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ORIGINAL RESEARCH

MJM BIOLABS

Virtual Screening of Antidiabetic Plant Phytochemicals for Analogues that Cause Beta Cell Regeneration

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ABSTRACT

Immune-mediated destruction of functional beta-cell mass is the key mechanism for pathogenesis of type 1 diabetes mellitus. Currently, the mainstay conventional treatment for Type 1 Diabetes Mellitus is use of exogenous insulin. These patients require lifelong insulin therapy which includes daily parenteral insulin administration, by single or multiple doses. Some plant phytochemicals have been shown to cause beta cell regeneration, gradually restoring insulin production in diabetic animal models. This would shift treatment from palliative care to more of curative approach.

this study aimed at screening analogs of these phytochemicals with better potency, pharmacokinetic and toxicological profiles.

Genistein and quercetin plant phytochemicals were selected to be screened by in-silico study design. ZINC database was used to obtain analogues, then top 20 selected for each, totaling 40 analogues, all which were prepared using the software Avogadro. Autodock vina embedded in Chimera was used for docking analysis at the epidermal growth factor receptor (erB1). Biovia Discovery studio was used to carry out ligand-enzyme interactions. SWISSADME was used for pharmacokinetic profiling and Protox for toxicological studies.

Three hundred and forty-four and 365 analogues were obtained for genistein and quercetin, respectively, with similarity scores ranging from 99.6%-100%. Genistein (docking score= -6.0) had 12 analogues that were better in terms of docking scores while quercetin had 5(docking score= -6.5). Among these, ZINC000038418848 was the best analogue for genistein (-6.7) and ZINC000004731234 for quercetin (-6.8). Both genistein and quercetin did not violate Lipinski rules, had high GI absorption, didn't permeate BBB, were not P-glycoprotein substrates and inhibited the enzymes CYP1A2, CYP2D6 and CYP3A4. LD50 for genistein and quercetin was 2500 mg/kg and 159 mg/kg respectively, indicating high toxicity for quercetin compared to genistein. Their analogues displayed similar pharmacokinetic and toxicological profiles, with few differences in docking scores, BBB permeation, synthetic accessibility, CYP enzyme inhibition and LD50.

In conclusion, 12 genistein analogues and 5 quercetin analogues had stronger binding affinity to erB1 than the parent compounds. The different ZINC compounds displayed varied pharmacokinetic and toxicological profiles, with a few having superior properties to their parent compounds.

Keywords: *Human Epidermal Growth Factor Receptor 1(erB1), Quercetin, Genistein, Type I Diabetes, Type II Diabetes*

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease characterized by elevated blood sugar levels. Diagnosis is made with repeated measurements of blood sugar levels where fasting blood glucose (FBG) is > 7.8 mmol/l or random blood glucose (RBG) > 11.1 mmol/L, glycated hemoglobin (HbA1c) > 6.5% or Oral Glucose Tolerance Test is >200mg/dl after 2 hours (Mathew et al., 2023). Once diagnosed, diabetes has been shown to have a poor recovery prognosis. It is associated with development of medical emergencies (such as diabetic ketoacidosis and hypoglycemia), microvascular complications (such as retinopathy, neuropathy and nephropathy) and macrovascular complications (such as CKD and stroke) (Tomic et al., 2022). Studies have reported diabetes as being immunosuppressive (Berbudi et al., 2020), therefore increasing vulnerability to infections in patients suffering from diabetes. All these reduce quality of life of these patients.

Diabetes is on the rise, postulated to reach 800 million cases by 2045 (International Diabetes Federation, 2022). This trend is not about to slow or stop, considering the drastic change in lifestyles in the world today. Of these cases, 85% is covered by type 2 diabetes(slow-onset) while 10% is by type 1 diabetes mellitus (early onset). In Africa approximately 24 million people are living with diabetes mellitus, and this is projected to shoot to 55 million by 2045 (International Diabetes Federation, 2022). In Kenya, for every 100 people, 4 have diabetes, yet two thirds of those living with diabetes are undiagnosed. These numbers indicate how silent a pandemic diabetes mellitus is (Mnif et al., 2022).

Currently, DM management (Alwan, 1994) is by two classes of drugs; exogenous insulin to replace diminished endogenous insulin, and use of oral hypoglycemics. Insulin, as a peptide molecule, requires storage in cool environment (Bahendeka et al., 2019). Patients without the capacity to provide these storage conditions (refrigerator, electricity) may not be able to have it in the best conditions. Injection site pain associated with administration of insulin may compromise patient compliance. Oral hypoglycemics in the market today include insulin sensitizers (biguanides) and insulin secretagogues (sulphonyl ureas) among others. These drugs cause adverse effects such as weight gain and vitamin B12 deficiency. In addition to that, some of these drugs cannot be used in specific conditions such as pregnancy, lactation and hepatorenal impairments. Clearly, several gaps can be identified in the management of diabetes in the world today. First, management is more palliative rather than curative. Secondly, once a diagnosis is made, diabetes mellitus management is lifelong. Also, diabetes poses an overwhelmingly great socioeconomic and psychological burden to the patients, their relatives and the society at large (Sharifirad et al., 2013). Therefore, this study aimed to screen the ZINC chemical database for analogues similar to specific plant phytochemicals that cause beta cells regeneration.

METHODS

Materials and Tools

Table 1 below lists the In-silico tools and materials utilized in each step of the study. These tools were used in a sequential manner, starting from obtaining the phytochemical analogues, to target receptor and analogues preparation, to docking analysis, and finally pharmacokinetic and toxicological studies.

