 4 and 5 illustrate the concentration-dependent inhibition patterns for both enzymes. The inhibition profiles demonstrate a consistent increase in enzyme inhibitory activity with increasing extract concentrations, following typical sigmoidal dose-response relationships. Both enzymatic assays confirmed the reproducibility of the inhibitory effects, with standard deviations indicating acceptable experimental precision across all tested concentrations.

Table a: The IC_{50} values of *H. niger extract* for *α*-glucosidase and *α*-amylase.

Activity	*H. niger*	Acrabose
α-glucosidase	35.85 ± 5 µg/mL	141.0 ± 5 µg/mL
α-amylase	44.56 ± 5 µg/mL	131.0 ± 10 µg/mL

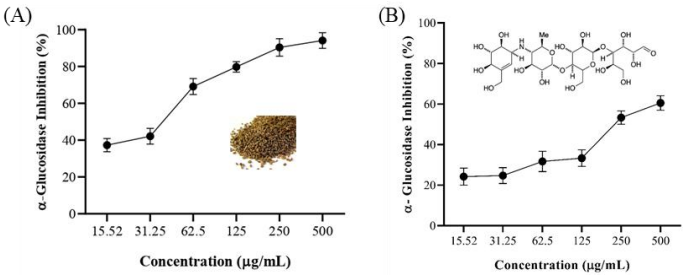

Figure 4. (A) % α-glucosidase inhibition by *H. niger* (seeds) methanol extract (B) Relative (%) α-glucosidase inhibition by acarbose as a positive control.

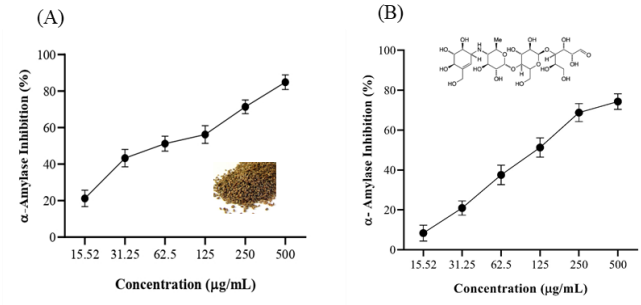

Figure 5. (A) % α-amylase inhibition by *H. niger* (seeds) methanol extract. (B) % α-amylase inhibition by acarbose as a positive control.

Molecular Docking Results

Virtual Screening and Binding Affinity Analysis

Following the promising *in vitro* inhibitory activity of *H. niger* extract, comprehensive molecular docking studies were conducted to elucidate the molecular mechanisms underlying the observed enzyme inhibition. Over 100 phytochemicals known to be present in *H. niger* were systematically screened against both α-amylase (PDB ID: 3BAJ) and α-glucosidase (PDB ID: 3L4Y) using PyRx virtual screening platform. The molecular docking approach allowed for detailed investigation of ligand-receptor interactions and identification of the most promising bioactive compounds based on their binding affinities and interaction patterns.

α-Amylase Interaction Analysis

The molecular docking results against α-amylase revealed several phytochemicals with exceptional binding affinities **(Table b)**. Pongamoside D emerged as the most promising candidate with a binding score of -9.3 kcal/mol. The docking analysis revealed that Pongamoside D formed stable interactions with five critical amino acid residues: TrpA:59, GluA:233, AspA:300, HisA:305, and AspA:356 (Figure 4). The binding pattern demonstrated multiple interaction types, including conventional hydrogen bonds and hydrophobic interactions, particularly with the highly conserved TrpA:59 residue.

