Computational Evaluation of the Anti-Obesity Potential of Phytochemicals Isolated from Kodo Millets Using Molecular Docking

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Abstract:

Kodo millet (*Paspalum scrobiculatum* L.) is a rich source of bioactive phytochemicals with potential anti-obesity and antihypercholesterolemic properties. LC–MS/MS analysis identified major phenolic acids and flavonoids, including hydroxybenzoic acid, ferulic acid, luteolin, apigenin, quercetin, and glycosylated flavones. Lipinski's Rule of Five, ADME properties calculate using SwissADME. Docking analysis was carry out against pancreatic triacylglycerol lipase (PTL) and HMG-CoA reductase using CB-Dock2, with photochemicals of kodo millets Atorvastatin and Orlistat used as positive controls. Apigenin exhibited the strongest binding affinity toward PTL (−9.3 kcal/mol), compared with the Orlistat (−5.7 kcal/mol) it indicates that the compound showed good anti lipase activity, while luteolin showed the highest affinity for HMG-CoA reductase (−9.7 kcal/mol), exceeding Atorvastatin (−8.2 kcal/mol), this speculated that it has excellent anticholesterol controlling capacity. The strong hydrogen-bonding and hydrophobic interactions observed suggest stable ligand–protein complexes. These findings indicate that Kodo millet phytochemicals possess significant potential to regulate lipid metabolism and may provide as promising functional food ingredients or nutraceutical candidates for the management of obesity and hypercholesterolemia. Further in vivo and clinical validation is crucial to establish the formulation of anti-obesity drug.

Keywords: Kodo millet (*Paspalum scrobiculatum* L.); Phytochemicals; Functional food; Molecular docking; Hypercholesterolemia; Nutraceuticals.

1. Introduction

Metabolic disorders are characterised by abnormalities in carbohydrate, protein, and lipid metabolism that impair normal physiological functions and promotes the onset of cardiovascular complications [1]. Currently, overweight and high blood pressure area global health problem because they contribute to the development of heart disease, type 2 diabetes, hypertension, dyslipidemia, non-alcoholic fatty liver disease and some cancers [2]. Excess weight is caused by excess fat accumulation due to an imbalance between energy intake and expenditure, while hypercholesterolemia is characterized by an increase in serum cholesterol, which increases atherosclerosis and coronary heart disease [3]. Pancreatic lipase plays an important role in the digestion of dietary fat, while3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase is the rate-limiting enzyme in the biosynthesis of cholesterol, because both enzymes are important therapeutic targets for the management of obesity and high cholesterol. Although drugs such as orlistat and atorvastatin are effective, their long-term use is associated with side effects, which shows the need for more natural and safe alternatives. Plant derived bioactive constituents, especially phenolic acids and flavonoids, and dietary fibers, have been reported to have an anti-lipase, anti-inflammatory, and cardioprotective properties [4].

Kodo millet (*Paspalum scrobiculatum* L.) is considered an essential functional food grain due to its high content ofdietary fibre, resistant starch, proteins, minerals, vitamins, phenolic acids, and flavonoids. These components are associated with several health-promoting properties, including lipid-modulating, high blood pressure and cardio vascular effects [5]. However, the specific phytochemicals responsible for these biological activities and their

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underlying mechanisms are not yet fully established. Therefore, the present study was undertaken to identify the bioactive phytochemicals present in kodo millet (*Paspalum scrobiculatum*L.) using LC–MS analysis and to evaluate their potential anti-obesity activity through molecular docking studies against two key therapeutic targets, HMG-CoA reductase and pancreatic lipase**.**

2. Methodology:

2.1 Sample Collection and Extraction:

Kodo millet (Paspalum scrobiculatum L.) was collected from a local organic farm in Bengaluru, Karnataka. For raw sample preparation millets were cleaned, sun-dried, and pulverised, while cooked samples, grains were cooked for 10 minutes, sun-dried for 12–24 hours, and powdered. Both samples were separately extracted with 70% ethanol (1:10, w/v) by cold maceration for 24 hours. The extracts were centrifuged at 5000 rpm for 10 minutes, and the supernatants were used for further assay.

