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GC-MS and molecular docking analysis of Kodo millet (*Paspalum scrobiculatum*) identifying the compounds with anti-diabetic potential

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ABSTRACT

Kodo millet (*Paspalum scrobiculatum*) is a nutritionally superior grain and a rich source of dietary fiber and protein. It helps in managing health and dietary issues such as malnutrition, diabetes, obesity, and celiac disease. Its low content of slowly digestible carbohydrates promotes a gradual release of glucose, helping to maintain stable blood glucose levels. The present study aims to screen and identify phytochemicals in kodo millet and to explore its antidiabetic properties through GC-MS and *in silico* molecular docking analyses. GC-MS-based metabolomics analysis was conducted to identify a diverse array of metabolites present in four different kodo millet cultivars, yielding 245 metabolites. A GC-MS-based metabolomics analysis identified 245 metabolites across four kodo millet cultivars. Subsequent pathway and enrichment analyses of these metabolites revealed several significantly enriched metabolic pathways, including fatty acid biosynthesis; amino sugar and nucleotide sugar metabolism; cysteine and methionine metabolism; phenylpropanoid biosynthesis; terpenoid backbone biosynthesis; starch and sucrose metabolism; and valine, leucine, and isoleucine biosynthesis. Further investigation into the pharmacological properties of these metabolites, followed by molecular docking analysis against α-amylase, revealed that several compounds possess antidiabetic activity. Collectively, our results demonstrate the basis of kodo millet's therapeutic potential, adding a layer of health-related significance to its consumption.

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INTRODUCTION

Kodo millet (*Paspalum scrobiculatum* L.), also called *Varagu* in Tamil and belonging to the Poaceae family, is one of the world's ancient grains. It originated in Africa and was later domesticated in India around 3,000 years ago. It is cultivated in southern regions of India, from Kerala and Tamil Nadu to northern parts such as Rajasthan, Uttar Pradesh, and West Bengal (Deshpande *et al.*, 2015). Kodo millet is notable for its high nutritional value, including the highest phosphorus content and significant radical scavenging activity, owing to its high phenol content. Consumption of Kodo millet has been found to reduce cardiovascular dysfunction in cardiac patients and to help control blood pressure in women, especially during the postmenopausal phase (Deepak *et al.*, 2018; Bunkar *et al.*, 2021). Diabetes is a chronic metabolic disorder characterized by hyperglycemia, arising from the body's inability to produce sufficient insulin or to utilize the hormone effectively, which is vital for regulating glucose metabolism (Chaudhary & Mudgal, 2020). Currently, the primary care for type 2 diabetes mellitus (T2DM) relies on pharmacological treatments such as insulin sensitizers, secretagogues, α-glucosidase inhibitors, incretin-based therapies, and SGLT2 inhibitors. However, the long-term use of these drugs can lead to side effects such as

nephrological disorders, gastrointestinal disturbances, fatigue, and diarrhea. The development of traditional antidiabetic treatments has become necessary due to the emergence of drug resistance and the adverse effects associated with prolonged use of synthetic oral antidiabetic medications, despite the availability of insulin (Alam *et al.*, 2022). These concerns emphasize the importance of exploring natural therapeutic products as alternative options for managing T2DM, with the potential to reduce side effects while offering significant therapeutic benefits (Jacob & Narendhirakannan, 2019; Blahova *et al.*, 2021).

Plants constitute a vital source of bioactive phytochemicals, many of which serve as precursors for the synthesis of modern pharmaceutical drugs used in the treatment of various diseases (Alam *et al.*, 2022). These compounds demonstrate a wide range of biological activities and are known to enhance human health without inducing significant adverse effects (Tran *et al.*, 2020). To date, approximately 410 medicinal plants have been identified with established antidiabetic properties; however, the complete mechanisms of action have been fully elucidated for only about 109 of these species (Prabhakar & Doble, 2008). Kodo millet (*Paspalum scrobiculatum* L.), a nutrient-dense minor millet, has attracted growing scientific interest for its potential efficacy in the management of diabetes mellitus. The inhibition of carbohydrate-hydrolyzing enzymes, particularly α-amylase, plays a crucial role in managing postprandial hyperglycemia. α-Amylase is responsible for breaking down complex carbohydrates into simple sugars, resulting in rapid glucose absorption and elevated blood sugar levels following meals. Therefore, understanding the interactions between phytochemicals present in Kodo millet and digestive enzymes like α-Amylase may offer valuable insights into its antidiabetic potential and support the development of millet-based therapeutic strategies. Previously, Phenolic compounds in Kodo millet have been reported to exhibit partial inhibitory activity against α-glucosidase and pancreatic α-amylase (Shobana *et al.*, 2009). Additionally, Chethan *et al.* (2008) suggest that enzyme inhibitors such as aldose reductase prevent sorbitol accumulation, thereby reducing the risk of diabetes-induced cataract formation. Bioactive compounds found in Kodo millet - including quercetin, ferulic acid, *p*-hydroxybenzoic acid, vanillic acid, and syringic acid - are believed to contribute to its antidiabetic properties. Consequently, regular consumption of Kodo millet may be beneficial for individuals with diabetes. Recent studies, such as that by Singh *et al.* (2022), have demonstrated that finger millet also exerts antidiabetic effects by inhibiting digestive enzymes and modulating the gut microbiota. Probiotic and prebiotic species such as *Faecalibacterium*, *Eubacterium*, and *Roseburia* utilize the prebiotic components of finger millet to produce colonic short-chain fatty acids (SCFAs), which are known for their antidiabetic properties. These findings support the potential of Kodo millet to confer similar metabolic benefits.

GC-MS (Gas Chromatography–Mass Spectrometry) based metabolomics analysis is a powerful approach for identifying and quantifying phytochemicals in complex plant matrices. It enables the detection of bioactive compounds that may contribute to health-promoting effects, such as antidiabetic properties. In silico molecular docking analysis further complements this by predicting the binding affinities and interactions between these phytochemicals and target enzymes like α-amylase. This integrated methodology aids in validating the functional role of dietary compounds and supports the development of millet-based interventions for metabolic disorders. With this back drop, the present study aims to screen and identify phytochemicals in kodo millet and to explore its antidiabetic properties through GC-MS and in silico molecular docking analyses. The obtained results will be useful to explore the mechanistic basis of kodo millet's therapeutic potential.

MATERIALS AND METHODS

Plant Materials

The seeds of four kodo millet cultivars - ATL 1, CO 3, TNAU 86, and RK 390-25 - were obtained from Tamil Nadu Agricultural University, Coimbatore, India, and used for GC-MS analysis.

