REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 1 (2024)
http://www.veterinaria.org
Article received- Revised- Accepted-

Exploring Network Pharmacology And Docking Analysis Of Bioactive Compounds In Kattuyanam Rice Extract Targeting Key Diabetes Pathways

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ABSTRACT
Background: Diabetes, a chronic metabolic disorder, poses significant health challenges globally. Recent research has highlighted the potential possible link between higher consumption of polished white rice and increased diabetes risk. On the other hand, pigmented rice varieties have gained attention due to their rich phytoconstituents and antioxidant properties. Kattuyanam, one such pigmented rice variety from Tamil Nadu is very well known for its antidiabetic property and low glycemic index.
Objectives: To evaluate the anti-diabetic potential of the Kattuyanam rice extract through *In vitro* and *In silico* studies.
Methods: Kattuyanam rice extract was analyzed for its inhibitory effects against α-amylase and α-glucosidase enzymes. Gas Chromatography-Mass Spectrometry (GC-MS) was employed to identify the phytochemical constituent of the extract. Additionally, Network Pharmacology analysis, including target gene prediction, and functional enrichment was conducted to explore the bioactive compounds and their mechanisms of action. Molecular docking studies were performed to assess the interactions between these bioactive compounds and target receptors.
Result and Conclusions: The Kattuyanam rice extract demonstrates potent inhibitory effects against α-amylase and α-glucosidase enzymes, crucial targets in diabetes management. GC-MS analysis of the rice extract unveiled the presence of number of Phytochemicals with therapeutic potentials. Through network pharmacology studies, a total of 204 target genes related toT2DM were identified primarily linked to responses to organic substances, regulation to biological quality, stress and chemical stimulus suggesting the involvement of PI3K-Akt and AGE-RAGE signaling pathways in the treatment of T2DM with Kattuyanam rice extract. Molecular docking studies supported these findings, revealing favourable interactions between the bioactive compounds of Kattuyanam rice and target receptors. These results offer insights into the potential of Kattuyanam rice as a complementary approach for diabetes mellitus management, paving the way for future dietary interventions.

Keywords- Diabetes, Coloured rice, Kattuyanam, Antioxidant, Anti-diabetic

1. Introduction

Diabetes mellitus, characterized by chronic hyperglycemia, represents a significant global health challenge. The prevalence of diabetes is escalating at an alarming rate worldwide, with projections estimating a staggering increase in affected individuals by 2045 (Saeedi et al., 2019). Diet plays a critical role in the etiology of this metabolic disorder, alongside genetic predisposition and lifestyle factors. Notably, higher consumption of rice is also attributed towards the development of Diabetes in particular Type II diabetes. Rice serves as the staple food for half of the world's population, with Asian countries, including India, being major consumers (Bahadoran et al., 2014). India, renowned for its rice cultivation, ranks second in production globally, with polished white rice being the predominant variety consumed by a significant portion of its population. However, the milling process involved in producing white rice results in the removal of the nutrient-rich bran layer, leaving behind a product high in starch but deficient in essential nutrients, including proteins, vitamins, minerals, and fibres. With the advancement of the grain processing technology, the outer bran of the intact rice grains (i.e., brown rice) was removed to make it more attractive and dazzling. The bran which contains plenty of nutrients are completely removed from the whole grain, thus making it full of starch and devoid of proteins, vitamins, minerals, functional nutrients and fibres (Rathna Priya et al., 2019). Research, notably from Harvard communiqued, has

linked regular consumption of white rice to an increased risk of Type II diabetes, irrespective of the Nationality and or family history of the disease (Hu et al., 2012). This predilection of White rice to raise the Blood Glucose levels after consumption makes it a food with High Glycemic index (GI). On the other hand, foods with high glycemic index gets easily absorbed which simultaneously increases the postprandial blood glucose level and insulin demand leading to Diabetes. Polished white rice falls under high glycemic foods category. Unlike white rice, pigmented rice varieties slowly releases sugars, which in turn helps to stabilize blood sugar sustainably. This trait makes it a better option for people who are suffering from diabetes mellitus (Nayar & Madhu, 2020).

India boasts a diverse array of traditional pigmented rice varieties renowned for their medicinal properties, encompassing hues of rad, brown, and black, delineated by the coloration of the paddy grains. These indigenous rice cultivars, prized for their superior nutritional profile compared to polished white rice, persistent in cultivation and consumption across various regions the country. Among these, Kattuyanam stands out as a prominent indigenous rice variety cultivated predominantly in the southern state of Tamil Nadu. Thriving particularly in coastal regions with elevated water salinity levels, Kattuyanam rice has garnered attention for its historical use in promoting bone marrow growth, enhancing immunity, bolstering vision, and preventing skin ailments, traditionally administered, notably to children (Dharshini K et al., 2021). Against this backdrop, the current investigation aims to elucidate the therapeutic attributes of Kattuyanam rice through network pharmacology and docking studies.

Plant-derived bioactive compounds, harbouring antioxidant properties alongside other pharmacological actions are often isolated from the crude plant extracts, offering promising avenues for medicinal development (Nasim et al., 2022). Chromatographic techniques, notably GC-MS, facilitates the initial screening and identification of bioactive compounds present in such extracts, encompassing phenolic acids, flavonoids, alcohols, esters, long-chain hydrocarbons, steroids, organic acids and amino acids (Aggarwal et al., 2020). In the context of antidiabetic potential, the rapid uptake of glucose in the intestine, facilitated by α-amylase and α-glucosidase enzymes, underscores the significance of inhibiting these enzymes as a pivotal strategy for managing postprandial hyperglycemia (Kajaria et al., 2013). In this study, the potential therapeutic mechanisms of Kattuyanam rice extract in treating T2DM (Type 2 Diabetes) was investigated through target gene screening, interaction network construction, and pathway analysis. Further, *In silico* studies targeting enzymes such as alpha amylase and alpha glucosidase further underscore their significance, as antidiabetic drugs targeting these enzymes typically exhibit fewer adverse effects. Effective inhibition of these carbohydrate-hydrolysing enzymes reduces glucose absorption rates, thereby mitigating postprandial blood glucose levels, offering promising avenues for diabetes treatment. Thus, these enzymes were selected as target proteins in the present study.

2. Methods

2.1 Material collection and sample extraction

Grains of Kattuyanam rice were procured directly from farmers in the Thanjavur district of Tamil Nadu. Subsequently, the rice variety underwent testing at the Rice Research Station in Ambasamudram to ensure their quality and suitability for the study. Initially, they were shade dried and then finely powdered using a motor and pestle. Subsequently, approximately 10 g of the powdered sample was mixed with 100 mL of 50% methanol for extraction using Soxhlet. After extraction, the resulting mixture was carefully filtered through Whatmann No.1 paper to remove any solid particles. The filtrate obtained was then concentrated using a rotary evaporator under reduced pressure at a controlled temperature below 45˚C. Finally, the concentrated extract was stored under refrigeration conditions for further analysis.

2.2 Quantitative analysis of Phytochemicals

The major phytochemicals including anthocyanins, phenols and flavonoids were determined based on standard procedures outlined by (Saikia et al., 2012).

2.3 In vitro antidiabetic activity analysis

2.3.1 α- Amylase inhibitory activity

To evaluate the inhibitory effect of Kattuyanam rice extract on α-amylase, a modified protocol derived from (Jemaa et al., 2017) was followed. Initially, 500µl of rice extract was mixed with 500µl of 0.02M sodium phosphate buffer (Ph 6.9) containing α-amylase, followed by a 10-minute incubation at 37°C. Subsequently, starch solution (500 µl, 0.5% w/v) was introduced, and the reaction mixtures were further incubated at 37°C for 10 minutes. The reaction was quenched by the addition of dinitrosalicylic acid colour reagent (1.0mL), followed by heating in a boiling water bath for 10 minutes and subsequent cooling. Absorbance was then measured at 540 nm to quantify the inhibition of α-amylase activity. Acarbose, a recognized α-amylase inhibitor, served as a positive control for comparative analysis. The results were calculated by the formula

Inhibitory activity (%) = $(1 – A_s/A_c) \times 100$

Where A_s is the absorbance in the test substance and A_c is the absorbance of the control

2.3.2 α- glucosidase inhibitory activity

For assessing α-glucosidase inhibition, a modified methodology based on (Bhatia et al., 2019) was employed. Initially, a solution of starch substrate was incubated with various concentrations of rice extract for 5 minutes at 37°C. Subsequently,

α-glucosidase enzyme was added, and the reaction mixture was further incubated for 10 minutes at 37°C. The reaction was halted with 3,5dinitro salicylic acid reagent and the absorbance was measured at 540 nm. Acarbose served as a positive control for this assay. The percentage of α-glucosidase inhibition was calculated based on the provided formula.
Inhibitory activity (%) = $(1 – A_s/A_c) \times 100$
Where A_s is the absorbance in the test substance and A_c is the absorbance of the control.

2.4 GC-MS analysis
For the GC-MS analysis, Kattuyanam rice extract underwent evaporation to dryness, followed by dissolution in n-hexane. The resulting n-hexane solutions were filtered to remove insoluble components and then dissolved in methanol. Subsequently, a small portion of the sample was injected into the auto-injector. Injection in split mode with a split ratio of 10 was performed, with approximately 2 µl of the sample injected. GC-MS analysis was conducted using PerkinElmer Gas Chromatograph Clarus 680 interfaced with a mass spectroscopy (PerkinElmer Mass Spectrometer Clarus SQ 8C). The capillary column used had a diameter of 5 micro meters (30m long × 0.250mm inner diameter × 1 micron). The analysis utilized an electron impact mode with an ionizing energy of 70eV and helium was employed as the carrier gas. The injector temperature remained constant at 250°C throughout the run, while the source temperature was set to 230°C. The oven temperature was programmed from 80°c initially and increased to 300°c at a rate of 5°C/min, with a total run time of 26.6 minutes. The electron ionization source temperature was maintained at 250°C. Identification of phytochemicals was based on comparison with the parameters such as retention time, peak area, peak height and mass spectral pattern with the NIST library, a spectral database for authentic compounds (Pradhan & Dubey, 2021).
***In silico* studies**

2.5 Network Pharmacology studies
2.5.1 Prediction of Potential Target Gene for the Quantitative Component and Disease
The Swiss Target Prediction tool (http://www.swisstargetprediction.ch/) was employed to forecast the target genes associated with the volatile compounds quantified through GC-MS analysis of the Kattuyanam rice extract. During the target prediction process, structural resemblance was assessed against a known compound. To predict the target gene related to disease, T2DiACod (https://t2diacod.igib.res.in/) and platforms such as OMIM (https://www.omim.org/) and GeneCards (https://www.genecards.org/) were utilized, using “type 2 diabetes mellitus” as the search term.