2.2Liquid Chromatography–Mass Spectrometry (LC–MS/MS):

Liquid chromatography-mass spectrometry (LC-MS-MS) assay have been conducted to determine the bioactive substances present in the Kodo millet ethanolic extracts. The chromatographic and mass spectrometric conditions have been optimised by means of preliminary experimental studies. The testing was carryout by Shimadzu HPLC (LC Solutions, Japan) coupled with the Waters Micro-mass Quattro Micro API triple-phase mass spectrometer, and performed with SCN805 Mass Lynx version 4.1 software. The chromatographic separation was done on the C18 Waters XBridge column (50 x 4.6 mm, particle size 3.5 µm). The mobile phase consisted of 0.1 percentage of formic acid in water (mobile phase A) and MS acetonitrile (mobile phase B) delivered at 1.0 ml per minute by gradient elution with a total running time of 15 min. The samples were prepared by the use of a solvent containing methanol, acetonitrile, water, 0.1 percentage points of formic acid and trifluoroacetic acid (TFAs). The detection was executed using UV-visible detector followed by mass analysis using an electrospray ionization (ESI) source. The optimized mass spectrometric condition incorporated a capillary voltage of 3.45 kV, cone voltage of 33 V, extractor voltage of 3.0 V, source temperature of 110°C, desolvation temperature of 350°C, desolvation gas flow of 750 L/h, cone gas flow of 50 L/h, and a scan time of 0.2 s. The acquired chromatographic and mass spectral data were resolute using the instrument software used for the identification of phytochemical constituents.

2.3 In Silico studies:

Seven phytochemicals of Kodo millet were selected for In silico studies. For structural identification, their 2D/3D structures and SMILES were acquired from PubChem (https://pubchem.ncbi.nlm.nih.gov/) [6], converted to PDB format using the CACTUS server (https://cactus.nci.nih.gov/) [7], and docking analysis was accomplished with CB Dock server(http://clab.labshare.cn/cb-dock/) [8]. Based on Lipinski's rule of five, the tool of SwissADME9 https://www.swissadme.ch/) [9] was used to estimate pharmacokinetic properties (ADME).

The target proteins of pancreatic triacyl glycerol lipase and HMG-CoA reductase (Fig 1a and 1b) were obtained from the Protein Data Bank (PDB) (https://www.rcsb.org/) [10] and were taken for molecular docking. CB-Dock, is a server which predicts ligand-protein interactions and finds active binding cavities. The standard anti-obesity drugs Atorvastatin and Orlistat were compared with the phytochemicals of Kodo millet which obeyed Lipinski rule.

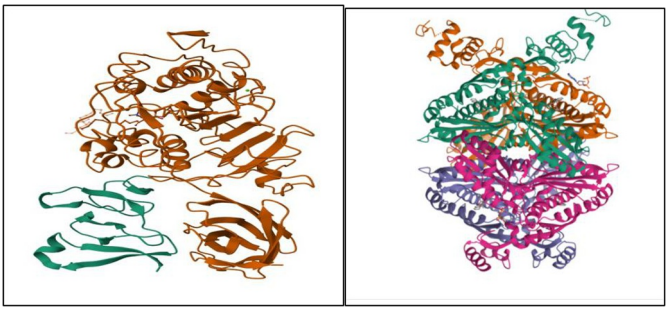


Figure 1 (a): Pancreatic triacyl glycerol lipase **Figure 1 (b): HMG-COA Reductase**

3. Result and Discussion

3.1 Liquid Chromatography–Mass Spectrometry (LC–MS/MS):

LC–MS Chromatographic Profile of Kodo millet Extract Showing Total Ion Chromatogram (TIC) and Base Peak Chromatogram (BPC) displayed in Figure 2.

