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RESEARCH ARTICLE

In-vitro and *in-silico* analysis of hesperidin and naringin for metabolic syndrome management

Duyu Taaza, Sunil S. Jalalpure*, Bhaskar Kurangi

Abstract

This study evaluates the *in-vitro* and *in-silico* activities of hesperidin and naringin in the context of metabolic syndrome. The compounds were assessed for their inhibitory effects on key metabolic enzymes, including lipase, α-amylase, and α-glucosidase, as well as their antioxidant properties through assays such as DPPH free radical scavenging, ferrous ion chelation, and nitric oxide scavenging. Molecular docking analysis was performed to predict the binding affinities of hesperidin and naringin with these target enzymes. The results revealed that hesperidin and naringin exhibited significant lipase inhibitory activity, comparable to orlistat, and effective inhibition of α-amylase and α-glucosidase, comparable to the standard acarbose. The molecular docking findings supported the *in-vitro* enzyme inhibition results, highlighting the strong binding interactions of these compounds with the enzymes. Additionally, the pronounced antioxidant potential of hesperidin and naringin underscores their therapeutic relevance in managing metabolic syndrome.

Keywords: Hesperidin, Naringin, Obesity, Diabesity, Metabolic syndrome, Molecular docking.

Introduction

Metabolic syndrome (MetS) has emerged as a major global public health concern affecting both developed and developing nations (Saklayen, 2018). Over the past three decades, obesity has reached pandemic levels, primarily driven by lifestyle changes. Currently, over 800 million people are affected by obesity, with healthcare costs associated with obesity-related conditions projected to exceed $1 trillion by 2025 (WHO, 2024). Type 2 diabetes mellitus (T2DM) significantly increases the risk of cardiovascular complications and mortality. Comorbidities such as dyslipidemia, hypertension, ischemic heart disease, and certain cancers exacerbate the health burden, especially when coexisting with T2DM (Rochlani *et al*., 2017; Jha *et al*., 2023). As a global health concern with rising prevalence, MetS presents a critical challenge, necessitating effective therapeutic strategies to mitigate its adverse effects (Shalaby *et al*., 2023). While lifestyle alterations, such as dietary changes and physical exercise, remain foundational for the management of MetS, pharmacological interventions targeting key metabolic pathways have emerged as valuable complementary approaches (Lim *et al*., 2014).

Diabesity, a form of diabetes driven by obesity, underscores the strong pathophysiological relationship between obesity and diabetes, predominantly arising from visceral adiposity. This condition induces insulin resistance, playing a central role in its pathogenesis. Effective management of diabesity requires strategies that simultaneously target obesity and diabetes (Astrup *et al*., 2000; Schmidt *et al*., 2003). One such approach involves modulating digestive enzymes like lipase, which is crucial for triglyceride metabolism and fatty acid absorption. Lipase inhibitors reduce fat digestion and absorption, aiding in obesity management while also improving cardiovascular health by lowering LDL levels and increasing HDL levels (Heck *et al*., 2000; Liu *et al*., 2020). Furthermore, enzymes such as α-amylase and α-glucosidase, which are key players in carbohydrate metabolism, influence energy balance, glucose regulation, and lipid profiles (Ćorković *et al*., 2022). Targeting these enzymes presents a promising strategy for the integrated management of diabetes and obesity. Oxidative stress, resulting from the accumulation of excess

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reactive oxygen species (ROS) and disrupted protein homeostasis, further contributes to metabolic disorders (Tangvarasittichai, 2015; Masenga *et al*., 2023). Flavonoids, plant-derived polyphenolic compounds, are increasingly recognized for their therapeutic potential due to their diverse biological activities. Hesperidin and naringin, found in citrus fruits, have shown promise in addressing multiple aspects of MetS through their antioxidant and enzyme-inhibitory properties (Sandoval *et al*., 2020; Mutha *et al*., 2021). This study evaluates the enzyme-inhibitory activities of hesperidin and naringin, focusing on their potential roles in managing MetS. Their efficacy was compared with standard enzyme inhibitors, orlistat and acarbose, using a combination of *in-vitro* assays and *in-silico* docking investigations. These methods assessed the inhibitory effects of hesperidin and naringin on lipase, α-amylase, and α-glucosidase, alongside analyses of their binding affinities and interactions with enzyme active sites. Furthermore, the antioxidant properties of hesperidin and naringin were investigated to understand their broader therapeutic implications.