2.5.2 Screening of the Common Target Genes and Mutual Network Construction
The common target genes associated with both the quantitative component and the disease were acquired, followed by the identification of Protein–protein interaction (PPI) using STRING. A threshold criterion of >0.9 (highest confidence) was applied for the selection of interactions.

2.5.3 Gene Ontology Analysis and KEGG Pathway Enrichment Analysis
GO analysis and KEGG pathway enrichment analysis of the common target genes was performed using ShinyGO.

2.5.4 Interaction Network Analysis
Using Cytoscape 3.8.2, we constructed a network illustrating the interaction between compound prescriptions, active components, diseases, target genes, and pathways. Within this network, each “node” corresponds to a drug, active component, disease, target gene, or pathway while “edge” represents the relationships among these nodes. Quality markers were assessed based on the degree centrality measure. Active components with degrees exceeding the network’s average were chosen for further screening.

2.6 Docking Studies
2.6.1 Ligand selection
The 3D structures of the 27 bioactive compounds identified from the GC-MS analysis of Kattuyanam rice were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and converted into pdb format.

2.6.2 Selection of Target protein
Target proteins alpha-amylase and alpha glucosidase were selected based on literature findings. Their 3D structures were retrieved from the PDB database (https://www.rcsb.org) with PDB Ids: 4GQR for alpha amylase and 3L4Y for alpha glucosidase. The active site of the target proteins were predicted using the online server CASTp.

2.6.3 Docking Studies
Ligand positioning within the enzyme’s active site was performed using AutoDock software, and the binding energy between ligand and receptor protein was calculated using a scoring algorithm. Ligand-protein interactions were analysed using Ligplot and Protein-Ligand Interaction Profiler.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
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2.7 Statistical analysis

Statistical analysis, conducted using Graphpad prism, involved paired student's t-test. Significance between rice varieties and standards tested is presented as $p<0.05$.

3. Results

3.1 Quantitative analysis of Phytochemicals

The quantitative assessment of phytochemicals, encompassing anthocyanin, flavonoid and phenol, is summarized in Table 1. Kattuyanam rice demonstrated higher levels of these phytochemicals. Specifically, the anthocyanin content in rice extract was measured at 213.21 mg/100g, while the flavonoid content was measured at 17.33 mg/mL. Furthermore, the phenolic content in Kattuyanam rice was determined to be 161.4 µg GAE/g.

Table 1 Quantitative analysis of Phytochemicals

Phytochemicals	Anthocyanin (mg/100g)	Flavonoid (mg/mL)	Phenols (µg GAE/g)
Kattuyanam Rice extract	213.21±0.14	17.33 ± 0.11	161.4±3.8

Values are mean ± standard error of three determinations.

3.2 Invitro antidiabetic analysis

3.2.1 α-amylase inhibitory assay

Figure 1(A) illustrates the α-amylase inhibitory activity of the rice extract alongside the standard. In our investigation, the methanol extract of Kattuyanam exhibited inhibitory effects at all tested concentrations (25, 50, 75 and 100 µg/mL), showing a concentration dependent increase with values ranging from 32.54±1.12 to 48.50±3.12%. The positive control Gallic acid, at a concentration of higher concentration of 100 µg/mL exhibited 48.50±1.80% inhibition.

3.2.2 α-Glucosidase inhibitory assay

Figure 1(B) illustrates the inhibition of the α-Glucosidase enzyme by rice extract compared to the standard. At a concentration of 25µg/mL, the rice extract showed an inhibition of 28.81±0.03% comparable with that of the positive control which showed 30.15±0.03% inhibition. At the final concentration of 100 µg/mL, the rice extract and the standard displayed increased inhibition percentages, reaching around 62.16±1.38% and 59.74±0.02% respectively.

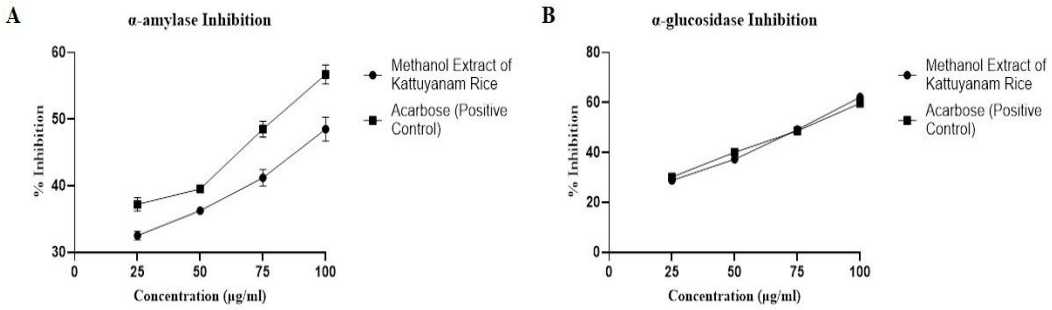

Figure 1: *In vitro* Antidiabetic analysis of the Kattuyanam rice extract. A. α-amylase inhibitory activity of the rice extract alongside the standard acarbose. B. α-glucosidase inhibitory activity of the rice extract alongside the standard acarbose.

3.3 GC-MS analysis of the Methanol extract of Kattuyanam rice

The GC-MS chromatogram (Figure 2) of Kattuyanam rice revealed the presence of 27 peaks, each corresponding to bioactive compounds. These compounds were identified by matching parameters such as retention time, peak area (%), height and mass spectral fragmentation with entries in the NIST Library. The identified metabolites in the methanol extract of Kattuyanam rice include Cyclopropanecarboxylic acid, Cinnamic acid, O-Coumaric acid, D-Allose, Benzaldehyde 2,4,-dimethyl, Terephthaldehyde dioxime, 1-Naphthalenol,4-methoxy, 1,9-Nonanediol dimethanesulfonate, 3-Hexadecyne, 1,4-Eicosadiene, Hexadecanoic acid methyl ester, 1,2-Benzenedicarboxylic acid, n-Hexadecanoic acid, Ferulic acid methyl ester, Furan 3-(4-methyl-3-pentenyl), Quercetin, Hexadecatrienoic acid, Phytol, 3-Dibenzofuranamine 2-methoxy, Diphthalimido-2-propanone, 2-propenoic acid, 1H-Indole 5-methyl-2-phenyl, Squalene, Benzaldehyde 3-nitro, 2H-1-Benzopyran-2-one, Benzamide and Lutein (Table 2).

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Table 2 List of metabolites in the methanol extract of Kattuyanam rice identified through GC-MS analysis

S.No	Retention Time (RT)	Compound Name	Molecular Formula	Molecular Weight (g/mol)
1	9.931	Cyclopropanecarboxylic acid	$C_4H_6O_2$	86.09
2	10.622	Cinnamic acid	$C_9H_8O_2$	148.16
3	11.690	O-Coumaric acid	$C_9H_8O_3$	164.16
4	11.898	D-Allose	$C_6H_{12}O_6$	180.16
5	14.197	Benzaldehyde 2,4,-dimethyl	$C_9H_{10}O$	134.17
6	14.275	Terephthalaldehyde dioxime	$C_8H_8N_2O_2$	164.16
7	14.453	1-Naphthalenol,4-methoxy	$C_{11}H_{10}O_2$	174.2
8	15.097	1,9-Nonanediol dimethanesulfonate	$C_{11}H_{24}O_6S_2$	316.4
9	15.308	3-Hexadecyne	$C_{16}H_{30}$	222.41
10	15.464	1,4-Eicosadiene	$C_{20}H_{38}$	278.5
11	15.841	Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	270.5
12	15.975	1,2-Benzenedicarboxylic acid	$C_8H_6O_4$	166.13
13	16.130	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42
14	16.561	Ferulic acid methyl ester	$C_{11}H_{12}O_4$	208.21
15	16.586	Furan 3-(4-methyl-3-pentenyl)	$C_{10}H_{14}O$	150.22
16	16.599	Quercetin	$C_{15}H_{10}O_7$	302.23
17	17.263	Hexadecatrienoic acid	$C_{16}H_{26}O_2$	250.38
18	17.341	Phytol	$C_{20}H_{40}O$	296.5
19	17.563	3-Dibenzofuranamine 2-methoxy	$C_{13}H_{11}NO_2$	213.23
20	17.663	Diphthalimido-2-propanone	$C_{19}H_{12}N_2O_5$	348.3
21	18.841	2-propenoic acid	$C_3H_4O_2$	72.06
22	21.396	1H-Indole 5-methyl-2-phenyl	$C_{15}H_{13}O$	207.27
23	22.074	Squalene	$C_{30}H_{50}$	410.7
24	22.618	Benzaldehyde 3-nitro	$C_7H_5NO_3$	151.12
25	22.774	2H-1-Benzopyran-2-one	$C_9H_{14}O_2$	154.21
26	23.329	Benzamide	C_7H_7NO	121.14
27	23.700	Lutein	$C_{40}H_{56}O_2$	568.9