Table 1:

Materials and Tools Used to Conduct the Study Methodology

Activity	Material/tool
Selection of study phytochemicals	• Literature review
Target validation	• SwissTargetPrediction
Structures of phytochemicals	• PubChem
Ligand-based virtual screening	• SwissSimilarity interface • PubChem • PubChem Sketcher v2.4
Structure-based virtual screening	• Avogadro (RRID:SCR_015983) • UCSF Chimera v1.16 (RRID:SCR_004097) • Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) (RRID:SCR_012820) • AutoDock Vina (RRID:SCR_011958)
Ligand-Receptor interaction visualization	• Biovia Discovery Studio
Pharmacokinetic analysis	• SwissADME
Toxicity analysis	• Protox-II (RRID:SCR_018506)

Study Design & Target Validation

This study was carried out at Kabarak University, school of pharmacy. *In-silico* study design was utilized for this study. Literature review narrowed the study to two phytochemicals, quercetin and genistein. To validate whether quercetin and genistein bind human epidermal receptor 1, target prediction was conducted using the online tool, SwissTargetPrediction. The predicted probability for binding of quercetin and genistein to human epidermal receptor 1 was found to be 1.00 (100%) for each phytochemical.

Ligand-Based Virtual Screening

For both Quercetin and Genistein, canonical smiles were obtained from PubChem website. Combined screening of the ZINC database (RRID:SCR_006082) for drug-like analogues was done using SwissSimilarity online tool, a database with open access and containing millions of chemical compounds with their structures. The results including 344 analogues for genistein and 365 analogues for quercetin were downloaded as excel files containing canonical smiles and similarity index of ZINC analogues. For each phytochemical, a sample size of 20 analogues with highest similarity index were isolated for further analysis, as agreed upon consensually by the authors. Therefore, a total of 40 ZINC analogues formed our study population. Using canonical smiles for each of the 40 ZINC analogues, sketching their structures was done using the online tool- PubChem Sketcher v2.4 . These were downloaded and saved as MDL molfile.

Structure-Based Virtual Screening

Using the software Avogadro (RRID:SCR_015983), all the sketched analogues and the phytochemicals Quercetin and Genistein were converted to their 3D format and optimized, at the set force field of MMFF94s. The optimized counterparts of ZINC analogues and the two phytochemicals were each minimized using the software-UCSF Chimera v1.16 (RRID:SCR_004097) to reduce their total energies.

Docking Analysis

Human epidermal receptor 1 structure was downloaded from Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) (RRID:SCR_012820) as a .pdb file. UCSF Chimera v1.16 (RRID:SCR_004097) was used to removenon-standardresiduesandnon-standardamino acids present in the protein, which was then saved as .pdb file. AutoDock Vina (RRID:SCR_011958) embedded in UCSF chimera was used to carry out surface binding analysis (docking) of the 40 ZINC analogues and the two phytochemicals to the standardized Human Epidermal Receptor 1. The corresponding docking scores for the 40 ZINC analogues, and the three phytochemicals were then recorded. BIOVIA Discovery Studio v21.1.0.20298 (RRID:SCR_015651) was used for visualization of complexes formed from ligand-receptor interactions between the ZINC analogues, Genistein and Quercetin with the Human Epidermal Receptor 1.

Pharmacokinetic Analysis

Prediction of pharmacokinetic profiles of the 40 ZINC analogues, Quercetin and Genistein was done using SwissADME online tool. Canonical smiles obtained from PubChem were entered into SwissADME, then run to obtain results that were later tabulated. Major focus was based on parameters such as conformity to Lipinski rules, gastrointestinal (GI) absorption, blood-brain barrier permeation, efflux by P-glycoprotein (P-gp) pump and interactions with cytochrome P450 (CYP450) enzymes.

Toxicology Analysis

Toxicology profiles of the 40 ZINC analogues and the two phytochemicals were predicted using Protox-II (RRID:SCR_018506) . This was also done by entering their canonical smiles to Protox-II online tool. Major focus was based on predicting the LD50, potential to cause hepatotoxicity, carcinogenicity, immunogenicity, mutagenicity, cytotoxicity and potential capability of activating pathways associated with nuclear signaling and stress signaling. The results obtained were then tabulated.

Ethical Consideration

Study approval was sought from the School of Pharmacy, Kabarak University. Ethical approval no. *KUREC-261022* was obtained from Kabarak University Institutional Scientific and Ethics Review Committee (KABU – ISERC). Permission to collect data (Research license no. *NACOSTI/P/23/2441*) was obtained from the National Commission for Science, Technology and Innovation (NACOSTI). Since this study was purely an *in-silico* study, no consent for participation was required.

RESULTS

Sampled Phytochemicals and Their Plant Sources

Table 2 below shows 100% predicted probability of both phytochemicals, Quercetin and Genistein binding to erB1 receptor. This validated literature review claims that both Quercetin and Genistein have high affinity for this receptor, thus target validation.

B. Structure of Quercetin and Genistein

Table 3 below illustrates Quercetin's and Genistein's 2-dimensional chemical structures. Both phytochemicals display great silimilarity, evident by possession of the chromene ring, which forms the major part of their structures. The positional substitution by the phenol rings on the chromene ring and number of hydroxyl groups differentiates the two.

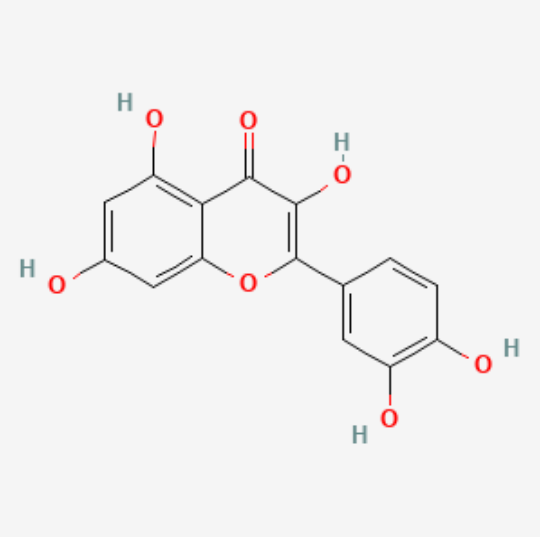
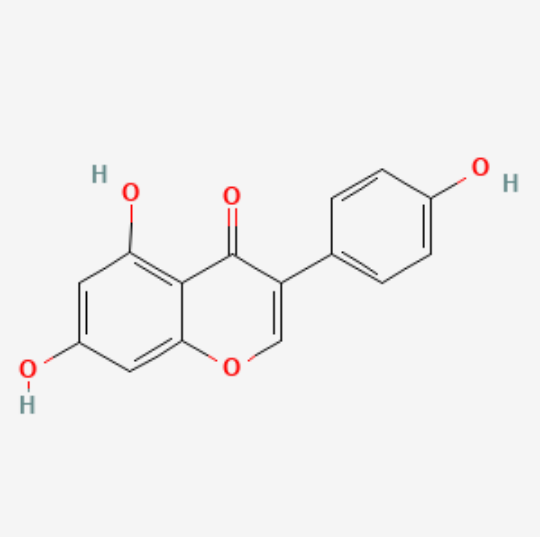
Table 2:

Predicted probability of Quercetin and Genistein binding to erB1.