Materials and Methods

Porcine Pancreatic Lipase Inhibitory Activity of Hesperidin and Naringin

The lipase inhibitory assay followed the protocol by Chedda *et al*. (2016). The pancreatic lipase inhibition assay utilized p-nitrophenyl butyrate (PNPB) as the substrate. The test samples and enzyme were combined in 1-mL of Tris-HCl buffer (pH 7.4) and incubated at 25°C for 15 minutes. Then, PNPB solution (100 µL) was added, and incubation continued at 37°C for 30 minutes. Enzyme activity was determined by measuring the hydrolysis of PNPB to p-nitrophenol at 400 nm, and IC_{50} values were calculated with orlistat as positive control.

α-Amylase Inhibitory Activity of Hesperidin and Naringin

The porcine pancreatic α-amylase inhibition assay was conducted following the method described by (Poovitha *et al*., 2016). The enzyme was dissolved in 0.1 M phosphate-buffered saline at pH 6.9. The samples were pre-incubated with the enzyme at 37°C for 10 minutes. The reaction was initiated by adding a substrate solution containing 0.1% starch to the incubation mixture. After 10 minutes of incubation, the reaction was stopped by adding 250 µL of dinitrosalicylic acid reagent. The mixture was then heated in a boiling water bath for 10 minutes to terminate the reaction. Following this, 250 µL of a 40% potassium sodium tartrate solution was added. After cooling the mixture to room temperature in a cold-water bath, the absorbance was measured at 540 nm, with acarbose serving as the positive control.

α-Glucosidase Inhibitory Activity of Hesperidin and Naringin

The α-glucosidase inhibitory activity was conducted following the method described by (Tsujii *et al*., 1996). The enzyme was dissolved in a 50 mM phosphate buffer (pH 6.9) and incubated with the test samples at 37°C for 10 minutes. The reaction was started by adding 50 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside, incubated for 30 minutes, and terminated with 1M sodium carbonate. The absorbance was recorded at 405 nm, and IC_{50} values were determined, with acarbose serving as the positive control.

In-vitro Antioxidant Activities of DPPH Free Radical Scavenging Assay

The antioxidant activity was assessed using the DPPH radical method described by(Sudarshan *et al*., 2019). A 2 mL sample was combined with 100 µM DPPH solution, and the total volume was adjusted to 3 mL with methanol. The mixture was kept in the dark at room temperature for 45 minutes before measuring the absorbance at 517 nm using a spectrophotometer. The results were reported as IC_{50} values, with L-ascorbic acid serving as a positive control.

Ferrous-Ion Chelating Assay

The ferrous ion-chelating activity was conducted using the method described by Lakshmegowda *et al*. (2020). Samples were mixed with 0.05 mL of FeCl2 (2 mM), and the reaction was initiated by the addition of 0.1 mL of ferrozine (5 mM). After incubation at room temperature for 10 minutes, the absorbance at 562 nm was measured to assess ferrous ion chelation. IC_{50} values were calculated using EDTA as a positive control.

Nitric Oxide Scavenging Activity

The nitric oxide scavenging activity was conducted using the method described by Sreejayan *et al*. (1997). In this assay, varying concentration of samples with sodium nitroprusside (10 mM in PBS, pH 7.4) is incubated for 150 minutes at room temperature to generate nitric oxide. Then, 0.5 mL of Griess reagent was added to detect nitrite production. After measuring the resulting chromophore's absorbance at 546 nm, the absorbance was compared to the control to determine the nitric oxide scavenging activity. The results, using L-ascorbic acid as a positive control, were presented as IC_{50} values.