Sample Preparation and Extraction

Metabolites were extracted from seeds of four kodo millet cultivars, namely ATL 1, CO 3, TNAU 86, and RK 390-25. Sample preparation was conducted as described by Fiehn (2016) and Lisec *et al.* (2006), with slight modifications. All the samples were ground into a fine powder using a sterilized, pre-cooled pestle and mortar. Approximately 0.3 g of the ground powder was transferred to 2 mL centrifuge tubes containing 1.4 mL of 100% methanol to halt enzymatic activity, and the samples were then vortexed. A total of 50 µL of ribitol (0.2 mg of ribitol in 1 mL deionized sterile water) was added as an internal standard and vortexed again. The samples were incubated at 70 °C with continuous shaking for 15 minutes and subsequently centrifuged at 12,000 rpm for 15 minutes at 4 °C. The supernatant was filtered through a 0.22 µm membrane filter, and 1.4 mL of deionized water along with 750 µL of chloroform were added to the filtrate. The mixture was centrifuged at 12,000 rpm for 15 minutes at 4 °C. The upper polar phase (supernatant) was transferred to a new Eppendorf tube and dried in a vacuum concentrator for 1.5 hours. After drying, the samples were stored at 80 °C until derivatization.

GC-MS Analysis

GC-MS analysis was performed using a Shimadzu triple quadrupole GCMS-TQ8040 NX Gas Chromatograph-Mass Spectrometer. At an injection temperature of 280 °C, 1 µL of the aliquot was injected at a split ratio of 1:20 into the GC column. The column (SH-Rxi-5Sil MS) temperature was initially set at 70 °C for 1 minute, then ramped to 320 °C and held for 10 minutes. The mass spectrometer was programmed with the following parameters: ion source temperature at 230 °C, interface temperature at 280 °C, solvent cut-off at 5.0 minutes, and a mass range of 50 to 650 m/z.

Data Pre-processing and Statistical Analysis

The GC-MS spectral raw data in NetCDF output format were pre-processed and analyzed using freely available AMDIS v2.7 software. Shimadzu post run analyzer v.2020 was used for metabolite profiling. For mass spectral interpretation and compound identification, both the National Institute of Standards and Technology (NIST) and Golm Metabolome database (GMD) were employed. The additional details such as CID, IUPAC name, Molecular Formula, Molecular Weight, SMILES and InCHIKey of identified metabolites were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) using a web scrapping Python script (Python v 3.11.5). Duplicate metabolites were removed from total metabolites obtained from GC-MS for further functional annotation. Chemical classification of identified metabolites was performed using the ClassyFire tool (Djoumbou Feunang *et al.*, 2016). Functional analysis of the identified metabolites was performed using MetaboAnalyst version 5.0 (Pang *et al.*, 2021). Statistical analysis, Pathway mapping and Enrichment based on Over Represented Analysis of accumulated metabolites was also summaryzed.

ADMET Analysis and Drug Likeliness

Compounds belonging to the classes polyphenols, flavonoids, alkaloids, and carboxylic acids were filtered and analyzed to evaluate their ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties using the pkCSM web server (https://biosig.lab.uq.edu.au/pkcsm/).

Selection of Targets

The target proteins associated with diabetes were selected through a comprehensive literature survey, and their three-dimensional X-ray crystal structures were retrieved from the Protein Data Bank (PDB; https://www.rcsb.org/).

Preparation of Ligands

The ligands consisted of metabolites identified through GC-MS analysis of kodo millet seeds, along with acarbose, a standard antidiabetic drug. Three-dimensional (3D) structures of the ligands in Structure Data File (SDF) format were retrieved from the PubChem database.

Molecular Docking Analysis

The anti-diabetic potential of kodo millet bioactive components was investigated using molecular docking in Discovery Studio 2022. The three-dimensional structures of proteins and ligands were cleaned and processed using the same platform. The CHARMm force field was applied to the protein structures to resolve steric clashes and stabilize conformations. Ligands were filtered based on Veber's and Lipinski's rules. Binding sites of the target proteins were identified using the receptor cavity method, based on the spatial arrangement and chemical relevance of amino acid residues within the binding pocket. In the docking analysis, each ligand was docked with both target proteins using the CDOCKER algorithm, which evaluates up to ten possible binding poses per ligand. CDOCKER, a molecular dynamics and simulated annealing-based algorithm, was employed to score the interacting compounds. Final poses were ranked based on total docking energy, which comprises the intramolecular energy of the ligand and the ligand–protein interaction energy. The structure with the lowest docking energy was considered the most stable and was selected for further analysis. Docking analysis results were interpreted using standard metrics provided by Discovery Studio, including CDOCKER energy, CDOCKER interaction energy, hydrogen bonding, and binding energy. Acarbose, a commonly used standard antidiabetic drug, was also docked with the selected protein targets for comparative analysis.

RESULTS

Metabolomics of Kodo Millet Grains

Metabolites profiling of derivatized samples

GC-MS based metabolite profiling of the kodo millet cultivars identified a total of 701 compounds. Specifically, 165 compounds were identified in ATL 1, 193 in CO 3, 180 in RK 390-25, and 131 in TNAU 86. Tetra Methyl Silane (TMS) and its derivatives, used as internal standards for GC-MS, along with outliers such as lactose (a non-plant metabolite), were excluded from the cumulative dataset. After removing duplicates, a total of 246 common metabolites were identified across all four cultivars.

Functional Annotation and Chemical Classification

Details retrieved from the PubChem database provided important features of 246 metabolites. SMILES notation was used to obtain functional annotations of these metabolites. While major metabolites were present across all kodo millet cultivars, their accumulation levels varied significantly. The log

