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Molecular Docking Analysis of Bioactive Constituents Identified by GC-MS from *Coprinus comatus* as α-Amylase and α-Glucosidase Inhibitors

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Abstract: *Diabetes mellitus remains a major global health concern characterized by chronic hyperglycemia and progressive metabolic dysfunction. One therapeutic strategy for controlling postprandial blood glucose involves inhibiting carbohydrate-hydrolyzing enzymes, particularly α-amylase and α-glucosidase. Natural products have gained increasing attention as potential enzyme inhibitors with improved safety profiles. Coprinus comatus, an edible mushroom, contains diverse bioactive constituents that may contribute to antidiabetic activity. This study aimed to evaluate the inhibitory potential of compounds identified by GC–MS from Coprinus comatus against α-amylase and α-glucosidase through molecular docking analysis. Seven compounds were selected: Methoxypromazine, Stepharinosine, Thioperoxydicarbonic diamide, Methylzinc propoxide, 2,4-Pentadienoic acid, 2-Hexenedioic acid, and Benzenesulfonamide, 2,4-dichloro-3-methyl-N-(3-pyridyl). Docking simulations were performed using the crystal structures of α-amylase (PDB ID: 1HNY) and α-glucosidase (PDB ID: 2ZE0). Acarbose was used as a reference inhibitor. Binding affinity values were calculated to assess ligand–protein interactions. The results demonstrated that acarbose exhibited binding affinities of −6.9 kcal/mol toward 1HNY and −7.0 kcal/mol toward 2ZE0. Among the identified compounds, Stepharinosine showed the strongest interaction with both targets, with binding affinities of −7.5 kcal/mol for α-amylase and −8.8 kcal/mol for α-glucosidase, exceeding the reference drug. Other compounds displayed moderate to weak binding energies. These findings suggest that Stepharinosine is a promising dual inhibitor of α-amylase and α-glucosidase and may contribute to the antidiabetic potential of C. comatus. Further in vitro and in vivo studies are required to validate these computational predictions.*
Keywords: *Molecular docking, Coprinus comatus, α-amylase inhibition, α-glucosidase inhibition, Stepharinosine*

1. INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia due to impaired insulin secretion, insulin action, or both. The disease disrupts carbohydrate, lipid, and protein metabolism and is associated with long-term complications affecting the cardiovascular system, kidneys, nerves, and retina. Type 2 diabetes mellitus (T2DM) accounts for the majority of cases globally and is strongly linked to lifestyle transitions, obesity, aging populations, and urbanization. The rapid increase in diabetes incidence represents a major public health concern, with substantial socioeconomic consequences.

Recent global estimates from the International Diabetes Federation indicate that more than 500 million adults are currently living with diabetes, and this number is projected to increase dramatically

over the next two decades. The growing burden is particularly evident in low- and middle-income countries, where healthcare systems face additional challenges in providing long-term disease management. Despite the availability of pharmacological therapies, optimal glycemic control remains difficult to achieve in many patients, and diabetes-related complications continue to contribute significantly to morbidity and mortality [1].

One important therapeutic strategy for controlling postprandial hyperglycemia involves the inhibition of carbohydrate-digesting enzymes, specifically α-amylase and α-glucosidase [2]. These enzymes catalyze the breakdown of complex carbohydrates into glucose, facilitating rapid absorption in the small intestine. Inhibiting these enzymes delays glucose release and attenuates postprandial glucose spikes [3]. Synthetic inhibitors such as acarbose are widely prescribed for this purpose. However, the clinical use of enzyme inhibitors and other first-line antidiabetic drugs, including metformin, is frequently associated with adverse gastrointestinal effects such as diarrhea, abdominal discomfort, flatulence, nausea, and bloating. Although metformin remains a cornerstone therapy, these side effects can reduce patient adherence and compromise long-term treatment success [4]. Consequently, there is a growing need to identify safer and well-tolerated alternatives, particularly from natural sources [5].

Natural products have historically served as a foundation for drug discovery, and edible and medicinal mushrooms represent an underexplored reservoir of bioactive compounds. Among them, *Coprinus comatus* (shaggy ink cap) is an edible mushroom traditionally consumed as food but insufficiently investigated for its pharmacological potential [6]. Previous studies suggest that *C. comatus* contains polysaccharides, phenolic compounds, sterols, and alkaloid-like constituents exhibiting antioxidant, anti-inflammatory, hypolipidemic, and potential hypoglycemic activities. Oxidative stress and chronic low-grade inflammation are key contributors to the pathogenesis of T2DM and its complications, highlighting the relevance of mushrooms with antioxidant and anti-inflammatory properties [7].