In-silico Molecular Docking

Schrodinger›s Glide extra precision mode (XP) was taken into consideration for docking in order to determine the inhibitors' orientation and binding interaction in the -amylase and α-glucosidase pocket (V. Ulaganathan *et al*., 2009). In the XP mode, false positives are eliminated using cutting-edge scoring and wide-range flexible sampling; penalties are taken into account in the scoring function;

conformational search sampling is used to rank the best poses; and interactions such as hydrogen bonding, hydrophobicity, and pi-pi stacking interaction are taken into consideration. Ligand structures were sketched using Maestro and prepared with Schrödinger's LigPrep module (Schrodinger, LLC, 2022). To minimise energy, the optimised potential for liquid simulations (OPLS4) force field was used. Torsional flexibility was specified to attain a better complimentary pose. The protein data bank (https://www.rcsb.org) provided the crystal structure coordinates of the binding sites for lipase (PDB ID: 1LPB), α-amylase (PDB ID: 4W93), and α-glucosidase (PDB ID: 3TOP) for *in-silico* investigations. The retrieved protein was prepared using the protein preparation wizard of Schrodinger's suit, and grid generation for docking was performed by defining the binding site based on the co-crystal ligand, allowing accurate targeting within the receptor. The pre-processing of protein included removing ligands and water, assigning bond orders, and determining pKa values at a pH of 7 ± 2. The proteins were subsequently optimized through energy minimization using the OPLS4 force field to ensure stability (H. Zhong *et al*., 2009; S. Kalva *et al*., 2014).

Results

Porcine Pancreatic Lipase Inhibitory Activity of Hesperidin and Naringin

The IC_{50} values for hesperidin and naringin were determined to be 163.22 ± 12.32 µg/mL and 128.26 ± 11.50 µg/mL, respectively, using orlistat as the standard, exhibiting an IC_{50} value of 64.20 ± 1.58 µg/mL as presented in Table 1.

α-Amylase inhibitory activity of Hesperidin and Naringin

The IC_{50} values for hesperidin and naringin were determined to be 217.39 ± 11.83 µg/mL and 205.86 ± 17.33 µg/mL, respectively, with acarbose, used as the standard, exhibiting an IC_{50} value of 55.52 ± 2.28 µg/mL, as detailed in the Table 2.

α-glucosidase Inhibitory Activity of Hesperidin and Naringin

The IC_{50} values for hesperidin and naringin were calculated to be 117.88 ± 7.04 µg/mL and 93.90 ± 5.29 µg/mL, respectively, while the standard, acarbose, exhibited an IC_{50} value of 45.49 ± 6.78 µg/mL, as presented in the Table 3.

In-vitro Antioxidant Activity of Hesperidin and Naringin

The antioxidant activity of hesperidin and naringin was assessed using three assays: DPPH radical scavenging, nitric oxide (NO) scavenging, and $FeCl_2$ ion chelation. The IC_{50} values for DPPH activity were 90.28 ± 5.39 µg/mL for hesperidin and 63.50 ± 6.78 µg/mL for naringin, compared to the reference antioxidant L-ascorbic acid, which showed IC_{50} value of 28.8 ± 0.75 µg/mL. For NO scavenging, hesperidin and naringin exhibited IC_{50} values of 268.06 ± 13.9 µg/mL and 255.84 ± 3.22 µg/mL, respectively, with L-ascorbic acid showing activity at 64.03 ± 9.0 µg/mL. In the $FeCl_2$ ion chelation assay, hesperidin and naringin demonstrated IC_{50} values of 139.01 ± 9.23 µg/mL and 119.7 ± 8.86 µg/mL, respectively, while EDTA, the standard chelating agent, showed the IC_{50} value of 42.07 ± 4.9 µg/mL. The IC_{50} values of both compounds for each antioxidant assay are summarized in Table 4.