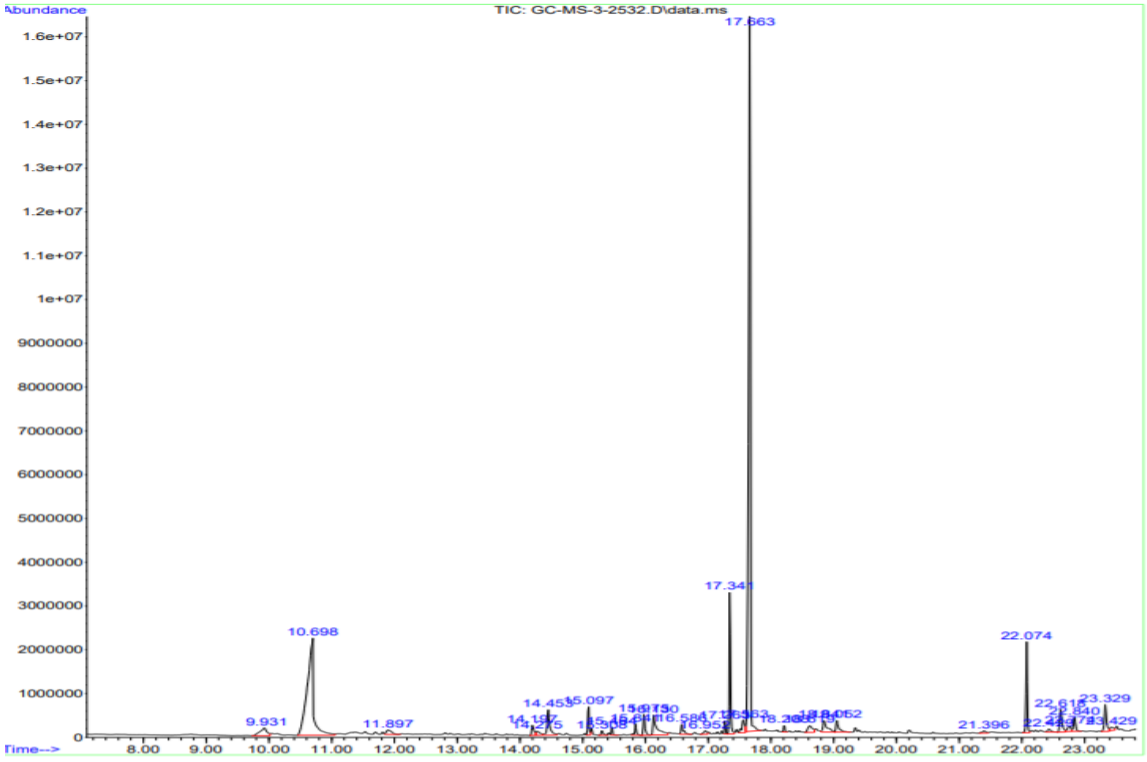

Figure 2: GC-MS spectrograms showing peaks of compounds of methanol extract of the Kattuyanam rice. In the present chromatogram, X axis represent the retention time (the time taken for the analyte to pass through the column and ultimately reaching the mass detector) based on which the bioactive compounds were analysed and identified while Y axis shows the Intensity counts which is a reflection of the amount of specific analyte that is present.

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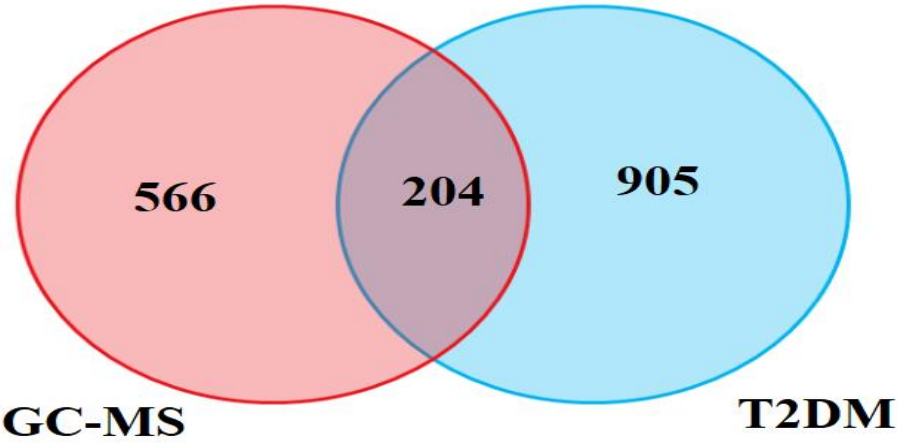
3.4 *In silico* studies

3.4.1 Target Gene screening and Interaction Network Construction

As shown in the Venn diagram in Figure 3, a total of 770 potential target genes were identified for the 27 bioactive compounds of the Kattuyanam rice extract. Concurrently, OMIM and GeneCards platforms yielded 1109 disease target genes associated with T2DM. Among these, 204 target genes were found to be common between the compounds of GC-MS and T2DM. The Protein-Protein Interaction (PPI) diagram (Figure 4(A)) depicting the common target genes revealed 204 nodes and 434 edges. The frequency of occurrence of the top 30 common target genes was shown in Figure 4(B). Notably target genes such as *STAT3*, *SRC*, *JUN*, *RELA*, *AKTI* and *PIK3CA* exhibited a high frequency of protein interaction, suggesting their potential as central nodes within the network. These findings indicate that the selected compounds from Kattuyanam rice extract exhibit substantial binding activity with these target genes, thus holding promise for their utilization in T2DM treatment.

Figure 3: Venn diagram represents common target genes for compound prescription and disease. The size denotes the number of the target genes, the blue circle symbolizes the target genes of T2DM, the red circle symbolizes the target genes of 27 bioactive compounds of Kattuyanam rice extract, and the coincident part depicts the common target genes.



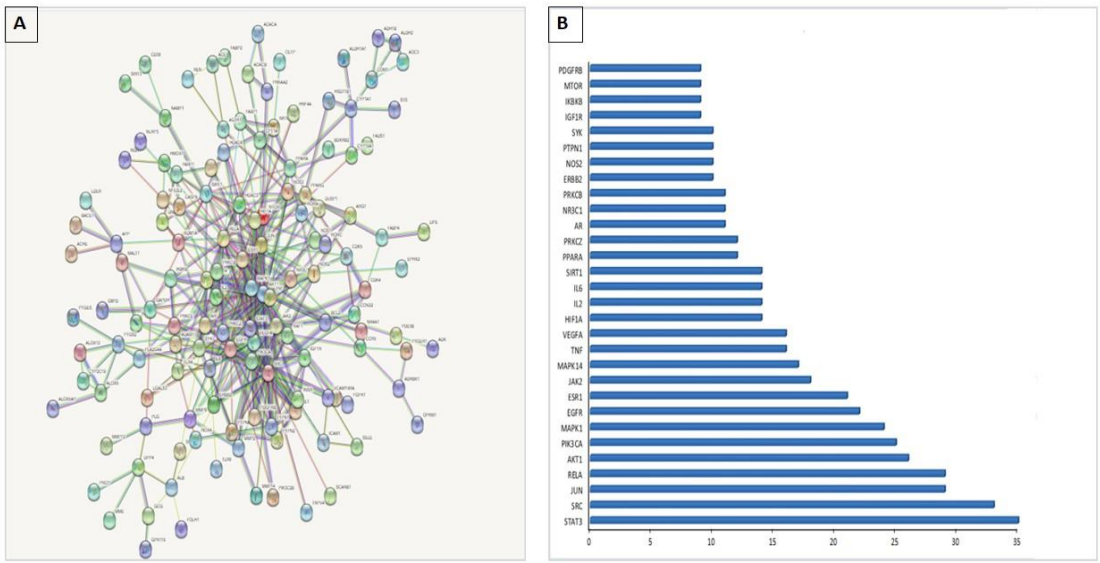


Figure 4: The outcome of the interaction network analysis for common target genes is presented as follows: (A) A Protein-Protein Interaction (PPI) network illustrating the connections between common target genes. Each node represents a target gene, with its interior displaying the 3D structure of the gene. The edges signify associations target genes, with different interactions depicted by various colours: cyan and purple denote known interactions,

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while green, red and blue purple indicate predicted interactions; chartreuse, black and light blue represent other interactions. (B) The frequency distribution of the top 30 common target genes.

3.4.2 Screening of Key Pathways of GC-MS for Treating T2DM

The Gene Ontology (GO) analysis conducted on the common target genes revealed their involvement primarily in biological processes such as Response to organic substance, Regulation of biological quality, Response to stress, and Cellular response to chemical stimulus (Figure 5). Additionally, KEGG pathway enrichment analysis of these common target genes (Figure 6) identified the top 20 signalling pathways after excluding broad pathways, detailed in Table 3. Notably, among these pathways, the 204 common target genes were predominantly associated with PI3K-Akt, AGE-RAGE, and various other multiple signalling pathways. This implies that the efficacy of Kattuyanam rice extract in treating T2DM may involve multiple pathways and complex interactions among them.