Table b. Binding scores of each top Phytochemicals with receptor protein

Sr. No	Ligands	PubChem ID	Binding Affinity (kcal/mol)	Interacting Amino Acids
α- Amylase Protein as Receptor				
01	Pongamoside D	44258684	-9.3	TrpA:59, GluA:233, AspA:300, HisA:305 and AspA:356
02	Pongamoside C	44258691	-9.0	TrpA:59, LeuA:165, ArgA:195, GluA:233 and HisA:305
03	Cleomiscosin B	156875	-8.1	TrpA:59, TyrA:62, LeuA:162, ThrA:163, ArgA:195, AlaA:198 and AspA:300
04	Raceanisodamine	71711121	-7.8	LeuA:162, LeuA:165, AspA:300,AsnA:352, AspA:353 and AspA:356
α- glucosidase Protein as Receptor				
01	Balanophonin	23252258	-9.0	AlaA:285, LeuA:286, ProA:287, SerA:288, AlaA:291, ArgA:520, LysA:513, IleA:523, MetA:567, HisA:695 and LysA:776
02	Pongamoside C	44258691	-8.9	ThrA:639, TyrA:733, IleA:734,LysA:765, GlyA:766
03	Pongamoside D	44258684	-8.3	ArgA:647, ArgA:653, HisA:657, GluA:658, ProA:676, GlyA:766 and GluA:767
04	Cleomiscosin B	156875	-8.1	ArgA:647, AspA:649, ArgA:653, GluA:658, ProA:676, LysA:765 and GluA:767

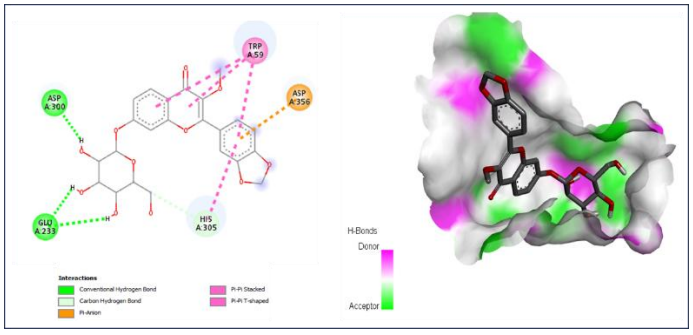

Figure 6. Interaction and binding pattern of Pongamoside D acid with α-amylase as a receptor.

The remaining compounds in the top five ranking showed progressively lower but still significant binding affinities: Pongamoside C (-9.0 kcal/mol), Cleomiscosin B (-8.1 kcal/mol), and Raceanisodamine (-7.8 kcal/mol). Interestingly, TrpA:59 appeared as a common interacting residue across multiple complexes, indicating its importance in ligand recognition and binding stability (Figure 6).

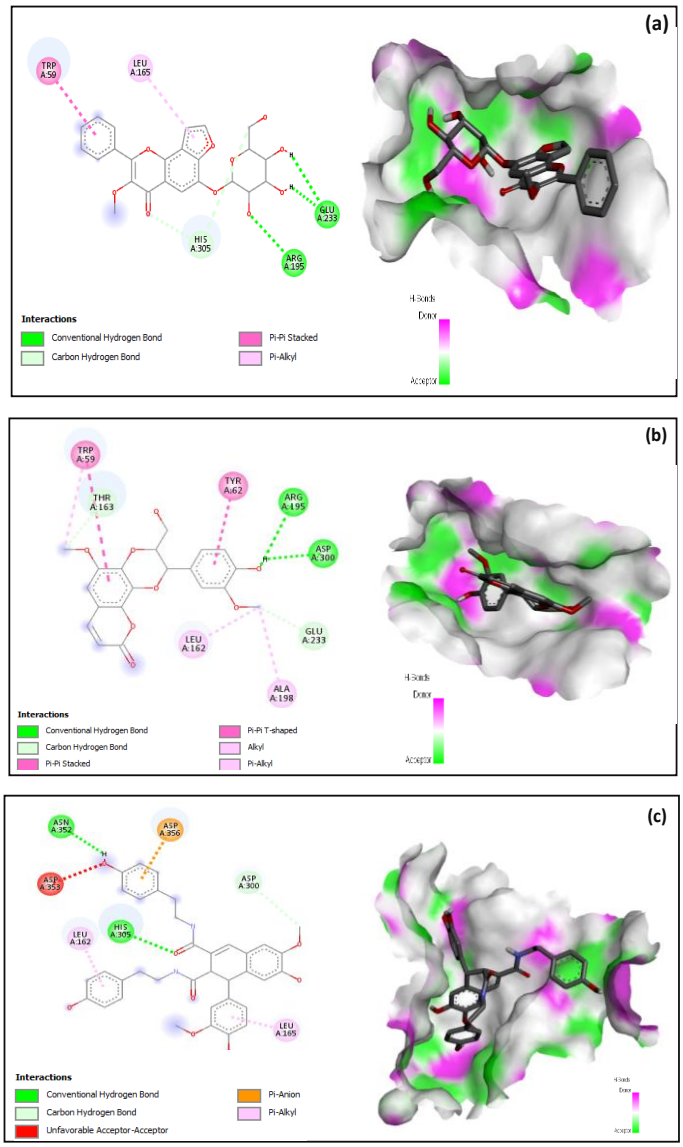

Figure 7. Two-dimensional interaction and 3D binding pattern with α-amylase as a receptor: (a) Pongamoside C, (b) Cleomiscosin B and (c) Raceanisodamine.