Plant Species	Phytochemical	Predicted Probability
Moringa oloifera	Quercetin	1
Glycine max	Genistein	1

Table 3:

Chemical structures of Quercetin and Genistein

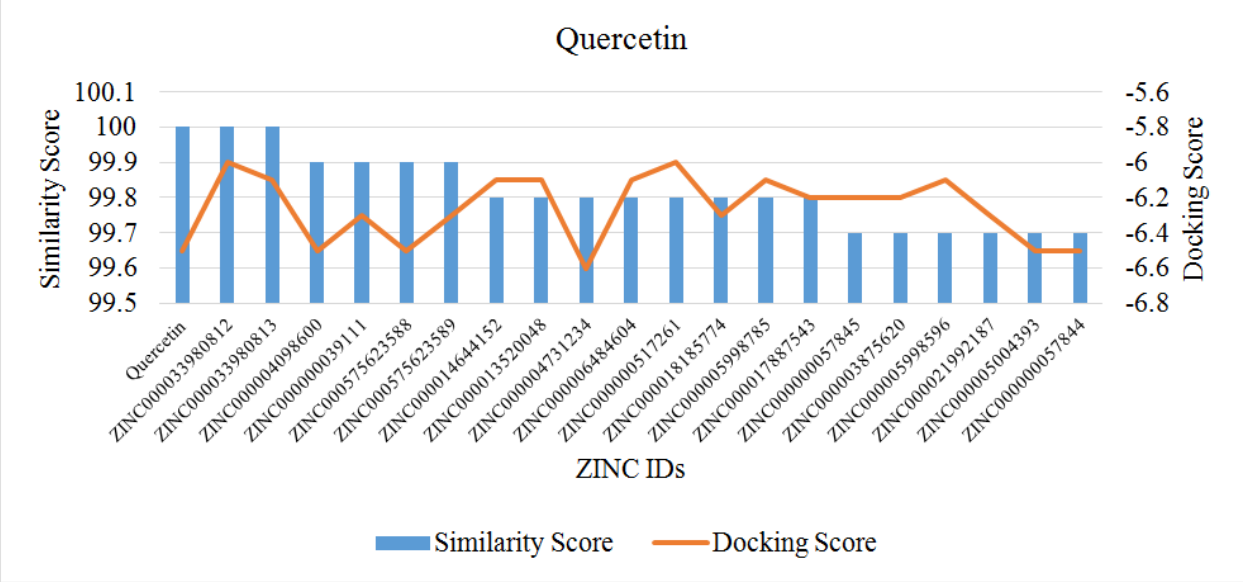
Quercetin	Genistein
	

C. Docking Scores of Selected Phytochemicals and Zinc Compounds

Quercetin

Figure 1 below shows that only ZINC000004731234 (-6.6) had a stronger docking score than that of quercetin (-6.5). Four ZINC analogues tied at -6.5 while only two analogues were 100% similar to quercetin.

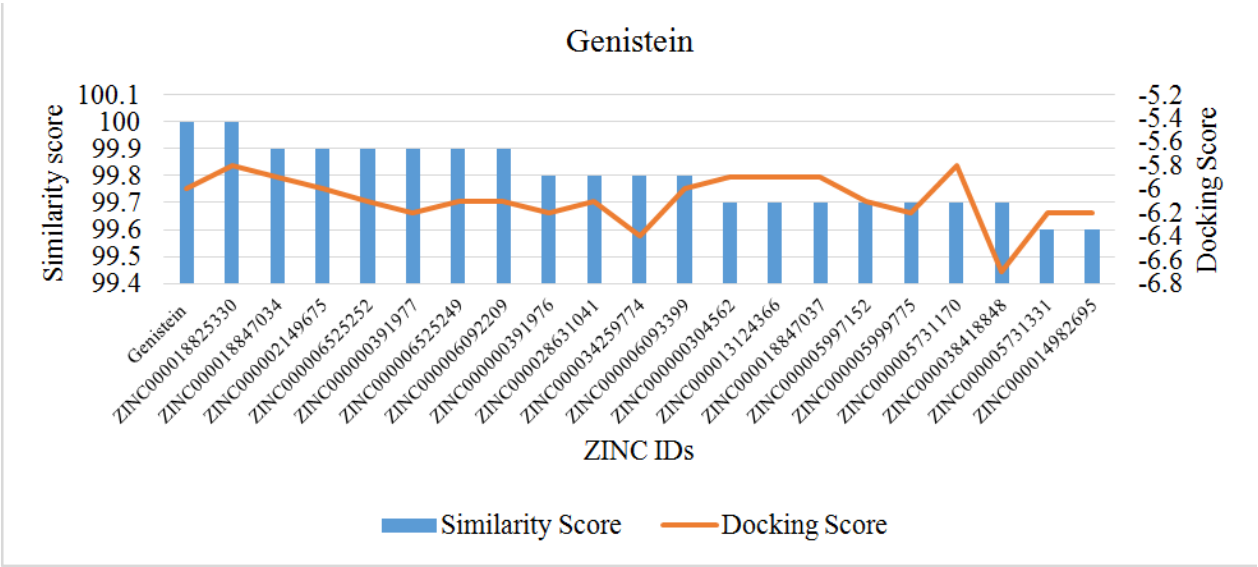
Figure 1:
Comparison of Similarity and Docking Scores of Quercetin and Its Zinc Analogues



Genistein

Figure 2 below shows that twelve compounds had superior docking scores than that of Genistein (-6.0) with only one being 100% similar to it. Evidently, ZINC000038418848 had the strongest docking (-6.7).

