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ORIGINAL RESEARCH

MJM BIOLABS

Computational Analysis of ZINC Analogues for Antidiabetic Phytochemical with Inhibitory Activity against Aldose Reductase Enzyme

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Abstract

The pathognomonic role of sorbitol pathway in development of microvascular complications cannot go underappreciated; while it may not be a sole independent initiator, it remains a key contributor to initiation and progression of diabetic microangiopathies particularly retinopathy, neuropathy and nephropathy. Sorbitol pathway is a two-step reaction process initiated by the rate-limiting enzyme aldose reductase. This study aimed to screen ZINC databases for chemical compounds similar to Ellagic acid, Kaempferol and Mangiferin and analyze their pharmacokinetic, toxicological and docking profile using computational methods. These phytochemicals were chosen based on literature review of ethnobotanical studies and validated via SwissTargetPrediction. An *in-silico* study design was employed using computational algorithms. Molecular structures of analogues were obtained from ZINC database and prepared using Avogadro software. Docking analysis was carried out using AutoDock Vina embedded in Chimera. Visualization of ligand-enzyme interactions was done using Discovery studio. SWISSADME and Protox-II were used to profile pharmacokinetic and toxicity of the analogs.

A total of 44 analogs were analyzed. Sulindac and parent phytochemicals were used as comparators. Kaempferol had strongest binding affinity (-8.7) followed by Ellagic acid and Mangiferin tying at -8.4. Kaempferol analogs had highest binding affinity compared to analogues of Ellagic acid and Mangiferin. In terms of Pharmacokinetic profile, analogues of Ellagic acid demonstrated a favorable profile with no Lipinski rule violations, high GI absorption, and inhibition limited to CYP1A2, which plays a minor role in drug metabolism compared to other enzymes. Toxicology predictions indicated that Kaempferol exhibited a higher safety profile compared to Ellagic acid and Mangiferin, with LD50 values of 3919 mg/kg, 2991 mg/kg, and 2 mg/kg, respectively. Ellagic acid analogues demonstrated chemical safety, absence of mutagenicity, hepatotoxicity, cytotoxicity, and activation of various pathways. Among the Kaempferol analogues, a subset was potentially carcinogenic and mutagenic, while all exhibited potential pathway activation. Mangiferin analogues, except for a few compounds, did not activate specific pathways, nor did they demonstrate hepatotoxicity or cytotoxicity. However, some analogues exhibited potential immunogenicity and mutagenicity.

Kaempferol and some of its ZINC analogues had strongest binding affinity compared to Ellagic acid, Mangiferin and their analogues. This can be attributed to the simpler structure of Kaempferol, allowing it to fit snugly into the hydrophobic pocket of aldose reductase. Ellagic acid, with its planar and rigid structure, interacted primarily with the outer surface of the hydrophobic pocket. Similarly, due to its complex and large structure, Mangiferin demonstrated a relatively lower affinity. analogues ZINC000005004393, ZINC000003872446 and ZINC000031156069 for Kaempferol, Ellagic acid and Mangiferin respectively depicted best optimal characteristics required for further development. We recommend an in vitro study be conducted to assess and validate the claims arrived at in this study.

Keywords: Aldose Reductase, Kaempferol, Mangiferin, Ellagic Acid, Type II Diabetes

INTRODUCTION

At a prevalence of 20.9 per 100,000 populations worldwide, diabetes mellitus is slowly turning to a pandemic. Currently with over 400 million individuals diagnosed with diabetes mellitus globally, and cases estimated to rise (World Health Organization, 2019), the future is distressing. Diabetes mellitus (DM) is a chronic metabolic disease that results from inability of tissue cells to uptake glucose from the blood circulation (Papatheodorou et al., 2015). Broadly, hyperglycemia results from either insufficient/ lack of insulin secretion in type 1 DM or development of insulin resistance by peripheral tissues in type 2 DM. This leads to accumulation of glucose in blood which damages the blood vessels especially those supplying the heart, renal tissue, retina and nerve tissue. Consequently, uncontrolled hyperglycemia leads to development of retinopathy, nephropathy and neuropathy in diabetic patients (Barrett et al., 2017). Such conditions are termed as microvascular complications since they result from persistent high blood sugar levels that damages micro blood vessels circulating the eyes, kidneys and nerves.

The development of microvascular complication is driven from multiple points involving complex biochemical processes. Uncontrolled high blood sugar levels lead to persistence of glucose in blood which in turn thickens the capillary basement membrane, and induce protein synthesis within the extracellular matrix (Giri et al., 2018). Additionally, blood glucose molecules become conjugated to proteins including those that make up the vasculature leading to formation of advanced glycated end-products (AGEs) which causes oxidative stress (Singh et al., 2014). Key to pathogenesis of microvascular complications and broadly studied is the sorbitol pathway (Yan, 2018). Altogether, AGEs, oxidative stress and the sorbitol pathway are significant in the development of diabetic microvascular complications. This study focused on the sorbitol pathway as a potential site for slowing progression of such complications via enzymatic inhibition.

The sorbitol pathway comprises a two-step reaction in which glucose is converted to fructose via sorbitol as intermediate byproduct. The first step of converting glucose to sorbitol is mediated by the enzyme aldose reductase while sorbitol dehydrogenase converts formed sorbitol to fructose. NADPH is oxidized it eh first step while NAD+ is reduced in the second reaction (Garg & Gupta, 2022). Aldose reductase is a non-specific rate-limiting enzyme in the process; by converting glucose to sorbitol intracellularly, it prevents escape of latter molecule from the cell (Jannapureddy et al., 2021). Glucose, sorbitol and fructose are osmotically active and when trapped intracellularly, they cause osmotic stress which forms part of the basis for development of these microangiopathies.

Evidently, not all cells in the body absorb glucose via an insulin-depended pathway. Cells such as lens epithelia, renal papilla and cortical cells, Schwann cells in peripheral nerves and islets of Langerhans in the pancreas absorb glucose in an insulin-independent fashion from the blood (Jannapureddy et al., 2021). Thus so long as glucose is presence is circulation, these cells will always absorb it for their cellular use. Notably, aldose reductase is highly localized in the lens, renal and Schwann cells worsening the problem. Consequently, in diabetic patients, uncontrolled high blood glucose levels provide a good substrate aldose reductase to act upon and induce cellular damage leading to rise in retinopathy, neuropathy and nephropathy.

Inhibition of the rate-limiting enzyme, aldose reductase has been demonstrated by use of phytochemicals and specific chemical and drug compounds. These include Ellagic acid, quercetin, Kaempferol, Mangiferin and curcumin from *Myrciaria dubia* (Ciddi & Dodda, 2014), *Trigonella-foenum graceum* (Nagulapalli Venkata et al., 2017), green leafy vegetables (Dabeek & Marra, 2019), *Salacia chinensis* (Vyas et al., 2016; Irondi et al., 2014), and *Curcuma longa* (Kondhare et al., 2019) respectively. These phytochemicals even at micro concentrations have potent inhibitory effect with phytochemicals such as Ellagic acid inhibiting both aldose reductase and sorbitol dehydrogenase (Ciddi & Dodda, 2014). Moreover, chemical and drug compounds have failed to meet safety levels at clinical trials and are not approved for public use. Their extraction and commercialization however is expensive and possess a threat to sustainable production. As such, this study aimed to screen online databases for chemical compounds similar to Ellagic acid, Kaempferol and Mangiferin and analyze their pharmacokinetic, toxicological and docking profile using computational methods.