To systematically identify bioactive constituents responsible for these effects, this study employed Gas Chromatography–Mass Spectrometry (GC–MS) analysis. GC–MS was selected because it is a highly sensitive, reproducible, and reliable analytical technique for the separation and identification of volatile and semi-volatile compounds in complex biological matrices. The gas chromatography component enables efficient separation of compounds based on volatility and polarity, while the mass spectrometry detector provides structural information through characteristic fragmentation patterns [8]. This dual capability allows precise compound identification through comparison with established spectral libraries. Compared to other analytical methods, GC–MS offers high resolution, excellent sensitivity, relatively rapid analysis, and robust qualitative profiling, making it particularly suitable for metabolite screening and phytochemical characterization. Its ability to detect minor constituents further strengthens its value in exploratory natural product research [8].

However, chemical identification alone does not establish biological relevance. Therefore, molecular docking was employed as an in silico approach to predict interactions between identified compounds and target enzymes involved in carbohydrate metabolism. Molecular docking enables the estimation of binding affinity, interaction stability, and potential inhibitory mechanisms at the molecular level. This computational strategy reduces time and cost compared to purely experimental screening while providing mechanistic insights into ligand–protein interactions. In the context of α-amylase and α-glucosidase inhibition, docking analysis helps prioritize promising candidates for further in vitro and in vivo validation [9].

The novelty of this study lies in integrating GC–MS-based metabolite profiling of *Coprinus comatus* with molecular docking analysis against key carbohydrate-hydrolyzing enzymes. While previous investigations have reported general bioactivities of mushroom extracts, comprehensive identification of individual compounds followed by structure-based computational evaluation remains limited. By combining advanced analytical chemistry with computational pharmacology, this research provides a rational framework for understanding the antidiabetic potential of *C. comatus*. Such an integrative approach not only expands scientific knowledge regarding underutilized edible mushrooms but also contributes to the discovery of safer and more effective enzyme inhibitors for diabetes management.

2. METHOD

a. Preparation of *Coprinus comatus* Simplicia

Fresh fruiting bodies of *Coprinus comatus* were collected, cleaned from adhering soil and debris, and washed with distilled water. The samples were sliced into small pieces to increase the surface area and then dried using a cabinet dryer at 40–45°C until a constant weight was achieved to prevent degradation of thermolabile compounds. The dried material was subsequently ground using a mechanical grinder to obtain a fine powder. The powdered mushroom (simplicia) was sieved to ensure uniform particle size and stored in an airtight container protected from light and moisture until further extraction [10].

b. Extraction by Maceration and Sonication

Extraction was carried out using a sequential maceration–sonication approach to enhance metabolite recovery. Approximately 200 g of powdered simplicia were immersed in 70% ethanol at a ratio of 1:10 (w/v) and subjected to maceration for 72 hours at room temperature with intermittent stirring to facilitate solvent penetration and diffusion of bioactive compounds. The mixture was filtered using Whatman No. 1 filter paper. The residue was re-extracted using fresh solvent under sonication for 30 minutes at 40 kHz to improve cell wall disruption and maximize compound release. Sonication enhances extraction efficiency by promoting cavitation, thereby accelerating solvent–matrix interaction. The combined filtrates were collected for further processing [11].

c. Concentration and Solvent Evaporation

The pooled extract was concentrated under reduced pressure using a rotary vacuum evaporator at 40–45°C to remove the solvent. The use of reduced pressure lowers the boiling point of the solvent, minimizing thermal degradation of sensitive constituents. The concentrated extract was further dried in a water bath to obtain a thick crude extract, and the yield percentage was calculated based on the initial dry weight of simplicia. The crude extract was stored at 4°C in a dark container prior to analysis [12].

d. Identification of Bioactive Compounds Using GC–MS

Chemical profiling of the crude extract was performed using Gas Chromatography–Mass Spectrometry (GC–MS). A small aliquot of the extract was diluted with analytical-grade solvent and filtered through a 0.22 µm membrane filter before injection. Separation was achieved using a capillary column under programmed temperature conditions. Helium was used as the carrier gas at a constant flow rate. Mass spectra were obtained in electron ionization (EI) mode at 70 eV, scanning within a mass range of 40–600 m/z. Compound identification was conducted by comparing the obtained spectra with reference spectra from the NIST library database. Compounds with high similarity index values were selected for further molecular docking analysis [13].

e. Preparation of Ligands and Target Proteins

The identified compounds were retrieved in 3D structure format from the PubChem database or drawn using ChemDraw and converted into PDB format. Ligand structures were energy-minimized using Open Babel integrated in PyRx to obtain stable conformations. The crystal structures of α-amylase (PDB ID: 1HNY) and α-glucosidase (PDB ID: 2ZE0) were downloaded from the Protein Data Bank (PDB). Protein preparation involved removal of water molecules, native ligands, and other heteroatoms. Polar hydrogens were added, and Kollman charges were assigned using AutoDock Tools. The prepared proteins were saved in PDBQT format for docking simulation [14].