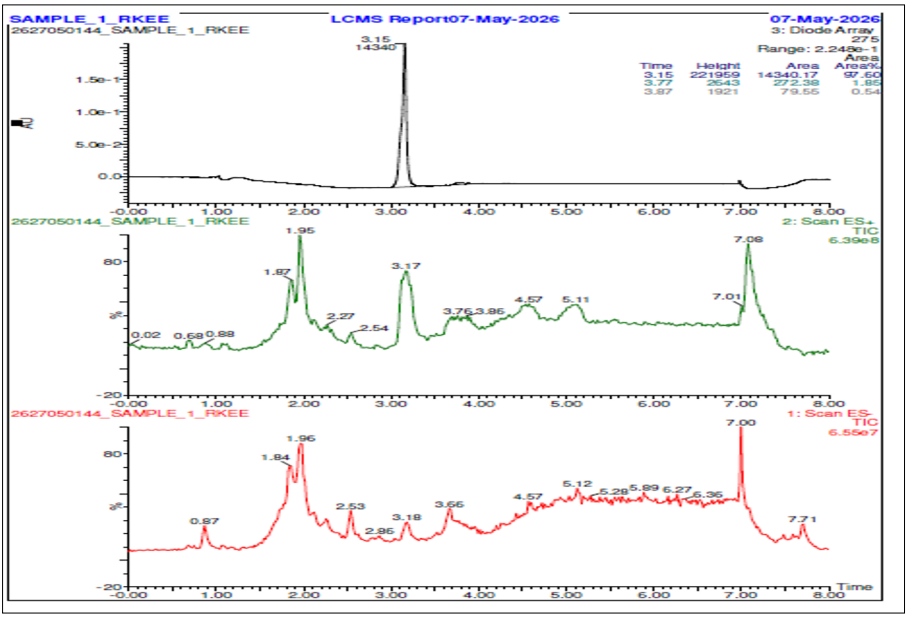


Figure 2: LC–MS Chromatographic Profile of Kodo millet Extract Showing Total Ion Chromatogram (TIC) and Base Peak Chromatogram (BPC)

It revealed the presence of phytochemical compounds such as phenolic acids and flavonoids, including hydroxybenzoic acid, quercetin, ferulic acid, apigenin, luteolin, and glycosyl flavone. Molecular docking indicated these compounds may inhibit pancreatic triacylglycerol lipase and HMG-CoA reductase. As a result, they also help to reduce triglyceride digestion, cholesterol absorption, LDL oxidation, and lipid accumulation. This suggests a potential role in improving lipid metabolism and preventing obesity, hypercholesterolemia, atherosclerosis, and CVD [11].

3.2 In Silico Studies:

The phytochemicals identified from Kodo millet (*Paspalum scrobiculatum L.*) structure were retrieved from the PubChem database and taken as ligands for molecular docking. The binding affinities of phytochemical compounds against pancreatic triacylglycerol lipase (PTL) and HMG-CoA reductase were evaluated using the CB-Dock2 server. Orlistat and Atorvastatin were used as positive controls.

3.2.1 Structural Identification of Phytochemical Components and Standard Drugs:

The six phytochemicals are chosen as ligands (hydroxybenzoic acid - 135, quercetin - 5280805, ferulic acid - 4445858, luteolin - 5280637, Glysoyl flavone - 5280441) for the docking, it's compared with the Orlistat (3034010).

Each compound is denoted by its PubChem ID, which acts as a distinctive reference for chemical data. The SMILES format illustrates the molecular composition in a format, while the respective 2D and 3D representations were created for ligand preparation and binding evaluation. These determine the identification of ligands and also facilitate a dependable comparison of binding interactions and affinity with the standard drug.

3.2.2 Lipinski's Rule of Five:

Lipinski's Rule of Five was used to assess the drug-likeness of the selected phytochemicals of Kodo millet (*Paspalum scrobiculatum L.*) based on their molecular weight, hydrogen bond donors (HBD), hydrogen bond acceptors (HBA), Log P, and the number of rule violations. Hydroxybenzoic acid (138.12 g/mol; Log P = 0.99) and Ferulic acid (194.18 g/mol; Log P = 1.00) exhibited no violations, indicating favourable oral drug-like properties (Table1). Apigenin (270.24 g/mol; HBD = 3, HBA = 5, Log P = 0.52) also satisfied all Lipinski criteria with zero violations. Orlistat, the standard drug (495.7 g/mol; Log P = 5.40) showed one violation, consistent with its elevated lipophilicity despite being an approved drug. In contrast, Quercetin (610.52 g/mol; HBD = 10, HBA = 16) exhibited three violations, while Luteolin (448.38 g/mol; HBD = 7, HBA = 11) and Glycosyl flavone (432.38 g/mol; HBD = 7, HBA = 10) showed two and one violations, respectively, suggesting comparatively lower oral bioavailability.