α-Glucosidase Interaction Analysis

For α-glucosidase, Balanophonin demonstrated the highest binding affinity with a score of -9.0 kcal/mol (table b), interacting with eleven amino acid residues: AlaA:285, LeuA:286, ProA:287, SerA:288, AlaA:291, ArgA:520, LysA:513, IleA:523, MetA:567, HisA:695, and LysA:776 (Figure 7).

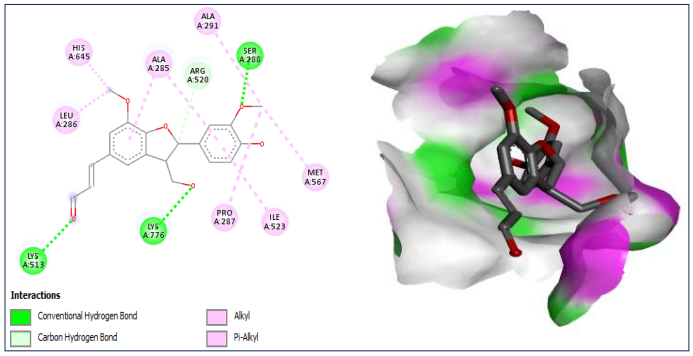

Figure 8. Interaction and binding Pattern of Balanophonin with α-glucosidase as a receptor.

The extensive interaction network formed by Balanophonin involved multiple hydrogen bonds and hydrophobic interactions, contributing to stable complex formation. Pongamoside C showed strong binding affinity of -8.9 kcal/mol with five key interactions, while Pongamoside D maintained consistent performance with -8.3 kcal/mol binding score, demonstrating its dual-target potential (Figure 8).

Against α-amylase pongamoside C, pongamoside D and cleomiscosin B showed a strong binding interaction in the orthosteric binding site as showed in the Figure 9.