f. Molecular Docking Simulation Using AutoDock Vina in PyRx

Molecular docking was performed using AutoDock Vina integrated in PyRx software to predict ligand–protein interactions. The grid box parameters were set to cover the active site region of each enzyme based on known catalytic residues. The exhaustiveness parameter was adjusted to ensure adequate conformational sampling. Each ligand was docked into the active site of α-amylase (1HNY) and α-glucosidase (2ZE0). Binding affinity values (kcal/mol) were recorded, and the best binding pose was selected based on the lowest binding energy and favorable interaction profile. Acarbose was used as a reference inhibitor to validate docking performance and provide comparative analysis [15].

g. Visualization and Interaction Analysis

The docking results were visualized and analyzed using BIOVIA Discovery Studio Visualizer. Hydrogen bonds, hydrophobic interactions, van der Waals forces, and other molecular interactions

between ligands and amino acid residues were examined. Two-dimensional (2D) and three-dimensional (3D) interaction diagrams were generated to illustrate binding modes within the enzyme active sites [16].

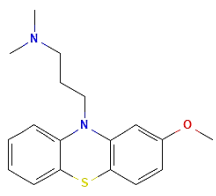
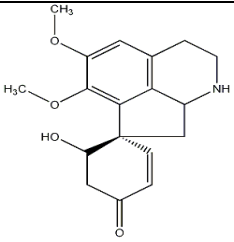
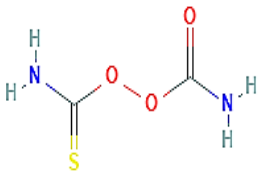
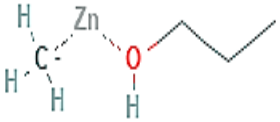
3. RESULTS AND DISCUSSION

Table. 1 Bioactive compounds of *Coprinus comatus*

No	Retention Time	% Area	% Height	Compounds	Molecular Weight (g/mol)	Formula
1	20.59	0.08	0.23	Methoxypromazine	314.4 g/mol	$C_{18}H_{22}N_2OS$
2	20.43	0.26	0.15	Stepharinosine	315.1 g/mol	$C_{18}H_{21}NO_4$
3	20.96	0.08	0.11	Thioperoxydicarbonic diamide	136.13 g/mol	$C_2H_4N_2O_3S$
4	19.52	0.19	0.22	Methylzinc propoxide	140.5 g/mol	$C_4H_{11}OZn^-$
5	19.22	0.05	0.14	2,4-Pentadienoic acid	234.25 g/mol	$C_{13}H_{14}O_4$
6	23.07	0.14	0.12	2-Hexenedioic acid	159.14 g/mol	$C_6H_9NO_4$
7	23.27	0.16	0.15	Benzenesulfonamide, 2,4-dichloro-3-methyl-N-(3-pyridyl)	317.2 g/mol	$C_{12}H_{10}Cl_2N_2O_2S$
Control				Acarbose	645.6 g/mol	$C_{25}H_{43}NO_{18}$

The bioactive compounds present in *Coprinus comatus* extract were identified using Gas Chromatography–Mass Spectrometry (GC–MS) analysis. As shown in Table 1, a total of seven compounds were detected with retention times ranging from 19.22 to 23.27 minutes, indicating the diversity of chemical constituents in the extract. The identified compounds include Methoxypromazine, Stepharmostine, Thioperoxydicarbamic diamide, Methylzinc propoxide, 2,4-Pentadienoic acid, 2-Hexenedioic acid, and a benzenesulfonamide derivative. Among the detected compounds, Stepharmostine exhibited the highest % area (0.26%), indicating it was the most relatively abundant compound in the chromatogram, while 2,4-Pentadienoic acid showed the lowest % area (0.05%). In terms of peak intensity, Methoxypromazine presented the highest % height (0.23%), whereas Thioperoxydicarbamic diamide showed the lowest % height (0.11%). Regarding molecular weight, the benzenesulfonamide derivative had the highest molecular weight (317.2 g/mol), while Thioperoxydicarbamic diamide had the lowest molecular weight (136.13 g/mol) among the identified compounds. Additionally, Acarbose (645.6 g/mol) was included as a control compound for comparison. Previous research has shown that ethanol extracts of *C. comatus* contain various compounds such as thiophosphoric acid, nitromethylene, methyl methoxycarbonyl, thiazole-4-carboxylic acid, furo quinoline, and hexanoic acid [17]. Meanwhile, in vivo experiments on carrageenan-induced inflammatory model mice showed that *C. comatus* extract was able to reduce pro-inflammatory cytokine levels such as interleukin 1-β (IL-1β) and tumor necrosis factor-α (TNF-α). The compounds contained in the *C. comatus* extract include piperazine, cholestan-3-ol, and stannane, which were identified using GC-MS. Meanwhile, qualitative identification results showed flavonoids, polyphenols, alkaloids, triterpenoids, and steroids [18].