Table 1: Lipinski's Rule of Five of kodo millet compounds

Compound name	Molecular weight (g/mol)	H bond donor	H bond acceptor	Log p	Violation
Hydroxybenzoic acid	138.12	2	3	0.99	0
Quercetin	610.52	10	16	-3.89	3
Ferulic acid	194.18	2	4	1.00	0
Apigenin	270.24	3	5	0.52	0
Luteolin	448.38	7	11	-2.10	2
Glycosyl flavone	432.38	7	10	-2.02	1

3.2.3 Pharmacokinetic Properties:

The pharmacokinetic (ADME) properties of the selected phytochemicals from Kodomillet(*Paspalum scrobiculatum L.*) are presented in Table 2. Hydroxybenzoic acid (ELogS = −1.17) and Ferulic acid (ELogS = −1.42) exhibited the most favourable pharmacokinetic profiles, with high gastrointestinal (GI) absorption, blood–brain barrier (BBB) permeability, and the highest bioavailability score of 0.85. Apigenin (ELogS = −3.72) also demonstrated high GI absorption with a bioavailability score of 0.55, although it predicted to be non-BBB permeable.

Table 2: Pharmacokinetic properties of phytochemicals of Kodo millet

Compound name	Elog S	Class	GI absorption	BBB	Bioavailability score
Hydroxybenzoic acid	-1.17	Soluble	High	Yes	0.85
Quercetin	0.18	Soluble	Low	No	0.17
Ferulic acid	-1.42	Soluble	High	Yes	0.85
Apigenin	-3.72	Soluble	High	No	0.55
Luteolin	-1.42	Soluble	Low	No	0.17
Glycosyl flavone	-1.69	Soluble	Low	No	0.55

In contrast, Quercetin (ELogS = 0.18), Luteolin (ELogS = −1.42), and Glycosyl flavone (ELogS = −1.69) showed low GI absorption, but lacked BBB permeability, and exhibited bioavailability scores of 0.17, 0.17, and 0.55, respectively. Orlistat, used as the positive control, exhibited moderate solubility (ELogS = −4.70), low GI absorption, no BBB permeability, and a bioavailability score of 0.55.

3.3 Molecular Docking:

The molecular docking investigation was carried out using pancreatic triacylglycerol lipase (PTL) and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase as the target proteins because of their crucial roles in lipid metabolism. Pancreatic triacylglycerol lipase (PTL) plays a key role in lipid digestion, by hydrolysing dietary triglycerides to produce free fatty acids and glycerol, whereas HMG-CoA reductase is the rate-limiting enzyme involved in cholesterol biosynthesis. For the docking analysis, the three-dimensional crystal structures of the target proteins were procured from the Protein Data Bank (PDB) (https://www.rcsb.org/) [10] and their FASTA sequences were obtained.

Molecular docking of phytochemicals identified from (*Paspalum scrobiculatumL.*) Kodo millets were performed against Pancreatic triacylglycerol lipase and HMG-CoA reductase, and the docked compounds are presented in Figure 3 and 4, respectively. The CB-Dock (Cavity-detection guided Blind Docking) server was employed to recognize the target proteins pancreatic triacylglycerol lipase (PTL) and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase a long with the ligands of *Paspalum scrobiculatum L.* The active binding pockets of both target proteins were automatically identified by the CB-Dock2 server, and the interactions between the ligands and the amino acid residues within the binding sites were analysed. The docking results, including the Auto Dock Vina

binding affinity scores and the corresponding interacting amino acid residues, were recorded and are presented in the respective table 3 and 4, while the ligand–protein interaction structures were visualized for further interpretation.