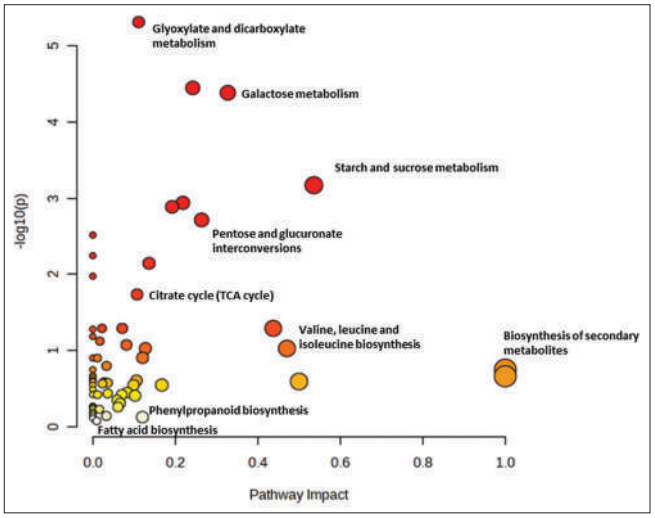

Figure 1: Pathway analysis of 246 metabolites

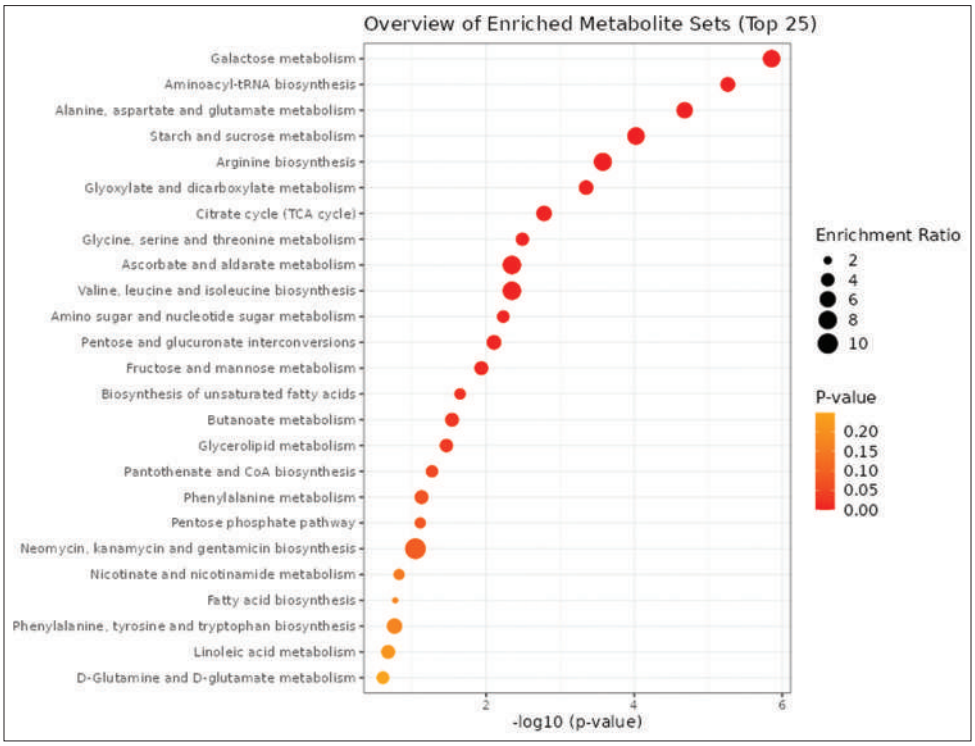

Figure 2: Enrichment dotplot of identified pathways

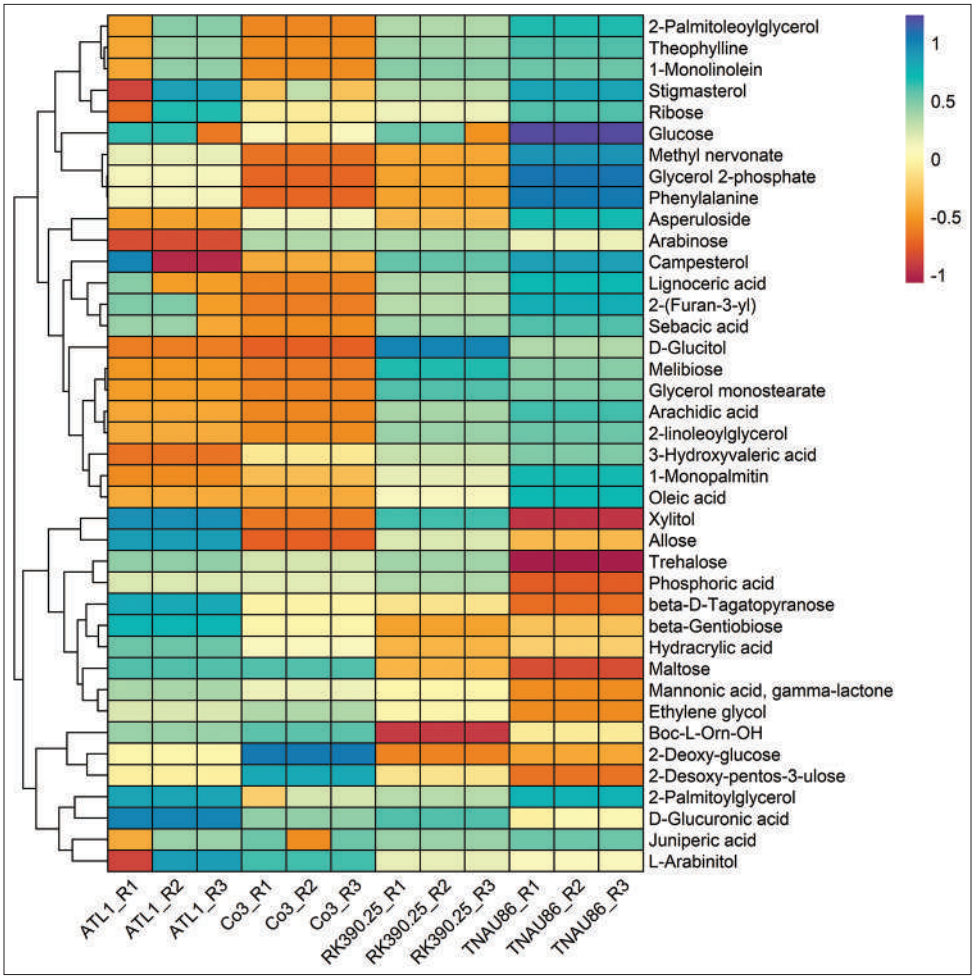

Figure 3: Differentially accumulated metabolites in kodo millet cultivars

fold change of metabolite levels between cultivars, indicating distinct accumulation patterns, is illustrated in a heat map (Figure 1). Functional annotation of the 246 metabolites revealed several key metabolic pathways with high impact scores and significant $-\log_{10}(p)$ values. These include fatty acid biosynthesis (56 metabolites), amino sugar and nucleotide sugar metabolism (50), cysteine and methionine metabolism (46), phenylpropanoid biosynthesis (46), terpenoid backbone biosynthesis (30), starch and sucrose metabolism (22), and valine, leucine, and isoleucine biosynthesis (22) (Figure 2). Enrichment analysis also indicated significant involvement of these metabolites in pathways related to the grain filling process (Figure 3). The 246 metabolites were chemically classified into organooxygen compounds, carboxylic acids and derivatives, fatty acyl compounds, polyphenols, steroids and their derivatives, isoflavonoids, and cinnamic acid derivatives, along with other important cyclic and non-cyclic compounds (Table 1). These classifications were used to select ligands for virtual screening studies.