METHODS

Table 1 below shows the websites and software used to conduct this study. Specific details on how they were used is elaborated within the various methodology subsections.

Table 1

Materials and Tools Used to Conduct the Study Methodology

Activity	Material/tool
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)
Pharmacokinetic analysis	• SwissADME
Toxicity analysis	• Protox-II (RRID:SCR_018506)

Study Design & Target Validation

This study utilized an *in-silico* study design and was carried out in the school of pharmacy, Kabarak University. To validate whether Ellagic acid, Kaempferol and Mangiferin bind aldose reductase, target prediction was conducted using the online tool, SwissTargetPrediction. The predicted probability for binding to aldose reductase for Ellagic acid, Kaempferol and Mangiferin was found to be 1.00 (100%) for each phytochemical. Sulindac was used as the comparator in this study due to its proven inhibitory activity against aldose reductase.

Ligand-Based Virtual Screening

Canonical smiles of Ellagic acid, Kaempferol and Mangiferin were each obtained from Pubchem website. Using the obtained canonical smiles, a combined screening of the ZINC database (RRID:SCR_006082) for drug-like analogues was done using SwissSimilarity online tool. The database is open access and contains millions of chemical compounds and their structure. Screening result was downloaded as an Excel file for each of the three phytochemicals and had canonical smiles and similarity index of ZINC analogues. A sample size of 20 analogues for each phytochemical for further analysis was agreed upon consensually by the authors. Sampling criteria was based on highest similarity index and therefore; the first 20 screened ZINC analogues with highest similarity index for each phytochemical were isolated for further analysis. Ellagic acid had a total of 4 ZINC analogues from the screening test and all were included for further analysis. Thus, a total of 44 ZINC analogues formed our study population. Canonical smiles for each of the 44 ZINC analogues were sketched using the online tool Pubchem Sketcher v2.4 and the sketched compounds saved and downloaded as MDL molfile.

Structure-Based Virtual Screening

Sketched ZINC analogues, sulindac and the three phytochemicals were converted to their 3D format and optimized using the software Avogadro (RRID:SCR_015983) at the set force field of the MMFF94s. Afterwards, the optimized counterparts of ZINC analogues, sulindac and the three phytochemicals were each minimized using the software-UCSF Chimera v1.16 (RRID:SCR_004097) to reduce their total energies.

Docking Analysis

Structure of the aldose reductase protein (PDB ID 3rx4) was downloaded from Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) (RRID:SCR_012820) as a .pdb file. Non-standard residues and non-standard amino acids present in the protein were removed using UCSF Chimera v1.16 (RRID:SCR_004097) and saved as .pdb file. AutoDock Vina (RRID:SCR_011958) embedded in UCSF chimera was used to carry out surface binding analysis (docking) of the 44 ZINC analogs, sulindac and the three phytochemicals to the standardized aldose reductase enzyme based on procedure stipulated by Eberhardt et al. (2021) and Trott & Olson (2009). The corresponding docking scores for the 44 ZINC analogs, sulindac and the three phytochemicals were then recorded. Selected complexes formed through interaction of the enzyme protein and the 44 ZINC analogs, sulindac and the three phytochemicals were visualized using BIOVIA Discovery Studio v21.1.0.20298 (RRID:SCR_015651).

Pharmacokinetic Analysis

The SwissADME online tool was used to predict pharmacokinetic profile of the 44 ZINC analogs, sulindac and the three phytochemicals. Specifically, parameters such as conformity with Lipinski rules, gastrointestinal (GI) absorption, blood-brain barrier permeation, efflux by P-glycoprotein (P-gp) pump and interactions with cytochrome P450 (CYP450) enzymes were examined. This was carried out by entering the canonical smiles of the 44 ZINC analogs, sulindac and the three phytochemicals into the SwissADME online tool. Results were then recorded in table format.

Toxicology Analysis

Further, Protox-II (RRID:SCR_018506) was used to predict toxicology profile of the 44 ZINC analogs, sulindac and the three phytochemical. This was also done by entering the canonical smiles of the 44 ZINC analogs, sulindac and the three phytochemicals into the Protox-II online tool. Toxicology profile assessed constituted predicting the LD50, potential to cause hepatotoxicity, carcinogenicity, immunogenicity, mutagenicity, cytotoxicity and potential capability of activating pathways associated with nuclear signaling and stress signaling. Obtained results were then recorded in table format.

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 collected data (Research license no. *NACOSTI/P/23/2441)* was obtained from the National Commission for Science, Technology and Innovation (NACOSTI). No consent for participation was required since this study was purely an *in-silico* study.

RESULTS

A. Sampled Plant Species and Their Phytochemicals

Table 2 below depicts the target validation results. All the three phytochemicals had a 100% predicted probability of binding to aldose reductase enzyme. This was used to validated claims obtained from literature asserting that Ellagic acid, Kaempferol and mangiferin bind and interact with aldose reductase enzyme.

Table 2

Predicted Probability of Ellagic acid, Kaempferol and Mangiferin binding to Aldose Reductase

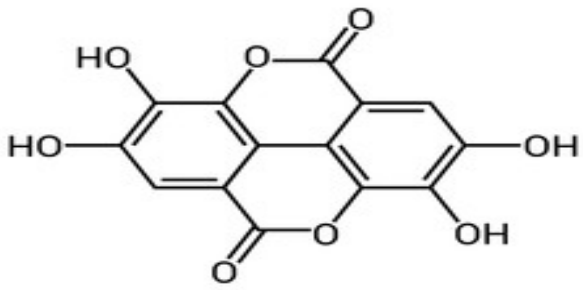
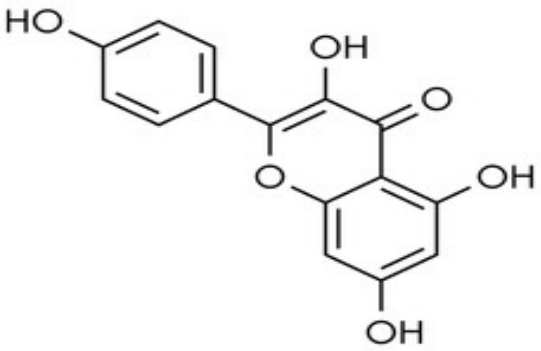
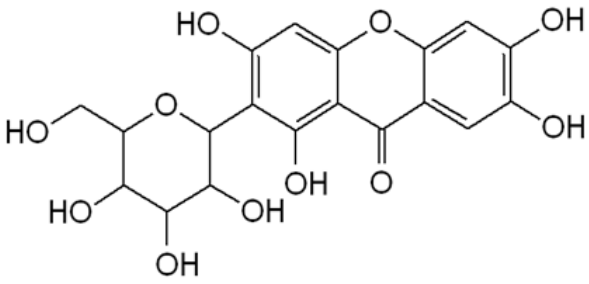
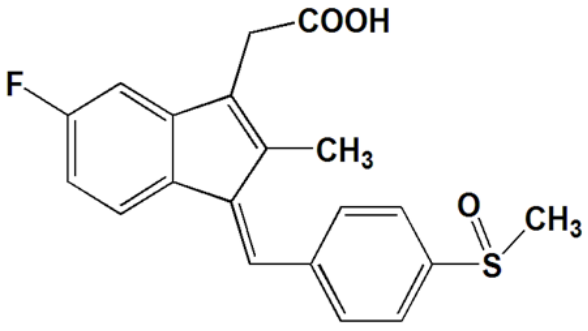
Plant species	Phytochemical	Predicted probability
Myrciaria dubia	Ellagic acid	1.00
Green leafy vegetables	Kaempferol	1.00
Salacia chinensis	Mangiferin	1.00