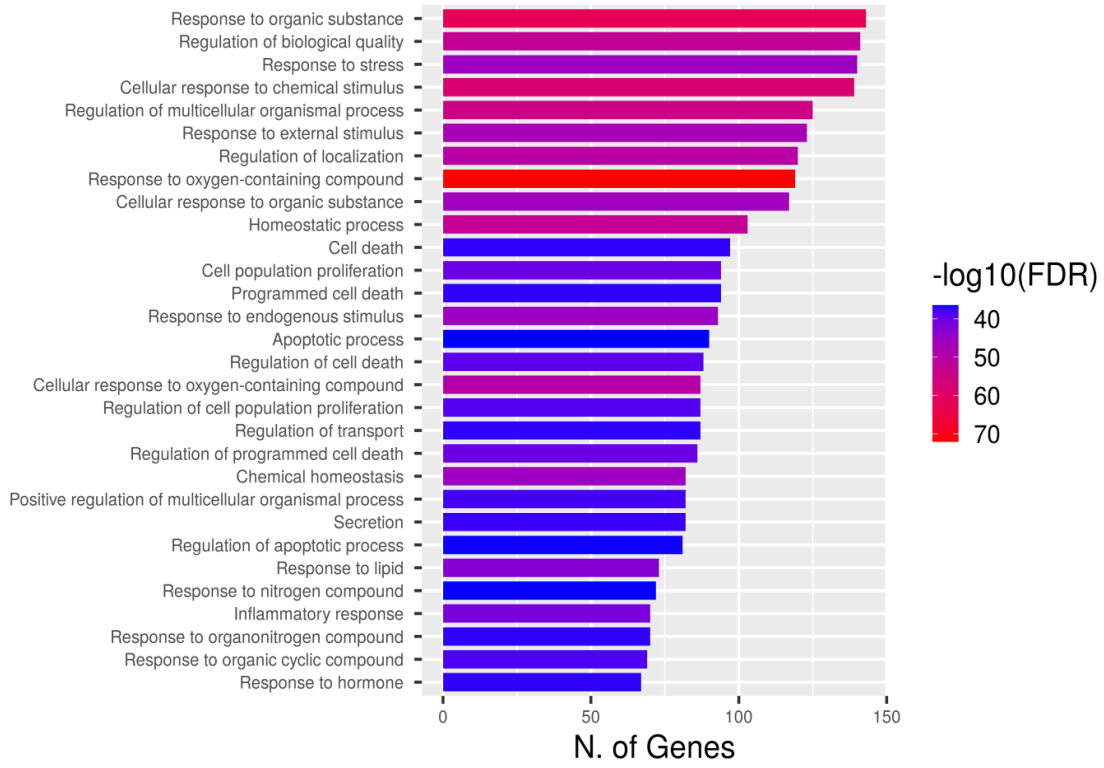

Figure 5: GO biological process analysis, focusing on the top 20 processes is depicted. Node length indicates the number of enriched target genes, while node colour, ranging from blue to red, signifies decreasing P values.

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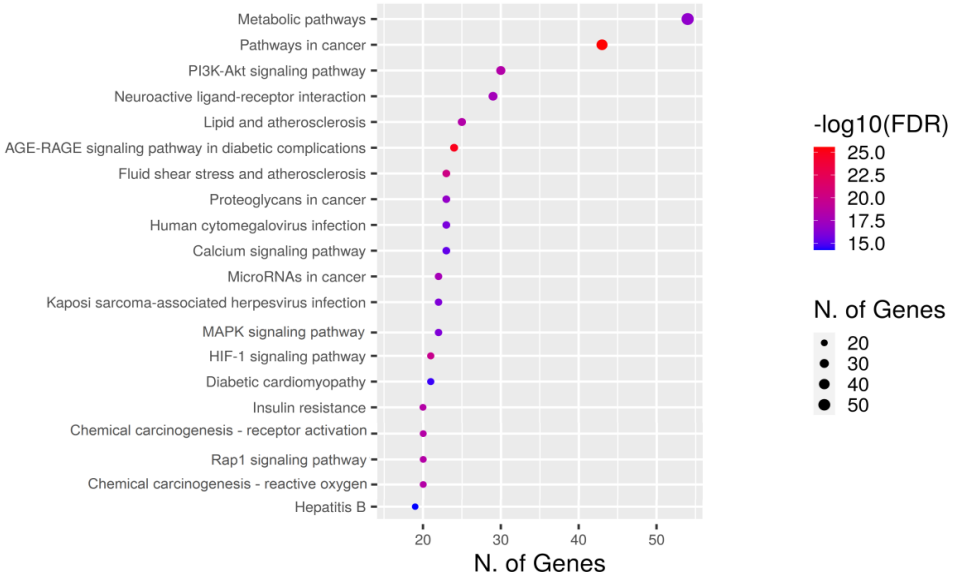

Figure 6: KEGG pathway enrichment analysis (top 20). The node size corresponds to the number of enriched target genes enriched, while the colour gradient from blue to red indicates decreasing P values, with darker shades representing higher significance.

Table 3: Pathway Enrichment Analysis (Top 20).

ID	***Pathway***	***Target Gene count***	***Enrichment FDR***
hsa01100	Metabolic pathways	54	1.97E-17
hsa05200	Pathways in cancer	43	2.70E-26
hsa04151	PI3K-Akt signaling pathway	30	4.25E-19
hsa04080	Neuroactive ligand-receptor interaction	29	2.85E-18
hsa05417	Lipid and atherosclerosis	25	4.05E-19
hsa04933	AGE-RAGE signaling pathway in diabetic complications	24	1.06E-25
hsa05418	Fluid shear stress and atherosclerosis	23	7.53E-21
hsa05205	Proteoglycans in cancer	23	1.53E-17
hsa05163	Human cytomegalovirus infection	23	1.25E-16
hsa04020	Calcium signalling pathway	23	5.44E-16
hsa05206	MicroRNAs in cancer	22	2.25E-18
hsa05167	Kaposi sarcoma-associated herpesvirus infection	22	8.47E-17
hsa04010	MAPK signaling pathway	22	2.53E-13
hsa04066	HIF-1 signaling pathway	21	1.79E-20
hsa05415	Diabetic cardiomyopathy	21	2.58E-15
hsa04931	Insulin resistance	20	3.42E-19
hsa05204	Chemical carcinogenesis - receptor activation	20	1.49E-14
hsa04015	Rap1 signaling pathway	20	4.74E-14
hsa05208	Chemical carcinogenesis - reactive oxygen	20	1.35E-13
hsa05161	Hepatitis B	19	5.44E-15

3.4.3 Compound Prescription-Active Component-Disease Target Gene-Pathway Interaction Network.
Figure 7 illustrates the findings of the compound prescription-active component-disease-target gene-pathway interaction network, comprising 234 nodes representing 204 target genes, 27 active components, 1 disease, 1 compound prescription,

and the top 20 KEGG pathways. Additionally, the interaction network results for the 27 active compounds are detailed in Table 4. The degree of Cyclopropanecarboxylic acid, Cinnamic acid, O-Coumaric acid, D-Allose, Benzaldehyde 2,4,-dimethyl, Terephthalaldehyde dioxime, 1-Naphthalenol,4-methoxy, 1,9-Nonanediol dimethanesulfonate, 3-Hexadecyne, 1,4Theicosadiene, Hexadecanoic acid methyl ester, 1,2-Benzenedicarboxylic acid, n-Hexadecanoic acid, Ferulic acid methyl ester, Furan 3-(4-methyl-3-pentenyl), Quercetin, Hexadecatrienoic acid, Phytol, 3-Dibenzofuranamine 2-methoxy, Diphthalimido-2-propanone, 2-propenoic acid,1H-Indole 5-methyl-2-phenyl,Squalene,Benzaldehyde 3-nitro,2H-1-Benzopyran-2-one,Benzamide and Lutein were 18, 26, 31, 22, 23, 24, 25, 31, 31, 41, 40, 10, 38, 27, 19, 26, 44, 24, 36, 27, 2, 25, 35, 26, 23 and 42 respectively. Excluding, Cyclopropanecarboxylic acid, 1,2-Benzenedicarboxylic acid, Furan 3-(4-methyl-3-pentenyl) and 2-propenoic acid, the remaining 24 bioactive compounds from Kattuyanam rice extract are deemed quality markers for treating T2DM. These findings suggest that quality markers within Kattuyanam rice extracts may influence the entire biological network system rather than acting on individual target genes.

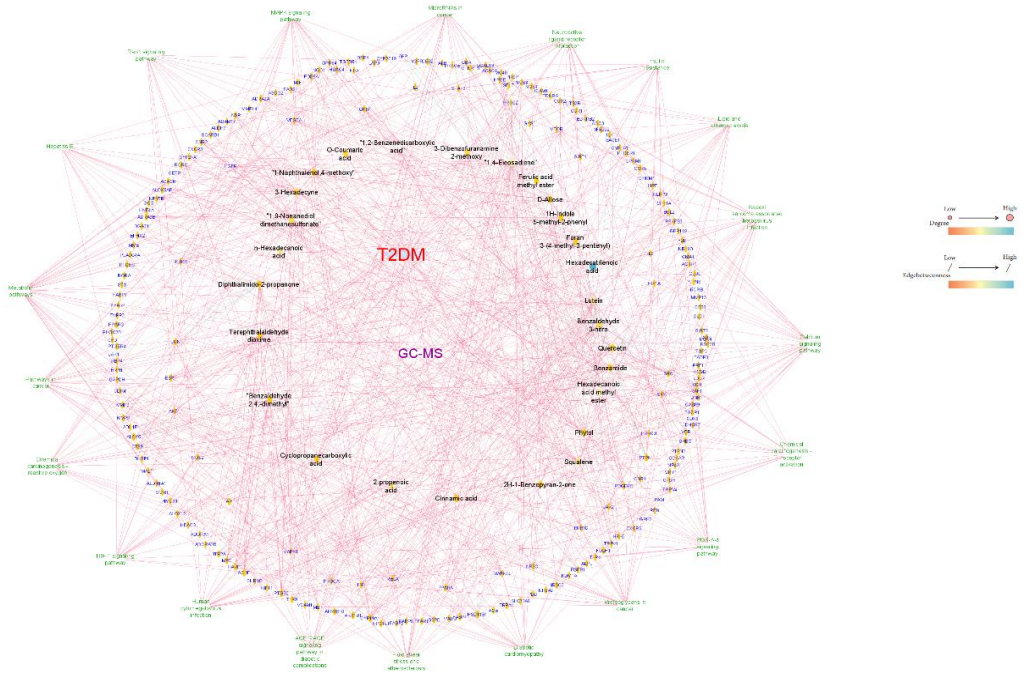

Figure 7: Compound prescription-active component-disease-target gene-pathway interaction network. In the network, node colours signify distinct groups: red for the disease; pink for the compound prescription; black for the active component; blue for the target gene and green for the pathway. Node size and colour reflect the degree, with smaller nodes in orange and larger nodes in cyan. Edge size and colour indicate edge betweenness, with thinner edges in orange and thicker edges in cyan.