Figure 2:
Comparison of Similarity and Docking Scores of Genistein and Its Zinc Analogues



Pharmacokinetic Profile of Selected Phytochemicals and ZINC Compounds

Quercetin

Table 4 below compares the pharmacokinetic profiles of all quercetin analogues. All analogues of quercetin: had high GI absorption, were not substrates for P-glycoprotein, did not permeate the blood brain barrier nor violate any of the five Lipinski rules, and inhibited only CYP-1A2, -2D6 and -3A4. Further, all had their logP values within recommended range for oral formulation and were easily synthesizable. Notably, only ZINC000006484604 inhibited CYP2C9.

Table 4:
Analysis of Pharmacokinetic profile of Quercetin analogues

Compounds	Log P	Log Kp (cm/s)	Lipinski rule of 5	GI absorption	Pgp substrate	BBB permeation	CYP enzyme inhibition					BA score	PAINS alert	Leadlikedness violation	Synthetic accessibility
							1A2	2C19	2C9	2D6	3A4				
ZINC000033980812	1.23	-7.05	0	High	No	No	Yes	No	No	Yes	Yes	0.55	1	0	3.23
ZINC000033980813	1.23	-7.05	0	High	No	No	Yes	No	No	Yes	Yes	0.55	1	0	3.23
ZINC000004098600	1.12	-7	0	High	No	No	Yes	No	No	Yes	Yes	0.55	1	0	3.21
ZINC000000039111	1.55	-6.65	0	High	No	No	Yes	No	No	Yes	Yes	0.55	1	0	3.16
ZINC000575623588	1.55	-6.65	0	High	No	No	Yes	No	No	Yes	Yes	0.55	1	0	3.16
ZINC000575623589	1.55	-6.65	0	High	No	No	Yes	No	No	Yes	Yes	0.55	1	0	3.16
ZINC000014644152	1.36	-6.6	0	High	No	No	Yes	No	No	Yes	Yes	0.55	1	0	3.12
ZINC000013520048	1.3	-6.6	0	High	No	No	Yes	No	No	Yes	Yes	0.55	1	0	3.05
ZINC000004731234	1.66	-6.43	0	High	No	No	Yes	No	No	Yes	Yes	0.55	1	0	3.42
ZINC000006484604	1.85	-6.13	0	High	No	No	Yes	No	Yes	Yes	Yes	0.55	0	0	3.26
ZINC000000517261	1.65	-6.9	0	High	No	No	Yes	No	No	Yes	Yes	0.55	0	0	3.26
ZINC000018185774	1.73	-6.25	0	High	No	No	Yes	No	No	Yes	Yes	0.55	1	0	3.02
ZINC000005998785	1.41	-7.29	0	High	No	No	Yes	No	No	Yes	Yes	0.55	1	0	3.33
ZINC000017887543	1.6	-6.24	0	High	No	No	Yes	No	No	Yes	Yes	0.55	1	0	3
ZINC000000057845	1.93	-6.3	0	High	No	No	Yes	No	No	Yes	Yes	0.55	0	0	3.14
ZINC000003875620	1.63	-6.9	0	High	No	No	Yes	No	No	Yes	Yes	0.55	1	0	3.3
ZINC000005998596	1.75	-6.31	0	High	No	No	Yes	No	No	Yes	Yes	0.55	1	0	3.29
ZINC000021992187	1.61	-6.85	0	High	No	No	Yes	No	No	Yes	Yes	0.55	1	0	3.24
ZINC000005004393	1.3	-6.4	0	High	No	No	Yes	No	No	Yes	Yes	0.55	0	0	3.09
ZINC000000057844	1.63	-6.34	0	High	No	No	Yes	No	No	Yes	Yes	0.55	1	0	3.07

Genistein

Table 5 below shows that all analogues of Genistein: had high GI absorption, were not substrates of P-glycoprotein, inhibited CYP1A2 and did not violate any of the five Lipinski rules. Ten compounds were modelled to penetrate the blood brain barrier while both CYP2D6 and CYP3A4 were inhibited by two compounds each. Further, only three analogues had logP values within recommended range for oral absorption while all were easily synthesizable. Only ZINC000000304562 inhibited CYP2C9.

Table 5:

Analysis of pharmacokinetic profile of Genistein analogues

Compounds	Log P	Log Kp (cm/s)	Lipinski rule of 5	GI absorption	Pgp substrate	BBB permeation	CYP enzyme inhibition					BA score	PAINS alert	Leadlikedness violation	Synthetic accessibility
							1A2	2C19	2C9	2D6	3A4				
ZINC000018825330	2.04	-6.05	0	High	No	No	Yes	No	No	Yes	Yes	0.55	0	0	2.87
ZINC000018847034	2.24	-6.1	0	High	No	Yes	Yes	No	No	Yes	Yes	0.55	0	0	2.79
ZINC000002149675	2.43	-5.71	0	High	No	Yes	Yes	No	No	Yes	Yes	0.55	0	0	2.84
ZINC000006525252	1.82	-6.45	0	High	No	No	Yes	No	No	Yes	Yes	0.55	0	0	2.89
ZINC000000391977	1.89	-6.45	0	High	No	No	Yes	No	No	Yes	Yes	0.55	1	0	2.86
ZINC000006525249	1.62	-6.41	0	High	No	No	Yes	No	No	Yes	Yes	0.55	0	0	2.95
ZINC000006092209	1.73	-6.25	0	High	No	No	Yes	No	No	Yes	Yes	0.55	1	0	2.99
ZINC000000391976	1.96	-6.45	0	High	No	No	Yes	No	No	Yes	Yes	0.55	1	0	2.92
ZINC000028631041	2.56	-5.93	0	High	No	Yes	Yes	No	No	Yes	Yes	0.55	0	0	2.89
ZINC000034259774	2.08	-6.19	0	High	No	No	Yes	No	No	Yes	No	0.55	0	0	2.91
ZINC000006093399	1.95	-6.45	0	High	No	No	Yes	No	No	Yes	Yes	0.55	1	0	2.93
ZINC000000304562	3.08	-5.7	0	High	No	Yes	Yes	No	Yes	Yes	Yes	0.55	0	1	2.84
ZINC000013124366	2.19	-6.3	0	High	No	No	Yes	No	No	Yes	Yes	0.55	0	0	3
ZINC000018847037	2.44	-5.91	0	High	No	No	Yes	No	No	Yes	Yes	0.55	0	0	2.89
ZINC000005997152	2.77	-5.75	0	High	No	Yes	Yes	No	No	Yes	Yes	0.55	0	0	2.83
ZINC000005999775	2.31	-6.1	0	High	No	Yes	Yes	No	No	Yes	Yes	0.55	1	0	2.84
ZINC000005731170	3	-5.47	0	High	No	Yes	Yes	No	No	Yes	Yes	0.55	0	1	2.81
ZINC000038418848	2.48	-5.84	0	High	No	Yes	Yes	No	No	No	No	0.55	0	0	2.85
ZINC000005731331	2.76	-5.75	0	High	No	Yes	Yes	No	No	Yes	Yes	0.55	0	0	2.88
ZINC000014982695	2.46	-5.84	0	High	No	Yes	Yes	No	No	No	No	0.55	0	0	2.87

Toxicological Analysis of Selected Phytochemicals and ZINC Compounds

Quercetin

Table 6 below shows toxicological analysis of quercetin-ZINC analogues. Fourteen analogues were predicted to be safe chemically as indicated by their LD50 values. Sixteen, eight and eleven of the analogues might be slightly carcinogenic, immunogenic and mutagenic respectively. All analogues were predicted to activate the AHR, ERα, ERLBD and MMP pathways. Four and seven analogues activated the aromatase and ATAD5 pathways respectively.

Table 6:

Predicted Toxicological analysis of Quercetin analogues.

Compounds	LD50	Toxicity class	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity	Aryl hydrocarbon Receptor (AhR)	Androgen Receptor (AR)	Androgen Receptor Ligand Binding Domain (AR-LBD)	Aromatase	Estrogen Receptor Alpha (ER)	Estrogen Receptor Ligand Binding Domain (ER-LBD)	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	Nuclear factor (erythroid-derived 2)-like 2/ antioxidant responsive element (nrf2/ARE)	Heat shock factor response element (HSE)	Mitochondrial Membrane Potential (MMP)	Phosphoprotein (Tumor Supressor) p53	ATPase family AAA domain-containing protein 5 (ATAD5)
ZINC000033980812	159 mg/kg	3	Inactive	Active	Inactive	Active	Inactive	Active	Inactive	Inactive	Inactive	Active	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive
ZINC000033980813	159 mg/kg	3	Inactive	Active	Inactive	Active	Inactive	Active	Inactive	Inactive	Inactive	Active	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive
ZINC000004098600	159 mg/kg	3	Inactive	Active	Active	Active	Inactive	Active	Inactive	Inactive	Inactive	Active	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive
ZINC000000039111	159 mg/kg	3	Inactive	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Active	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive
ZINC000575623588	159 mg/kg	3	Inactive	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Active	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive
ZINC000575623589	159 mg/kg	3	Inactive	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Active	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive
ZINC000014644152	4000 mg/kg	5	Inactive	Active	Inactive	Active	Inactive	Active	Inactive	Inactive	Inactive	Active	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive
ZINC000013520048	3919 mg/kg	5	Inactive	Active	Inactive	Active	Inactive	Active	Inactive	Inactive	Inactive	Active	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive
ZINC000004731234	5000 mg/kg	5	Inactive	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Active	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive
ZINC000006484604	5000 mg/kg	5	Inactive	Inactive	Active	Inactive	Inactive	Active	Inactive	Inactive	Active	Active	Active	Inactive	Inactive	Inactive	Active	Inactive	Active
ZINC000000517261	5000 mg/kg	5	Inactive	Inactive	Active	Inactive	Inactive	Active	Inactive	Inactive	Active	Active	Active	Inactive	Inactive	Inactive	Active	Inactive	Active
ZINC000018185774	3919 mg/kg	5	Inactive	Active	Inactive	Active	Inactive	Active	Inactive	Inactive	Inactive	Active	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive
ZINC000005998785;	5000 mg/kg	5	Inactive	Active	Active	Active	Inactive	Active	Inactive	Inactive	Inactive	Active	Active	Inactive	Inactive	Inactive	Active	Inactive	Active
ZINC000017887543	3919 mg/kg	5	Inactive	Active	Active	Active	Inactive	Active	Inactive	Inactive	Inactive	Active	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive
ZINC000000057845	4000 mg/kg	5	Inactive	Inactive	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Active	Active	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive
ZINC000003875620	5000 mg/kg	5	Inactive	Active	Inactive	Active	Inactive	Active	Inactive	Inactive	Inactive	Active	Active	Inactive	Inactive	Inactive	Active	Inactive	Active
ZINC000005998596	5000 mg/kg	5	Inactive	Active	Active	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Active	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive
ZINC000021992187	5000 mg/kg	5	Inactive	Active	Active	Active	Inactive	Active	Inactive	Inactive	Inactive	Active	Active	Inactive	Inactive	Inactive	Active	Inactive	Active
ZINC000005004393	3919 mg/kg	5	Inactive	Inactive	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Active	Active	Active	Inactive	Inactive	Inactive	Active	Active	Active
ZINC000000057844	3919 mg/kg	5	Inactive	Active	Active	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Active	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive

Genistein

Table 7 below shows the toxicological analysis of Genistein ZINC analogues. Most compounds were mildly toxic falling into toxicity class 5 based on their LD50. Some of the compounds were carcinogenic and mutagenic. Most compounds were predicted to activate AhR, aromatase, ER, ERLBD proteins and also the MMP, p53 and ATAD5 pathways related to stress response.