Table 1: *In-vitro* lipase inhibitory activity of hesperidin and naringin

S.No	*Test agent*	*IC_{50} (µg/mL)*
1	Hesperidin	163.22 ± 12.32
2	Naringin	128.26 ± 11.5
3	Orlistat (Standard)	64.20 ± 1.58

Table 2: *In-vitro* α-amylase inhibitory activity of hesperidin and naringin

S. No	*Test agents*	*IC_{50} (µg/mL)*
1	Hesperidin	217.39 ± 11.83
2	Naringin	205.86 ± 17.33
3	Acarbose (standard)	55.52 ± 2.28

Table 3: *In-vitro* α-glucosidase inhibitory activity of hesperidin and naringin

S. No	*Test agents*	*IC_{50} (µg/mL)*
1	Hesperidin	117.88 ± 7.04
2	Naringin	93.90 ± 5.29
3	Acarbose (standard)	45.49 ± 6.78

In-silico Molecular Docking Study for Lipase, α-Amylase, and α-Glucosidase

Maestro graphical user interface 13.2 was used to analyze and visualize the molecular docking study conducted in Schrodinger's Small molecule drug discovery suit.

Lipase (1LPB)

The standard compound orlistat exhibits a binding affinity of -8.22 kcal/mol and a glide energy of -55.35 kcal/mol, forming hydrogen bond interactions with *HIE263* and *PHE77*. Hesperidin on the other hand, demonstrates a binding affinity of -9.73 kcal/mol and a glide energy of -49.63 kcal/mol, establishing hydrogen bonds with *HIE263, ASP79*, and *PHE215*. Similarly, naringin shows a binding affinity of -9.14 kcal/mol and glide energy of -46.83 kcal/mol, interacting *via* hydrogen bonds with *PHE77* and *ASP79* (Figure 1). These variations in binding affinities and hydrogen bonding interactions are attributed to the distinct binding orientations of these ligands within the lipase binding pocket, as summarized in the Table 5.

Table 4: *In-vitro* antioxidant activity of hesperidin and naringin

IC_{50} (µg/mL) of test agents			IC_{50} (µg/mL) of reference sample	
Antioxidant activity	*Hesperidin*	*Naringin*	*L-ascorbic acid*	*EDTA*
DPPH	90.28 ± 5.39	63.50 ± 6.78	28.8 ± 0.75	-
$NO^{\cdot}$	268.06 ± 13.9	255.84 ± 3.22	64.03 ± 9.0	-
$FeCl_2$	139.01 ± 9.23	119.7 ± 8.86	-	42.07 ± 4.9

α-amylase (4W93)

Acarbose, the standard reference compound, exhibited a binding affinity of -10.95 kcal/mol and a glide energy of -60.34 kcal/mol. It established hydrogen bond interactions with key residues *HIE201, THR163, GLU233*, and *ASP300* within the α-amylase binding pocket, indicative of its strong inhibitory potential. Hesperidin demonstrated a binding affinity of -8.22 kcal/mol and a glide energy of -63.27 kcal/mol. It formed hydrogen bonds with multiple residues, including *GLU233, ASP197, GLN63, HIE299*, and *ASP300*. This extensive interaction network suggests that hesperidin's structural characteristics facilitate strong binding within the enzyme's active site. Naringin exhibited a binding affinity of -7.30 kcal/mol and a glide energy of -53.05 kcal/mol. Its hydrogen bonding interactions involved *ASP300, HIE299, GLU233*, *HIE201*, and *LYS200* (Figure 2). A summary of these findings is provided in Table 6.