Table 4: Interaction network details of 27 active components.

Component	Degree	Target gene
Cyclopropanecarboxylic acid	18	*HDAC3,FABP4,PPARA,FABP5,PPARD,FFAR1,FABP2,HSD11B1,AR,VDR,AKR1B10,FOLH1,AKR1B1,CYP19A1,SHBG,G6PD,NPC1L1,SLC22A6*
Cinnamic acid	26	*AKR1B1,TLR4,TRPA1,ALOX5,MMP9,MMP2,PTPN1,AKR1B10,FOLH1,EGFR,PPARA,ESR1,PAM,PTGER4,CCR2,HSD11B1,RELA,GRK2,ACE,REN,NR4A1,ACHE,BACE1,TLR9,ERBB2,MAPK1*
O-Coumaric acid	31	*ACE,ADORA1,ADORA2B,AKR1B1,AKR1B10,ALOX5,ALPL,APP,CYP2C19,DPP4,EGFR,ERBB2,ESR1,FOLH1,FTO,HSD11B1,LAP3,MAPK1,MIF,MME,MMP2,MMP9,NGFR,PTGER4,PTGS2,PTPN1,PTPN2,SRC,TLR4,TLR9,TRPA1*
D-Allose	22	*ADRA2A,ADRA2B,AKR1B1,APP,EGFR,FOLH1,GBA,HPSE,HTR2A,IL2,LAP3,LGALS3,MME,MMP2,NPC1L1,PRKCE,RORC,STAT3,TLR9,TRPV1,VDR,VEGFA*

Benzaldehyde 2,4,-dimethyl	23	*ACHE,ADH1B,ALDH1A1,ALOX12,ALOX5,CHRM3,COMT,CTSS,CYP19A1,DUSP1,FABP4,FOLH1,HMOX1,HSD11B1,MALT1,MAPK14,MPO,NOS2,PARP1,PTGS2,PTPN1,TGM2,TRPA1*
Terephthalaldehyde dioxime	24	*ACE,ACHE,ACHE,AKT1,ALDH1A1,ALOX12,ALOX5,COMT,CTSS,CYP19A1,DUSP1,ESR1,FOLH1,HMOX1,HSD11B1,MALT1,MAPK14,MMP2,MMP9,PARP1,PTGS2,PTPN1,TGM2,TRPA1*
1-Naphthalenol,4-methoxy	25	*ADORA1,AKR1B1,ALPL,AR,CNR1,COMT,CXCR2,CYP19A1,FADS1,FGFR1,GPR84,HDAC4,JAK2,MAPK14,MIF,MMP2,MMP9,NQO1,PARP1,PDE5A,SHBG,SRC,TGFBR1,TLR9,VEGFA*
1,9-Nonanediol dimethanesulfonate	31	*ABCG2,ADAM17,ADRA2A,ADRA2B,ALDH2,ALOX5AP,AR,CETP,CNR1,CNR2,CTSS,CYP19A1,EGFR,FGFR1,HSD11B1,INSR,KDM1A,MAPK14,MMP14,MMP2,MMP9,MPO,NPY5R,NR3C1,NR3C2,PTGS2,RORC,SCARB1,SRC,STS,TACR1*
3-Hexadecyne	31	*ACACB,ACHE,ADAM17,ADORA1,ALDH2,ALOX5AP,CETP,CHRM3,CNR1,CNR2,CXCR3,CYP19A1,CYP24A1,EGFR,EPHX2,HSD11B1,INSR,MAPK14,MPO,NPY5R,NR3C1,NR3C2,NR5A2,PTGES,PTGS2,RORC,SCARB1,SHBG,SRC,TACR1,TSPO*
1,4-Eicosadiene	41	*ACACA,ACACB,ACHE,ADORA1,ADRA2A,ADRA2B,AKT1,ALOX12,AR,CETP,CHRM3,CNR2,CYP19A1,CYP24A1,CYP2C19,EGFR,ERBB2,ESR1,FABP1,FABP4,FABP5,FFAR1,HRH2,HSD11B1,HTR2A,MC4R,MTNR1B,NPC1L1,NPY5R,NR3C1,NR3C2,PDGFRB,PLA2G4A,PPARA,PPARD,PPARG,PRCP,PTPN1,PTPN2,SHBG,TRPV1*
Hexadecanoic acid methyl ester	40	*ACACB,ADK,ADORA1,ADORA2B,AKR1B10,ALOX5,ALOX5AP,APP,AR,BCL2,CNR1,CNR2,CPT1A,CYP19A1,DGAT1,F2R,FABP2,FABP3,FABP4,FFAR1,FGFR1,G6PD,GPR119,HSD11B1,LDLR,NPC1L1,NPY5R,PAM,PFKFB3,PLA2G4A,PPARA,PPARD,PRF1,PTPN1,RAF1,RORC,SHBG,STS,TRPV1,VDR*
1,2-Benzenedicarboxylic acid	10	*ALB,CES2,DPP4,FABP4,FOLH1,FTO,IGF1R,PARP1,SLC22A6,TLR4*
n-Hexadecanoic acid	38	*ADRA2B,AKR1B10,ALOX12,AR,CYP19A1,DGAT1,ENPP2,EPHX2,FABP1,FABP2,FABP4,FABP5,FFAR1,FFAR4,G6PD,GCG,HNF4A,HSD11B1,MAPK1,MAPK14,MME,MMP2,NPC1L1,PLA2G4A,PLG,PPARA,PPARD,PPARG,PTGER4,PTGES,PTPN1,RBP4,RORA,SHBG,SLC22A6,STS,TRPA1,VDR*
Ferulic acid methyl ester	27	*ADORA1,ADORA2B,AKR1B1,ALOX5,ALPL,APP,BACE1,CXCR2,CYP19A1,CYP1A1,EGFR,HSD11B1,ILK,JAK2,KDM1A,MET,MMP2,MMP9,MTOR,NFE2L2,PARP1,PTGS2,PTPN1,RELA,SIRT1,STAT3,TLR4*
Furan 3-(4-methyl-3-pentenyl)	19	*ADRA2A,ADRA2B,ALOX5,CHRM4,CNR1,CNR2,CTSS,CYP19A1,EGFR,HSD11B1,LIPE,MAPK14,MPO,MTNR1B,NLRP3,NOS2,PDGFRB,PTGS2,PTPN1*
Quercetin	26	*ABCG2,ACHE,ADORA1,AKR1B1,AKR1B10,AKT1,ALOX12,ALOX5,APP,BACE1,CD38,CYP19A1,EGFR,GLO1,IGF1R,INSR,MET,MMP12,MMP2,MMP9,MPO,NOX4,PARP1,SRC,SYK,XDH*
Hexadecatrienoic acid	44	*AGTR1,AKR1B10,ALOX12,ALOX5,AR,CES2,CMA1,CNR1,CPT1A,CYP19A1,EDNRA,FABP1,FABP4,FABP5,FFAR1,FFAR4,G6PD,GLUL,HDAC3,HSD11B1,IL6,MIF,MME,MMP2,MMP9,NOS2,NPC1L1,NR1H3,NR3C1,NR3C2,PPARA,PPARD,PPARG,PTGER1,PTGER4,PTGES,PTGS2,PTPN1,PTPN2,PTPN6,RBP4,RORC,SHBG,TRPV1*
Phytol	24	*APP,AR,CCR1,CCR5,CDK6,CNR2,CXCR3,CYP24A1,DGAT1,DHCR7,EPHX2,F2R,IGF1R,MTNR1B,NPC1L1,PFKFB3,PRKCB,PRKCE,PTPN1,RORA,RORC,SHBG,SRC,TACR1*
3-Dibenzofuranamine 2-methoxy	36	*ADORA1,ADORA2B,AKR1B1,ALOX5,ALPL,AOC3,AR,BDKRB2,CCR1,CCR2,CYP19A1,EDNRA,EGFR,ERBB2,ESR1,FADS1,FGFR1,GYS1,HDAC3,HSD11B1,ICAM1,IGF1R,INSR,JAK2,MAPK14,MTNR1B,NOS1,NOS2,NOS3,NR3C2,PARP1,PIK3CA,PRKCZ,PTPN1,SELE,UTS2R*