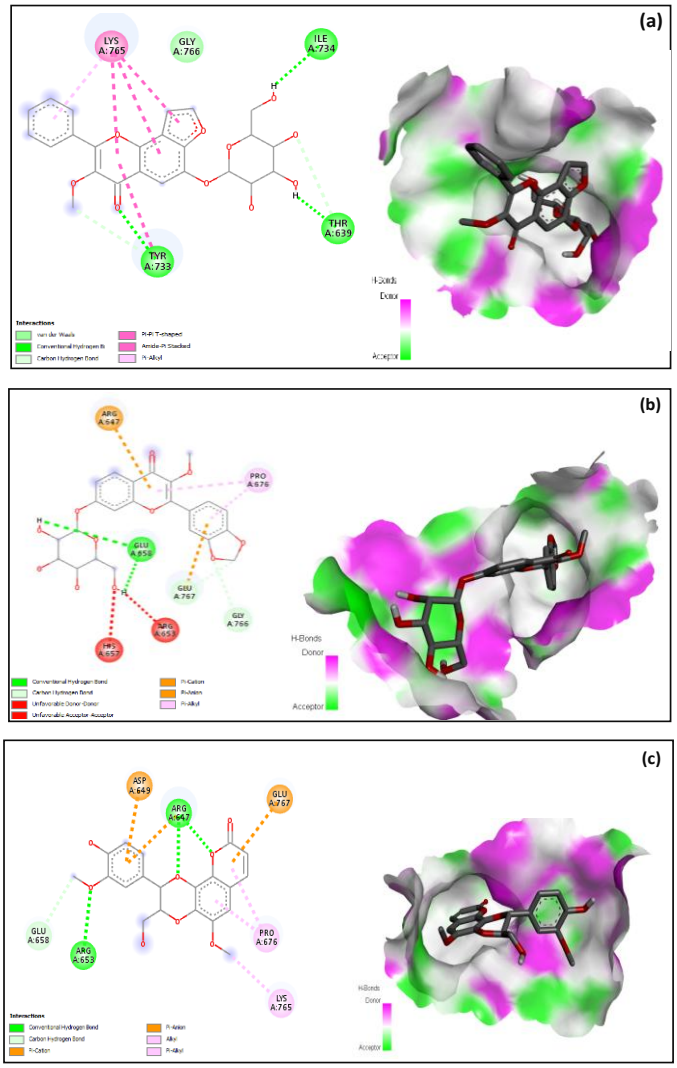

Figure 9. Two-dimensional interaction and 3D binding pattern with α-amylase as a receptor: (**a**) Pongamoside C, (**b**) Pongamoside D and (**c**) Cleomiscosin B.

Cross-Target Analysis

The analysis revealed Pongamoside D as the most promising dual-target inhibitor with binding scores of -9.3 kcal/mol for α-amylase and -8.3 kcal/mol for α-glucosidase. This dual activity presents significant therapeutic advantages for comprehensive carbohydrate digestion inhibition. Cleomiscosin B also showed balanced dual activity with binding scores of -8.1 kcal/mol for both enzymes.

Drug-likeness Assessment (Lipinski's Rule of Five)

The pharmacokinetic evaluation of the top-ranking compounds revealed varying degrees of drug-likeness compliance (Table c). Pongamoside D violated one Ro5 parameter with 11 hydrogen bond acceptors (threshold ≤10), while maintaining acceptable values for molecular weight (474.41 Da), hydrogen bond donors (4), LogP (3.36), and molecular refractivity (114.62).

Pongamoside C showed better compliance with all parameters within acceptable ranges: molecular weight (470.43 Da), 4 hydrogen bond donors, 10 hydrogen bond acceptors, LogP (2.46), and molecular refractivity (118.33).

Cleomiscosin B emerged with the most favorable drug-likeness profile, fully satisfying Lipinski's Rule of Five with molecular weight of 386.35 Da, 2 hydrogen bond donors, 8 hydrogen bond acceptors, LogP of 3.0, and molecular refractivity of 98.82.

The remaining compounds showed multiple Ro5 violations. Raceanisodamine demonstrated acceptable profiles with no violations, while Balanophonin maintained good drug-likeness with no parameter violations. Radar plot physiochmeical properties of thes ehit comouns are also shown in figure 10.

Table c. Physiochemical Properties of Hit Phytochemicals

Molecular Properties							
Ligands	**Molecular Mass (≤500 Dalton)**	**Hydrogen Bond Donor (≤5)**	**Hydrogen Bond Acceptor (≤10)**	**Number of Rotatable Bonds (≤10)**	**Log P (≤5)**	**Molar Refractivity (40-130)**	**Violations (Lipinski)**
Pongamoside D	474.41	4	11	5	3.36	114.62	1
Pongamoside C	470.43	4	10	5	2.46	118.33	0
Cleomiscosin B	386.35	2	8	4	3.0	98.82	0
Raceanisodamine	305.37	2	5	5	2.67	85.67	0
Balanophonin	356.37	2	6	6	2.79	96.35	0

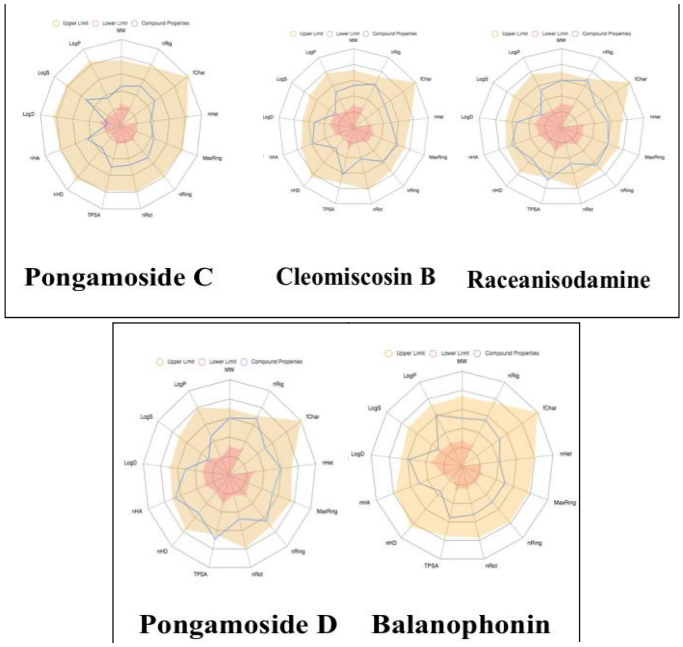

Figure 10. Radar plot demonstrates physiochemical properties of hit compounds against **α**-amylase and **α**-glucosidase Brown Area: Upper Limit for each property. Blue area: Compound Property, Pink area: Lower limit of physiochemical Property.