B. Structure of Ellagic Acid, Kaempferol, Mangiferin and Sulindac

Table 3

Table 3 below shows the chemical structures of Ellagic acid, Kaempferol, mangiferin and sulindac in 2D format. Evidently, the phytochemicals are made of rings with Ellagic acid having rigid compact rings which give it a planar structure. Notably, all have a chromene portion within their structure.

Chemical Structures of Ellagic Acid, Kaempferol, Mangiferin and Sulindac

Ellagic Acid	Kaempferol
	
Mangiferin	**Sulindac**
	

Image(s) used obtained from Shutterstock.com.

C. Docking Scores of Selected Phytochemicals and ZINC compounds

Figure 1 below shows the similarity indices and docking scores of ZINC analogues in comparison with Ellagic acid and sulindac. *ZINC000003872446* had 100% similarity to Ellagic acid. On docking analysis, *ZINC000003872446* (-8.6) and *ZINC000005784243* (-8.6) were modelled to have slightly stronger binding strength than Ellagic acid (-8.4).

Figure 1

Comparison of Docking and Similarity Scores of ZINC Compounds, Ellagic Acid and Sulindac

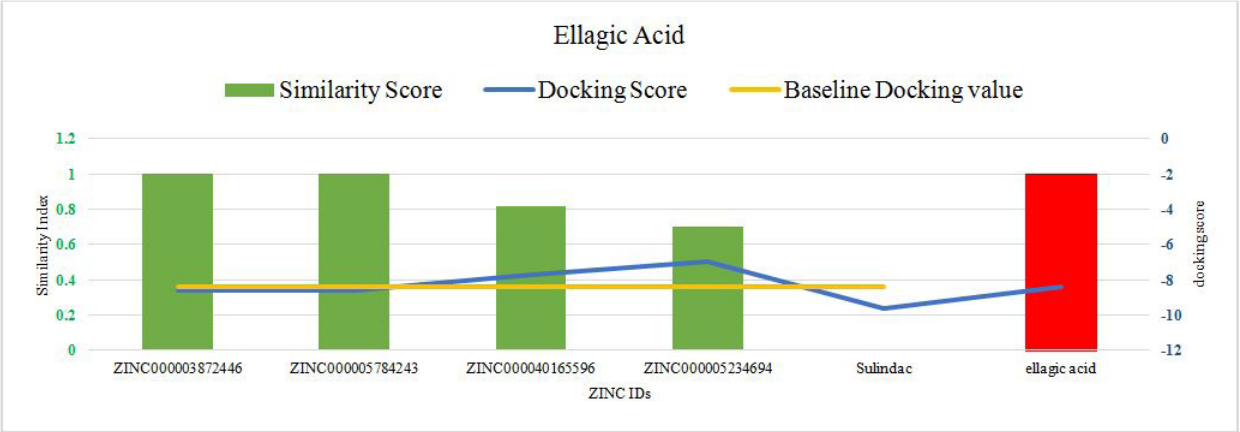


Figure 2 below shows the similarity indices and docking scores of ZINC analogues in comparison with Kaempferol and sulindac. On analysis, four ZINC analogues *(ZINC000006093351, ZINC000033980812, ZINC000033980813* and *ZINC000012359395)* were 99.9% similar to Kaempferol; ten ZINC analogues were 99.8% similar to Kaempferol while the remaining ZINC analogues were 99.7% similar to Kaempferol. Docking analysis showed that 14 out of the 20 analogues had docking scores below that of Kaempferol (-8.7). Eight of these compounds *(ZINC000033980812, ZINC000033980813, ZINC000012359395, ZINC000004098600, ZINC000003871576, ZINC000005004393, ZINC000006411540* and *ZINC000005842416)* had docking scores ≤-10 while the remaining six were distributed between -8.7 and >-10. Comparatively, ZINC000575623588 and the above eight stated analogues had binding scores lower than sulindac (-9.6).

Figure 2

Comparison of Docking and Similarity Scores of ZINC Compounds, Kaempferol and Sulindac

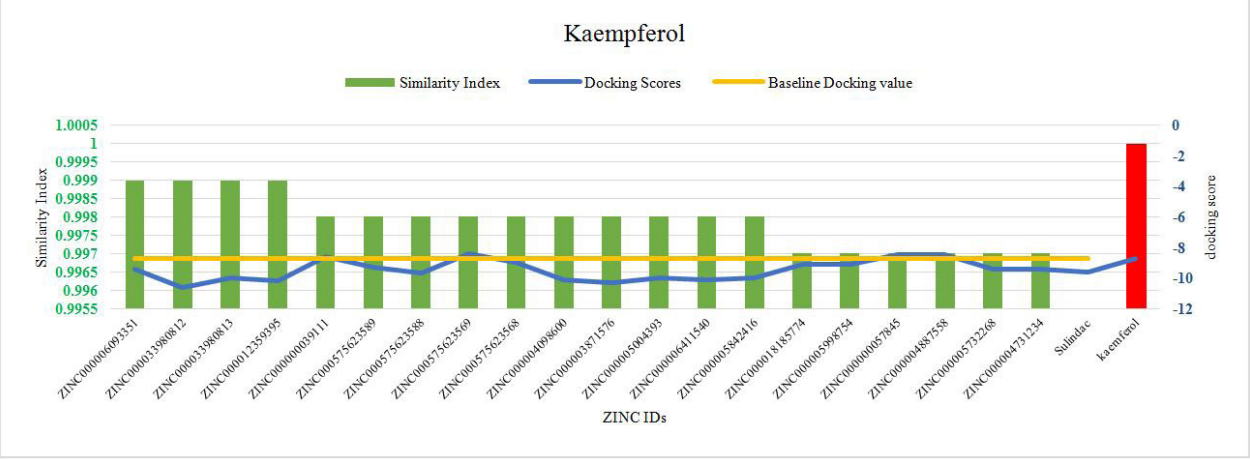
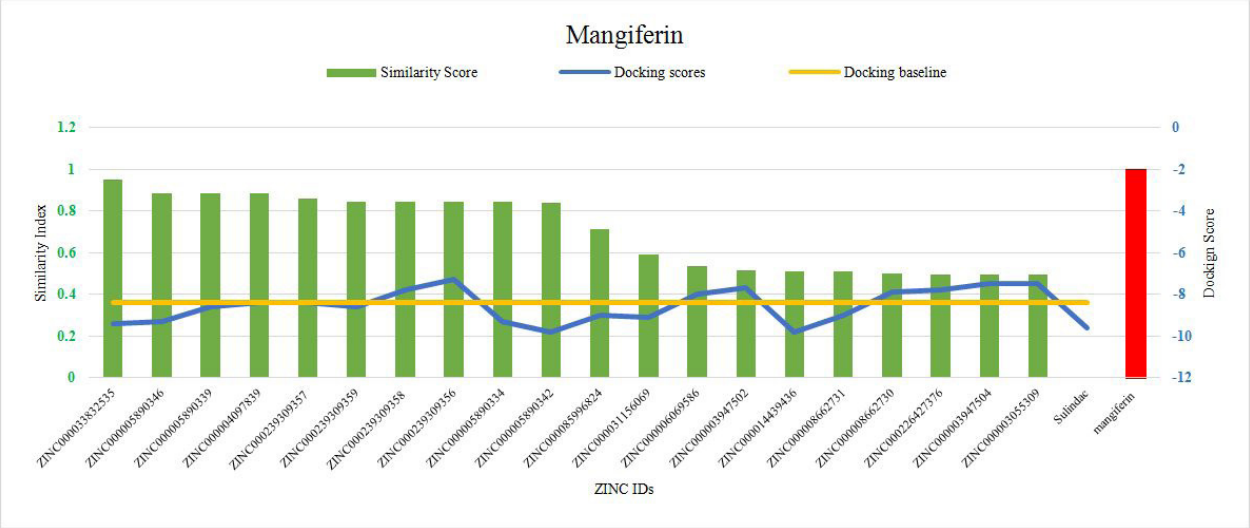