Molecular Docking Analysis

Compounds belonging to the classes of polyphenols, flavonoids, alkaloids, carboxylic acids, cinnamic acids, and quinolines were filtered and analyzed for ADMET properties, resulting in the identification of 40 metabolites (Table 2). All 40 compounds complied with Lipinski's Rule of Five. Each compound possessed at least one hydrogen bond acceptor, except for stigmast-5-ene. Similarly, hydrogen bond donors were absent in benzaldehyde, oxazole, stigmast-5-ene, and tetrahydrofuran. The ADMET properties namely water solubility, intestinal absorption percentage, blood-brain barrier (BBB) permeability, central nervous system (CNS) permeability, total clearance, and hepatotoxicity were considered critical factors in the pharmacokinetic evaluation. These parameters are presented in Table 3. The major compounds exhibiting strong interactions with α-amylase belonged to the classes of vitamins, phenols, coumarins, and steroid derivatives. Molecular docking of 40 ligands, along with acarbose - a standard drug used against the

Table 1: Classification of metabolites based on their structure and physicochemical properties

S. No.	Compounds	PubChem (CID)	Molecular Formula	Molecular Weight (g/mol)	Average Percent area	Class
1	(2-Ethoxyethoxy) acetic acid	192850	$C_6H_{12}O_4$	148.16	141805	Carboxylic acids and derivatives
2	1-O-trans-p-Coumaroylglycerol	5319874	$C_{12}H_{14}O_5$	238.24	3737078	Cinnamic acids and derivatives
3	2-Hydroxyphenylacetic acid	11970	$C_8H_8O_3$	152.15	690554	Benzene and substituted derivatives
4	2-Ketoglutaric acid	51	$C_5H_6O_5$	146.1	411130	Keto acids and derivatives
5	3-Hydroxyisobutyric acid	87	$C_4H_8O_3$	104.1	796914	Hydroxy acids and derivatives
6	4-Coumaric acid	637542	$C_9H_8O_3$	164.16	917302	Cinnamic acids and derivatives
7	5-O-Methylgenistein	5748551	$C_{16}H_{12}O_5$	284.26	794656	Isoflavonoids
8	7-Hydroxyquinolin-2 (1H)-one	10975787	$C_9H_7NO_2$	161.16	2182393	Quinolines and derivatives
9	Aconitic acid	643757	$C_6H_6O_6$	174.11	181889	Carboxylic acids and derivatives
10	Allantoin	204	$C_4H_6N_4O_3$	158.12	253905	Azoles
11	Benzaldehyde	240	C_7H_6O	106.12	2143041	Benzene and substituted derivatives
12	beta-Tocopherol	6857447	$C_{28}H_{48}O_2$	416.7	269841	Prenol lipids
13	Caffeic acid	689043	$C_9H_8O_4$	180.16	12818741	Cinnamic acids and derivatives
14	Campesterol	173183	$C_{28}H_{48}O$	400.7	10988529	Steroids and steroid derivatives
15	Carbamic acid	277	CH_3NO_2	61.04	2483853	Organic carbonic acids and derivatives
16	Catechol	289	$C_6H_6O_2$	110.11	1585725	Phenols
17	Ferulic acid	445858	$C_{10}H_{10}O_4$	194.18	695196	Cinnamic acids and derivatives
18	Fumaric acid	444972	$C_4H_4O_4$	116.07	168260	Carboxylic acids and derivatives
19	Gamma-Tocopherol	92729	$C_{28}H_{48}O_2$	416.7	855982	Prenol lipids
20	Glycolic acid	757	$C_2H_4O_3$	76.05	626586	Hydroxy acids and derivatives
21	Grevillic acid	6442618	$C_9H_8O_4$	180.16	36049123	Cinnamic acids and derivatives
22	Hydracrylic acid	68152	$C_3H_6O_3$	90.08	695147	Hydroxy acids and derivatives
23	Hypotaurine	107812	$C_2H_7NO_2S$	109.15	2601625	Sulfinic acids and derivatives
24	L-Tryptophan	6305	$C_{11}H_{12}N_2O_2$	204.22	1464069	Indoles and derivatives
25	Magnolioside	3084335	$C_{16}H_{18}O_9$	354.31	2998801	Coumarins and derivatives
26	Malic acid	525	$C_4H_6O_5$	134.09	4887268	Hydroxy acids and derivatives
27	Malonic acid	867	$C_3H_4O_4$	104.06	293594	Carboxylic acids and derivatives
28	Methanesulfinic acid	87251	CH_4O_2S	80.11	151437	Sulfinic acids and derivatives
29	Oxalic acid	971	$C_2H_2O_4$	90.03	20122710	Carboxylic acids and derivatives
30	Oxazole	9255	C_3H_3NO	69.06	216275	Azoles
31	Phenol	996	C_6H_6O	94.11	385526	Phenols
32	Phenoxyethanol	31236	$C_8H_{10}O_2$	138.16	151508	Phenol ethers
33	Protocatechoic acid	72	$C_7H_6O_4$	154.12	9475719	Benzene and substituted derivatives
34	Pyruvic acid	1060	$C_3H_4O_3$	88.06	684084	Keto acids and derivatives
35	Quininic acid	345824	$C_{11}H_9NO_3$	203.19	389468	Quinolines and derivatives
36	Stigmast-5-ene	21771614	$C_{29}H_{50}$	398.7	23581985	Steroids and steroid derivatives
37	Stigmasterol	5280794	$C_{29}H_{48}O$	412.7	12657605	Steroids and steroid derivatives
38	Succinic acid	1110	$C_4H_6O_4$	118.09	2313977	Carboxylic acids and derivatives
39	Tetrahydrofuran	8028	C_4H_8O	72.11	5749900	Tetrahydrofurans
40	Trisaminol	6503	$C_4H_{11}NO_3$	121.14	10007636	Organonitrogen compounds
41	Vanillic Acid	8468	$C_8H_8O_4$	168.15	2033130	Benzene and substituted derivatives

Table 2: Molecular properties of filtered 40 metabolites

S. No.	Compounds	M.W (g/mol)	No. of Rotatable Bonds	No. of Acceptors	No. of Donors
Standard	Acarbose	645.60	9	19	14
1	(2-Ethoxyethoxy) acetic acid	148.15	6	3	1
2	beta-Tocopherol	416.69	12	2	1
3	gamma-Tocopherol	416.69	12	2	1
4	1-O-trans-p-Coumaroylglycerol	238.23	5	5	3
5	2-Hydroxyphenylacetic acid	152.14	2	2	2
6	2-Ketoglutaric acid	146.09	4	3	2
7	3-Hydroxyisobutyric acid	104.10	2	2	2
8	4-Coumaric acid	164.16	2	2	2
9	5-O-Methylgenistein	284.26	2	5	2
10	7-Hydroxyquinolin-2 (1H)-one	161.16	0	2	2
11	Aconitic acid	174.10	4	3	3
12	Allantoin	158.11	1	3	4
13	Benzaldehyde	106.12	1	1	0
14	Caffeic acid	180.15	2	3	3
15	Campesterol	400.69	5	1	1
16	Carbamic acid	61.04	0	1	2
17	Catechol	110.11	0	2	2
18	Ferulic acid	194.18	3	3	2
19	Glycolic acid	76.051	1	2	2
20	Grevillic acid	180.15	2	3	3
21	Hypotaurine	109.15	2	2	2
22	L-Tryptophan	204.22	3	2	3
23	Magnolioside	354.31	4	9	4
24	Malic acid	134.08	3	3	3
25	Malonic acid	104.06	2	2	2
26	Methanesulfinic acid	80.10	0	1	1
27	Oxalic acid	90.03	0	2	2
28	Oxazole	69.06	0	2	0
29	Phenol	94.11	0	1	1
30	Phenoxyethanol	138.16	3	2	1
31	Protocatechoic acid	154.12	1	3	3
32	Pyruvic acid	88.06	1	2	1
33	Quininic acid	203.19	2	3	1
34	Stigmast-5-ene	398.71	6	0	0
35	Stigmasterol	412.70	5	1	1
36	Succinic acid	118.08	3	2	2
37	Tetrahydrofuran	72.10	0	1	0
38	Trisaminol	121.13	3	4	4
39	Vanillic Acid	168.14	2	3	2
40	Fumaric acid	116.07	2	2	2