α-glucosidase (3TOP)

Acarbose, the standard reference compound, exhibited a binding affinity of -10.40 kcal/mol and a glide energy of -75.19 kcal/mol. It formed hydrogen bond interactions with several key residues, including *ASP142, ASP156, ASP137, LYS146, ASP115*, and *HIE158,* within the α-glucosidase binding pocket, reflecting its strong binding potential. Hesperidin demonstrated a binding affinity of -9.53 kcal/mol and a glide energy of -56.40 kcal/mol. It interacted with the binding site through hydrogen bonds involving residues *ASP208, TYR209*, and *GLU134*. These interactions suggest that hesperidin's specific molecular structure facilitates effective binding to critical residues in the α-glucosidase active site. Naringin displayed a binding affinity of -8.05 kcal/mol and a glide energy of -49.32 kcal/mol. It established hydrogen bond interactions with residues *ASP106, LYS105, TRP103, GLU198, SER205, GLN335*, and *ARG320*. The distinct binding orientation of naringin, compared to acarbose, likely accounts for its unique interaction profile and relatively lower binding affinity (Figure 3). A summary of these results is presented in (Table 6), providing insights into the structural and functional aspects of ligand binding.

Table 5: Binding affinity and interactions of compounds with 1LPB (Lipase) using the Glide module of Schrodinger's

Compound	*Docking score*	*Glide energy*	*Amino Acid residue*
Orlistat	-8.22	-55.35	*HIE263, PHE77*
Hesperidin	-9.74	-49.63	*HIE263*, ASP79, PHE215
Naringin	-9.14	-46.83	*PHE77*, ASP79

Table 6: Binding affinity and interactions of compounds with 4W93: (α-amylase) using the Glide module of Schrodinger's

Compound	*Docking score*	*Glide energy*	*Amino acid residue*
Acarbose	-10.95	-60.34	*HIE201*, THR163, *GLU233*, *ASP300*
Hesperidin	-8.22	-63.27	*GLU233*, ASP197, GLN63, HIE299, *ASP300*
Naringin	-7.30	-53.05	*ASP300*, HIE299, *GLU233*, *HIE201*, LYS200

Discussion

This study investigates the enzyme-inhibitory activities of hesperidin and naringin in the context of MetS management, comparing their efficacy with standard inhibitors such as orlistat and acarbose. The analysis encompasses *in-vitro* assays to assess their inhibitory potential on lipase, α-amylase, and α-glucosidase, in conjunction with *in-silico*

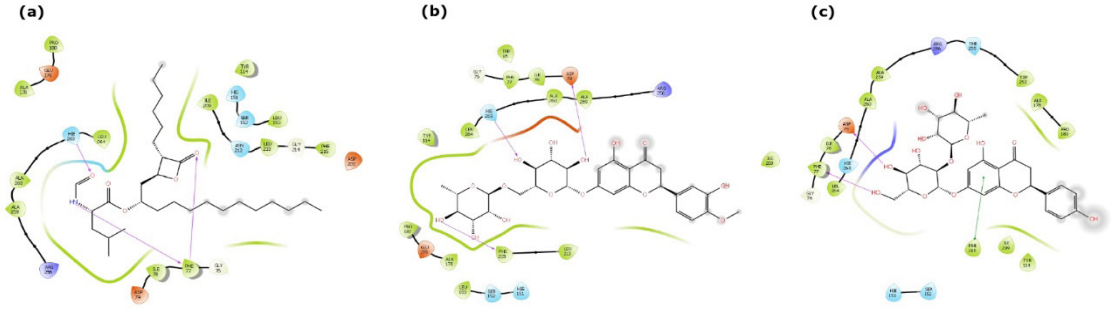

Figure 1: 2D orientation of (a) Orlistat (b) Hesperidin and (c) Naringin in lipase binding pocket

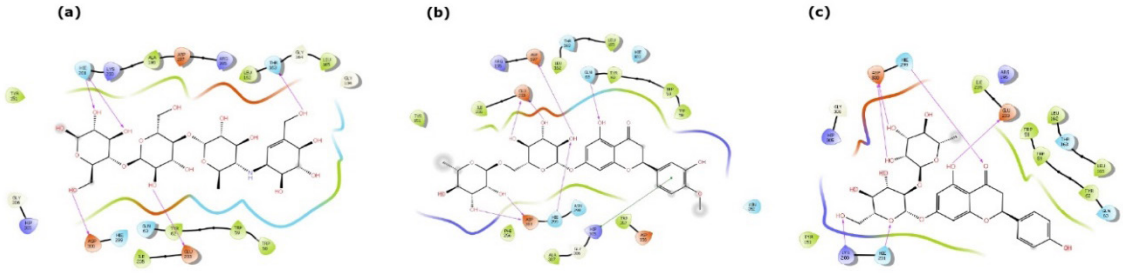

Figure 2: 2D orientation of (a) Acarbose (b) Hesperidin and (c) Naringin in α- amylase binding pocket