Figure 3 below shows the similarity indices and docking scores of ZINC analogues in comparison with Mangiferin and sulindac. On analysis: the similarity indices of the 20 analogues was widely distributed with highest and lowest similarity indices being 94.8% and 49.4% respectively. Docking analysis showed that 12 out of the 20 analogues had docking scores below or equal to that of Mangiferin (-8.4). None of the 12 compounds registered a docking score ≤ -10. Comparatively, only *ZINC000005890342* and *ZINC000014439436* had docking scores lower than that of sulindac (-9.6).

Figure 3

Comparison of Docking and Similarity Scores of ZINC Compounds, Mangiferin and Sulindac



D. Pharmacokinetic and Toxicology Profile of Selected Phytochemicals and ZINC Compounds

Table 4 describes the modelled pharmacokinetic profile of Ellagic acid and its *ZINC* analogues in comparison with that of sulindac. All the analogues and parent compound were predicted to: obey Lipinski rule of 5 without any violation, have a high gastrointestinal absorption, neither penetrated blood brain barrier nor was a P-glycoprotein substrate, and inhibited the enzymes *CYP1A2* (except *ZINC000005234694*). Only *ZINC000040165596* inhibited CYP3A4 and neither analogues nor Ellagic acid inhibited *CYP2C19, CYP2C9* and *CYP2D6*. Comparatively, sulindac inhibited all analyzed enzymes except *CYP1A2* and *CYP2D6*.

All analogues were predicted to be safe chemically as indicated by their toxicology class and LD50 values; were not mutagenic and neither caused hepatotoxicity nor cytotoxicity. All analogues except *ZINC000005234694* were moderately carcinogenic with *ZINC000040165596* have a very high activity confidence score (0.84). All analogues did not activate AR, aromatase, ARLBD, ERα, ERLBD, PPARδ, ARE, HSFRE, MMP, TS-P53 and ATAD5 pathways. Additionally, only *ZINC000005784243* was predicted to be a moderate activator of AHR pathway.

Table 4

Analysis of Pharmacokinetic and Toxicology Profile of Ellagic Acid and Sulindac in Comparison with Its (Ellagic Acid) ZINC Analogues

		Sulindac	Ellagic acid	*ZINC000003872446*	*ZINC000005784243*	*ZINC000040165596*	*ZINC000005234694*
Similarity index			1	1	0.999	0.817	0.698
Docking scores		-9.6	-8.4	-8.6	-8.6	-7.7	-7.0
LD50 [mg/kg] (toxicology class)		264 (3)	2991 (4)	2991 (4)	3200 (5)	5370 (6)	3200(5)
Lipinski violation		0	0	0	0	0	0
GI absorption		High	High	High	Low	High	High
P-gp substrate		No	No	No	No	No	No
BBB permeation		No	No	No	No	Yes	No
CYP-enzyme inhibition	*CYP1A2*	No	Yes	Yes	Yes	Yes	No
	CYP2C19	Yes	No	No	No	No	No
	CYP2C9	Yes	No	No	No	No	No
	CYP2D6	No	No	No	No	No	No
	CYP3A4	Yes	No	No	No	Yes	No
Hepatotoxicity		Active (0.8)	Inactive	inactive	inactive	inactive	inactive
Carcinogenicity		Inactive	Active (0.59)	active (0.59)	active (0.54)	active (0.84)	inactive
Immunogenicity		Inactive	Inactive	inactive	inactive	inactive	inactive
Mutagenicity		Inactive	Inactive	inactive	inactive	inactive	inactive
Cytotoxicity		Inactive	Inactive	inactive	inactive	inactive	inactive
Activation of NRSPs		Inactive	Inactive	Inactive	Inactive	Active (AHR)	Inactive
Activation of SRPs		Inactive	Inactive	Inactive	Inactive	Active (MMP)	Active (MMP)

Table 5 describes the modelled pharmacokinetic profile of Kaempferol and its *ZINC* analogues in comparison with that of sulindac. All the analogues and parent compound were predicted to: obey Lipinski rule of 5 without any violation, have a high GI absorption, neither penetrated blood brain barrier nor were P-glycoprotein substrate, and inhibited the enzymes *CYP1A2, CYP2D6* and *CYP3A4*. Neither analogues nor Kaempferol inhibited *CYP2C19*, and *CYP2C9*. Fourteen analogues were predicted to be safe chemically while remaining six were slightly toxic as indicated by their toxicology class and LD50 values. All analogues were predicted to be non-hepatotoxic, non-immunogenic and non-cytotoxic. Eleven and six of the analogues might be slightly carcinogenic and mutagenic respectively. All analogues did not activate AR, ARLBD, PPARδ (except *ZINC000003871576*), ARE, HSFRE, TS-P53 (except *ZINC000003871576* and *ZINC000005004393*) and ATAD5 pathways. All analogues were predicted to activate the AHR, ERα, ERLBD and MMP pathways. Nine, four and two analogues activated the aromatase, ATAD5 and TS-P53 pathways respectively.