α-amylase enzyme - yielded the best CDOCKER interaction energy of −60.60 kcal/mol. Among the metabolites, β-tocopherol and γ-tocopherol, major forms of vitamin E, exhibited the most favorable interaction energies following acarbose, with values of −44.99 kcal/mol (pose 5) and −44.73 kcal/mol (pose 10), respectively. Other phytochemicals, including campesterol, stigmast-5-ene, stigmasterol, magnolioside, 1-O-trans-p-coumaroylglycerol, 5-O-methylgenistein, L-tryptophan, and quinic acid, also revealing strong binding affinities (Table 4). The 3D structural models illustrate the binding pocket and ligand interaction sites of α-amylase with the identified phytocompounds. The interacting amino acids that form bonds within the protein-ligand complex can be visualized using 2D interaction diagrams (Figure 4). The strong binding of these ligands within the α-amylase active site suggests inhibition of the enzyme's activity. This inhibition likely reduces α-amylase-mediated hydrolysis of starch into glucose, supporting the antidiabetic potential of kodo millet metabolites.

DISCUSSION

Kodo millet, a small-grained cereal crop, has garnered increasing attention in recent years due to its significant agronomic, nutritional, and ecological importance. According to Deshpande *et al.* (2015), Kodo millet thrives in conditions where other crops may struggle, showcasing its resilience to adverse environmental conditions. Its rich micronutrient content contributes to its role in improving the nutritional quality of diets. As research continues to uncover its potential, Kodo millet is emerging as a crucial player in sustainable agriculture and food security efforts, emphasizing the need for further exploration of its genomics and potential applications in crop improvement and human nutrition. GC-MS analysis is recognized as one of the most effective analytical methods for identifying components of volatile substances. Notably, the use of GC-MS has been comparatively more prevalent in determining the bioactive phytochemicals present in medicinal plants. In this study,

Table 3: Pharmacokinetic (ADMET) properties of filtered 40 metabolites

S. No.	Compounds	Absorption		Distribution		Metabolism		Excretion	Toxicity
		Water Solubility	Intestinal absorption (human)	BBB permeability	CNS permeability	CYP3A4 substrate	CYP3A4 inhibitor	Total Clearance	Hepatotoxicity
1	(2-Ethoxyethoxy) acetic acid	0.083	95.557	-0.266	-3.067	No	No	0.921	No
2	beta-Tocopherol	-7.417	89.452	0.915	-1.638	Yes	No	0.808	No
3	gamma-Tocopherol	-7.602	90.043	0.739	-1.669	Yes	No	0.821	No
4	1-O-trans-p-Coumaroylglycerol	-0.913	31.44	-0.934	-3.175	No	No	0.53	No
5	2-Hydroxyphenylacetic acid	-2.125	82.116	-0.27	-2.525	No	No	0.309	No
6	2-Ketoglutaric acid	-0.9	25.01	-0.247	-3.112	No	No	0.834	No
7	3-Hydroxyisobutyric acid	0.116	81.601	-0.332	-3.025	No	No	0.692	No
8	4-Coumaric acid	-2.378	93.494	-0.225	-2.418	No	No	0.662	No
9	5-O-Methylgenistein	-3.307	95.62	-0.305	-2.218	Yes	Yes	0.301	Yes
10	7-Hydroxyquinolin-2 (1H)-one	-2.269	93.759	-0.352	-3.152	No	No	0.549	No
11	Aconitic acid	-1.671	0	-0.886	-3.156	No	No	0.99	No
12	Allantoin	-2.028	51.948	-0.566	-3.679	No	No	0.559	No
13	Benzaldehyde	-1.198	96.246	0.082	-1.75	No	No	0.243	No
14	Caffeic acid	-2.33	69.407	-0.647	-2.608	No	No	0.508	No
15	Campesterol	-7.068	94.543	0.774	-1.758	Yes	No	0.572	No
16	carbamic acid	0.66	81.508	-0.483	-3.401	No	No	0.551	No
17	Catechol	-0.762	86.856	-0.318	-2.076	No	No	0.147	No
18	Ferulic acid	-2.817	93.685	-0.239	-2.612	No	No	0.623	No
19	Glycolic acid	0.838	83.237	-0.372	-3.414	No	No	0.711	No
20	Grevillic acid	-2.335	64.491	-0.677	-2.587	No	No	0.669	No
21	Hypotaurine	-2.888	83.138	-0.41	-3.448	No	No	0.913	No
22	L-Tryptophan	-2.891	77.224	-0.495	-2.622	No	No	0.64	No
23	Magnolioside	-2.351	49.012	-1.27	-3.619	No	No	0.73	Yes
24	Malic acid	-1.381	13.831	-0.788	-3.523	No	No	0.81	No
25	Malonic acid	-1.038	77.912	-0.054	-3.061	No	No	0.708	No
26	Methanesulfinic acid	0.716	99.201	-0.333	-2.69	No	No	0.706	No
27	Oxalic acid	-0.948	79.42	0.017	-3.415	No	No	0.766	No
28	Oxazole	0.359	100	-0.082	-2.606	No	No	0.652	No
29	Phenol	-0.723	93.055	-0.222	-1.824	No	No	0.208	No
30	Phenoxyethanol	-0.742	85.558	-0.125	-2.159	No	No	0.249	No
31	Protocatechoic acid	-2.069	71.174	-0.683	-3.305	No	No	0.551	No
32	Pyruvic acid	0.169	92.305	-0.337	-2.985	No	No	0.757	No
33	Quininic acid	-2.326	99.313	0.004	-2.344	No	No	0.649	No
34	Stigmast-5-ene	-6.136	96.88	0.994	-0.973	Yes	No	0.618	No
35	Stigmasterol	-6.682	94.97	0.771	-1.652	Yes	No	0.618	No
36	Succinic acid	-0.66	71.748	-0.163	-3.06	No	No	0.722	No
37	Tetrahydrofuran	-0.368	100	0.022	-2.637	No	No	0.517	No
38	Trisaminol	0.15	59.874	-1.047	-4.678	No	No	0.951	No
39	Vanillic Acid	-1.838	78.152	-0.38	-2.628	No	No	0.628	No
40	Fumaric acid	-0.642	71.771	-0.127	-3.046	No	No	0.89	No