Table 2. Chemical structure of several bioactive compounds of *Coprinus comatus*

 **Methoxypromazine**	 **Stepharinosine/ 1-O-Acetylnorpluviine**	 **Thioperoxydicarbonic diamide**	 **Methylzinc propoxide**

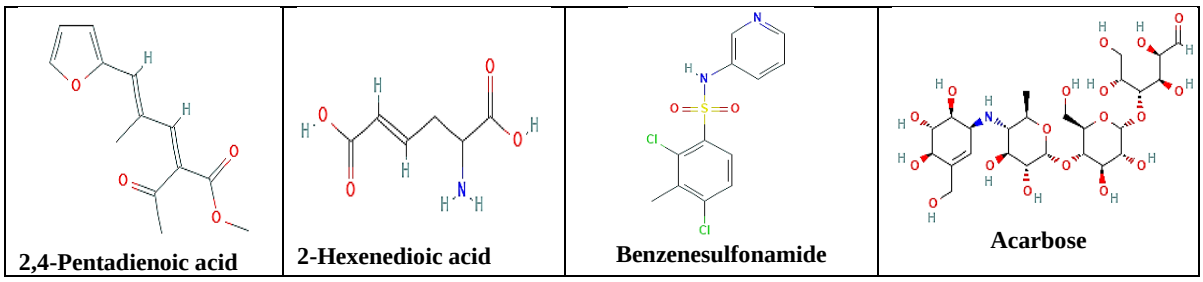


Table 2 presents the chemical structures of several bioactive compounds identified in *Coprinus comatus* through GC–MS analysis. The illustrated compounds include Methoxypromazine, Stepharmostine (1-O-acetylhorphyrine), Thioperoxydicarbamic diamide, Methylzinc propoxide, 2,4-Pentadienoic acid, 2-Hexenedioic acid, and a benzenesulfonamide derivative, which represent diverse chemical classes that may contribute to the biological activity of *C. comatus*. The structures reveal the presence of several important functional groups, such as aromatic benzene rings, amine (–NH/–NH_2) groups, methoxy (–OCH_3) groups, carboxylic acid (–COOH) groups, amide (–CONH–) groups, sulfonamide (–SO_2NH–) groups, double bonds (C=C), and sulfur-containing groups (–S–). In addition, Methylzinc propoxide contains a metal–oxygen (Zn–O) coordination bond, indicating the presence of a metal-containing organic structure. These functional groups are important because they can influence the chemical reactivity, stability, and potential biological activities of the compounds. For comparison, the chemical structure of Acarbose is also included as a reference control compound, which contains multiple hydroxyl (–OH) groups and glycosidic linkages, commonly associated with α-glucosidase inhibitory and antidiabetic activity. The structural information therefore provides insight into the molecular features that may contribute to the bioactivity of the identified compounds. Penelitian sebelumnya juga menunjukkan bahwa beberapa senyawa yang teridentifikasi dengan menggunakan high-performance liquid chromatography (HPLC) menunjukkan bahwa jamur *C. comatus* menggandung senyawa α-tokoferol, asam askorbat, dan rutin yang banyak mengandung gugus hidroksil (-OH) [19]. Beberapa senyawa dari jamur *C. comatus* yang diidentifikasi dengan menggunakan HPLC lainnya juga banyak berasal dari golongan flavones, flavonols, flavanones, flavanols, biflavonoids, isoflavonoids, dan coumarins. Hasil pengujian terhadap aktivitas antioksidan juga menunjukkan bahwa *C. comatus* dapat mengahambat radikal bebas DPPH (2,2-diphenyl-1-picrylhydrazyl), dan FRAP (Ferric Reducing Antioxidant Power) [20].

Table 3. Molecular docking results analysis of several compounds of *Coprinus comatus* mushrooms

Protein Target	Ligand/ Compounds	*Binding Affinity* kcal/mol	Hidrophobic Bonds	Amino Acids Residu
α-amylase (1HNY)	Methoxypromazine	-6.7 -6.6	**ASP_{300}**	TYR_{62}, ALA_{198}, GLU_{233}, ASP_{197}, TRP_{58}, ASP_{300}, ALA_{307}, ILE_{236}, TRP_{59}, HIS_{305}
	Stepharinosine/ 1-O-Acetylnorpluviine	-7.5 -7.1	HIS_{305}, GLU_{233}	HIS_{305}, GLU_{233}, TRP_{58}, TRP_{59}, ASP_{300}, ARG_{195}, LEU_{162}, ALA_{198}, LEU_{165}, TYR_{62}
	Thioperoxydicarbonic diamide	-4.9 -4.7	**ARG_{421}**, **GLY_{334}**	ARG_{398}, VAL_{401}, ARG_{421}, HIS_{331}, ASP_{402}, GLY_{334}, PRO_{332}
	Methylzinc propoxide	-1.3 -1.3	-	-
	2,4-Pentadienoic acid	-6.0 -6.0	HIS_{299}, ASP_{197}, **ASP_{300}**, HIS_{101}, **THR_{163}**, **GLN_{63}**	ARG_{195}, TYR_{62}, LEU_{165}, LEU_{162}, TRP_{59}, HIS_{299}, ASP_{197}, ASP_{300}, HIS_{101},