Table 5

Analysis of Pharmacokinetic and Toxicology Profile of Kaempferol and Sulindac in Comparison with Its (Kaempferol) ZINC Analogues

		Sulindac	Kaempferol	*ZINC000006093351*	*ZINC000033980812*	*ZINC000033980813*	*ZINC000012359395*	*ZINC000000039111*	*ZINC000575623589*	*ZINC000575623588*	*ZINC000575623569*	*ZINC000575623568*	*ZINC000004098600*	*ZINC000003871576*	*ZINC000005004393*	*ZINC000006411540*	*ZINC000005842416*	*ZINC000018185774*	*ZINC000005998754*	*ZINC000000057845*	*ZINC000004887558*	*ZINC000005732268*	*ZINC000004731234*
Similarity index			1	0.999	0.999	0.999	0.999	0.998	0.998	0.998	0.998	0.998	0.998	0.998	0.998	0.998	0.998	0.997	0.997	0.997	0.997	0.997	0.997
Docking scores		-9.6	-8.7	-9.4	-10.6	-10	-10.2	-8.6	-9.3	-9.7	-8.4	-9	-10.1	-10.3	-10	-10.1	-10	-9.1	-9.1	-8.5	-8.5	-9.4	-9.4
LD50 (toxicology class)		264 (3)	3919 (5)	4000 (5)	159 (3)	159 (3)	3919 (5)	159 (3)	159 (3)	159 (3)	3919 (5)	3919 (5)	159 (3)	2500 (5)	3919 (5)	3919 (5)	3919 (5)	3919 (5)	4000 (5)	4000 (5)	3919 (5)	3919 (5)	5000 (5)
Lipinski violation		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GI absorption		High	High	High	High	High	High	High	High	High	High	High	High	High	High	High	High	High	High	High	High	High	High
P-gp substrate		No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
BBB permeation		No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
CYP-enzyme inhibition	*CYP1A2*	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	CYP2C19	Yes	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
	CYP2C9	Yes	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
	CYP2D6	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	CYP3A4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Hepatotoxicity		Active (0.8)	inactive	inactive	inactive)	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive
Carcinogenicity		Inactive	Inactive	inactive	active (0.68)	active (0.68)	active (0.71)	active (0.71)	active (0.71)	active (0.71)	inactive	inactive	active (0.68)	inactive	inactive	inactive	active (0.68)	active (0.68)	inactive	inactive	active	inactive	active (0.60)
Immunogenicity		Inactive	Inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	active (0.55)	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive
Mutagenicity		Inactive	Inactive	inactive	active (0.51)	active (0.51)	inactive	inactive	inactive	inactive	inactive	inactive	active (0.51)	inactive	inactive	inactive	active (0.51)	active (0.51)	inactive	inactive	active (0.51)	inactive	inactive
Cytotoxicity		Inactive	Inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive
Activation of NRSPs		Inactive	Active	Active (AHR, ERα, ERLBD)																			
Activation of SRPs		Inactive	Active	Active (MMP)																			

Table 6 describes the modelled pharmacokinetic profile of Mangiferin and its *ZINC* analogues in comparison with that of sulindac. All the analogues and parent compound were predicted to: obey Lipinski rule of 5 without any violation (except Mangiferin itself that had > 10 and 5 N or H and NH or OH atoms respectively), have a low GI absorption (except *ZINC000085996824*), did not penetrated blood brain barrier and did not inhibit the enzymes *CYP1A2, CYP2C19, CYP2C9*, and *CYP2D6*. Eight of the 20 analogues were P-gp substrate while seven inhibited the enzyme CYP3A4. Eight analogues were predicted to be safe chemically while eleven were slightly toxic as indicated by their toxicology class and LD50 values. *ZINC000014439436* had a very high toxic level (7mg/kg). All analogues were predicted to be non-hepatotoxic, non-cytotoxic and non-carcinogenic (except *ZINC000085996824*). Nine and eleven of the analogues might be slightly immunogenic and mutagenic respectively. All analogues did not activate AR, AHR (except *ZINC000033832535*), aromatase, ARLBD, ERα, PPARδ (except *ZINC000003871576*), ARE, HSFRE, MMP (except *ZINC000085996824* and *ZINC000014439436*) and ATAD5 pathways. Only four analogues were predicted to slightly activate TS-P53 pathway.

Table 6

Analysis of Pharmacokinetic and Toxicology Profile of Mangiferin and Sulindac in Comparison with Its (Mangiferin) ZINC Analogues

		Sulindac	Mangiferin	*ZINC000033832535*	*ZINC000005890346*	*ZINC000005890339*	*ZINC000004097839*	*ZINC000239309357*	*ZINC000239309359*	*ZINC000239309358*	*ZINC000239309356*	*ZINC000005890334*	*ZINC000005890342*	*ZINC000085996824*	*ZINC000031156069*	*ZINC000006069586*	*ZINC000003947502*	*ZINC000014439436*	*ZINC000008662731*	*ZINC000008662730*	*ZINC000226427376*	*ZINC000003947504*	*ZINC000003055309*
Similarity index				0.948	0.885	0.883	0.883	0.859	0.846	0.844	0.844	0.843	0.839	0.712	0.592	0.536	0.516	0.511	0.511	0.498	0.496	0.496	0.494
Docking scores		-9.6	-8.4	-9.4	-9.3	-8.6	-8.4	-8.4	-8.6	-7.8	-7.3	-9.3	-9.8	-9	-9.1	-8	-7.7	-9.8	-9	-7.9	-7.8	-7.5	-7.5
LD50 (toxicology class)		264 (3)	2 (1)	832 (4)	832 (4)	832 (4)	832 (4)	832 (4)	832 (4)	832 (4)	832 (4)	832 (4)	832 (4)	1213 (4)	5000 (5)	5000 (5)	2500 (5)	7 (2)	5000 (5)	5000 (5)	5000 (5)	2500 (5)	5000 (5)
Lipinski violation		0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
GI absorption		High	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	High	Low	Low	Low	Low	Low	Low	Low	Low	Low
P-gp substrate		No	No	No	No	No	No	No	No	No	No	No	No	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
BBB permeation		No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
CYP-enzyme inhibition	*CYP1A2*	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
	CYP1A2	Yes	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
	CYP1A2	Yes	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
	CYP1A2	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
	CYP1A2	Yes	No	No	No	No	No	No	No	No	No	No	No	Yes	No	No	Yes	No	Yes	Yes	Yes	Yes	Yes
Hepatotoxicity		Active	Inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive
Carcinogenicity		Inactive	Inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	active (0.52)	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive
Immunogenicity		Inactive	Inactive	active (0.5)	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	active (0.80)	inactive	inactive	active (0.66)	active (0.99)	active (0.82)	active (0.82)	active (0.82)	active (0.66)	active (0.82)
Mutagenicity		Inactive	Active	inactive	active (0.54)	active (0.54)	active (0.54)	active (0.54)	active (0.54)	active (0.54)	active (0.54)	active (0.54)	active (0.54)	inactive	active (0.75)	active (0.75)	inactive	inactive	inactive	inactive	inactive	inactive	inactive
Cytotoxicity		Inactive	Inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive	inactive
Activation of NRSPs		Inactive	Inactive	Inactive																			
Activation of SRPs		Inactive	inactive	Inactive																			

E. Model Visualization of Interaction of Selected Phytochemicals, *ZINC* Compounds with Aldose Reductase Enzyme

Table 6 below displays the pictorial representation of aldose reductase in 3D model. Figure 5A shows the peptide chains (as different colors) that constitute the entire enzyme protein. Figure 5B displays the hydrophobic surface of the active binding site of aldose reductase. Figure 5C shows how the molecule sulindac fits into the binding pocket while figure 5D shows bond interaction between sulindac and enzyme amino acids.