ADMET Profiling

The comprehensive ADMET analysis provided crucial insights into the pharmacological potential of the selected compounds (Table d). All top compounds showed no blood-brain barrier (BBB) permeability, which is advantageous for diabetes treatment as central nervous system effects are not desired for this therapeutic application.

Pongamoside D showed ADMET characteristics with moderate HIA, appropriate Caco-2 permeability (-5.595), but demonstrated positive AMES toxicity (++) and high carcinogenicity (+++), raising safety concerns. Balanophonin presented a more favorable safety profile with no AMES toxicity and positive carcinogenicity (+), along with good pharmacokinetic properties.

The clearance values ranged from 3.287 to 9.953, with half-lives varying from 0.186 to 0.712, indicating reasonable pharmacokinetic profiles for most compounds.

Table d. ADMET-related drug-like parameters of the best-selected phytochemicals against α-amylase and α-glucosidase

Phytochemicals					
	Pongamoside D	**Pongamoside C**	**Cleomiscosin B**	**Raceanisodamine**	**Balanophonin**
Absorption and Distribution					
BBB	NO	No	No	No	NO
HIA	---	-	---	--	---
Caco-2 Permeability	-5.595	-5.810	-4.927	-5.206	-4.802
PGS	-	-	---	---	---
PGI	---	---	+++	---	---
Metabolism					
CYP1A2 inhibitor	---	---	--	---	+++
CYP1A2 substrate	-	---	++	---	---
CYP2C19 inhibitor	---	---	--	---	+++
CYP2C19 substrate	--	--	-	++	---
CYP2C9 inhibitor	---	---	-	---	---
CYP2C9 substrate	+	--	++	+	---
CYP2D6 inhibitor	++	---	-	-	--
CYP2D6 substrate	+	--	++	++	+
CYP3A4 inhibitor	+	---	+	---	++
CYP3A4 substrate	---	---	++	++	+++
Excretion					
CL	3.287	3.372	8.428	9.953	4.855
$T_{1/2}$	0.186	0.350	0.560	0.305	0.489
Toxicity					
AMES Toxicity	++	+	--	---	+
Carcinogenicity	+++	++	+	---	+

The prediction probability values for classification models are denoted by several symbols, including 0–0.1(—), 0.1–0.3(–), 0.3–0.5(-), 0.5–0.7(+), 0.7–0.9(++), and 0.9–1.0(+++). Usually, the token '+++' or '++' denotes the molecule that is more likely to be toxic or defective, while '–' or '-' represents nontoxic or appropriate.

H. niger's remarkable antidiabetic properties through carbohydrate-digesting enzyme inhibition. Our *in vitro* results demonstrate that *H. niger* methanolic extract exhibits superior inhibitory potency against both α-glucosidase (IC_{50}: 35.85 ± 5 µg/mL) and α-amylase (IC_{50}: 44.56 ± 5 µg/mL), significantly outperforming acarbose by 3.9-fold and 2.9-fold respectively. This exceptional activity positions *H. niger* among the most potent natural enzyme inhibitors reported in recent literature, surpassing many well-studied medicinal plants including Gymnema sylvestre, Momordica charantia, and Acacia nilotica [27].

The comprehensive molecular docking screening of over 100 phytochemicals initially identified Pongamoside D as the most promising dual-target inhibitor, demonstrating exceptional binding affinities of -9.3 kcal/mol against α-amylase and -8.3 kcal/mol against α-glucosidase. The compound's strong binding stems from its extended conjugated system and stratpegically positioned hydroxyl groups, facilitating extensive hydrogen bonding and π-π interactions with enzyme active sites. Against α-amylase, Pongamoside D engages critical residues including TrpA:59, GluA:233, AspA:300, HisA:305, and AspA:356, while forming stable interactions with multiple subsites in α-glucosidase.