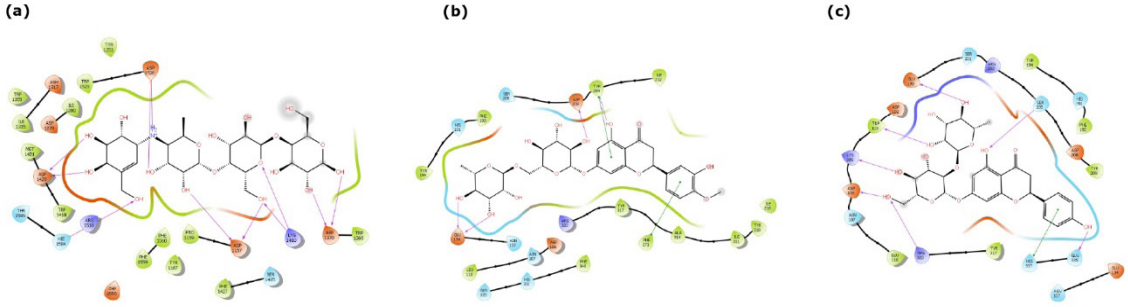

Figure 3: 2D orientation of (a) Acarbose (b) Hesperidin and (c) Naringin in α- glucosidase binding pocket.

Table 7: Binding affinity and interactions of compounds with 3TOP (α-glucosidase) using the Glide module of Schrodinger's

Compound	*Docking score*	*Glide energy*	*Amino acid residue*
Acarbose	-10.40	-75.19	ASP142, ASP156, ASP137, LYS146, ASP115, HIE158
Hesperidin	-9.53	-56.40	ASP208, TYR209, GLU134
Naringin	-8.05	-49.32	ASP106, LYS105, TRP103, GLU198, SER205, GLN335, ARG320

docking studies to explore their binding affinities and interaction profiles (Table 7). The varied class of polyphenolic chemicals originating from plants, known as flavonoids, have garnered substantial attention as potential therapeutic agents due to their multifaceted biological activities (Dias *et al*., 2021; Jucá *et al*., 2020). Hesperidin and naringin, two flavonoids predominantly found in citrus fruits, exhibit promising properties for addressing multiple aspects of MetS. Their antioxidant and enzyme-inhibitory capabilities make them suitable candidates for managing the complex pathophysiology of MetS (Sandoval *et al*., 2020; Mutha *et al*., 2021)

Additionally, the antioxidant properties of these flavonoids are assessed to understand their broader therapeutic implications. Lipase inhibitors, such as orlistat, function by reducing triglyceride hydrolysis, thereby decreasing fat absorption and contributing to improved lipid profiles and weight management (Vangoori *et al*., 2019). The inhibition of pancreatic lipase by hesperidin and naringin highlights their potential in managing lipid metabolism, a critical aspect of metabolic syndrome (Heck *et al*., 2000; Liu *et al*., 2020). While orlistat exhibits the highest inhibitory potency, hesperidin and naringin demonstrate significant activity as natural inhibitors. The similar IC_{50} values observed for hesperidin and naringin can be attributed to their structural similarity, including their hydroxylation patterns and glycosidic linkages. These flavonoids have a C6-C3-C6 structure, which features two benzene rings connected by a three-carbon bridge, typically forming a heterocyclic pyran ring (Dias *et al*., 2021). This structural framework supports diverse biological activities, including antioxidant (Agati *et al*., 2020), anti-diabetic (Al-Ishaq *et al*., 2019), and anti-obesity (Gómez-Zorita *et al*., 2017) effects.