Diphthalimido-2-propanone	27	*ACHE,ADORA1,ADORA2B,AKR1B10,CDK4,CFD,CHRM3,GAPDH, HRH1,HTR2A,IKBKB,JAK1,JUN,KIF11,MAPK1,MET,MPO,PARP1, PDGFRB,PIK3C2B,PIK3CA,PTGS2,RELA,TLR9,TNF,TSPO,VCAM1*
2-propenoic acid	2	*SLC22A6,TRPA1*
1H-Indole 5-methyl-2-phenyl	25	*ADORA1,ADRA2A,ADRA2B,ALOX5,AR,CCR1,CCR2,CCR5,CXCR2, CXCR3,CYP19A1,ESR1,FADS1,HSD11B1,HTR2A,KIF11,MAPK14,N R5A2,OPRM1,PARP1,PDGFRB,PLG,PPARG,PTGER1,PTGES*
Squalene	35	*ACACB,ADRA2A,ADRA2B,AGTR1,ALOX5AP,BACE1,BCL2,CCKAR, CCR5,CHRM3,CNR2,CYP24A1,EGFR,ENPP2,EPHX2,ERBB2,F2R, GPR119,HIF1A,HRH2,HRH2,HSD11B1,HTR2A,INSR,MC4R,NR3C1 ,NR3C2,NR5A2,P2RY1,PPARA,PTGES,RORA,TACR1,TSPO,VDR*
Benzaldehyde 3-nitro	35	*ACHE,AKR1B1,ALDH1A1,ALOX5,AOC3,APP,CASP9,CES2,CHRM3 ,COMT,CYP19A1,ESR1,HMOX1,ICAM1,IGF1R,KIF11,MALT1,MAP K1,MME,MMP12,MPO,NOS1,NOS1,NOS3,PARP1,PTGES,PTGS2,P TPN1,SELE,SIRT3,STAT3,TGM2,TLR9,VCAM1,XDH*
2H-1-Benzopyran-2-one	26	*ACHE,BDKRB2,CCR5,CPT1A,CTSH,CTSS,CYP19A1,EGFR,HMOX1 ,HRH2,HSD11B1,LIPE,MAPK14,NOS1,NR3C2,OPRM1,PAM,PARP 1,PDE5A,PPARD,PRKCE,PTGES,PTPN1,PTPN2,TRPV4,VDR*
Benzamide	23	*ADH1B,ADORA1,ADORA2B,ADRA2B,ALOX5,CTSS,CYP19A1,DNM T1,DUSP1,HDAC3,HTR2A,MALT1,MPO,NOS2,NOS3,NQO1,PARP1 ,PDE3B,PDE5A,PTPN1,SIRT3,TGM2,TSPO*
Lutein	42	*ADORA1,ADRA2A,AKR1B1,ALOX12,ALOX5,AR,BCL2,CES2,CHRM 3,CXCR3,CYP19A1,ENPP2,ESR1,FABP4,FFAR1,G6PD,HIF1A,HSD 11B1,MAPK1,MAPK14,MTOR,NPC1L1,NR1H3,NR3C1,NR3C2,OPR M1,PIK3CA,PPARA,PPARD,PPARG,PTGER1,PTGER4,PTPN1,RBP 4,RORA,RORB,RORC,SHBG,TLR9,TNF,TRPA1,VDR*

3.5 Docking study of the bioactive compounds with the selected receptor proteins

Table 5 shows the binding energies of bioactive compounds identified in Kattuyanam rice and the reference compound acarbose against the major diabetic receptors such as alpha amylase (4GQR) and alpha glucosidase (3L4Y). The top five bioactive compounds for each enzyme were chosen for interactive analysis based on the active site interactions, lowest binding energies and binding poses. Lutein, Quercetin, Cinnamic acid, O-coumaric acid and Ferulic acid methyl ester are the top five bioactive compounds that have docked to the alpha amylase enzyme (4GQR). Their binding affinity values are -11.35, -10.89, -9.72, -9.20 and -8.93 kcal/mol respectively when compared to the reference compound acarbose which had a binding affinity value of -12.52 kcal/mol. Meanwhile, the top five docked bioactive compounds against alpha glucosidase (3L4Y) are Lutein, Quercetin, Hexadecatrienoic acid, O-coumaric acid, and Cinnamic acid with binding affinity values of -11.05, -10.60, -9.59, -9.45 and -9.19 kcal/mol respectively when compared to the standard acarbose (-14.39 kcal/mol) employed in the docking study. The docking results indicated that compounds like Lutein, Quercetin, Cinnamic acid and O-coumaric acid possess significant multitargeting potential, being effective against both carbohydrate metabolizing enzymes.

Table 5 Binding energy values of the 32 different ligands with the two receptor proteins 4GQR and 3L4Y

Ligands	**Binding Energy (kcal/mol)**	
	4GQR	**3L4Y**
Cyclopropanecarboxylic acid	-5.88	-5.14
Cinnamic acid	-9.20	-9.19
O-Coumaric acid	-9.72	-9.45
D-Allose	-5.93	-5.90
Benzaldehyde 2,4,-dimethyl	-6.57	-6.86
Terephthalaldehyde dioxime	-5.12	-5.73
1,9-Nonanediol dimethanesulfonate	-6.28	-5.14
3-Hexadecyne	-4.11	-5.17
1,4-Eicosadiene	-5.60	-6.41
Hexadecanoic acid methyl ester	-6.95	-6.32
1,2-Benzenedicarboxylic acid	-4.78	-5.20
n-Hexadecanoic acid	-5.62	-5.44
Ferulic acid methyl ester	-8.93	-7.86

Furan 3-(4-methyl-3-pentenyl)	-4.56	-4.91
Quercetin	-10.89	-10.60
Hexadecatrienoic acid	-8.05	-9.59
Phytol	-7.41	-8.01
3-Dibenzofuranamine 2-methoxy	-6.52	-7.17
Diphthalimido-2-propanone	-5.14	-5.62
2-propenoic acid	-5.06	-4.77
1H-Indole 5-methyl-2-phenyl	-6.43	-6.11
Squalene	-6.34	-7.74
Benzaldehyde 3-nitro	-5.42	-5.75
2H-1-Benzopyran-2-one	-4.33	-5.14
Benzamide	-6.11	-6.54
Lutein	-11.35	-11.05
Acarbose (Standard)	-12.52	-14.39

3.5.1 Active site of the receptor protein
The active site of the two receptor proteins 4GQR and 3L4Y were predicted through the online server CASTp and is given below.

4GQR
TRP-58, TRP-59, TYR-62, GLN-63, HIS-101, ALA-106, VAL-107, SER-108, SER-112, TYR-151, LEU-162, THR-163, LEU-165, ARG-195, ASP-197, ALA-198, LYS-200, HIS-201, LYS-208, ASP-212, GLU-233, ILE-235, GLU-240, LYS-243, ASN-250, PHE-256, LYS-257, ASN-298, HIS-299, ASP-300, ASN-301, ARG-303, HIS-305, GLY-306, ALA-307, ASP-356, TRP-357.

3L4Y
GLU-114, SER-118, ASP-203, ARG-283, PRO-284, ALA-285, LEU-286, PRO-287, SER-288, ALA-291, GLU-404, PHE-450, VAL-506, ALA-509, GLU-510, ALA-512, LYS-513, ARG-520, SER-521, PHE-522, ILE-523, ARG-526, GLY-533, LYS-534, PHE-535, ALA-536, ALA-537, ASP- 542, ILE-565, PRO-566, MET-567, PHE-641, HIS-645, THR-775, LYS-776, ASP-777, THR-778, VAL-779, ALA-780.

3.5.2 Aminoacid interaction of top docked compounds with α-amylase and α-glucosidase
The interaction of the top docked bioactive compounds and the reference acarbose with the active site residues of the target enzymes α-amylase and α-glucosidase is represented in Table 6 and 7 and Figure 8 and 9 respectively. The majority of the interactions between the ligand groups and the enzyme residues were hydrophobic, with a small number of hydrogen bonds below 3.95 Å.

3.5.2.1 Interaction of the compounds with α-amylase (4GQR)
The interaction between Lutein and α-amylase was found to be stabilized through four hydrogen bonds with the residues TYR-52, ASN-53, LYS-200 and GLU-240 at a distance of 3.33, 3.83, 2.85 and 3.10 Å respectively. And hydrophobic contacts were formed with the residues VAL-49, ILE-51, VAL-107, TYR-151, LEU-162, THR-163, LEU-165 and ILE-235. Quercetin that showed least binding affinity with the α-amylase enzyme formed hydrogen bond with the residues ALA-50, TYR-52, GLN-63, ALA-106, SER-108 and SER-112 at a distance of 2.82, 3.43, 2.67, 3.50, 3.25 and 2.99 Å respectively. It also established hydrophobic interactions with the enzyme residues VAL-49, ILE-51, TRP-59 and VAL-107. Cinnamic acid interacted with α-amylase by forming hydrogen bonds with residues LYS-208, ASP-212 and LYS-227 at a distance of 2.61, 2.88 and 3.13 Å respectively, while also making hydrophobic contacts with PCA-1, LEU-211, LEU-214, LYS-227, PRO-228 and ILE-230. Similarly, O-coumaric acid formed hydrogen bonds with residues LYS-208, ASP-212 and ASN-250 at a distance of 2.61, 3.04 and 3.17 Å respectively, and exhibited hydrophobic contact with the same residues as Cinnamic acid, albeit at slightly different distances. For instance, the hydrophobic contact with residue LEU-211 was at a distance of 3.04 Å for O-coumaric acid compared to 3.61 Å for cinnamic acid. Ferulic acid methyl ester formed hydrogen bonds with residues ASP-197 (3.68 Å), HIS-201 (2.77 Å) and ILE-235 (3.06 Å), along with hydrophobic interactions with residues LEU-162, ALA-198 and ILE-235. Finally, the reference compound acarbose interacted with α-amylase through hydrogen bonds with residues TYR-62, THR-163, ARG-195, ASP-197, GLU-233 and ASP-300 at a distance of 4.05, 3.19, 3.42, 2.60, 3.05 and 2.76 Å respectively and made hydrophobic contact with LEU-162 alone.