	2-Hexenedioic acid	-5.5 -5.3	**ARG421**, PRO322, **GLY334**, SER289, ARG252, ASP402, **GLN63**	THR163, GLN63 GLY403, ARG398, ALA292, ARG421, PRO322, GLY334, SER289, ARG252, ASP402
	Benzenesulfonamide, 2,4-dichloro-3-methyl-N-(3-pyridyl)	-7.1 -7.1	**ASP300**	ASP300, TYR62, TRP58, TRP59
	Acarbose	-6.9 -6.8	**THR163**	THR162, HIS305, ASP300
	Methoxypromazine	-7.7 -7.3	-	GLN167, ASP60, PHE144, ASN61, ARG411, ASP382, TYR63, ASP326, HIS325, ASN324, ARG197, GLU256, ALA200, HIS103. PHE163
	Stepharinosine/ 1-O-Acetylnorpluviine	-8.8 -7.6	ARG411, **TYR63**	ASN324, HIS325, ASP326, ARG407, ARG411, ASN61, ASP60, GLU256, ARG197, ASP199, TYR63, ALA200, HIS103, GLN167
	Thioperoxydicarbonic diamide	-5.0 -5.0	MET355, ASN318, ASN309	TRP308, LEU312, LEU320, LEU305, MET355, ASN318, ASN309
	Methylzinc propoxide	-1.4 -1.3	-	-
α-glucosidase (2ZE0)	2,4-Pentadienoic acid	-6.5 -6.2	**TYR63**, **ARG197**, HIS325, ASP326, GLN167	ASN324, ASP199. ALA200, GLU256, ASP60, TRP49, TYR63, ARG197, HIS325, ASP326, GLN167
	2-Hexenedioic acid	-5.2 -5.1	HIS103, **GLU256**, **ASP199**	TRP49, ARG197, VAL100, ASP98, TYR63, GLN197, ASP199, GLU256, ALA200, HIS103, PHE163
	Benzenesulfonamide, 2,4-dichloro-3-methyl-N-(3-pyridyl)	-7.7 -7.7	**GLU256**, ALA200, **ASP199**	TRP49, ARG197, HIS325, ASP326, ASN324, ASN61, ASP60, GLN167, TYR63, HIS103, PHE163, GLU256, ALA200, PHE282, ASP199
	Acarbose	-7.0 -6.9	**ASP199**, **GLU256**, **ARG197**, ASP226, ARG407, **TYR63**	LEU327, ASN324, ALA200, TRP49, HIS325, GLN167, ASP60, PHE163, ILE143, ASP199, GLU256, ARG197, ASP226, ARG407, TYR63

The docking results show that several compounds from *Coprinus comatus* exhibit notable binding affinities toward both target enzymes. Binding affinity values are expressed in kcal/mol, where more negative values indicate stronger predicted binding between the ligand and the protein active site.

For α-amylase (1HNY), the compound Stepharinosine / 1-O-acetylstepharinosine demonstrates one of the strongest interactions with binding affinity values of approximately −7.5 and −7.1 kcal/mol, respectively. These values are comparable to or stronger than the reference drug acarbose, which shows binding energies around −6.9 to −6.8 kcal/mol. Other compounds such as Benzenesulfonamide, 2,4-dichloro-3-methyl-N-(3-pyridyl) and Methoxypromazine also show relatively strong binding interactions with the enzyme active site. In addition to binding energy, the table highlights hydrophobic interactions and key amino acid residues

involved in stabilizing ligand binding. For α-amylase, several residues appear repeatedly in ligand interactions, including ASP_{300}, THR_{163}, HIS_{305}, and GLU_{233}. These residues are known to be part of or near the catalytic pocket of the enzyme and play an important role in substrate recognition and catalytic activity. The presence of hydrogen bonds and hydrophobic contacts with these residues suggests that the compounds may inhibit enzyme activity by occupying the catalytic region and preventing substrate access.

For α-glucosidase (2ZE0), the docking results also reveal strong interactions with several compounds from *C. comatus*. Notably, Stepharinosine / 1-O-acetylstepharinosine again exhibits the most favorable binding affinity, reaching values around −8.8 kcal/mol, which is stronger than the reference inhibitor acarbose (approximately −7.0 to −6.9 kcal/mol). Other compounds, such as 2,4-pentadienoic acid and 2-hexenedioic acid, show moderate binding interactions with energies around −6.5 to −5.1 kcal/mol. The interaction analysis indicates that residues such as ASP_{199}, ASP_{206}, ARG_{407}, TYR_{63}, GLU_{256}, and ASN_{324} contribute to ligand stabilization through hydrogen bonds, hydrophobic interactions, and van der Waals forces. These interactions play a crucial role in determining the stability and inhibitory potential of the compounds within the enzyme binding pocket.