Table 4: Top bioactive compounds and their interaction energy

S. No.	Compounds	CDOCKER Interaction Energy (kcal/mol)	Pose number
1	Acarbose (Standard)	-60.61	5
2	Beta-Tocopherol	-45.00	8
3	Gamma-Tocopherol	-44.73	10
4	Campesterol	-44.52	1
5	Stigmast-5-ene	-39.67	2
6	Stigmasterol	-38.92	6
7	Magnolioside	-38.28	3
8	1-O-trans Coumaroylglycerol	-33.45	1
9	5-O-Methylgenistein	-31.37	1
10	L-Tryptophan	-25.54	2
11	Quininic acid	-24.96	1
12	Aconitic acid	-24.79	1
13	Grevillic acid	-24.58	4
14	Caffeic acid	-24.34	2
15	Ferulic acid	-24.12	3

important metabolites such as tocopherols, squalene, 1-O-trans-p-coumaroylglycerol, 2-hydroxyphenylacetic acid, campesterol, and stigmasterol were identified in Kodo millet cultivars. A study on the antioxidant activity of phenolic acids found that caffeic acid, ferulic acid, quinic acid, and vanillic acid are present in medicinal plants such as *Cissus quadrangularis* (Kaur *et al.*, 2022). Numerous metabolites have previously demonstrated therapeutic applications. For instance, methyl esters exhibit anti-cancer, hypocholesterolemic, anti-arthritic, hepatoprotective, anti-androgenic, anti-acne, 5-alpha-reductase inhibitory, nematicidal, antihistaminic, and anti-coronary properties. Additionally, 5-hydroxymethylfurfural displays antioxidant and antiproliferative activities (Mishra *et al.*, 2022).

The inhibition of α-amylase, a key enzyme responsible for starch hydrolysis, plays a pivotal role in regulating postprandial glucose levels. Phenolic compounds have been identified as

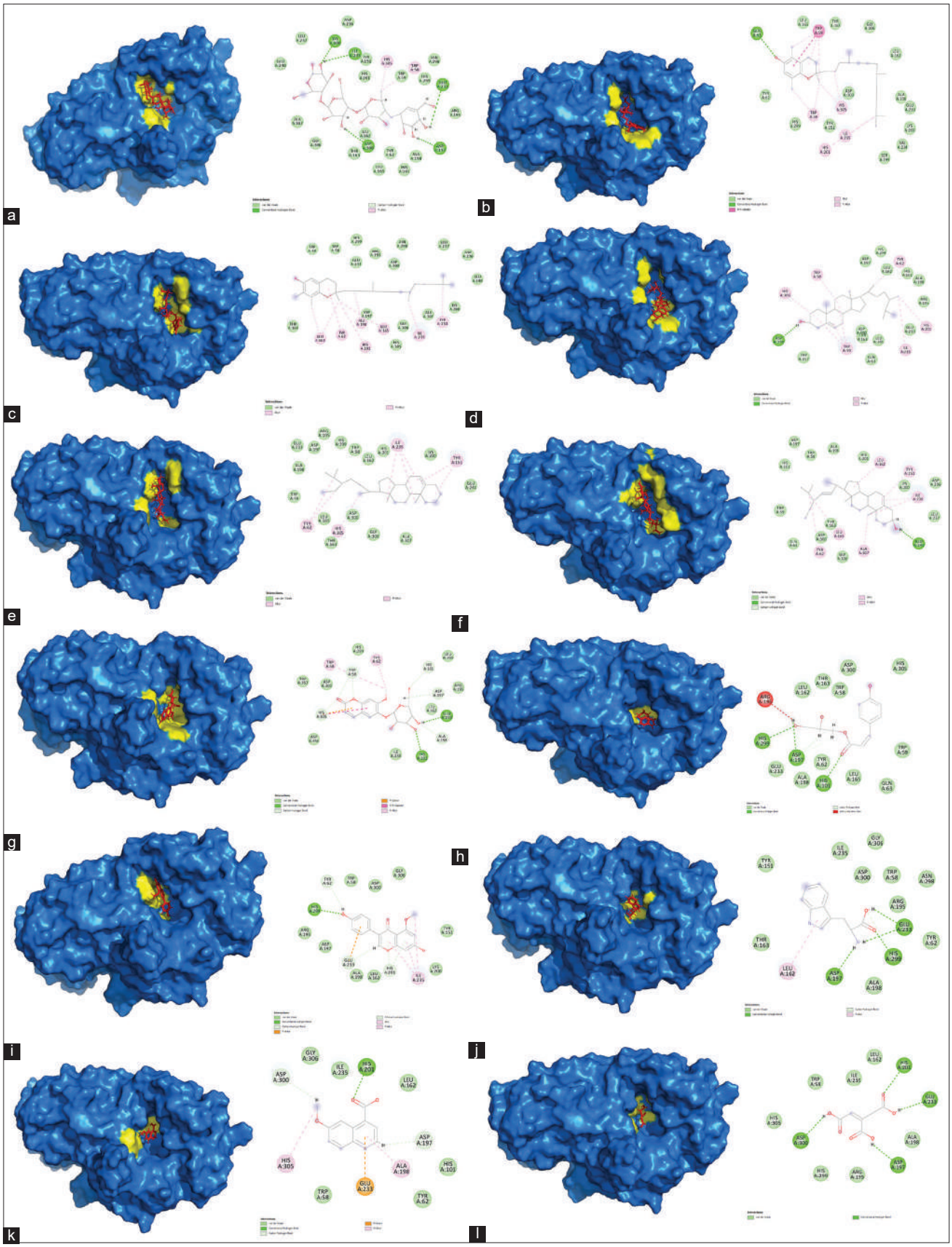

Figure 4: 3D diagram of binding pockets and 2D interaction plot. a) Acarbose, b) beta-tocopherol, c) gamma-tocopherol, d) Campesterol, e) Stigmast-5-ene, f) Stigmasterol, g) Magnolioside, h) 1-O-trans-p-Coumaroylglycerol, i) 5-O-Methylgenistein, j) L-Tryptophan, k) Quininic acid, l) Aconitic acid, m) Grevillic acid, n) Caffeic acid and o) Ferulic acid