3.5.2.2 Interaction of the compounds with α-glucosidase (3L4Y)
The stabilization of the interaction between Lutein and α-glucosidase is evidenced by the formation of three hydrogen bonds with residues SER-118, SER-288, and SER-521 at a distance of 2.90, 2.86 and 2.49 Å, respectively. Additionally hydrophobic contacts were observed with residues GLN-117, ALA-285, ALA-509, ARG-520, PHE-522, ILE-523 and

PHE-535. Quercetin, displaying notable binding affinity with the α-glucosidase enzyme, establishes hydrogen bonds with residues ALA-285, SER-288, ALA-509, SER-521, ILE-523, GLY-533 and LYS-776 at a distance of 3.95, 3.71, 3.41, 2.48, 3.95, 2.72 and 3.11 Å respectively. Hydrophobic interactions are formed with residues ILE-523 and MET-567. Hexadecatrienoic acid formed hydrogen bonding with the enzyme residues ARG-520 (3.34 Å), THR-775 (2.49 Å), ASP-777 (3.80 Å), THR-778 (3.12 Å) and VAL-779 (2.89 Å) and hydrophobic interactions with residues PRO-284, ALA-285, LEU-286, PRO-287, ARG-520, PRO-566 and MET-567. Meanwhile, O-coumaric acid forms hydrogen bonds with ILE-523, PHE-535, ALA-537 and LYS-776 residues at a distance of 3.01, 2.89, 3.72 and 2.66 Å respectively and establishes hydrophobic contact with the residues PRO-287, ARG-520, PHE-522 and ILE-523. Cinnamic acid exhibited hydrogen bonding with α-glucosidase residues SER-288, SER-521 and ILE-523 at a distance of 3.12, 2.79 and 3.06 Å respectively and hydrophobic contacts with PRO-284, ALA-285, PRO-566, MET-567 and PHE-641. In comparison the reference compound acarbose formed hydrogen bond with the residues ASP-203, GLU-404, PHE-450, ARG-526 and ASP-542 at a distance of 2.87, 2.74, 2.56, 4.09 and 3.27 Å respectively and made hydrophobic contact with ASP-203, TRP-406, and LYS-480.

Table 6 H-bond, Hydrophobic and other Interactions of the top docked ligands with the alpha amylase receptor protein

Protein	**Ligand**	**H-bond Interactions (with distance in Å)**	**Hydrophobic Interactions**
α-amylase (4GQR)	Lutein	TYR-52 (3.33 Å), ASN-53 (3.83 Å), LYS-200 (2.85 Å) and GLU-240 (3.10 Å).	VAL-49, ILE-51, VAL-107, TYR-151, LEU-162, THR-163, LEU-165 and ILE-235
	Quercetin	ALA-50 (2.82 Å), TYR-52 (3.43 Å), GLN-63 (2.67 Å), ALA-106 (3.50 Å), SER-108 (3.25 Å) and SER-112 (2.99 Å)	VAL-49, ILE-51, TRP-59 and VAL-107
	O-Coumaric acid	LYS-208 (2.61 Å), ASP-212 (3.04 Å) and ASN-250 (3.17 Å).	PCA-1, LEU-211, LEU-214, LYS-227, PRO-228 and ILE-230
	Cinnamic acid	LYS-208 (2.61 Å), ASP-212 (2.88 Å) and LYS-227 (3.13 Å)	PCA-1, LEU-211, LEU-214, LYS-227, PRO-228 and ILE-230
	Ferulic acid methyl ester	ASP-197 (3.68 Å), HIS-201 (2.77 Å) and ILE-235 (3.06 Å)	LEU-162, ALA-198 and ILE-235
	Acarbose (Standard)	TYR-62 (4.05 Å), THR-163 (3.19 Å), ARG-195 (3.42 Å), ASP-197 (2.60 Å), GLU-233 (3.05 Å), and ASP-300 (2.76 Å)	LEU-162

Table 7 H-bond, Hydrophobic and other Interactions of the top docked ligands with the alpha glucosidase receptor protein

Protein	**Ligand**	**H-bond Interactions (with distance in Å)**	**Hydrophobic Interactions**
α-glucosidase (3L4Y)	Lutein	SER-188 (2.90 Å), SER-288 (2.86 Å) and SER-521 (2.49 Å).	GLN-117, ALA-285, ALA-509, ARG-520, PHE-522, ILE-523 and PHE-535
	Quercetin	ALA-285 (3.95 Å), SER-288 (3.71 Å), ALA-509 (3.41 Å), SER-521 (2.48 Å), ILE-523 (3.95 Å), GLY-533 (2.72 Å) and LYS-776 (3.11 Å)	ILE-523 and MET-567
	Hexadecatrienoic acid	ARG-520 (3.34 Å), THR-775 (2.49 Å), ASP-777 (3.80 Å), THR-778 (3.12 Å) and VAL-779 (2.89 Å)	PRO-284, ALA-285, LEU-286, PRO-287, ARG-520, PRO-566 and MET-567
	O-Coumaric acid	ILE-523 (3.01 Å), PHE-535 (2.89 Å), ALA-537 (3.72 Å) and LYS-776 (2.66 Å)	PRO-287, ARG-520, PHE-522 and ILE-523
	Cinnamic acid	SER-288 (3.12 Å), SER-521 (2.79 Å) and ILE-523 (3.06 Å)	PRO-284, ALA-285, PRO-566, MET-567 and PHE-641
	Acarbose (Standard)	ASP-203 (2.87 Å), GLU-404 (2.74 Å), PHE-450 (2.56 Å), ARG-526 (4.09 Å and ASP-542 (3.27 Å)	ASP-203, TRP-406, and LYS-480

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 1 (2024)
http://www.veterinaria.org
Article received- Revised- Accepted-

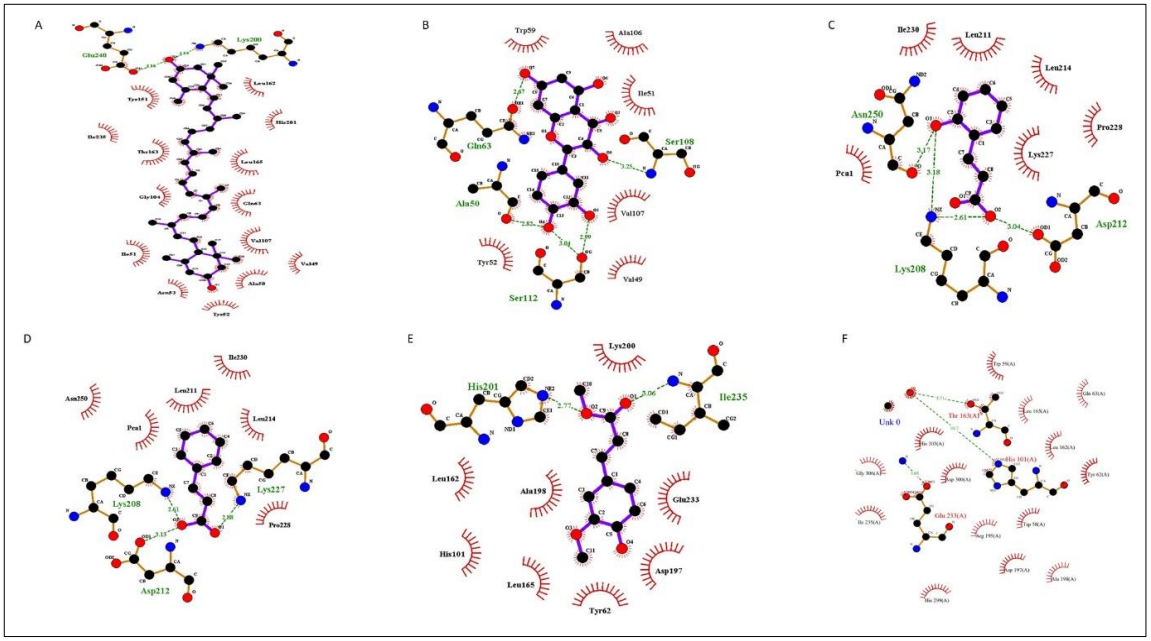

Figure 8: Two dimensional interaction images of the top docked ligands with α-amylase. A. Interaction of Lutein with α-amylase, B. Quercetin and α-amylase, C. O-coumaric acid and α-amylase, D. Cinnamic acid and α-amylase, E. Ferulic acid methyl ester and α-amylase, F. Acarbose (standard) and α-amylase.

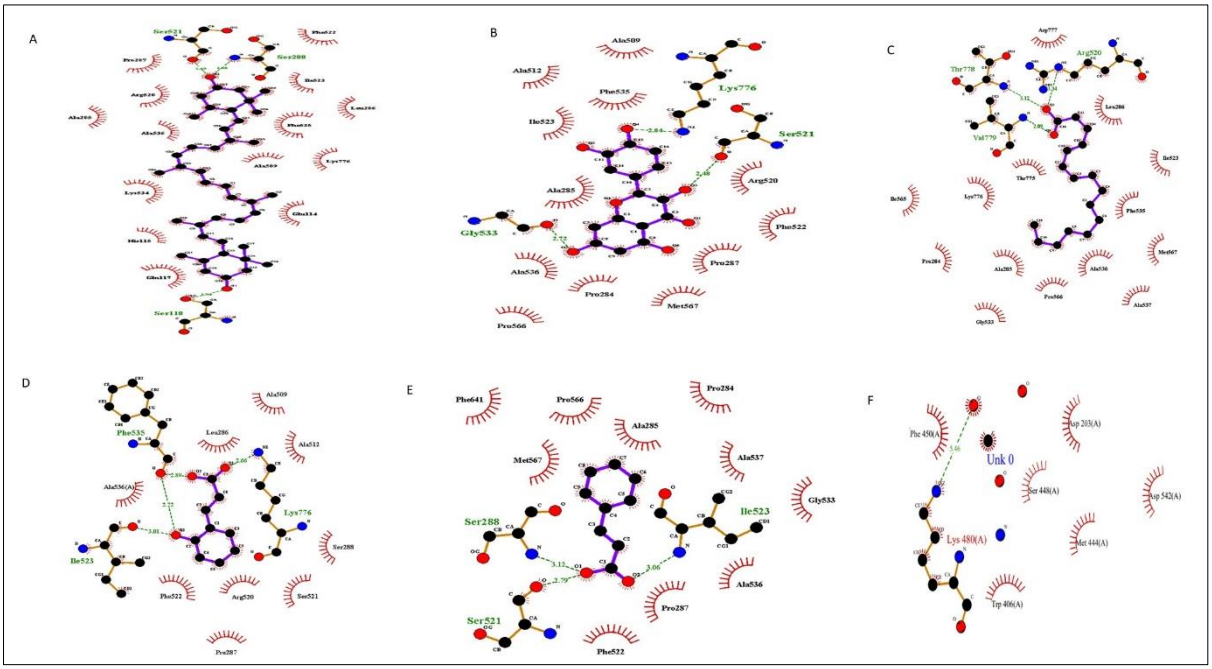

Figure 9: Two dimensional interaction images of the top docked ligands with α-glucosidase. A. Interaction of Lutein with α-glucosidase, B. Quercetin and α-glucosidase, C. Hexadecatrienoic acid and α-glucosidase, D. O-Coumaric acid and α-glucosidase, E. Cinnamic acid and α-glucosidase, F. Acarbose (standard) and α-glucosidase.