However, detailed ADMET profiling revealed significant safety concerns with Pongamoside D. The compound violated Lipinski's Rule of Five with 11 hydrogen bond acceptors (threshold ≤10) and demonstrated positive AMES toxicity (++) and high carcinogenicity (+++), raising serious questions about its therapeutic potential. Additionally, the compound showed concerning pharmacokinetic properties including extensive cytochrome P450 interactions and potential for drug-drug interactions.

Given these safety limitations, Cleomiscosin B emerges as the most viable therapeutic candidate from our screening. This flavonoid demonstrated excellent dual-target inhibitory activity with binding scores of -8.1 kcal/mol against both α-amylase and α-glucosidase, indicating balanced enzyme inhibition. Cleomiscosin B exhibits a more favorable safety profile with full compliance with Lipinski's Rule of Five: molecular weight of 386.35 Da, 2 hydrogen bond donors, 8 hydrogen bond acceptors, LogP of 3.0, and molecular refractivity of 98.82.

The ADMET analysis strongly supports Cleomiscosin B's therapeutic potential. The compound shows no AMES toxicity (--) and minimal carcinogenicity (+), representing a significantly safer profile compared to Pongamoside D. Its moderate Caco-2 permeability (-4.927) suggests reasonable intestinal absorption, while the absence of blood-brain barrier permeability eliminates concerns about central nervous system effects. The compound's cytochrome P450 interaction profile shows fewer concerning interactions compared to Pongamoside D, with moderate CYP2C9 substrate activity (++) and CYP2D6 substrate activity (++), which are more manageable in clinical settings.

Cleomiscosin B belongs to the flavonoid class of compounds, specifically a methylenedioxyflavonoid, commonly found in Solanaceae species [28]. These compounds are known for their diverse biological activities, including anti-inflammatory, antioxidant, and enzyme inhibitory effects [29]. The structural features of Cleomiscosin B, including its planar aromatic system and strategic hydroxyl substitutions, contribute to its dual enzyme inhibitory activity through multiple interaction mechanisms including hydrogen bonding, hydrophobic interactions, and aromatic stacking with enzyme active sites [30].

The balanced inhibitory profile of Cleomiscosin B against both enzymes offers therapeutic advantages over selective inhibitors. This dual activity could provide comprehensive carbohydrate digestion control while potentially reducing the severe gastrointestinal side effects associated with complete α-amylase inhibition by acarbose. The compound's moderate inhibitory strength allows for physiological regulation and reduces hypoglycemic risks compared to more potent irreversible inhibitors [31].

From a pharmaceutical development perspective, Cleomiscosin B's favorable drug-likeness properties position it as an excellent lead compound for optimization. The molecular framework provides multiple sites for structural modification to enhance potency, selectivity, or pharmacokinetic properties while maintaining the core safety profile. The compound's natural origin also supports traditional medicine practices and may facilitate regulatory approval processes for botanical drug development.

CONCLUSION

This comprehensive study establishes *H. niger* as a valuable source of dual-target carbohydrate-digesting enzyme inhibitors for diabetes management. While *H. niger* extract demonstrated superior inhibitory activity compared to acarbose in *in vitro* assays, detailed molecular analysis revealed important considerations among lead compounds. Although pongamoside D showed the highest binding affinities (-9.3 and -8.3 kcal/mol), its predicted ADMET violations including positive AMES toxicity and carcinogenicity concerns based on computational analysis suggest potential limitations for therapeutic development. Cleomiscosin B emerged as the optimal lead compound, combining excellent dual-target inhibitory activity (-8.1 kcal/mol for both enzymes) with favorable predicted drug-likeness properties and superior computational safety profile. However, it is important to note that the safety assessments in this study are based solely on *in vitro* enzymatic assays and computational ADMET predictions. Therefore, definitive conclusions regarding the safety of *H. niger* compared to synthetic antidiabetic agents cannot be established without comprehensive *in vivo* toxicity studies and clinical evaluation. The integrated computational-experimental approach successfully identified promising natural product candidates that warrant further investigation. Future *in vivo* studies, advanced computational analyses, and comprehensive toxicological evaluations are essential to validate the antidiabetic potential and establish the actual safety profile of *H. niger* metabolites for potential therapeutic applications in postprandial glucose control.

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