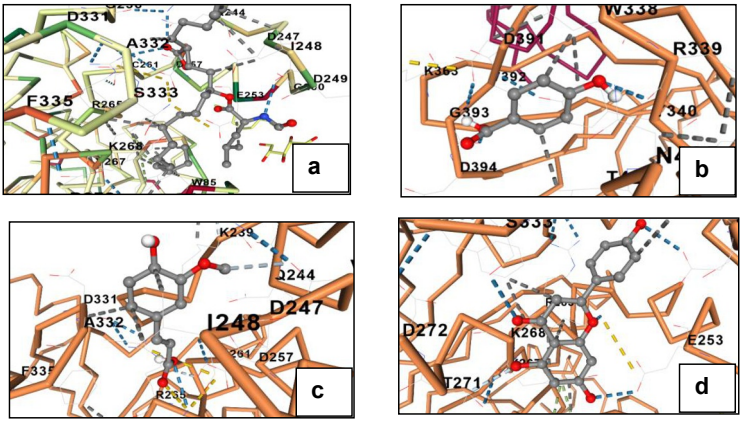


Figure 3: Docking image of ligands with Pancreatic triacyl glycerol lipase from the CB DOCK tool (A. Orlistat, B. Hydroxybenzoic acid, C. Ferulic acid, D. Apigenin).

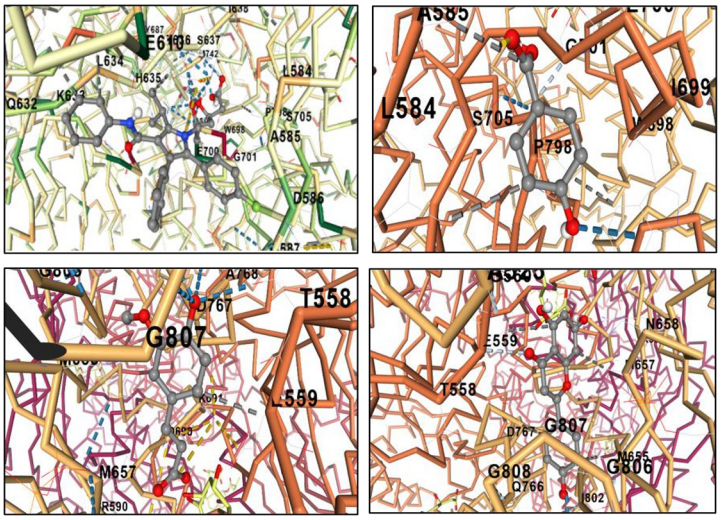


Figure 4: Docking image of ligands with HMG-COA Reductase from the CB DOCK tool (A. Atorvastatin, B. Hydroxybenzoic acid, C. Ferulic acid, D. Apigenin).

Molecular docking study exhibited that apigenin have the strongest binding affinity toward the Pancreatic triacyl glycerol lipase (target protein), with the lowest Vina score of −9.3 kcal/mol, compared with −6.6 kcal/mol for orlistat (positive control) and ferulic acid and −6.0 kcal/mol for hydroxybenzoic acid. All compounds were docked in the same cavity (size 400) centred at X = 13, Y = 23, and Z = 49. Apigenin interacted with several important residues, including Ile209, Phe215, Pro180, Tyr114, Leu153, Ser152, His151, Phe77, Gly76, Asp79, His203, Leu264, Ala260, Ala259, and Arg256. These residues provide hydrophobic, polar, hydrogen-bonding, and electrostatic contacts that can stabilize the ligand within the binding pocket [13, 14]. The lesser Vina score and multiple residue interactions recommend that apigenin has a more favourable and stable interaction with the target protein than the other compounds. Overall, apigenin was identified as the most promising ligand, although further experimental studies are required to validate its binding and biological activity.