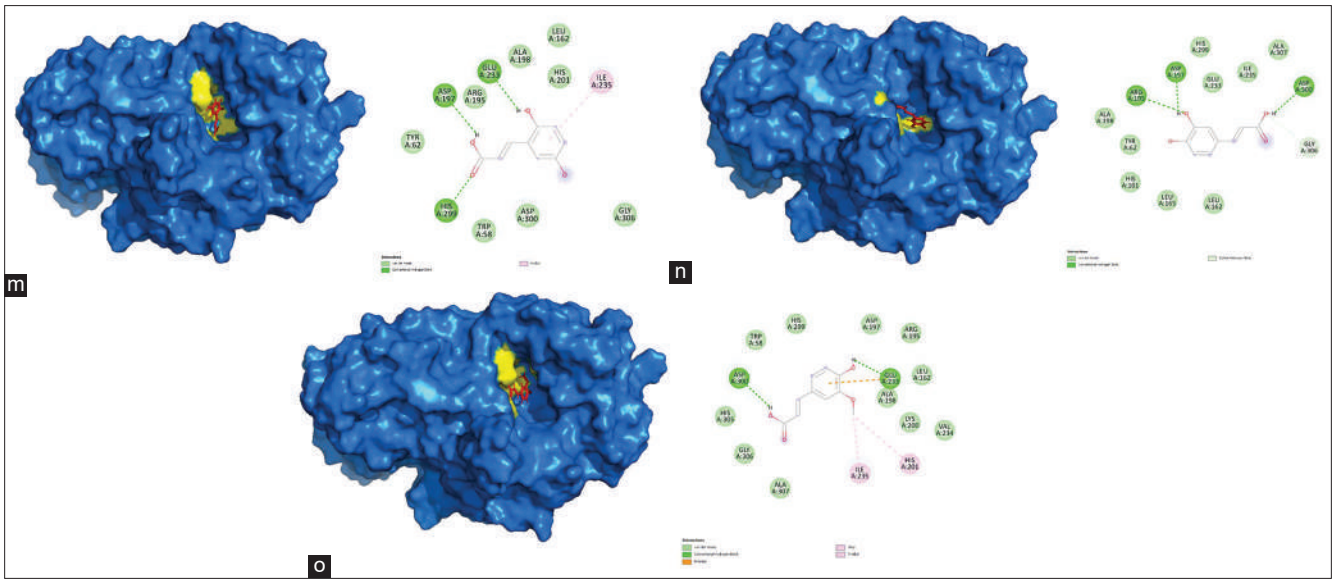

Figure 4: *(Continued)*

potential α -amylase inhibitors derived from plant sources. A wide range of plant-derived compounds - mainly alkaloids, glycosides, galactomannan gum, polysaccharides, hypoglycans, peptidoglycans, guanidine, steroids, glycopeptides, and terpenoids - have demonstrated bioactivity against hyperglycemia. *Syzygium cumini* L. and *Psidium guajava* L. are widely used in traditional systems of medicine to treat diabetes in India (Shah *et al.*, 2018). Polyphenols from pearl millet have been found to inhibit α-amylase activity and regulate hepatic glucose uptake (Krishnan *et al.*, 2022). Vanillic acid, ferulic acid, and kaempferol from foxtail millet were confirmed to inhibit α-amylase through a molecular docking approach (Reddy *et al.*, 2021). It was reported that the metabolites of Kodo millet for their antidiabetic properties by docking them with α-amylase, using a distinct metabolite profile derived from a single cultivar. Similarly, in the present study, molecular docking analysis was employed to investigate the potential inhibitory action of metabolites identified through GC-MS across various Kodo millet cultivars. The results revealed a significant inhibitory effect on α-amylase activity by several metabolites, highlighting their potential as natural inhibitors of starch digestion and regulators of postprandial glucose levels.

Among the identified metabolites, β-tocopherol, γ-tocopherol, campesterol, stigmast-5-ene, stigmasterol, magnolioside, 1-O-trans-p-coumaroylglycerol, 5-O-methylgenistein, L-tryptophan, quinic acid, aconitic acid, grevillic acid, caffeic acid, and ferulic acid demonstrated notable inhibitory activity against α-amylase, as indicated by their high binding affinities. This wide array of inhibitory compounds highlights the richness and diversity of bioactive constituents within Kodo millet cultivars, which may contribute to their potential health benefits, particularly in managing postprandial hyperglycemia. The presence of plant sterols - including campesterol, stigmast-5-ene, and stigmasterol - is noteworthy, as these compounds have been associated with cholesterol-lowering effects and may exhibit a similar mechanism of action in inhibiting α-amylase. This potential dual functionality could aid in the modulation of postprandial blood glucose levels, as observed in animal models (Batta *et al.*, 2006). Furthermore, the presence of magnolioside, 1-O-trans-p-coumaroylglycerol, 5-O-methylgenistein, L-tryptophan, quinic acid, aconitic acid, grevillic acid, caffeic acid, and ferulic acid underscores the complexity of the bioactive profile in Kodo millet cultivars. Findings by Oboh *et al.* (2015) confirm the enzymatic inhibition of α-amylase by caffeic and chlorogenic acids. β-Tocopherol and γ-tocopherol, both forms of vitamin E (Azzi, 2019), have been previously studied for their antioxidant properties but remain less explored for their potential roles in carbohydrate metabolism (Reiter *et al.*, 2007). Vitamin E has been shown to control hyperglycemia and reduce HbA1c levels by inhibiting oxidative stress pathways in diabetic rats (Ihara *et al.*, 2000). Our findings suggest a novel avenue for investigating these compounds as potential α-amylase inhibitors, which may have implications for diabetes management and glycemic regulation. Notably, leaf extracts of *Tapinanthus cordifolius* have been reported to contain tocopherol derivatives that inhibit α-amylase activity, aiding in the management of type 2 diabetes. It is important to acknowledge that the inhibitory potential demonstrated in this *in silico* docking study warrants further *in vitro* and *in vivo* investigations to validate these findings. Moreover, the precise mechanisms through which these metabolites inhibit α-amylase remain to be elucidated in order to fully understand their therapeutic potential. The present study, in alignment with previous research, provides compelling evidence of the inhibitory activity of various metabolites identified in Kodo millet cultivars against α-amylase. These findings highlight the potential of Kodo millet as a valuable dietary component for glycemic control and support continued research into the health-promoting properties of its bioactive compounds, particularly in the context of diabetes management.

AUTHORS' CONTRIBUTION

S.N. led the conceptualization. I.R., S.N., and S.B. performed the experiments and data analysis. V.M., S.Na., and M.P. provided suggestions. S.N., I.R., S.B., and K.A. were involved in the writing, review, and editing of the manuscript. All authors have reviewed and approved the submitted version of the manuscript.

REFERENCES

Alam, S., Sarker, M. M. R., Sultana, T. N., Chowdhury, M. N. R., Rashid, M. A., Chaity, N. I., Zhao, C., Xiao, J., Hafez, E. E., & Khan, S. A. (2022). Antidiabetic phytochemicals from medicinal plants: prospective candidates for new drug discovery and development. *Frontiers in Endocrinology, 13*, 800714. https://doi.org/10.3389/fendo.2022.800714

Aye, M. M., Aung, H. T., Sein, M. M., & Armijos, C. (2019). A review on the phytochemistry, medicinal properties and pharmacological activities of 15 selected Myanmar medicinal plants. *Molecules, 24*(2), 293. https://doi.org/10.3390/molecules24020293

Azzi, A. (2019). Tocopherols, tocotrienols and tocomonoenols: Many similar molecules but only one vitamin E. *Redox Biology, 26*, 101259. https://doi.org/10.1016/j.redox.2019.101259

Batta, A. K., Xu, G., Honda, A., Miyazaki, T., & Salen, G. (2006). Stigmasterol reduces plasma cholesterol levels and inhibits hepatic synthesis and intestinal absorption in the rat. *Metabolism, 55*(3), 292-299. https://doi.org/10.1016/j.metabol.2005.08.024