Further, molecular docking analysis reveal that orlistat forms key hydrogen bonds with active site residues, enhancing its inhibitory effect. Hesperidin and naringin, interact with critical residues such as ASP79 and PHE215, suggesting their structural suitability for lipase inhibition. These findings align with prior reports emphasizing

flavonoid's role in regulating lipid metabolism and their potential for combating obesity (Gómez-Zorita *et al*., 2017; Jucá *et al*., 2020)

Similarly, α-amylase and α-glucosidase inhibitors, such as acarbose, prolongs carbohydrate digestion and glucose absorption, effectively controlling postprandial hyperglycemia (Tundis *et al*., 2010). Despite their clinical efficacy, these conventional enzyme inhibitors often come with limitations, including gastrointestinal side effects and variability in patient response, prompting the exploration of alternative natural compounds (Alqahtani *et al*., 2019). Inhibiting α-amylase is a strategic approach to controlling postprandial hyperglycemia, a hallmark of metabolic syndrome (Ogunyemi *et al*., 2022). Hesperidin and naringin demonstrate moderate α-amylase inhibition compared to acarbose, the standard inhibitor. Docking studies reveal acarbose's strong binding interactions with residues such as GLU233 and ASP300, correlating with its inhibitory efficacy. The ability of hesperidin and naringin to modulate carbohydrate metabolism is consistent with their structural properties and supports their use as dietary interventions or therapeutic agents for the management of metabolic syndrome (Al-Ishaq *et al*., 2019). Similarly, targeting α-glucosidase offers a mechanism to slow carbohydrate digestion and absorption, reducing glycemic spikes associated with metabolic syndrome (Kashtoh *et al*., 2022). Acarbose remains the most potent inhibitor due to its strong binding with key residues such as ASP142, ASP156, and HIE158. Hesperidin and naringin form significant interactions with residues, including ASP208 and TYR209 (hesperidin) and LYS105 and TRP103 (naringin). These structural interactions underline the potential of flavonoids as α-glucosidase inhibitors. The observed inhibitory activities align with literature highlighting flavonoid derivatives' role in glycemic control and their relevance in metabolic syndrome management (He *et al*., 2919). The inhibitory action of flavonoids on enzymes like α-amylase and α-glucosidase can differ, including competitive, non-competitive, or uncompetitive inhibition, based on the number and placement of hydroxyl groups and other substituent modifications (Lam *et al*., 2024).

Additionally, oxidative stress is a key aspect in the pathogenesis of metabolic syndrome, influencing insulin resistance, dyslipidemia, and vascular dysfunction (Tangvarasittichai, 2015; Masenga *et al*., 2023). The antioxidant properties of hesperidin and naringin, assessed through various assays, demonstrate their capacity to mitigate oxidative damage. While less potent than standards such as L-ascorbic acid or EDTA, these compounds exhibit notable radical scavenging and metal ion chelation activities. This antioxidant potential is crucial in alleviating oxidative stress-induced cellular damage associated with metabolic syndrome. Flavonoids are well known to neutralize free radicals and enhance cellular antioxidant defenses, as corroborated by studies on their therapeutic implications (Hassanpour *et al*., 2023)

Molecular docking analyses deliver valuable insights into the binding interaction of hesperidin and naringin with lipase, α-amylase, and α-glucosidase. These studies suggest that both ligands exhibit favorable binding orientations and interactions with active site residues. The structural adaptability of hesperidin and naringin underscores their suitability as templates for designing novel inhibitors targeting metabolic syndrome. These findings are consistent with computational studies that emphasize the predictive power of molecular docking in drug discovery (Bhagat *et al*., 2021; Aguiar *et al*., 2024).

The results of this study add to the increasing evidence supporting the use of natural compounds in the management of MetS. This study provides insights into their potential as multifunctional agents for mitigating the metabolic and oxidative stress associated with MetS. Such an integrated approach underscores the value of natural compounds in developing effective and safer therapeutic options for complex metabolic disorders.

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