Overall, the docking results suggest that several metabolites identified from *Coprinus comatus*, particularly stepharinosine derivatives, demonstrate strong binding interactions with both α-amylase and α-glucosidase. These findings indicate that the mushroom may serve as a promising natural source of enzyme inhibitors with potential antidiabetic activity by reducing carbohydrate digestion and glucose absorption. Penelitian sebelumnya menunjukkan bahwa senyawa *C. comatus* yang diidentifikasi dengan menggunakan GC-MS pada pengujian in vivo tikus model inflamasi yang diinduksi complete freund's adjuvant (CFA) mengandung berbagai senyawa seperti vallesamidine, naphthalene, 4-o-methyl-ursuline, pyrimidinone, dan propionic acid methyl ester [21].

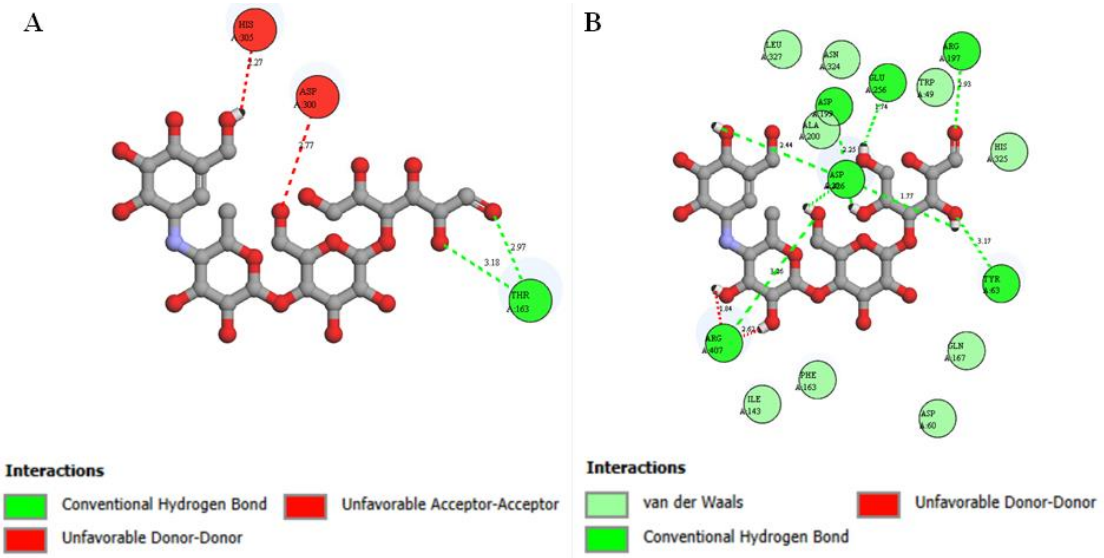


Figure 1. Amino Acids Interaction Between Acarbose and α-Amlyase (A) and α-Glucosidase (B) Enzyme

In Figure 1A, acarbose interacts with the active site of α-amylase through several amino acid residues. Hydrogen bonding interactions are observed with residues such as THR_{163}, while other residues including HIS_{305} and ASP_{300} show unfavorable interactions. These interactions indicate that acarbose can bind within the catalytic pocket of α-amylase and potentially inhibit the enzyme's activity by blocking substrate access. In Figure 1B, acarbose shows a more extensive interaction network with α-glucosidase. Multiple amino acid residues, such as ASP_{256}, ASP_{206}, ARG_{407}, ARG_{63}, and TYR_{63}, participate in conventional hydrogen bonds with the ligand. Additional residues, including LEU_{327}, ASN_{324}, TRP_{406}, HIS_{325}, and PHE_{463}, contribute through van der Waals interactions that stabilize the ligand within the binding pocket. One unfavorable donor–donor

interaction is also observed. Overall, the interaction patterns suggest that acarbose forms stable binding within the active sites of both enzymes, particularly α-glucosidase, through hydrogen bonding and van der Waals forces. These interactions support the role of acarbose as an inhibitor that can reduce carbohydrate hydrolysis and consequently delay glucose absorption.