Table 7:

Predicted Toxicological Analysis of Genistein-ZINC Analogues

Compounds	LD50	Toxicity class	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity	Aryl hydrocarbon Receptor (AhR)	Androgen Receptor (AR)	Androgen Receptor Ligand Binding Domain (AR-LBD)	Aromatase	Estrogen Receptor Alpha (ER)	Estrogen Receptor Ligand Binding Domain (ER-LBD)	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	Heat shock factor response element (HSE)	Mitochondrial Membrane Potential (MMP)	Phosphoprotein (Tumor Supressor) p53	ATPase family AAA domain-containing protein 5 (ATAD5)
ZINC000018825330	2500 mg/kg	5	Inactive	Inactive	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Active	Active	Active	Inactive	Inactive	Inactive	Active	Active	Active
ZINC000018847034	2340 mg/kg	5	Inactive	Inactive	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Active	Active	Active	Inactive	Inactive	Inactive	Active	Inactive	Active
ZINC000002149675	2500 mg/kg	5	Inactive	Inactive	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Active	Active	Active	Inactive	Inactive	Inactive	Active	Active	Active
ZINC000006525252	2430 mg/kg	5	Inactive	Inactive	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Active	Active	Active	Inactive	Inactive	Inactive	Active	Active	Active
ZINC000000391977	2500 mg/kg	5	Inactive	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Active	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive
ZINC000006525249	600 mg/kg	4	Inactive	Inactive	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Active	Active	Active	Inactive	Inactive	Inactive	Active	Active	Active
ZINC000006092209	2500 mg/kg	5	Inactive	Active	Inactive	Active	Inactive	Active	Inactive	Inactive	Inactive	Active	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive
ZINC000000391976	2500 mg/kg	5	Inactive	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Active	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive
ZINC000028631041	2500 mg/kg	5	Inactive	Inactive	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Active	Active	Active	Inactive	Inactive	Inactive	Active	Inactive	Active
ZINC000034259774	500 mg/kg	4	Inactive	Inactive	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Inactive	Active	Active	Active
ZINC000006093399	2500 mg/kg	5	Inactive	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Active	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive
ZINC000000304562	2500 mg/kg	5	Inactive	Inactive	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Active	Active	Active
ZINC000013124366	2500 mg/kg	5	Inactive	Inactive	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Active	Active	Inactive	Inactive	Inactive	Active	Inactive	Active
ZINC000018847037	2500 mg/kg	5	Inactive	Inactive	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Active	Active	Active	Inactive	Inactive	Inactive	Active	Active	Active
ZINC000005997152	2500 mg/kg	5	Active	Inactive	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Active	Active	Active
ZINC000005999775	2500 mg/kg	5	Inactive	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Active	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive
ZINC000005731170	2500 mg/kg	5	Inactive	Inactive	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Active	Active	Active
ZINC000038418848	500 mg/kg	4	Inactive	Inactive	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Inactive	Active	Inactive	Active
ZINC000005731331	2500 mg/kg	5	Active	Inactive	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Active	Active	Active
ZINC000014982695	500 mg/kg	4	Inactive	Inactive	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Inactive	Active	Active	Active

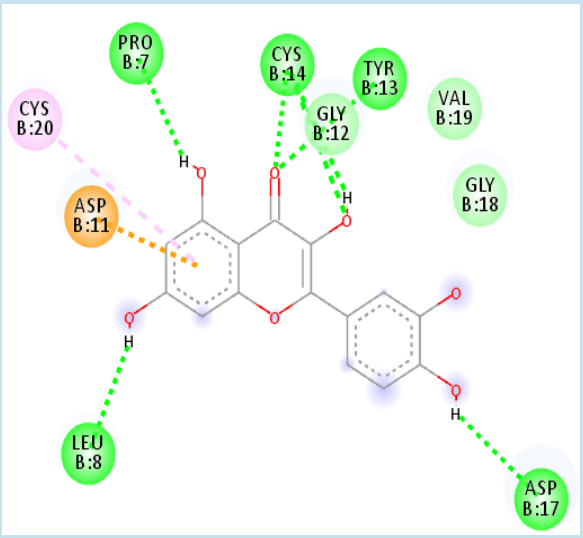
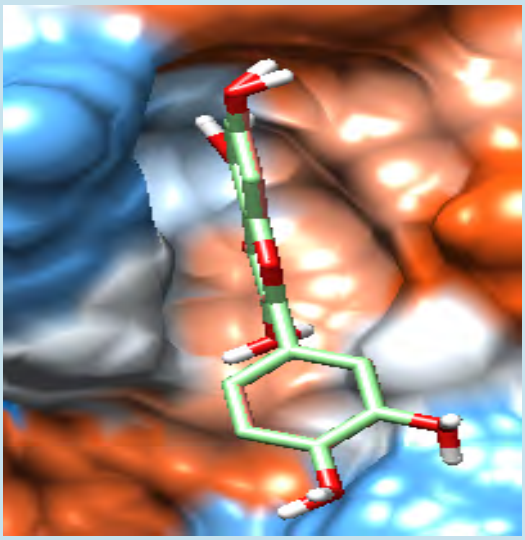
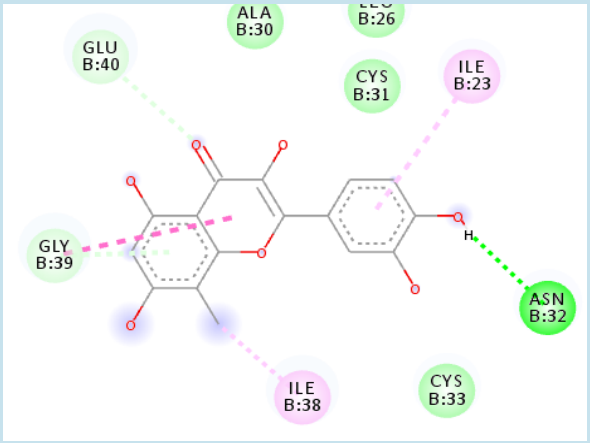
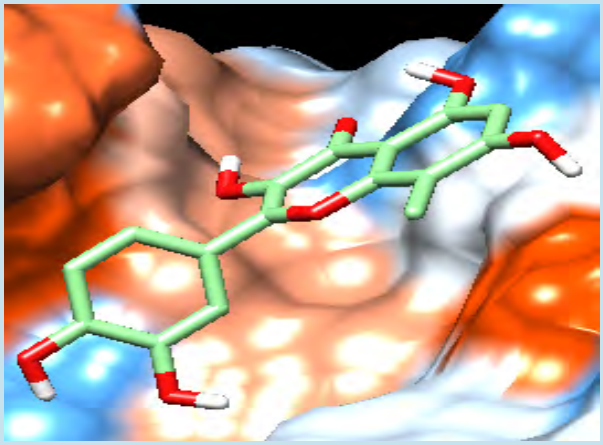
Model Visualization of Interaction of Selected Phytochemicals, ZINC Compounds with Human Epidermal Receptor 1