Table 7

Model Visualization of Aldose Reductase Enzyme and Sulindac

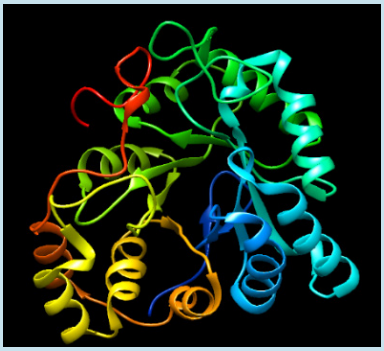
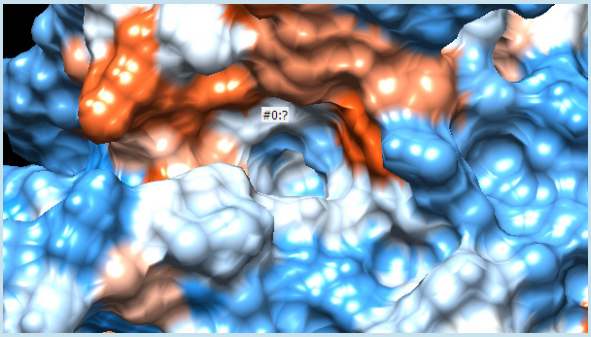
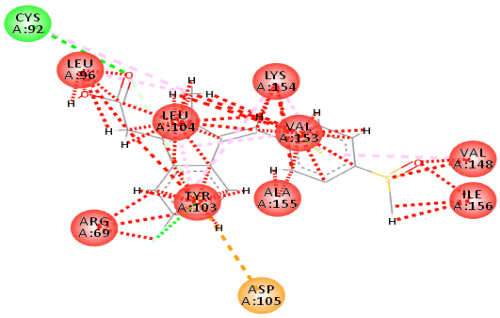
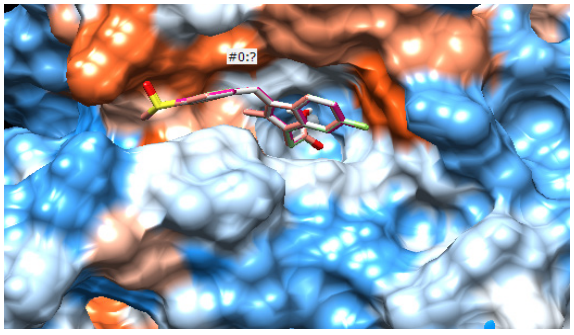
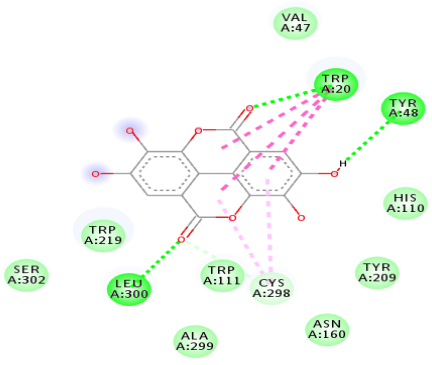
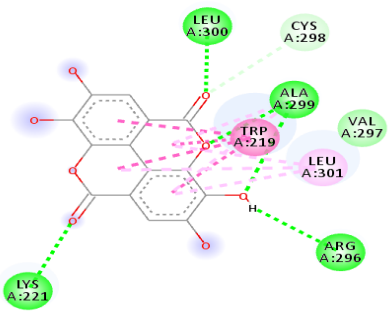
Aldose reductase	
3D receptor	Hydrophobicity surface (binding site)
	
Sulindac	
Ligand-receptor interaction	Hydrophobicity surface
	

Table 8 below shows interaction of Ellagic acid and its highest binding analogue- *ZINC000003872446* with aldose reductase enzyme. Main bond interactions are conventional hydrogen bonding and pi-pi stacking. Aldose reductase amino acids that were involved in bonding were TRP A:20, LEU A:300, CYS A:298 and TYR A:48 in Ellagic acid. For *ZINC000003872446*, LEU A:300, A:301, CYS A:298, LYS A:221, ARG A:296, TRP A:219 and ALA A:299.

Table 8

Model Visualization of Interaction Between Ellagic Acid and Its Overall Best Similar ZINC with Aldose Reductase

Ligand-receptor interaction	
Ellagic acid	*ZINC000003872446*
	

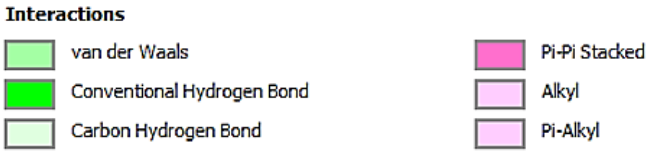


Table 9 below shows interaction of Kaempferol and its highest binding analogue; *ZINC000005004393* with aldose reductase enzyme. Man bond interactions were hydrogen bonding, pi-pi stacking and pi-sulfur bonding. Enzyme amino acids involved in bonding were TYR A:209, TRP A:20, GLN A:183, ILE A:260 and CYS A:298. For *ZINC000005004393*, ASP A:224, LYS A:221, TRP A:219, LEU A:301, CYS A:298 and ALA A:299.

Table 9

Model Visualization of Interaction Between Ellagic Acid, Its Overall Best Similar ZINC with Aldose Reductase