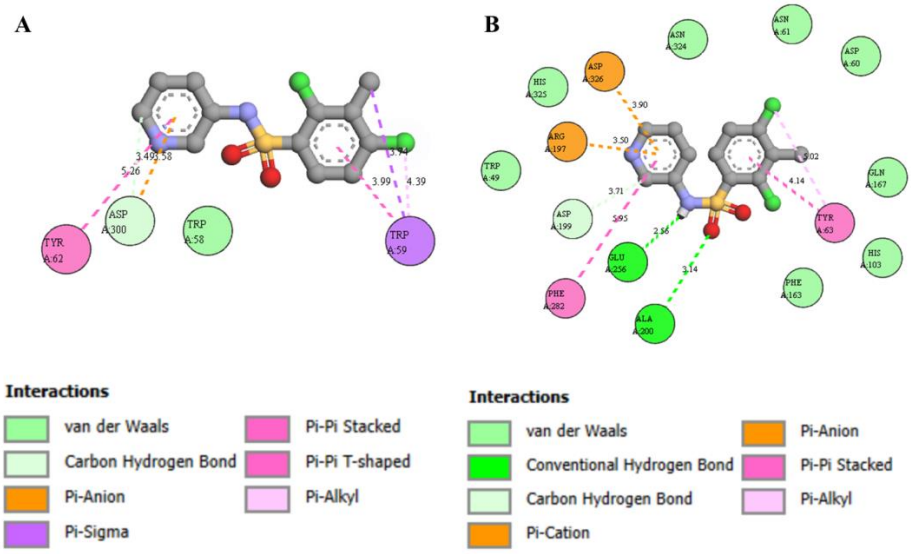


Figure 2. Amino Acids Interaction Between Methoxypromazine and α-Amlyase (A) and α-Glucosidase (B) Enzyme

In Figure 2A, the interaction between methoxypromazine and α-amylase is mainly stabilized by van der Waals interactions with the amino acid residues ASP_{300} and TRP_{58}, which help maintain the ligand within the enzyme binding pocket through weak intermolecular forces. In addition, a carbon hydrogen bond is observed with the residue ASP_{300}, indicating a weak hydrogen interaction between the ligand and the enzyme. These interactions contribute to the stabilization of methoxypromazine within the active site region of α-amylase and may influence the inhibition of the enzyme's catalytic activity. In Figure 2B, methoxypromazine interacts with α-glucosidase through several residues that contribute to ligand stabilization. Van der Waals interactions occur with residues ASN_{324}, ASP_{460}, ASN_{461}, TRP_{49}, GLN_{167}, His103, and PHE_{163}, indicating multiple weak contacts surrounding the ligand in the binding pocket. A carbon hydrogen bond interaction is observed with the residue ASP_{199}, suggesting additional stabilization between the ligand and the enzyme. Furthermore, conventional hydrogen bonds, which are stronger and more specific interactions, are formed with GLU_{256} and ALA_{200}. These hydrogen bonds play an important role in maintaining the proper orientation of the ligand within the active site of α-glucosidase.

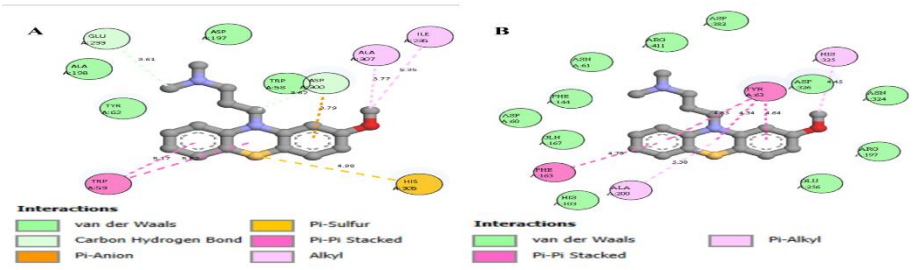


Figure 3. Amino Acids Interaction Between Benzenesulfonamide, 2,4-dichloro-3-methyl-N-(3-pyridyl) and α-Amlyase (A) and α-Glucosidase (B) Enzyme

In Figure 3A, the interaction between benzenesulfonamide derivative and α-amylase is mainly supported by van der Waals interactions with several amino acid residues surrounding the ligand, including GLU_{233}, ASP_{197}, ALA_{198}, TYR_{62}, TRP_{58}, and ASP_{300}. These residues create a stabilizing environment around the ligand through weak intermolecular interactions that help maintain its position in the enzyme binding site. In addition, a carbon hydrogen bond is formed with the residue TRP_{58}, contributing additional stabilization between the ligand and the protein. The presence of these interactions indicates that the compound can occupy the binding pocket of α-amylase and potentially interfere with substrate binding. In Figure 3B, the interaction between the compound and α-glucosidase also involves several residues through van der Waals interactions, including ASN_{61}, ARG_{411}, ASP_{382}, ASP60, GLN_{167}, PHE_{144}, HIS_{103}, ASN_{324}, ASP_{326}, ARG_{197}, and GLU_{256}. These residues surround the ligand within the active site cavity and contribute to the stabilization of the ligand–enzyme complex through weak intermolecular forces. The presence of multiple van der Waals contacts suggests that the ligand is well accommodated within the binding pocket of α-glucosidase. Overall, the interaction patterns observed in both enzymes indicate that the compound forms stable complexes with α-amylase and α-glucosidase through van der Waals forces and carbon hydrogen bonding. These interactions may contribute to the inhibitory potential of the compound against carbohydrate-digesting enzymes, which are important targets in the management of postprandial hyperglycemia.