Quercetin

Table 8 shows that interaction between quercetin and HER 1 is mediated majorly by hydrogen bonding. However, such hydrogen bonding is reduced in ZINC000004731234 which has a notable increase in van der Waals interaction and additional carbon-hydrogen interaction. The second part visualizes binding of quercetin and ZINC000004731234 to the hydrophobic surface of the active binding site of Human Epidermal Receptor 1.

Table 8:

Visual representation of Quercetin and ZINC000004731234 bound to Human Epidermal Receptor 1 and the predicted bonds formed

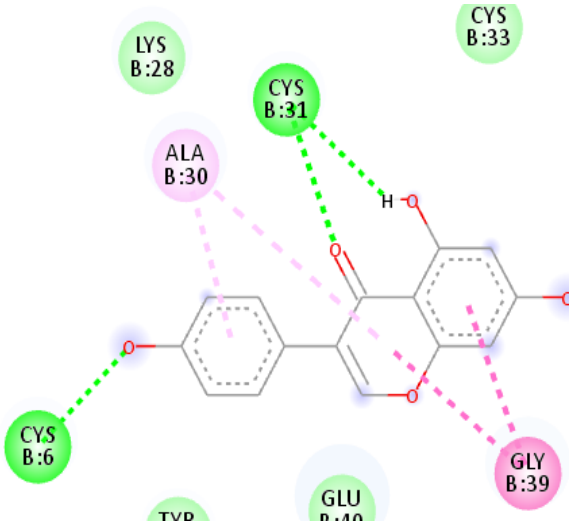
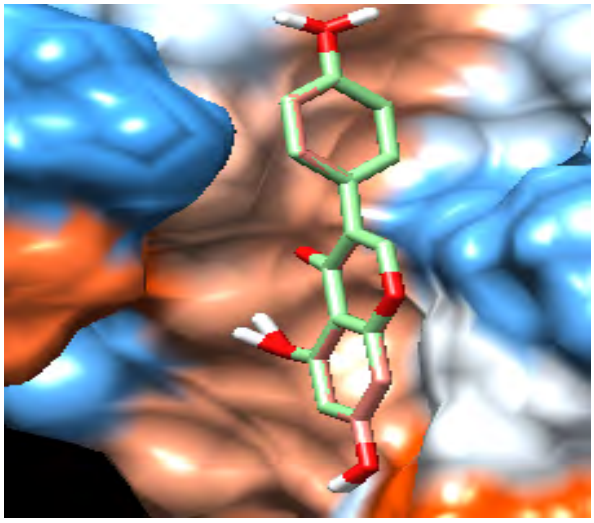
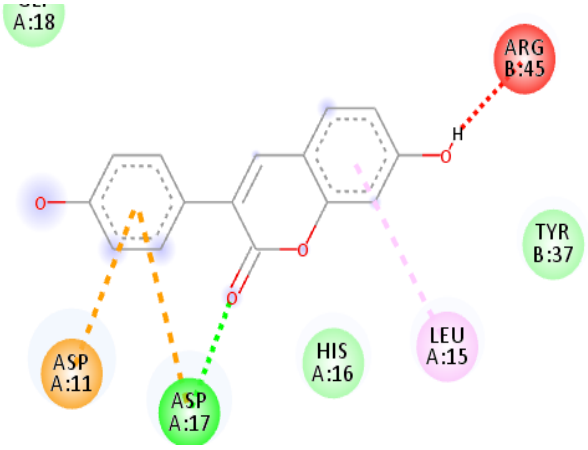
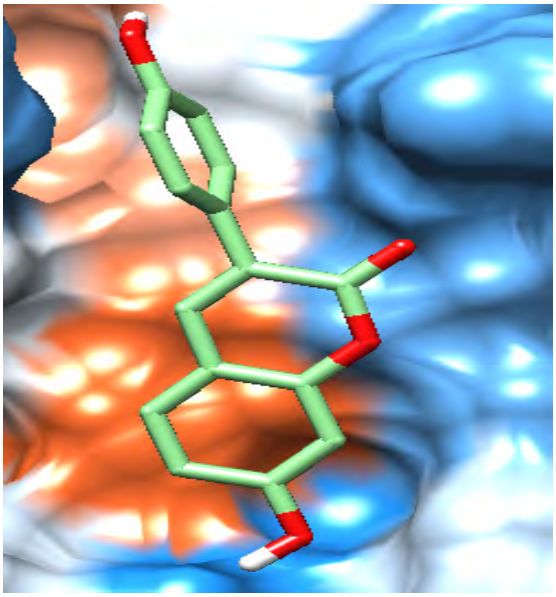
Quercetin	
3D receptor	Hydrophobicity surface (binding site)
	
ZINC000004731234	
Ligand-receptor interaction	Hydrophobicity surface
	

Genistein

Table 9 below shows that Genistein interacts with HER 1 vial hydrogen bonding, Pi-Pi stacking and van der Waals forces. Notably, there is a decrease in interaction between ZINC000038418848 and the receptor despite docking the strongest. The second part visualizes binding of Genistein and analogue ZINC000038418848 to the hydrophobic surface of the active binding site of Human Epidermal Receptor 1.