Kaempferol (Ligand-receptor interaction)	
Kaempferol	*ZINC000005004393*

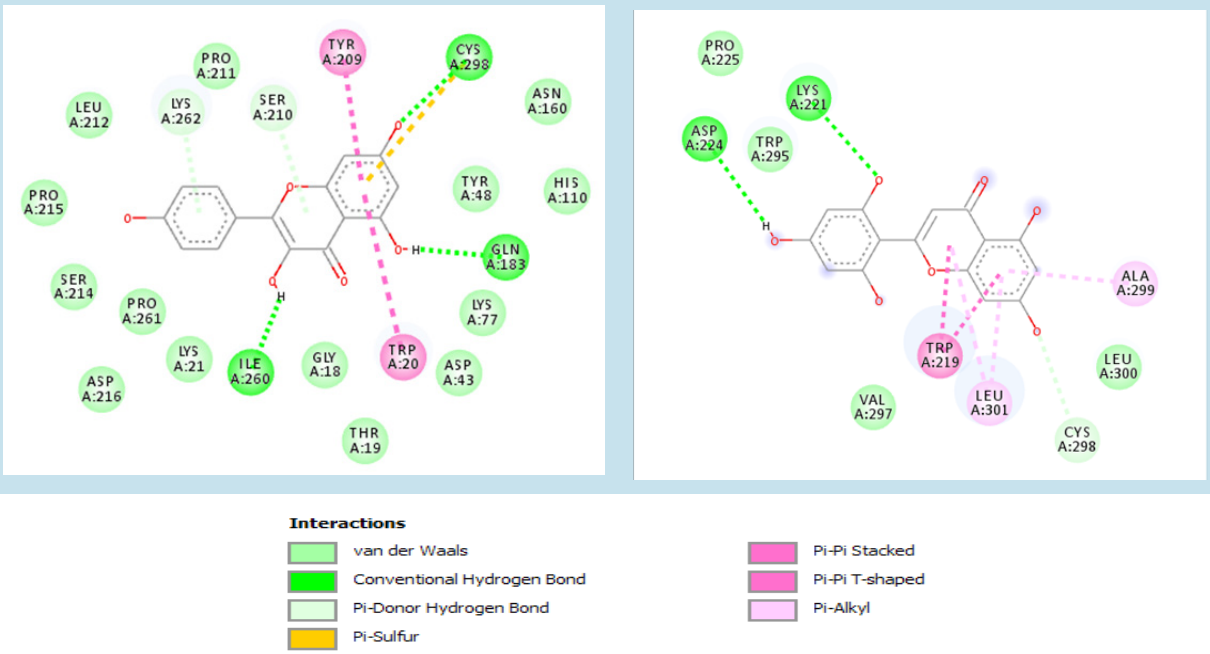
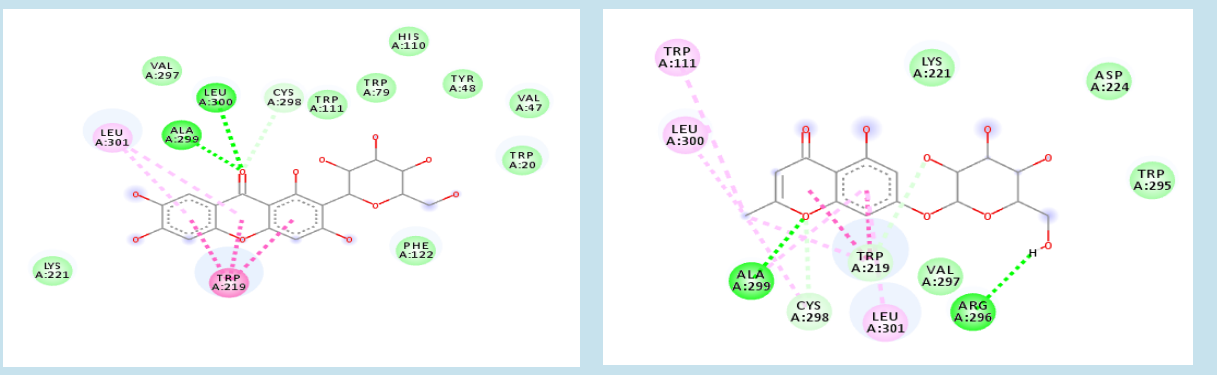


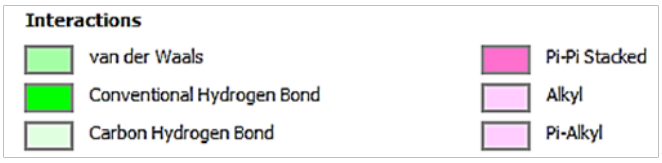
Table 10 below shows interaction of Mangiferin and its highest binding analogue; *ZINC000031156069* with aldose reductase enzyme. Amino acids involved in bonding were TRP A:219, LEU A:300, ALA A:299, LEU A:301 and CYS A:298. For *ZINC000031156069*, TRP A:111, A:219, LEU A:300, A:301, ALA A:299, CYS A:298 and ARG A:296.

Table 10

Model Visualization of Interaction Between Ellagic Acid, Its Overall Best ZINC Analogue with Aldose Reductase

Ligand-receptor interaction	
Mangiferin	*ZINC000031156069*





DISCUSSION

The sorbitol pathway though quiescent in normoglycemic conditions, its highly active in hyperglycemic conditions of DM. This is because, as earlier indicated, the rate limiting enzyme for this two-step process is highly localized in body tissues that do not require insulin to uptake glucose from circulation (Jannapureddy et al., 2021). Thus the high levels of glucose provide an ever present pool of substrate to be acted upon. For this reason, we can be almost sure that a person who develops DM and their sugars is not well-controlled will always develop either retinopathy, nephropathy or neuropathy sometime in the future. Since the '90s, development of aldose reductase inhibitors (ARIs) has been ongoing but till date, none has been approved for use in microvascular diabetic complications (Singh Grewal et al., 2015). This is largely due to their toxic side effects which raises concerns for patient toxicity. Studied ARIs commonly are derivatives of spirosuccinimide, carboxylic acids and pyridazones (Singh Grewal et al., 2015). Such derivatives in most cases are required in high amount to achieve the inhibitory effect required yet as such concentrations, they lead to development of toxic effects. Commonly known drugs such as acetylsalicylic acid (Zhu, 2013) and sulindac (Cousido-Siah et al., 2015) have inhibitory activity towards aldose reductase but in a clinical setting higher concentrations than the stipulated therapeutic doses are required to elicit such inhibitory effect. Unlike such synthetic compounds, specific plant phytochemicals are well demonstrated to potently inhibit aldose reductase even at micro molar concentrations. Phytochemicals such as quercetin, luteolin, Ellagic acid, Mangiferin and Kaempferol are currently known to be potent ARIs (Julius et al., 2022). Similarly, our target prediction revealed that Ellagic acid, Kaempferol and Mangiferin had a 100% probability for binding to aldose reductase.

Structurally, Ellagic acid, Kaempferol and Mangiferin comprises aromatic rings and polar groups such as hydroxyl and carbonyl moieties [Table 3]. Studies looking at structural activity relationship of aldose reductase have elucidated that ARIs need to: have a primary lipophilic group which in most cases is the aromatic ring and either a carbonyl or thiocarbonyl moiety that is 2.8 to 3.8A from the center of the primary lipophilic group (Rendell & Kirchain, 2000). Acetylsalicylic acid and sulindac conform to such specifications and thus the latter was used as our study comparator. Correspondingly as shown in Table 3, the three phytochemicals all conform to such SAR specifications validating their ARI activity. We thus sought to screen for similar compounds that are commercially available online. A total of 44 ZINC compounds were obtained: 4 for Ellagic acid while Kaempferol and Mangiferin had each 20 similar compounds.

Docking analysis showed that Kaempferol (-8.7) had strongest affinity for binding to aldose reductase with Ellagic acid and Mangiferin tying at -8.4 [*the more negative the docking scores the stronger the binding affinity*]. This could be attributed to the simpler structure of Kaempferol which enables it to fit in the hydrophobic pocket of aldose reductase. Ellagic acid is planar and rigid structurally and as such it only interacted with outer surface of the hydrophobic pocket. Similarly, Mangiferin is structurally complex and large. However, sulindac had stronger affinity (-9.6) than all three phytochemicals. Two *ZINC* compounds similar to Ellagic acid: *ZINC000003872446* and *ZINC000005784243* had a slightly stronger affinity (-8.6) than parent phytochemical [fig 1]. Fourteen of the 20 *ZINC* compounds similar to Kaempferol had stronger affinity (<-8.7) with 8 of the compounds scoring ≤-10 [fig 2]. Lastly, 12 of the 20 compounds similar to Mangiferin had stronger affinity than parent phytochemical (<-8.4) but none scored ≤-10 [fig 3]. Evidently, Kaempferol similar compounds had highest binding affinity compared to analogues of Ellagic acid and Mangiferin.