Blahova, J., Martiniakova, M., Babikova, M., Kovacova, V., Mondockova, V., & Omelka, R. (2021). Pharmaceutical drugs and natural therapeutic products for the treatment of type 2 diabetes mellitus. *Pharmaceuticals, 14*(8), 806. https://doi.org/10.3390/ph14080806

Bunkar, D. S., Goyal, S., Meena, K. K., & Kamalvanshi, V. (2021). Nutritional, functional role of kodo millet and its processing: a review. *International Journal of Current Microbiology and Applied Sciences, 10*(01), 1972-1985. https://doi.org/10.20546/ijcmas.2021.1001.229

Chaudhary, J. K., & Mudgal, S. (2020). Antidiabetic and hypolipidaemic action of finger millet (*Eleusine coracana*)-enriched probiotic fermented milk: An in vivo rat study. *Food Technology and Biotechnology, 58*(2), 192. https://doi.org/10.17113/ftb.58.02.20.6308

Chethan, S., Dharmesh, S. M., & Malleshi, N. G. (2008). Inhibition of aldose reductase from cataracted eye lenses by finger millet (*Eleusine coracana*) polyphenols. *Bioorganic & Medicinal Chemistry, 16*(23), 10085-10090. https://doi.org/10.1016/j.bmc.2008.10.003

Deepak, Teggelli, R. G., & Thakur, V. (2018). Minor millets-their potential health benefits and medicinal properties: A review. *International Journal of Pure & Applied Bioscience, 6*(1), 1677. https://doi.org/10.18782/2320-7051.6466

Djoumbou Feunang, Y., Eisner, R., Knox, C., Chepelev, L., Hastings, J., Owen, G., Fahy, E., Steinbeck, C., Subramanian, S., & Bolton, E. (2016). ClassyFire: automated chemical classification with a comprehensive, computable taxonomy. *Journal of Cheminformatics, 8*, 1-20. https://doi.org/10.1186/s13321-016-0174-y

Fiehn, O. (2016). Metabolomics by gas chromatography–mass spectrometry: Combined targeted and untargeted profiling. *Current Protocols in Molecular Biology, 114*(1), 30.4.1-30.4.32. https://doi.org/10.1002/0471142727.mb3004s114

Ihara, Y., Yamada, Y., Toyokuni, S., Ban, N., Kuroe, A., & Seino, Y. (2000). Antioxidant [Alpha]-Tocopherol Improves Glycemic Control of GK Rats, a Model of Type 2 Diabetes. *Diabetes, 49*(5), A429-A429. https://doi.org/10.1016/s0014-5793(00)01489-7

Kaur, J., Dhiman, V., Bhadada, S., Katare, O., & Ghoshal, G. (2022). LC/MS guided identification of metabolites of different extracts of *Cissus quadrangularis*. *Food Chemistry Advances, 1*, 100084. https://doi.org/10.1016/j.focha.2022.100084

Krishnan, V., Verma, P., Saha, S., Singh, B., Vinutha, T., Kumar, R., Kulshreshta, A., Singh, S., Sathyavathi, T., & Sachdev, A. (2022). Polyphenol-enriched extract from pearl millet (Pennisetum glaucum) inhibits key enzymes involved in post prandial hyper glycemia (α-amylase, α-glucosidase) and regulates hepatic glucose uptake. *Biocatalysis and Agricultural Biotechnology, 43*, 102411. https://doi.org/10.1016/j.bcab.2022.102411

Lisec, J., Schauer, N., Kopka, J., Willmitzer, L., & Fernie, A. R. (2006). Gas chromatography mass spectrometry–based metabolite profiling in plants. *Nature Protocols, 1*(1), 387-396. https://doi.org/10.1038/nprot.2006.59

Mishra, V., Tomar, S., Yadav, P., Vishwakarma, S., & Singh, M. P. (2022). Elemental analysis, phytochemical screening and evaluation of antioxidant, antibacterial and anticancer activity of *Pleurotus ostreatus* through in vitro and in silico approaches. *Metabolites, 12*(9), 821. https://doi.org/10.3390/metabo12090821

Oboh, G., Agunloye, O. M., Adefegha, S. A., Akinyemi, A. J., & Ademiluyi, A. O. (2015). Caffeic and chlorogenic acids inhibit key enzymes linked to type 2 diabetes (in vitro): a comparative study. *Journal of Basic and Clinical Physiology and Pharmacology, 26*(2), 165-170. https://doi.org/10.1515/jbcpp-2013-0141

Pang, Z., Chong, J., Zhou, G., de Lima Morais, D. A., Chang, L., Barrette, M., Gauthier, C., Jacques, P.-É., Li, S., & Xia, J. (2021). MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights. *Nucleic Acids Research, 49*(W1), W388-W396. https://doi.org/10.1093/nar/gkab382

Prabhakar, P. K., & Doble, M. (2008). A target based therapeutic approach towards diabetes mellitus using medicinal plants. *Current Diabetes Reviews, 4*(4), 291-308. https://doi.org/10.2174/157339908786241124

Reddy, B. V., Reddy, C., Sekhar, A. C., Reddy, P. C. O., & Srinivasulu, K. (2021). A new insights and novel targets for hyperglycemia from foxtail millet (*Setaria italica* L.) using molecular docking studies. *Current Trends in Biotechnology and Pharmacy, 15*(2), 213-219. https://doi.org/10.5530/ctbp.2021.2.23

Reiter, E., Jiang, Q., & Christen, S. (2007). Anti-inflammatory properties of α-and γ-tocopherol. *Molecular Aspects of Medicine, 28*(5-6), 668-691. https://doi.org/10.1016/j.mam.2007.01.003

Shah, S. B., Sartaj, L., Ali, F., Shah, S. I. A., & Khan, M. T. (2018). Plant extracts are the potential inhibitors of α-amylase: a review. *MOJ Bioequiv Availab, 5*(5), 270-273. https://doi.org/10.15406/mojbb.2018.05.00113

Shobana, S., Sreerama, Y., & Malleshi, N. (2009). Composition and enzyme inhibitory properties of finger millet (*Eleusine coracana* L.) seed coat phenolics: Mode of inhibition of α-glucosidase and pancreatic amylase. *Food Chemistry, 115*(4), 1268-1273. https://doi.org/10.1016/j.foodchem.2009.01.042

Singh, V., Lee, G., Son, H., Amani, S., Baunthiyal, M., & Shin, J.-H. (2022). Anti-diabetic prospects of dietary bio-actives of millets and the significance of the gut microbiota: A case of finger millet. *Frontiers in Nutrition, 9*, 1056445. https://doi.org/10.3389/fnut.2022.1056445

Tran, N., Pham, B., & Le, L. (2020). Bioactive compounds in anti-diabetic plants: From herbal medicine to modern drug discovery. *Biology, 9*(9), 252. https://doi.org/10.3390/biology9090252