Table 9:

Visual representation of Genistein and ZINC000038418848 bound to Human Epidermal Receptor 1 and the predicted bonds formed

Genistein	
3D receptor	Hydrophobicity surface (binding site)
	
ZINC000038418848	
Ligand-receptor interaction	Hydrophobicity surface
	

DISCUSSION

The main pathognomonic characteristic of diabetes mellitus is hyperglycemia caused by either insufficient insulin secretion or insulin resistance by peripheral cells (Sameer et al., 2020). Insufficient insulin secretion is a major issue as patients have to rely on exogenous insulin to live a normal life. While this may be an alternative route, it is often associated with side effects such as lipodystrophy and may be uncomfortable for patient who fear needles. Beta cells that secrete insulin have the ability to regenerate and increase their numbers within the islet of Langerhans (Ji et al., 2022). This could be beneficial to improve insulin secretion in type 1 diabetes and avoid use of exogenous insulin. Beta cell regeneration occurs primarily by replication of beta cells. Other modes include neogenesis especially during early development, trans-differentiation and stem cell differentiation (Jin et al., 2020).

The ability of beta cells to regenerate requires specific signals that activate specific pathways associated with the process. Literature review points out that, the *HER 1* (Oh et al., 2011), *VEGFR 1* (De Leu et al., 2013) and tyrosine kinase (Welsh et al., 2000) are some of the proteins associated with beta-cell regeneration. Consequently, this study aimed to investigate ZINC analogues of specific plant phytochemicals that have been shown to have beta-cell regeneration based on ethnobotanical and in vitro studies using an in-silico approach. Analogues of Genistein and Quercetin were analyzed for pharmacodynamic, pharmacokinetic and toxicological analysis.

Model Pharmacodynamic Analysis

For both, all 20 analogues were above 99% similar to the parent phytochemicals. Model ligand-receptor interaction showed that the phytochemicals together with their highest docking analogues majorly interacted with their receptor via conventional hydrogen bonding and Pi-Pi stacking. Contrary, *HER 1* had surface pockets as active sites. Consequently, the ligand-receptor analysis shows that the active sites may not necessarily require a rigid pharmacophore for compounds that bind it rather presence of hydrophilic moieties substituted on ring core structures.

Model Pharmacokinetic Analysis

SWISSADME analysis modelled that all analogues of the two phytochemicals had: high GI absorption indicating that bioavailability through the oral route would be relatively high. None violated any of the five Lipinski rules hence they would be orally active. None were substrates for P-glycoprotein (the multi-drug resistance protein) hence have ability to penetrate cells without the efflux pump reducing their penetrability. They inhibited only *CYP-1A2, -2C9, -2D6* and *-3A4* indicating the analogues bear potential for drug-drug interactions, and requiring necessary dose adjustments if co-administered with other drugs, and were easily synthesizable. Some were predicted to cross the blood brain barrier, hence would require formulation into conventional dosage forms that do not penetrate the BBB. All had their logP values above 1 and below 3 indicating relatively moderate solubility, renal clearance and membrane permeability. Noteworthy though is that the predicted logP of some of the compounds were above 1.8 yet, for oral absorption, it is recommendation that logP be between 1.3 and 1.8.

Model Toxicology Analysis

Using Protox server to determine LD5o of the analogues, toxicological prediction shows that most compounds fell in toxicity class five. Notably, some of the compounds were very toxic as their LD5O values were as low as 159mg/kg especially analogues of quercetin. Additionally, most were modelled to be carcinogenic, mutagenic activated AhR, ER, ERLBD pathways required for nuclear signaling and MMP, and ATAD5 pathways associated with stress response. The potential for carcinogenicity, mutagenicity and nuclear signaling could be attributed to the fact that induction of beta cell regeneration requires activation of cellular division via mitosis which can results in mutations and cancers. A few of the analogues were immunotoxic but none was predicted to be cytotoxic. Quercetin analogues were mostly toxic while Genistein analogues had less strong affinity for receptor binding. Analogues *ZINC000038418848* (-6.7) *and ZINC000004731234* (-6.6) were optimally best under Genistein and Quercetin respectively.

CONCLUSION

In conclusion

- 40 analogues out of the 709 were with highest similarity score, were chosen for further analysis, with 12/20 and 5/20 analogues with

better docking scores than Genistein and Quercetin, respectively.

- SWISSADME and Protox analysis showed relatively acceptable pharmacokinetic and toxicological properties, requiring only minimal alterations for optimization.
- Analogues ZINC000038418848 and ZINC000004731234 were optimally best under Genistein and Quercetin respectively, based on their docking scores, pharmacokinetic and toxicological analysis.

RECOMMENDATIONS

Although SWISSADME analysis indicates that the analogues have favorable pharmacokinetic features, optimization attempts to increase some parameters, including Log-P values within the approved range for oral absorption, might be necessary. Optimizing the chemical structures of the analogues may maximize their therapeutic efficacy by improving their bioavailability, distribution, metabolism, and excretion profiles. This study recommends that consumers of plants bearing these phytochemicals should be aware of the potential toxicological profile outlined above especially for Quercetin. Modifications to the structure to lessen the generation of hazardous metabolites or focusing on particular pathways linked to negative effects, are two possible strategies to lower these hazards. Furthermore, thorough preclinical safety evaluations are necessary to determine the possible hazards and long-term effects of administering the analogs. From the discussion and conclusions derived from this study, we recommend further in vitro and in vivo studies on analogues ZINC000038418848 and ZINC000004731234 to be conducted to assess and validate the claims made in this research.

DECLARATION

Competing Interests

The authors declare that they have no competing interests.

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