On *in silico* pharmacokinetic (PK) analysis, all three phytochemicals: were not substrates for P-glycoprotein (P-gp), did not cross the blood-brain barrier (BBB) and were not inhibitors of *CYP-2C19* and *-2C9*. Both Ellagic acid and Kaempferol had a high predicted gastrointestinal (GI) absorption

and inhibited *CYP1A2*. Only Kaempferol was predicted to inhibit *CYP2D6* and *CYP2A4* while Mangiferin had low GI absorption, did not inhibit any of the Cytochrome enzymes and violated two of the Lipinski rules [had > 10 N or H and > 5 NH or OH atoms]. Pharmacokinetic analysis of *ZINC* analogues for Ellagic acid showed similar PK results as their parent phytochemical except *ZINC000040165596* and *ZINC000005234694* that inhibited CYP3A4; evidently also, by virtue of their less binding affinity (>-8.4) they may not be considered for further analysis [table 4]. Similarly, all *ZINC* analogues for Kaempferol also depicted PK results similar to their parent phytochemical compound [table 5]. In so doing, by virtue of them inhibiting CYP 1A2, CYP2D6 and CYP3A4, potential drug interactions are inevitable if they are to be formulated to a drug. *ZINC* analogues of Mangiferin had a low GI absorption (except *ZINC000085996824*), neither penetrated BBB nor inhibited *CYP1A2, CYP2C19, CYP2C9,* and *CYP2D6*. However, 8 of the 20 analogues were P-gp substrate while 7 analogues inhibited the enzyme *CYP3A4* [table 6]. While analogues of Mangiferin had reduced enzyme interactions, most of the analogues had a low predicted GI absorption [table 6]; further though analogues of Kaempferol had strongest affinity, each was predicted to at least inhibit three of the cytochrome P450 enzymes [table 5. Thus in terms of PK, analogues of Ellagic acid depicted better profile with no Lipinski violation, high GI absorption and ignition of only CYP1A2 which plays minor role in drug metabolism as compared to the other enzymes.

Toxicology prediction showed that Kaempferol was safer followed by Ellagic acid and finally Mangiferin since their corresponding LD50 were 3919 mg/kg, 2991 mg/kg and 2 mg/kg respectively. Evidently, Mangiferin is too toxic to be even considered for formulation. Further, toxicity model report showed that Ellagic acid could be potentially carcinogenic while Mangiferin could be mutagenic. Kaempferol as shown in table 5 was predicted to potentially activate nuclear receptor signaling pathways [specifically aryl hydrocarbon receptor, aromatase, estrogen receptor alpha and estrogen receptor ligand binding domain] and stress response pathways [mitochondrial membrane potential]. This strongly puts Kaempferol on the watch out and could be disregarded for further development. All Ellagic acid analogues were predicted: to be safe chemically as indicated by their LD50 values; were not mutagenic and neither caused hepatotoxicity nor cytotoxicity, did not activate AR, aromatase, ARLBD, ERα, ERLBD, PPARδ, ARE, HSFRE, MMP, TS-P53 and ATAD5 pathways [table 4]. However, three analogues were predicted to be potentially carcinogenic while *ZINC000005784243* suggested to have activity in the AhR pathway.

All Kaempferol analogues were predicted to be non-hepatotoxic, non-immunogenic and non-cytotoxic [table 5]. Only 14 analogues were predicted to be safe as per their LD50 values. Further, 11 of the 20 Kaempferol ZINC analogues might be potentially carcinogenic while six could be mutagenic. All analogues could potentially activate nuclear receptor and stress response pathways. Lastly, all Mangiferin *ZINC* analogues did not activate nuclear receptor and stress response pathways except *ZINC000033832535*, *ZINC000003871576*, *ZINC000085996824* and *ZINC000014439436* compounds. Additionally, none was hepatotoxic or cytotoxic. However, 9 analogues were potentially immunogenic while 8 were mutagenic [table 6].

Overall, most if not all analogues for the three phytochemicals are potentially toxic based on their model toxicity reports. However, analyzing their docking and similarity scores, PK profile and toxicity report, analogues: *ZINC000005004393*, *ZINC000003872446* and *ZINC000031156069* for Kaempferol, Ellagic acid and Mangiferin respectively offer optimal characteristics required for further development. As depicted in table 8 to 10, these compounds majorly interact with amino acids in aldose reductase using hydrogen bonds and Pi-Pi-stacking. This shows that their interaction is reversible unlike if they were covalent bonds.

CONCLUSION

In conclusion:

- Kaempferol and *ZINC* analogues were predicted to have stronger binding affinity compared to Ellagic acid, Mangiferin and their analogues.
- Analogues of Ellagic acid were predicted to have better PK profile unlike analogues of Kaempferol and Mangiferin.
- Most analogues of the three phytochemicals are predicted to be potentially toxic but in mild to moderate severity.
- Overall, analogues *ZINC000005004393*, *ZINC000003872446* and *ZINC000031156069* for Kaempferol, Ellagic acid and Mangiferin respectively depicted best optimal characteristics required for further development.

RECOMMENDATIONS

While analogues ZINC000005004393, ZINC000003872446 and ZINC000031156069 depicted the optimal characteristics for further analysis, we recommend that all the 44 analogues to undergo in vitro analysis to validate the claims made by this prediction.

LIST OF ABBREVIATIONS

AGEs	Advanced Glycated End products
AHR	Aryl Hydrogen receptor
ALA	Alanine
AR	Androgen Receptor
ARE	Nuclear factor (erythroid-derived 2)-like 2/antioxidant
ARG	Arginine
ARIs	Aldose Reductase Inhibitors
ARLBD	Androgen Receptor Ligand Binding Domain
ASP	Aspartate
ATAD5	ATPase family AAA domain-containing protein 5
CYP	Cytochrome P450 enzyme
CYS	Cysteine
DM	Diabetes Mellitus
ERLBD	Estrogen Receptor Ligand Binding Domain
ERα	Estrogen receptor α
GI	Gastrointestinal
GLN	Glutamine
HSFRE	Heat shock factor response element
ILE	Isoleucine
LD50	Lethal dose
LEU	Leucine
LYS	Lysine
MMP	Mitochondrial Membrane Potential
NAD+	nicotinamide adenine dinucleotide phosphate
NADPH	Reduced nicotinamide adenine dinucleotide phosphate

PK	Pharmacokinetic
PPARδ	Peroxisome Proliferator Activated Receptor Gamma
TRP	Tryptophan
TS-P53	Phosphoprotein (Tumor Suppressor) p53
TYR	Tyrosine

DECLARATION

Availability of Data and Materials

The datasets generated and/or analyzed during the current study are available in the HAVARD repository, https://doi.org/10.7910/DVN/DCKULS.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contribution

FO, SM and CK contributed to conception, design, acquisition, analysis, interpretation and approval of submitted version.

RK contributed to conception, design, formatting, analysis and approval of submitted version.

TS contributed to design, formatting and approval of the submitted version.

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