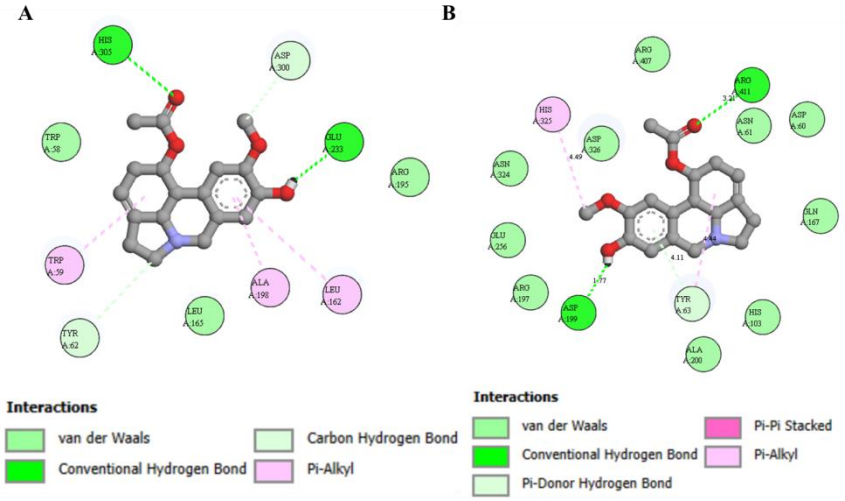


Figure 4. Amino Acids Interaction Between Stepharinosine/ 1-O-Acetylnorpluviine and α-Amlyase (A) and α-Glucosidase (B) Enzyme

In Figure 4A, the interaction between stepharinosine/1-O-acetylnorpluviine and α-amylase is stabilized by several amino acid residues through van der Waals interactions, including TRP_{58}, TYR_{62}, LEU_{165}, ARG_{195}, and ASP_{300}. These residues surround the ligand within the enzyme binding cavity and contribute to stabilizing the ligand–protein complex through weak intermolecular forces. In addition, conventional hydrogen bonds are formed with the residues HIS_{305} and GLU_{233}, which play an important role in maintaining the ligand orientation within the active site of α-amylase. A carbon hydrogen bond is also observed with the residue ASP300, providing additional stabilization between the ligand and the enzyme.

In Figure 4B, the interaction between stepharinosine/1-O-acetylnorpluviine and α-glucosidase involves several amino acid residues forming van der Waals interactions, including ASN_{324}, ASN_{61}, ASP_{60}, GLN_{167}, TYR_{63}, HIS_{103}, ALA_{200}, ARG_{197}, ARG_{407}, and GLU_{256}. These residues create a stabilizing environment around the ligand within the enzyme binding pocket. Furthermore, conventional hydrogen bonds are formed with ARG_{411} and ASP_{199}, which help anchor the ligand within the catalytic region of α-glucosidase. These hydrogen bonds are important because they contribute to the stability and proper positioning of the ligand in the active site. Overall, the interaction patterns indicate that stepharinosine/1-O-acetylnorpluviine can form stable complexes with both α-amylase and α-glucosidase through multiple van der Waals interactions, carbon hydrogen bonding, and conventional hydrogen bonds. These interactions suggest that the compound has the potential to inhibit the activity of carbohydrate-hydrolyzing enzymes by occupying their active sites and

interfering with substrate binding. Overall, these findings highlight *C. comatus* as a promising natural source of enzyme inhibitors with potential antidiabetic properties. In particular, stepharinosine may act as a dual inhibitor of α-amylase and α-glucosidase. However, further experimental validation through in vitro, in vivo, and pharmacological studies is necessary to confirm its efficacy, safety, and mechanism of action before potential therapeutic application.

4. CONCLUSION

The molecular docking analysis demonstrated that several bioactive compounds identified from *Coprinus comatus* have the potential to inhibit carbohydrate-hydrolyzing enzymes associated with postprandial hyperglycemia. The studied compounds showed varying degrees of interaction with both α-Amylase (PDB ID: 1HNY) and α-Glucosidase (PDB ID: 2ZE0), which are important targets in the management of Diabetes Mellitus. Among the tested ligands, Stepharinosine exhibited the most favorable binding affinity toward both enzymes, with values of −7.5 kcal/mol for α-amylase and −8.8 kcal/mol for α-glucosidase, surpassing the reference inhibitor Acarbose. The docking interactions indicated that stepharinosine formed stable complexes with key amino acid residues within the catalytic pockets of both enzymes, suggesting its ability to interfere with carbohydrate digestion and glucose release. Although other compounds identified from *C. comatus* showed moderate to weak binding affinities, their interactions still indicate potential contributions to the overall antidiabetic activity of the mushroom. Further research could be conducted by performing in vitro tests on the mushroom extract and its fractions to assess their antidiabetic potential against the enzyme using the enzyme-linked immunosorbent assay (ELISA) method, as well as in vivo tests using laboratory animals.

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