Compound name	**Vina score**	**Cavity size**	**Centre**			**Size**			**Amino acid**
			X	**Y**	**Z**	**X**	**Y**	**Z**	
Orlistat	-6.6	400	13	23	49	29	29	29	Trp252, Thr255, Arg256, Ala259, Ala260, Asp79, Ile78, Phe77, Gly76, Tyr114, Pro180, Leu153, Ile209, Phe215,Ser152, His151, His263, Leu264
Hydroxybenzoic acid	-6.0	400	13	23	49	17	17	17	Ile209, Phe215, His263, Gly216, Glu179, Ala178, Ser152, Leu153, Phe77, Tyr114, Pro180
Ferulic acid	-6.6	400	13	23	49	19	19	19	His263, Phe815, Ile209, Gly216, Pro177, Ala178, Glu179, Pro180, Tyr114, Phe77, Ser152
Apigenin	-9.3	400	13	23	49	21	21	21	Ile209, Phe215, Pro180, Ala179, Tyr114, Leu153, Ser152, His151, Phe77, Gly76, Asp79, His203, Leu264, Ala260, Ala259, Arg256

Table 3. Molecular Docking Scores and Interacting Amino Acid Residues of Kodo Millet-Derived Ligands against Pancreatic triacyl glycerol lipase.

Docking analysis showed that apigenin revealed the strong binding affinity toward the HMG CoA reductase (target protein), with the lowest Vina score of −9.2 kcal/mol, followed by atorvastatin (positive control) (−8.2 kcal/mol), ferulic acid (−6.8 kcal/mol), and hydroxybenzoic acid (−6.1 kcal/mol). Apigenin and ferulic acid were docked in the same cavity of size 1318, centered at X = 44, Y = 16, Z = 32, with apigenin showing interactions with residues including GLY559, ALA225, MET657, GLY808, ILE802, and ASP767. Atorvastatin also showed favourable binding, interacting with GLU610, SER637, PHE615, LEU634, HIS635, ILE691, TYR687, and LYS606. Overall, the lower docking score of apigenin indicates a more favourable projected interaction and potentially greater binding

stability compared with the other tested compounds. Thus, apigenin can be considered the capable ligand in this docking analysis, although experimental endorsement is essential to confirm its biological activity.

Compound name	Vina score	Cavity size	Centre			Size			Amino acid
			X	Y	Z	X	Y	Z	
Atorvastatin	-8.2	4063	20	10	0	26	26	26	GLU610, SER637, PHE615, LEU634, HIS635, ILE691, TYR687, LYS606
Hydroxybenzoic acid	-6.1	4663	33	-6	-12	35	34	17	THR758, ILE762, ALA556, LEU562, ASN755, ALA759
Ferulic acid	-6.8	1318	44	16	32	19	19	19	GLY808, GLY766, GLN765, THR558, GLU559, LYS691, MET655, ASN658
Apigenin	-9.2	1318	44	16	32	21	21	21	GLY559, ALA225, MET657, GLY808, ILE802, ASP767,

Table 4. Molecular Docking Scores and Interacting Amino Acid Residues of Kodo Millet-Derived Ligands against HMG-CoA Reductase

HMG-CoA reductase (KEGG: Steroid biosynthesis – map00100) regulates cholesterol biosynthesis by converting HMG-CoA to mevalonate [15]. Its inhibition reduces endogenous cholesterol and lipid synthesis, preventing lipid accumulation. Pancreatic lipase (PNLIP) (KEGG: map04975) hydrolyses dietary triglycerides into absorbable fatty acids; its inhibition decreases fat absorption and storage [16]. Hence, these phytochemicals help to modulation of both pathways contributes to anti-obesity effects by reducing lipid synthesis and dietary fat uptake**.**

4. Conclusion:

The present study showed that Kodo millet (*Paspalum scrobiculatum L.*) is a functional food rich in bioactive phytochemicals with promising anti-obesity and anti-hypercholesterolemia potential. LC-MS analysis identified the plant-derived phytochemical compounds such as phenolic acids, apigenin, luteolin, quercetin and tricin. Swiss ADME predictions suggest drug similarity and favourable pharmacokinetic properties for most of the identified phytochemicals. Molecular docking revealed that apigenin had the highest binding affinity to pancreatic triacylglycerol lipase, while luteolin showed the highest affinity to HMG-CoA reductase. These results indicate that Kodo millet is a promising natural resource for the management of obesity and hypercholesterolemia through several bioactive mechanisms. Further in vivo studies, mechanistic investigations, and well-designed clinical trials are needed to validate efficacy, safety, and therapeutic dosing. Future research

should also focus on improving bioavailability, evaluating long-term toxicity, and developing formulations for functional food or nutraceutical applications.

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