4. Discussion

Rice is the staple food for a significant portion of the Asian population. Eventhough it is easily digestible, it is also recognized for its higher glycemic index, making it unsuitable for individuals with compromised glucose metabolism (Fukagawa & Ziska, 2019). This study hence seeks to investigate the therapeutic properties of Kattuyanam, a traditional rice variety of Tamil Nadu with purported anti-diabetic properties. In general, the medicinal properties of plants and grains largely stem from their bioactive chemical composition. Anthocyanins, potent antioxidants, are commonly found in pigmented rice varieties (Mackon et al., 2021). Kattuyanam rice exhibited notably higher amount of anthocyanins (213.21±0.14 mg/100g). Flavonoids, also recognized for their antioxidant potentials, exert protection against platelet

aggregation; tumours, hepatotoxins, ulcers and other biological functions are also associated with it (Ullah et al., 2020). The methanol extract of Kattuyanam was found to harbour a higher content of flavonoids of 17.33 ± 0.11mg/mL. Phenolic compounds plays an important role in exerting antioxidant, anti-inflammatory, anti-carcinogenic and free radical scavenging ability of the plant (Rahman et al., 2021). The total phenolic content in Kattuyanam was (161.4±3.8 µg GAE/g). Diet rich in Polyphenols protects the development and progression of Diabetes and other disorders (Pandey & Rizvi, 2009).

Alpha amylase and α-glucosidase are crucial carbohydrate-hydrolysing enzymes responsible for postprandial hyperglycemia. Alpha amylase initiates carbohydrate metabolism by breaking down polysaccharides into disaccharides through hydrolysis of 1,4-glycosidic linkages (Kajaria et al., 2013). Subsequently, α-glucosidase catalyses the hydrolysis of disaccharides into monosaccharides, leading to an increase in blood glucose levels and subsequent postprandial hyperglycemia. Therefore, inhibitors of these enzymes play a pivotal role in controlling hyperglycemia by delaying carbohydrate digestion and reducing postprandial plasma glucose levels. In our study, as expected there persists a dose dependent relationship between the percentage inhibitions of the enzyme activity. The rice extract exhibited percentage inhibition comparable with that of the positive control. This could be attributed to the higher presence of phenols, flavonoids and anthocyanins in the methanol extract of Kattuyanam rice. Higher levels of phenols reduce the activity of carbohydrate-hydrolysing enzymes by inhibiting specific sites on the enzyme (Hanhineva et al., 2010). Inhibition of the α-glucosidase enzyme is a crucial strategy in managing hyperglycemia (Kajaria et al., 2013). In the present study, Kattuyanam rice extract showed the highest inhibition, with 62.16±1.38%, slightly surpassing the activity of the positive control (Gallic acid) which showed 59.74±0.02% inhibition at 100µg/ mL. This finding aligns with the established knowledge that phenolic-enriched extracts or compounds are effective in inhibiting the α-glucosidase enzyme.

The GC-MS profiling of Kattuyanam rice revealed the presence of Phytochemicals with potent biological activity, contributing to the medicinal property of the rice variety. Phytochemicals such as phenols, flavonoids, steroids, terpenoids, carotenoids, fatty acids and other compounds were identified in the methanol extract of Kattuyanam rice. Among the phytochemicals, Cinnamic acid, a phenolic acid was found to stimulate insulin secretion and delay carbohydrate digestion and glucose absorption, thereby lowering blood glucose levels (Adisakwattana, 2017). O-Coumaric acid, another polyphenolic compound, plays a significant role in the prevention and treatment of obesity, diabetes and related disorders (Alam et al., 2016). Ferulic acid, also present in Kattuyanam rice, was reported to suppress weight gain in high-fat diet-induced obesity in experimental mouse models (de Melo et al., 2017). Some Phytochemicals found in Kattuyanam rice belongs to the class of saturated fatty acids. While, saturated fatty acids were previously associated with impaired insulin sensitivity and glucose intolerance, recent studies have identified higher levels of odd chain saturated fatty acids to be associated with a lower risk of Type 2 Diabetes (Huang et al., 2019). Hexadecanoic acid methyl ester, a saturated fatty acid identified in Kattuyanam rice, plays a role in insulin stimulation and exhibits antidiabetic effect (Rizvi et al., 2023). Quercetin, another phytochemical present in the rice variety, is an important flavonoid with anti-diabetic activity. Studies have shown that quercetin lowers serum glucose levels and significantly improves triglyceride levels in Streptozotocin-induced experimental diabetic rats (Vessal et al., 2003). Lutein, a carotenoid found in Kattuyanam rice, has been reported to prevent the development of diabetic retinopathy (Fathalipour et al., 2020). In addition to its anti-diabetic potential, several phytochemicals present in Kattuyanam rice exhibit various biological activities, including anti-inflammatory, antioxidant and antimicrobial properties. Squalene, for instance, is known for its anticancer, antimicrobial, antioxidant and anti-tumour properties (Udhaya Nandhini et al., 2023). Other major phytochemicals identified in Kattuyanam rice belong to the classes of alcohols, esters and sugars, further contributing to its therapeutic potential.

The integration of target gene screening and interaction network construction offers valuable insights into the potential therapeutic mechanism of Kattuyanam rice extract in managing type 2 diabetes mellitus. Initially, a total of 770 potential target genes were identified, indicating the rich bioactivity of the extract. Notably, 204 of these genes overlapped with disease target genes associated with T2DM, suggesting a specific relevance to diabetes management. Further exploration via Protein-Protein Interaction (PPI) analysis revealed key genes such as STAT3, SRC and PIK3CA, which exhibited frequent protein interactions, underscoring their potential as central nodes within the network. Additionally, Gene Ontology (GO) analysis elucidated the involvement of common target genes in essential biological processes such as stress response and chemical stimulus, providing insights into the mode of action of Kattuyanam rice extract. Furthermore, KEGG pathway enrichment analysis identified multiple signaling pathways, with notable associations to T2DM treatment including PI3K-Akt and AGE-RAGE pathways. The PI3K-Akt signalling pathway is known to regulate various life processes, including cell proliferation, differentiation, growth and apoptosis, and it plays significant roles in the pathogenesis of T2DM, obesity and inflammation. This pathway has been extensively studied due to its involvement in multiple physiological and pathological conditions (He et al., 2021). Additionally, the AGE-RAGE signaling pathway has been implicated in cell damage and endothelial dysfunction in coronary arterioles. Studies have shown its mediation of cellular damage and its contribution to various pathological processes, highlighting its importance in disease mechanism (Yue et al., 2022). The compound prescription-active component-disease-target gene-pathway interaction network further illustrated the intricate interplay between bioactive compounds, target genes and pathways. Some compounds emerged as potential quality markers for T2DM treatment, emphasizing their influence on the entire biological network system rather than individual target genes. These findings underscore the multifaceted nature of Kattuyanam rice extract's therapeutic effects, suggesting its potential utility in T2DM management through modulation of diverse biological processes and

pathways. Further investigation is warranted to validate these findings and unravel the underlying mechanism driving the observed interactions within the biological network.

Molecular docking investigations of the bioactive compounds of Kattuyanam rice extract revealed inhibitory activity of the α-amylase and α-glucosidase enzymes. The objective of the docking study was to decipher the underlying mechanisms of the rice bioactive compounds as an anti-diabetic agent and propose it as an alternative approach for managing Diabetes mellitus. The findings identified four key compounds from the Kattuyanam rice extract, namely cinnamic acid, O-coumaric acid, Lutein and quercetin, as potential inhibitors of both the enzymes. Additionally, Ferulic acid methyl ester exhibited inhibitory activity against α-amylase, while Hexadecatrienoic acid inhibited α-glucosidase. These compounds demonstrated favourable binding affinity values and low binding energy, indicating strong enzyme inhibitory activity through easy ligand-receptor binding. Moreover, the interactions and potency were comparable to the standard Acarbose. The compounds were found to strongly bind to the active site residues of the enzymes, suggesting a potential impact on supressing postprandial levels. Hydrogen bonding played a crucial role in ligand binding specificity (Wade & Goodford, 1989), and in this study, the rice compounds formed favourable hydrogen bonding with the active site residues of both enzymes, indicating their potential for inhibition of the enzymes.

CONCLUSION

In conclusion, the study provides strong evidence for the therapeutic potential of Kattuyanam rice, a traditional variety from Tamil Nadu, in the management of diabetes. Its potential therapeutic efficacy is supported by the presence of bioactive compounds such as quercetin, lutein, cinnamic acid, and O-coumaric acid identified through GC-MS analysis. Additionally, target gene screening and interaction network analysis provided insights into its pharmacological actions. Molecular docking studies revealed the ability of rice bioactive compounds to inhibit key carbohydrate- hydrolysing enzymes, α-amylase and α-glucosidase, with comparable efficacy to standard anti-diabetic drug Acarbose. These findings suggest that Kattuyanam rice hold promise as a natural alternative for managing diabetes mellitus, offering potential benefits in mitigating associated complications through its multifaceted bioactive profile. Further research and clinical trials are necessary to validate its efficacy and explore its full therapeutic potential in the context of diabetes management.

Acknowledgement

The authors of the manuscript acknowledge Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu for the support throughout the research work.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this research article.

Data availability

Data will be made available on request.

Funding: Not Applicable

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